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전북대학교
약학대학
신약개발연구소
정례 세미나



전북대학교
JEONBUK NATIONAL UNIVERSITY

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2022.09.14.

2022년 2학기 신임 교수 연구 내용 발표

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전북대학교 약학대학
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2022.09.14

RESEARCH



PHARMACOKINETIC STUDIES

- INTRODUCTION
- RESEARCH SUMMARY
- ONGOING RESEARCH
- RESEARCH PLAN

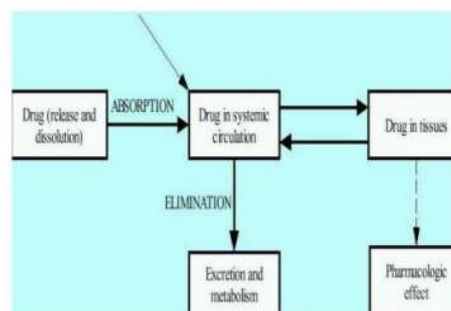
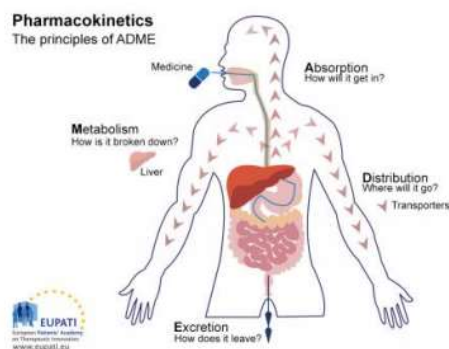
Pharmacokinetics

- 시간에 따른 약물의 흡수, 분포, 대사, 배설에 대한 학문
- What does the body do on the drug?
- **ADME: Absorption, Distribution, Metabolism, Excretion**
 - Absorption
 - Movement of a drug from the site of administration to bloodstream
 - Distribution
 - Reversible transfer of drug from one location to another w/i the body
 - Metabolism
 - Process of transformation of a drug within the body to make it more hydrophilic so that it can be excreted out from the body by the kidneys
 - Excretion
 - Removal of the substance from the body

Pharmacokinetics

Pharmacokinetics

The principles of ADME



투여된 용량에서의 시간에 따른 약물 농도 경과
Kinetics = **Motion**

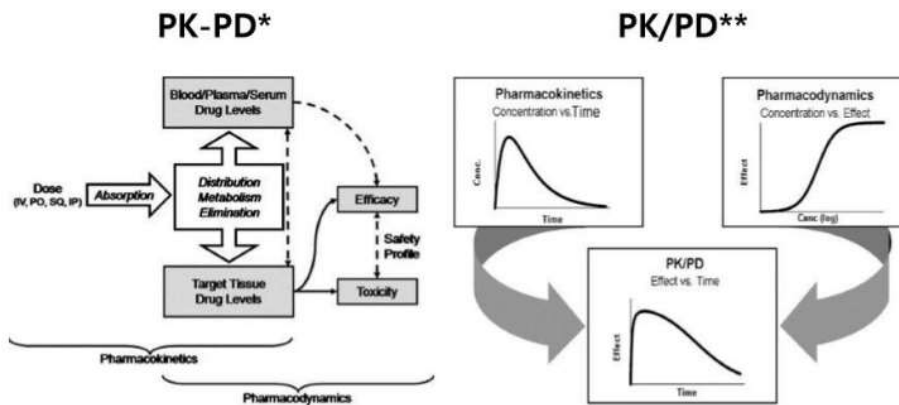
Clinical Pharmacokinetics

- Clinical: bedside (임상, 臨床)
- 각각의 환자에게 안전하고 효과적인 약물투여를 하기 위해 약동학적 원리를 적용
 - 개인 맞춤형 약물용량용법 (individualized dosage regimens)
 - 약물 치료하는 환자에게 약효를 높이고 독성을 낮추기 위함

Pharmacodynamics

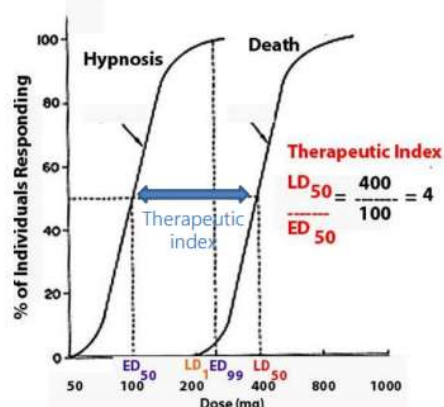
- 약물이 작용하는 위치의 약물의 농도와 치료효과와의 관계
- What does the drug do on the body?
- 시간에 따른 치료효과와 부작용 사이의 관계도 포함
- 수용체의 약물 농도 ~ 약효의 세기
 - 이 밖의 요인 : 세포 표면의 수용체 정도, 이차전령(second messenger)에 의해 신호가 세포 내로 전달 되는 기전, 유전자 번역(gene translation), 단백질 생산을 조절하는 요인

PK-PD versus PK/PD



Therapeutic Drug Monitoring (TDM)

- 혈장 약물 농도를 분석하는 과정과 분석된 농도를 해석하여, 안전하고 효과적인 투약법에 응용하는 것
- 경험적으로 투약하는 경우보다, 치료약물농도(Therapeutic Window)에 더 빠르고 안전하게 도달할 수 있게 됨

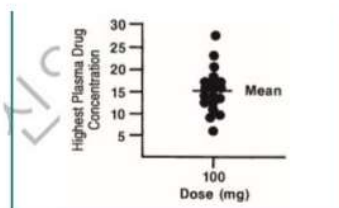


Therapeutic Drug Monitoring (TDM)

TDM is valuable when:

1. The drug's **desired pharmacologic effects** cannot be assessed readily
2. A **good correlation** exists between the **pharmacologic response** and **plasma concentration**.
3. The drug has a **narrow therapeutic index**
4. **Wide inter-subject variation** in plasma drug concentrations results from a given dose.

Therapeutic Drug Monitoring (TDM)



Inter-individual
variability
(개인간 차이)

■ 농도의 개인차 요인

- 약물 간의 상호작용 (Drug-Drug Interaction)
- 약물 대사와 제거에 대한 개개인의 능력 차이 (예. 유전적 요인)
- 약물 흡수, 분포, 대사, 제거를 변화시키는 질병 상태 (예. 신장, 간, 또는 심장), 생리학적 상태 (예. 비만, 나이)

Inter-individual variability

■ 나이

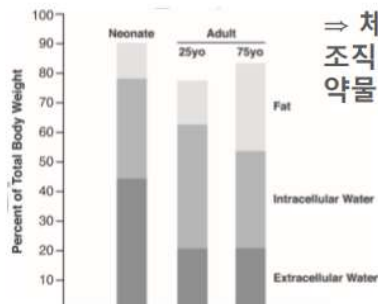
- **고령:** 주요 장기의 기능이 젊은 성인에 비해 현격히 떨어짐
 - ✓ 40살 이후 신장의 기능은 대략적으로 연간 1 mL/분씩 감소함.
- **신생아(특히 미숙아)와 노인:** 신장의 기능과 약물배설 능력이 많이 떨어짐. 간 기능 또한 매우 낮음.
 - ✓ 신생아의 경우, 유아기까지 신장의 기능이 성인과 동일한 수준까지 또는 그 이상으로 발달.
 - ✓ 소아 환자의 경우 성인에 비해 약의 대사속도가 빠르기 때문에 청소율이 높음. 더 자주 투여해야 할 수도 있음.

⇒ 약물을 제거하는 장기의 기능이 저하되면 해당 장기에서 제거되는 약물의 "투여간격"을 늘려야 함.

Inter-individual variability

■ 나이

- **체내 구성의 차이**
 - ✓ 신생아는 성인과 비교할 때, 체내수분 비율이 높고 지방비율은 상대적으로 낮음.
 - ✓ 나이가 들면서 체내수분 비율은 낮아지고, 조직의 지방함량은 높아짐.
 - ✓ 약물의 분포와 작용에 영향



⇒ 체내 수분과 지질의 함량이 달라지면, 조직에 분포하는 약물의 양이 달라지기 때문에 약물의 "투여용량"을 조절 해야함.

Inter-individual variability

■ 질병 상태

• 주로 간질환과 신장질환

- ✓ 약물의 청소율 저하, 약물의 체내 반감기 길어짐
→ 청소율만 감소 시, 투여용량은 비슷하게 투여간격만 늘림.

- ✓ 말단에 체액이 정체되어 약물 분포 용적이 커짐
→ 분포용적만 증가 시, 투여용량을 분포용적에 비례하게 변경. (투여 간격은 변화 없음)

Inter-individual variability

■ 유전적 요인과 약물유전학

- 약물 유전학(pharmacogenetics): 약물 효과의 다양성을 유발하는 유전자 전체의 다양성을 연구 하는 학문
- 유전적인 결손 (돌연변이): 1% 이하의 인구에서 드물게 나타남
- 유전적인 다양성: 인류 1% 이상에서 나타남.
- 약물대사효소의 유전적인 다양성
 - ✓ CYP2C9과 **warfarin**
 - ✓ VKORC1과 **warfarin**
 - ✓ CYP2C19와 **clopidogrel**

Inter-individual variability

■ 비만

• Thiopental

- 지용성, 지방 조직으로 잘 분포하는 약물
- 비만 환자에서 더 고용량을 투여해야 함.

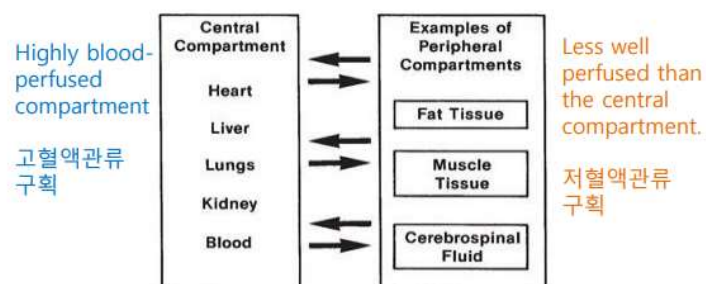
■ 약물상호작용

• Cimetidine

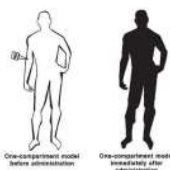
- Theophylline을 대사시키는 간효소를 저해하여 theophylline의 청소율을 감소시킴
- Theophylline의 투여간격을 늘려야 함.

Pharmacokinetic model

■ Compartment model



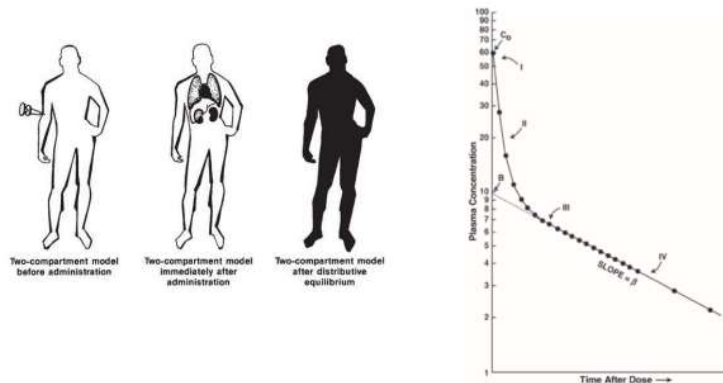
- One-compartment model: 가장 단순한 모델로 약물은 투여 후 즉각적으로 체내 전체로 분포



Pharmacokinetic model

Compartment model

- 2-Compartment model: 간, 신장과 같이 혈액이 많이 관류하는 장기로 빠르게 분포된 후, 비교적 느린 속도로 다른 장기에 분포. 약물의 소실은 Central에서만. Central과 Peripheral 평형 유지 위해 구획들 사이 이동



Pharmacokinetic model

Linear VS. Nonlinear pharmacokinetics

- Nonlinear pharmacokinetics : 약물의 투여량에 따라 약물동태가 변화

Process	Agent	Mechanism
Absorption	Riboflavin, methotrexate, gabapentin	Saturable gut wall transport
	Penicillins	Saturable decomposition in GI tract
Distribution	Methotrexate	Saturable transport into and out of tissues
	Salicylates	Saturable protein binding
Renal elimination	Penicillin G	Active tubular secretion
	Ascorbic acid	Active reabsorption
Extrarenal elimination	Carbamazepine	Enzyme induction
	Theophylline, phenytoin	Saturable metabolism

TABLE. Drugs Having Dose- or Time-Dependent Pharmacokinetics

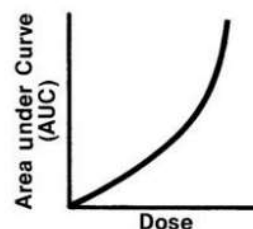


FIGURE. Relationship of AUC to drug dose with dose-dependent PKs.

RESEARCH

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RESEARCH SUMMARY ①

Extracorporeal membrane oxygenation (ECMO)

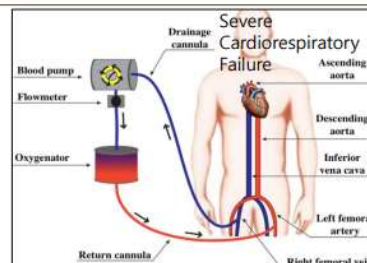


CICU (Cardiovascular intensive care unit) ward rounds
Pharmacist-led drug intervention

<http://www.workforcex.org>



Growing # of ECMO patients



ECMO could affect drug PK

No drug dosing Guideline



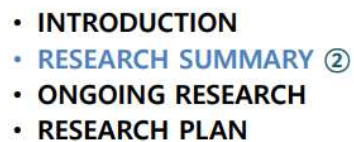
Optimal drug dose???

RESEARCH SUMMARY^①



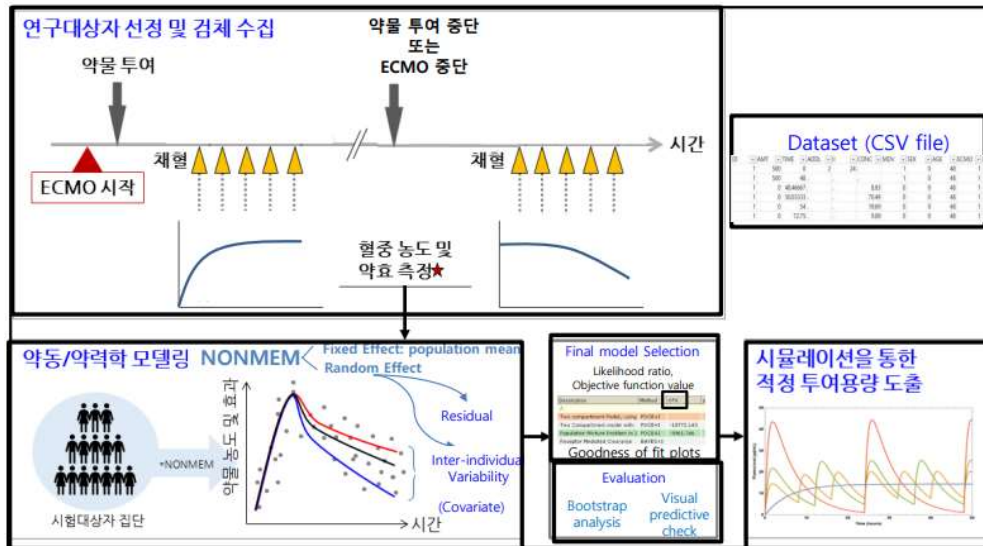
Study Design:

PHARMACOKINETIC STUDIES



PROSPECTIVE PK STUDY DESIGN

Study Design

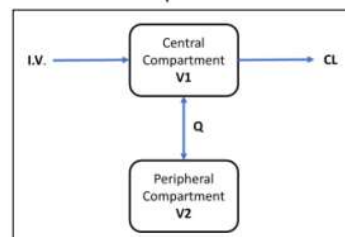


SUFENTANIL PK CHANGES DURING ECMO

Study Results: PK model

	Number/median (range) (n = 20)
Age (years)	55 (23-88)
Male	16
Body weight (kg)	69.4 (52.9-92.5)
Lean body weight (kg)	55.3 (36.8-58.6)
Body mass index (kg m ⁻²)	24.7 (20.5-31.8)
Indication for VA-ECMO	
Acute myocardial infarction	4
ST-elevation myocardial infarction	12
Non-ST-elevation myocardial infarction	4
APACHE II	29 (15-36)
Time between onset of MI and initiation of ECMO (h)	2 (0.5-12)
Blood chemistry, serum levels	
Total plasma protein (g dL ⁻¹)	4.5 (2.1-6)
Total bilirubin (mg dL ⁻¹)	1.9 (0.3-6.6)
Blood urea nitrogen (mg dL ⁻¹)	21.3 (7.5-58)
Serum creatinine (mg dL ⁻¹)	1.4 (0.4-4.9)
Partial pressure of carbon dioxide (mmHg)	29.1 (13.5-46.7)
Tympanic body temperature (°C)	36.9 (33-38.7)
Use of continuous venovenous hemodialfiltration	9
Duration of VA-ECMO (h)	138 (52.9-263)
ECMO flow rate (L min ⁻¹)	3 (0.6-4.1)
Duration of sufentanil infusion (h)	110 (34-260)
Sufentanil infusion rate	
17.5 µg h ⁻¹	15
12.5 µg h ⁻¹	5

Two compartment model



Final PK model

$$CL = 37.8 \times \text{EXP} (0.207 \times (\text{temperature} - 36.9)) \text{ L h}^{-1}$$

$$V1 = 229 \text{ L}$$

$$V2 = 1640 \times (\text{total plasma protein}/4.5)^{2.46} \text{ L}$$

$$Q = 41 \text{ L h}^{-1}$$

Body temperature ↑ ⇒ Clearance ↑
Plasma protein ↑ ⇒ Peripheral volume ↑

SUFENTANIL PK CHANGES DURING ECMO

Study Results: Evaluation

Parameter	Structural model (RSE%) (shrinkage%)	Final model (RSE%) (shrinkage%)	Bootstrap Median	95% CI (2.5–97.5%)
Fixed effects				
θ_{CL}	39.5 (11)	37.8 (9)	37.4	27.0, 47.8
θ_{V1}	220 (23)	229 (19)	223	66.2, 352
θ_{V2}	1620 (26)	1640 (9)	1629	755, 4304
θ_{CL}	35.8 (17)	41 (22)	42.1	21.5, 67.6
$\theta_{T_{1/2}}$	—	0.207 (5)	0.21	0.066, 0.437
$\theta_{T_{1/2,ECMO}}$	—	2.46 (7)	2.51	0.892, 5.12
Random effects				
Inter-individual variability				
ω_{CL}^2	0.196 (40) [15]	0.167 (57) [20]	0.197	0.03, 0.484
ω_{V1}^2	1.41 (35) [21]	1.13 (48) [22]	0.968	0.081, 2.08
Residual variability				
$\sigma^2_{\text{proportional}}$	0.0015 (138) [16]	0.0041 (55) [8]	0.0743	0.047, 0.116

Internal validity
(stable)

Table 2 Parameter estimates and bootstrap confidence intervals

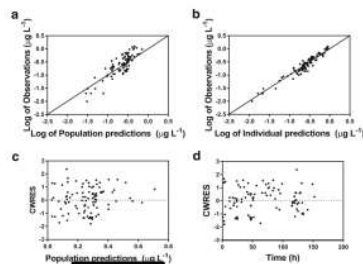


Fig. 1 Goodness-of-fit plots for the final population pharmacokinetic model

Unbiased & No model misspecification

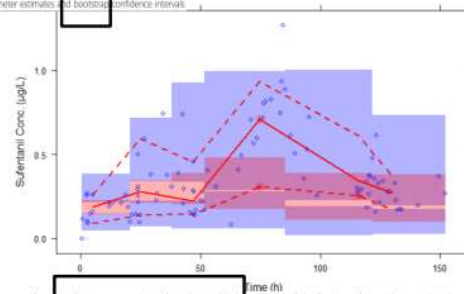


Fig. 2 Prediction-corrected visual predictive check (pc-VPC) of the final population pharmacokinetic model

Good precision & prediction

SUFENTANIL PK CHANGES DURING ECMO

Study Results: M-C simulation

Monte Carlo Simulation

Scenario

- Covariate: Body temperature (33, 35, 36.7, 38, 39 °C)
Total plasma protein level (2, 4, 6, 8 g dL⁻¹)
- Dosing: Infusion rate (12.5 $\mu\text{g h}^{-1}$, 17.5 $\mu\text{g h}^{-1}$)
Infusion duration (96 h, 120 h, 168 h)

 $\Rightarrow 5 \times 4 \times 2 \times 3 = 120 \text{ scenarios}$

covariate dosing

Simulated 1000 times

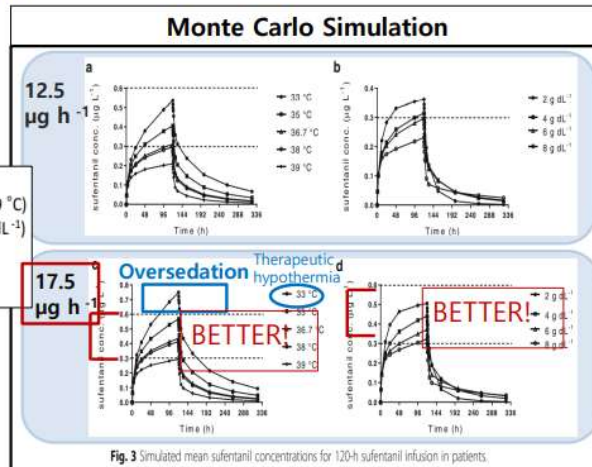
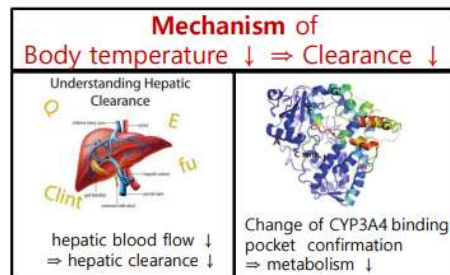
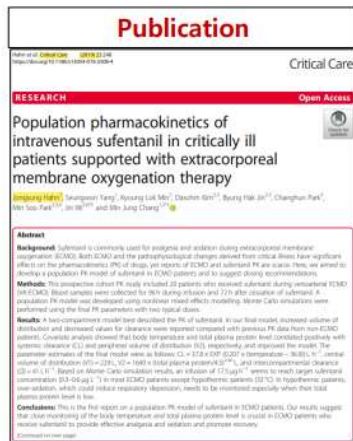


Fig. 3 Simulated mean sufentanil concentrations for 120-h sufentanil infusion in patients

SUFENTANIL PK CHANGES DURING ECMO



RESEARCH

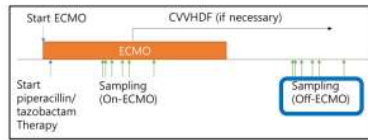
PHARMACOKINETIC STUDIES



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RESEARCH SUMMARY③

PIPERACILLIN/TAZOBACTAM PK DURING ECMO



Final PK model

$$CL (L/h) = 9.4 \times (1 - 0.092 \times ECMO) + [0.115 \times (CrCL - 54.7)]$$

$$V1 (L) = 6.56 \times (1 + 1.46 \times CRRT)$$

$$V2 (L) = 14.2 \quad Q (L/h) = 17.2$$

ECMO ⇒ Clearance ↓
CRRT (신대체요법) ⇒ Central Volume ↑

Dosing Regimen

Category of Patients

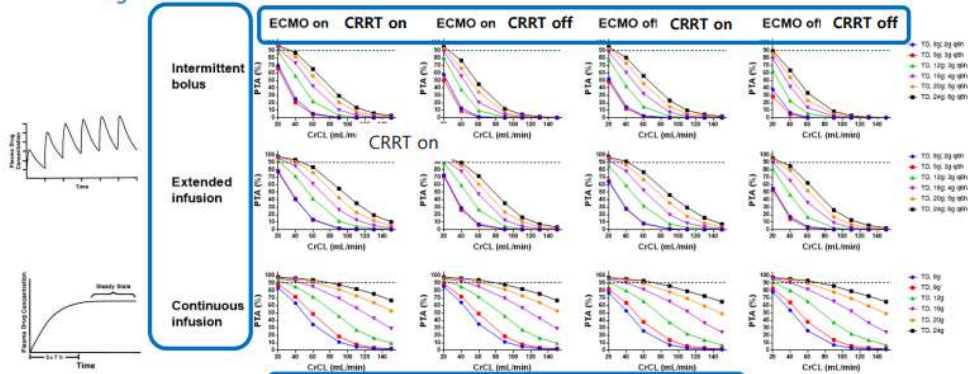


Figure 1. Probability of PD target attainment (100% fT > MIC of 16 mg/L) for piperacillin. TD, total dose

RESEARCH SUMMARY③

PIPERACILLIN/TAZOBACTAM PK DURING ECMO

Study Result

TABLE 4. Proposed empirical piperacillin-tazobactam dosage regimen					
Category of patients	Dosing regimen				
CrCL (mL/min)	Infusion method	ECMO on, CVVHDF on	ECMO on, CVVHDF off	ECMO off, CVVHDF on	ECMO off, CVVHDF off
40<	Intermittent bolus	5 g q6h-6 g q6h	5 g q6h-6 g q6h	5 g q6h-6 g q6h	6 g q6h*
	Extended infusion	3 g q6h-4 g q6h	4 g q6h-5 g q6h	4 g q6h-5 g q6h	4 g q6h-5 g q6h
	Continuous infusion	12 g/day	12 g/day	12 g/day	12 g/day
40-60	Extended infusion	5 g q6h-6 g q6h	6 g q6h*	6 g q6h*	Not recommended
	Continuous infusion	16 g/day-20 g/day	16 g/day-20 g/day	16 g/day-20 g/day	16 g/day-20 g/day
60-90	Continuous infusion	20 g/day-24 g/day	20 g/day-24 g/day	20 g/day-24 g/day	20 g/day-24 g/day
≥90	Continuous infusion	>24 g/day*	>24 g/day*	>24 g/day*	>24 g/day*

*This dosage regimen might not achieve adequate PTA despite the high total daily dose of 24 g/day. Dosing regimen suggestions were based on the achievement of 90% PTA (target: 100% fT_{MIC} of 16 mg/L) for piperacillin and 50% PTA (target: 63% fT > 2 mg/L) for tazobactam. ECMO, extracorporeal membrane oxygenation; CVVHDF, continuous venovenous hemodiafiltration; CrCL, creatinine clearance.

- ✓ Preliminary insights : **incremental** effects of critical illness, ECMO and CRRT on PK
- ✓ **Proposed Empirical Dosage Regimen**
- * **Continuous infusion** should be considered (CrCL > 60 mL/min) *

Publication



Thank you for Listening.

2022.09.21.

우수연구자 초청 세미나

충남대 의대

이충재 교수님

Development of Novel Botanical Drugs for Regulating Airway Mucin Hypersecretion in Inflammatory Pulmonary Diseases: Lessons from Anecdotes of Ancestors

Choong Jae Lee
Department of Pharmacology
School of Medicine
Chungnam National University

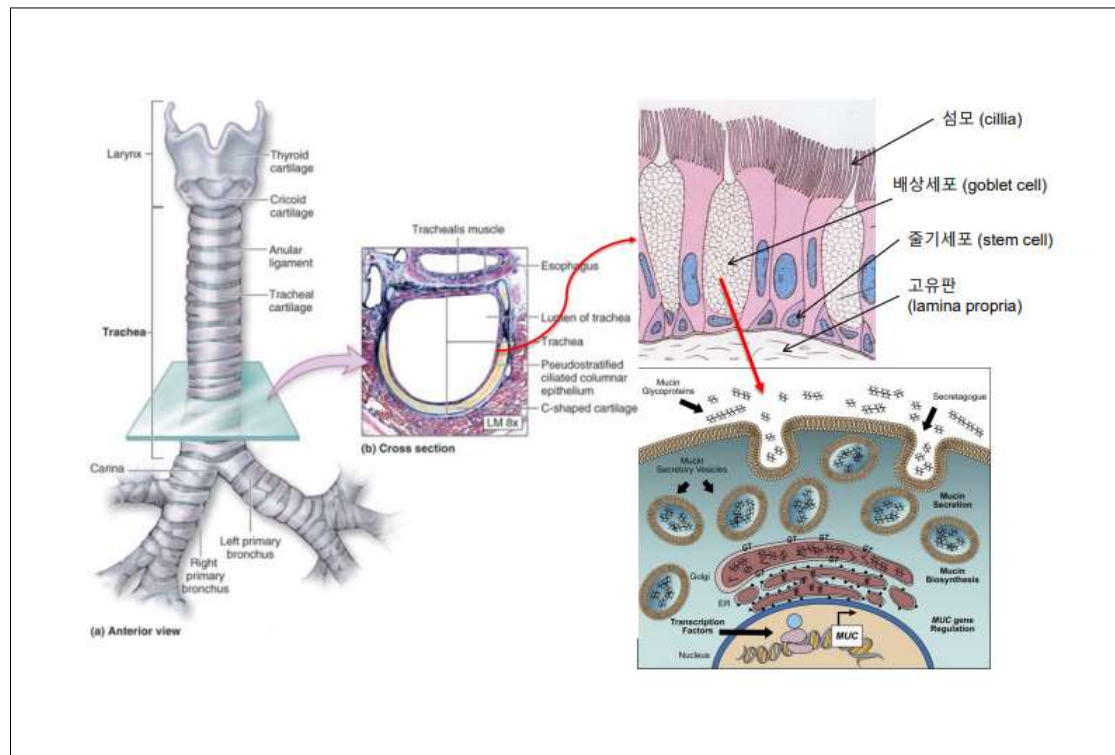


My Research Projects Based on Ideas from Traditional Asian Herbal Medicine



1. 천식 및 COPD에서 기도 객담 과다분비 조절용 천연물/혁신신약 후보물질 탐색 및 작용기전 규명 :
진해 및 거담성 생약(본초학적으로 화담지해평천약), 항균성, 항염증성 생약(본초학적으로 청열해독약)에 관한 지식과 정보로부터 도출된 연구과제
2. 골관절염 및 류마티스성 관절염 개선 목적의 천연물 신약 후보물질 개발:
진통성, 항염증성 생약(본초학적으로 거풍습약, 청열약 등) 에 관한 지식과 정보로부터 도출된 연구과제
3. 수종의 약초로부터 가축사료 첨가용 항생제 대체물질 개발 및 실용화 :
항균성, 항염증성 생약(본초학적으로 청열해독약)에 관한 지식과 정보로부터 도출된 연구과제
4. 만성 비세균성 전립선염 개선 및 전립선암 진행 억제 효과를 발현하는 천연물 신약 후보물질 개발 :
항균성, 항염증성 생약(본초학적으로 청열해독, 청열사화약)에 관한 지식과 정보로부터 도출된 연구과제



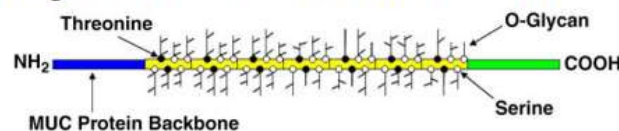


Mucus (점액, 粘液)

1. Gel comprising macromolecules, ions, proteins, and water (= products of inflammation, including neutrophil-derived deoxyribonucleic acid (DNA) and filamentous actin (F-actin), effete cells, bacteria, mucin, and cell debris)
2. Sputum; expectorated mucus (객담, 喀痰)
3. Playing a crucial role in defense mechanisms against a multitude of pathogens through a mechanism called the mucociliary clearance

Mucin (점액소, 粘液素)

1. Major macromolecular components of mucus produced by epithelial goblet cells and submucosal mucous cells



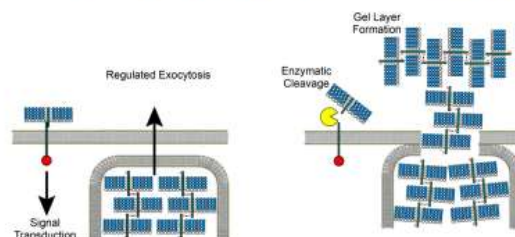
2. The protective function of mucus is due to the viscoelastic property of mucous glycoproteins or mucins

MUC gene??

1) 뮤신의 peptide backbone을 coding 하는 유전자

2) 지금까지 MUC 1, 2, 3A, 3B, 4, 5AC, 5B, 6, 7, 8, 9, 11, 13, 16, 17, 18, 19, 20 등 약 20여종의 mucin gene (MUC)이 확인됨

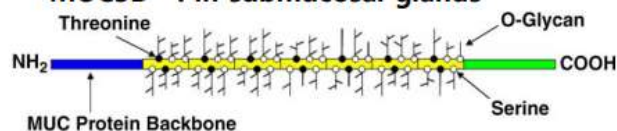
세포막 결합 뮤신과 겔 형성 뮤신



Respiratory mucin : gel-forming (secreted) mucin

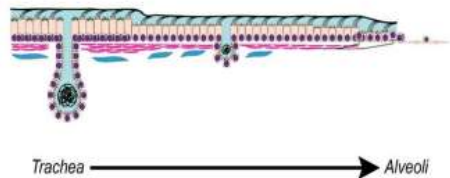
*MUC5AC : in airway epithelial goblet cells

*MUC5B : in submucosal glands



기도 점액 과다분비 질환의 병태생리

Healthy Airways



1. 기도 점액 과다분비가 관찰되는 질환군

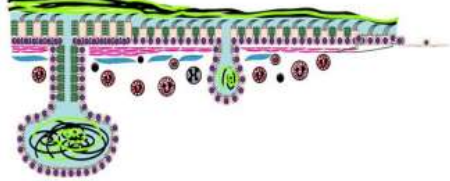
천식, 만성 폐쇄성 폐질환, 낭포성 섬유증, 호흡기 감염증

2. 증상 및 징후

1) 섬모운동 저해/정지, 배상세포수의 증가, 점액 분비량 및 점도의 증가

2) 과다분비 점액으로 인한 기도 폐쇄, 감염 시 배농 장애 및 극심한 감염 악화

Allergic Airways



Mucoactive agents (점액작용 약물군)

1. Pharmacological agents affecting mucus properties and promoting clearance of mucus, intended either to increase the ability to expectorate sputum or to decrease mucus hypersecretion
2. Expectorants, mucolytics, mucoregulatory, mucospissic, mucokinetic drugs

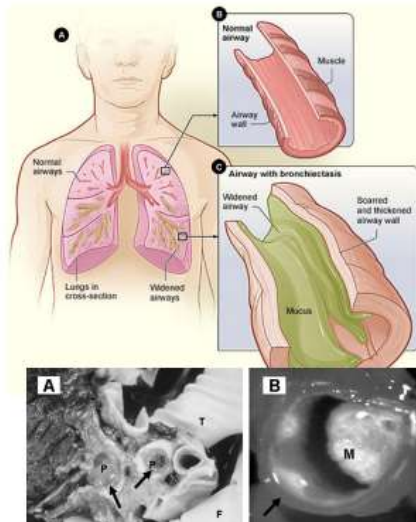
Expectorants, mucolytics

1. Expectorants (거담제, 祛痰劑); agents improving the ability to expectorate purulent secretions (increasing the volume of airway secretion, promoting cough efficiency)
2. Mucolytics (점액 용해제, 粘液 溶解劑); agents changing the biophysical properties of secretions by degrading the mucin polymers, DNA, fibrin, or F-actin in airway secretions, generally decreasing viscosity

Mucoactive agents under clinical use

2-mercaptoethane sulfonate sodium (MESNA)
ambroxol, azithromycin, bromhexine, erdosteine
glucocorticoids, myrtol, glyceryl guaiacolate
hypertonic saline solution, letocysteine, dornase alfa
mannitol dry powder, N-acetyl L-cysteine (NAC)
S-carboxymethyl cysteine, sobrerol, thymosin β -4

기도 점액 과다분비 질환의 치료



1. 기도 점액 분비의 조절

→ 증상 경감, 질환의 예후에 있어 대단히 중요

2. 현행 치료 원칙

1) 점액 점도 저하 후 물리적 흡인배출

→ 점액 용해제(mucolytics) 사용

→ 단점 : 점액 점도 감소 후 물리적 흡인 배출 시 기도벽을 자극, 반사 기전에 의한 극심한 점액분비 유발

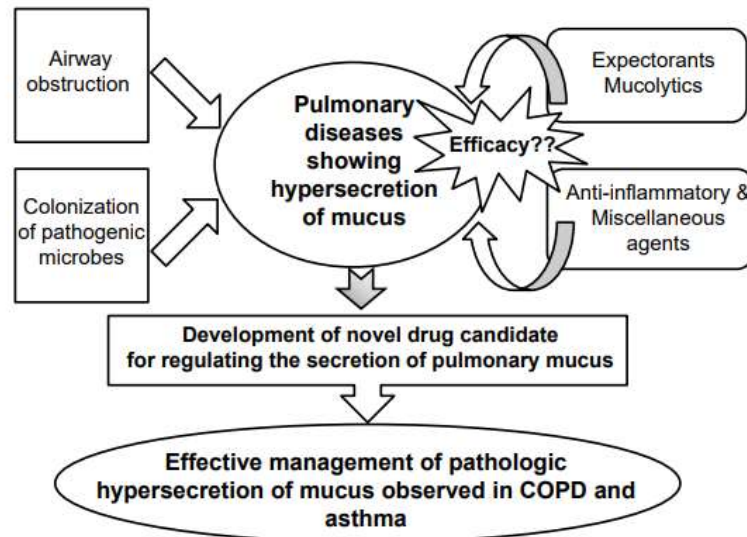
→ 지나친 점도 감소 시 점액의 원위 기관지 유입으로 기관지 폐쇄

2) 점액 배출 자극을 통한 배출 촉진

→ 거담제(expectorants) 사용, 약효 및 작용기전 모호, 부작용 빈발

Needs for the development of
novel agent :

Agent that can specifically
inhibit(regulate)
hypersecretion and/or production of
airway mucus (sputum)



기도 점액 분비 조절효능 검색 대상의 선정

1. 복합 조성 천연물 (방제, 탕제)의 조 추출물
2. 각 방제를 구성하는 단일 생약(본초)의 추출물
3. 각 생약에 함유된 주요 약효발현 예상 단일 성분들
4. 약효 발현 단일 천연물(single compounds)의 화학구조 수식 및 변형체들 (structurally-modified compounds)

복합 조성 천연물 (방제, 탕제)의 조 추출물

치효산 가미방, 청금강화탕, 소청룡탕,
과루지실탕, 취연탕, 통규탕 가미, 청폐탕,
형방패독산, 필용방 감길탕, 청화보음탕,
정천탕, 선방패독탕, 배농산급탕, 은교산...

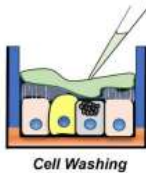
가미 사물탕, 가미 이중탕, 금수육군전,
가미 육군자탕, 가미 신기탕, 팔미환료,
청상보하탕, 맥문동탕, 이음전, 생맥산,
육미환료, 가미 보중익기탕.....

각 방제를 구성하는 단일 생약(본초)의 추출물 및 약효발현 예상 단일성분들

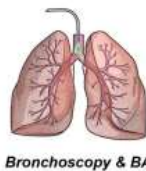
오미자 중의 schizandrin, schizandrin A, gomisin A / 감초 중의 carbenoxolone, prunetin, glycyrrhizin
황금 중의 baicalin, baicalein, wogonin, apigenin / 산수유 중의 ursolic acid, oleanolic acid
울금 중의 curcumin, tumerone / 갈근 중의 genistein, puerarin, daidzein
백지, 강활 중의 imperatorin, oxypeucedanin, marmesinin / 지실 중의 Hesperidin, kaempferol
화피 중의 betulin / 당귀 중의 decursin, coumarin / 대추 중의 betaine / 황련 중의 berberine
길경 중의 platycodin / 맥문동 중의 opiophogonin / 감국 중의 cynarin, linarin, cynaroside
사삼 중의 lupenone, lupeol, taraxerol / 상백피 중의 morusin / 반하 중의 homogentisic acid
식방풍 중의 pteryxin, anomalin / 소엽 중의 myristicin, rosmarinic acid / 전호 중의 umbelliferone
천문동 중의 dioscin / 결명자 중의 obtusifolin / 패모 중의 verticine / 관동화 중의 tussilagone
어성초 중의 hyperoside / 기타 fisetin, ferulic acid, quercitrin, hesperetin, eriodictiol, tangertin....

Summary of broadly categorized strategies to study mucus and mucins

Collection Methods



Cell Washing



Bronchoscopy & BAL

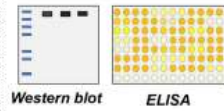
Visual/Imaging Methods



Lectins & Antibodies

Histological stains

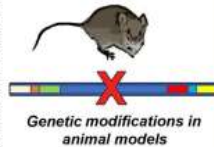
Protein Methods



Western blot

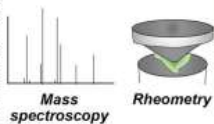
ELISA

Molecular Methods



Genetic modifications in animal models

Biochemical/Biophysical Methods



Mass spectroscopy

Rheometry

각 약물의
기도 점액 과분비
및
점액의 주
생화학적
구성성분인
뮤신의
분비, 생성 및
유전자 발현에
대한 효과는 ?

In vivo research methodology



Apparatus for sulfur dioxide exposure



호흡기 염증 및 뮤신 과다 생성/분비 동물모델

- 1) 흰쥐에의 이산화황 (sulfur dioxide) and/or 담배연기 추출물 혼합 증기 흡입 투여 모델
- 2) 흰쥐 기도에 endotoxin인 lipopolysaccharide (LPS) 흡입 투여 모델
- 3) 각 실험군 간 기관-폐포 세척액 (broncho-alveolar lavage fluid, BALF) 내의 뮤신 함량 측정 (ELISA)
- 4) 각 동물의 기관 (trachea) 적출 후 조직병리학적 검사 (Periodic Acid Schiff (PAS)-alcian blue 염색)로 각 실험군 간 배상세포 내 점액 함유 정도 비교

In vitro research methodology

Hamster or Rat Tracheal Surface Epithelial(RTSE) cell culture (primary culture system)

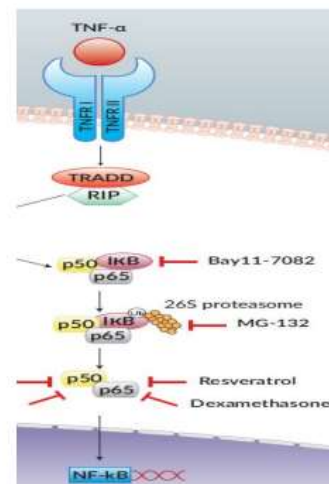
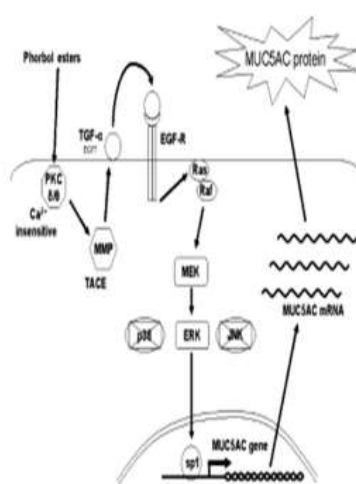
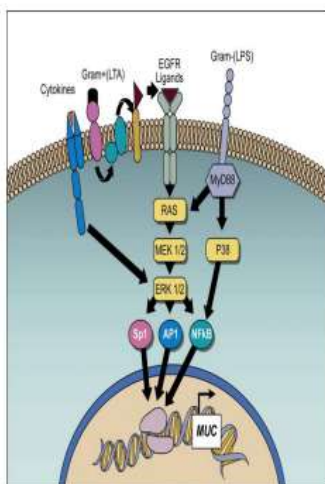
Immortal cell line - the human airway epithelial NCI-H292 cells

Inducers of mucin gene expression/production/secretion

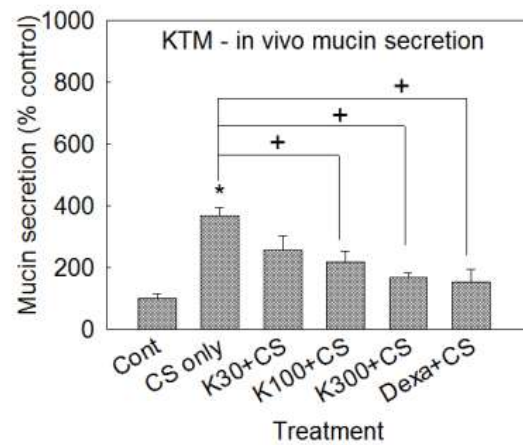
Adenosine triphosphate (ATP), phorbol ester (PMA), epidermal growth factor (EGF), tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β).....

- 1) 배양세포에 각 약물을 30분간 전 처리한 후 이어지는 24시간 동안 PMA 등 inducer를 투여, 뮤신 생성, 분비 및 뮤신 유전자 발현을 자극시킴
- 2) 자극된 상태의 MUC5AC 뮤신 생성/분비 및 유전자 발현에 대한 약물의 영향을 ELISA (뮤신 생성 및 분비) 및 RT-PCR (mRNA 발현) 로 각각 검증함

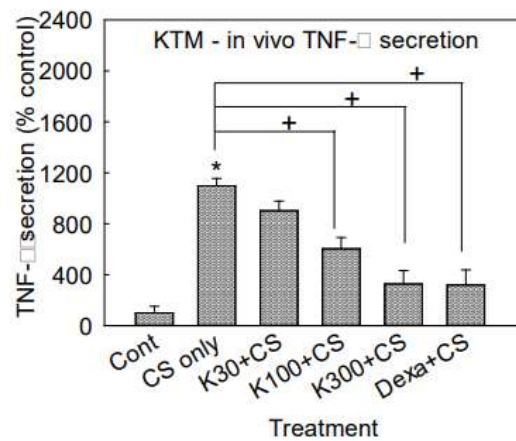
EGF, PMA, TNF- α 에 의한 MUC5AC 뮤신 유전자 활성화 신호전달 경로



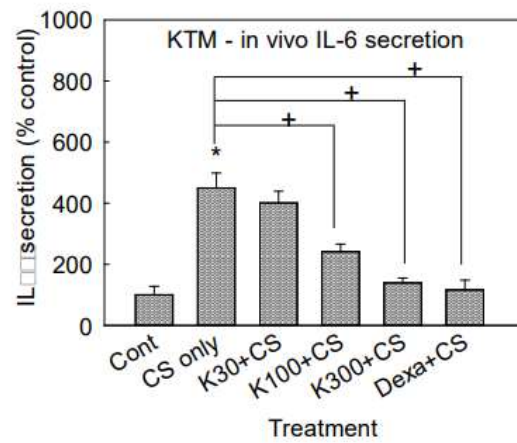
Effect of KTM on secretion of *in vivo* airway mucin from rats exposed to cigarette smoke extract plus sulfur dioxide



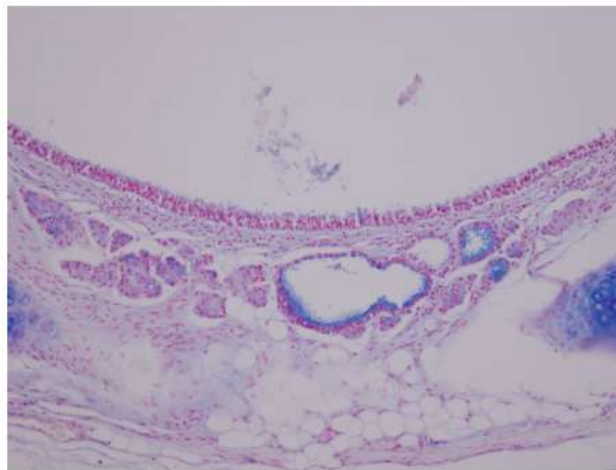
Effect of KTM on the secretion level of TNF- α in BALF from rats exposed to cigarette smoke extract plus sulfur dioxide



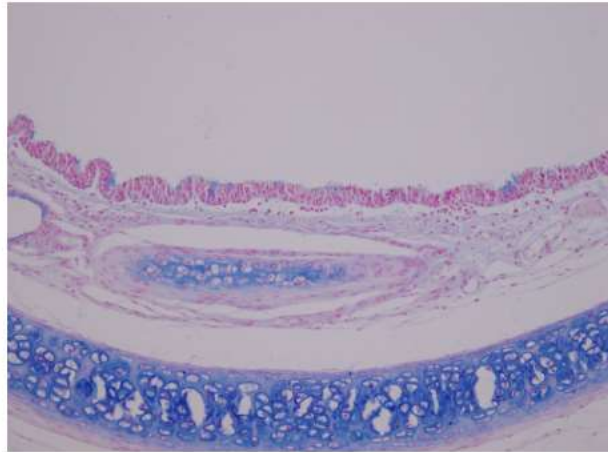
Effect of KTM on the secretion level of IL-6 in BALF from rats exposed to cigarette smoke extract plus sulfur dioxide



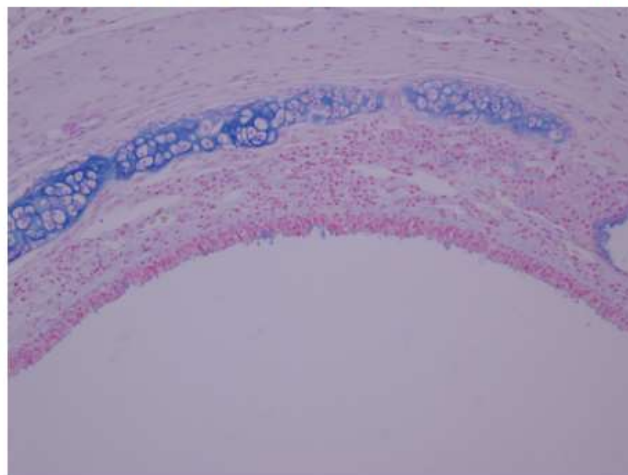
대조군



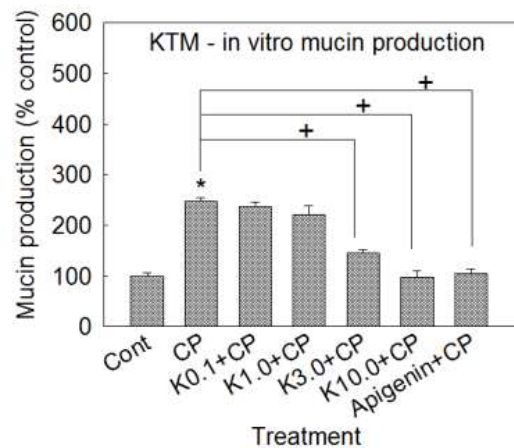
이산화황 단독 흡입군



이산화황 흡입 + 약물 투여군

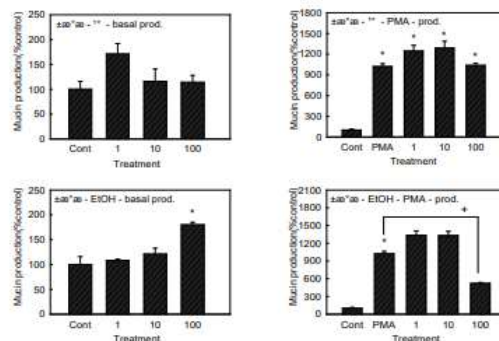


Effect of KTM on cigarette smoke extract plus phorbol ester-induced MUC5AC mucin production in vitro



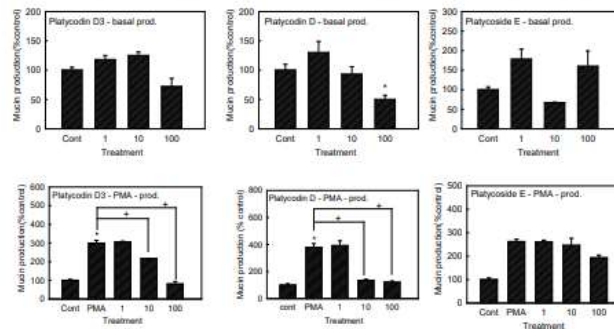
길경 추출물의 호흡기 점액 생성에 대한 영향

- 길경의 H₂O 추출물은 기초적 및 PMA로 자극된 뮤신 생성에 뚜렷한 영향이 없었음
- 그러나, 길경의 70% EtOH 추출물은 기초적 뮤신 생성은 증가시켰으며, 호흡기 염증 상태에서의 뮤신 과다생성을 모사한, 즉 PMA로 자극된 뮤신 생성은 억제하는 경향을 보여주었음.



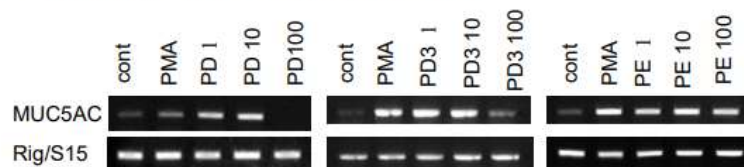
길경 활성성분의 호흡기 점액 생성에 대한 영향

- Platycodin D3는 기초적 뮤신 생성에 대해서는 유의한 영향이 없었으나, PMA로 자극된 뮤신생성은 억제하였음. Platycodin D의 경우, PMA로 자극된 뮤신 생성 뿐 아니라 기초적 뮤신 생성도 억제하였음
- Platycoside E는 뮤신 생성에 대해 뚜렷한 작용을 나타내지 않았음



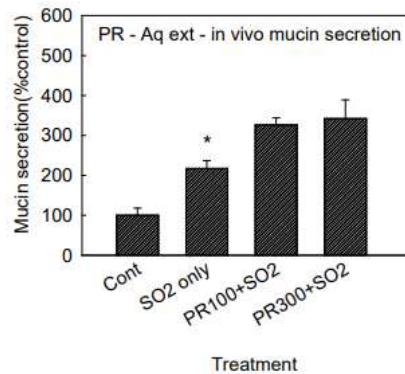
길경 활성성분의 호흡기 뮤신 유전자 발현에 대한 영향

- 길경에서 분리된 Platycodin D3와 Platycodin D 공히 호흡기 염증 상태에서의 뮤신 과다생성을 모사한, 즉 PMA로 자극된 호흡기 뮤신 MUC5AC 유전자의 발현을 억제하였음
- 그러나, Platycoside E는 유의한 영향이 없었음

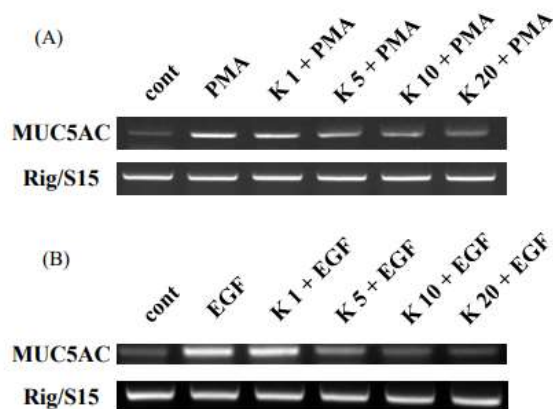


길경 추출물의 호흡기 점액 배출(분비)에 대한 영향

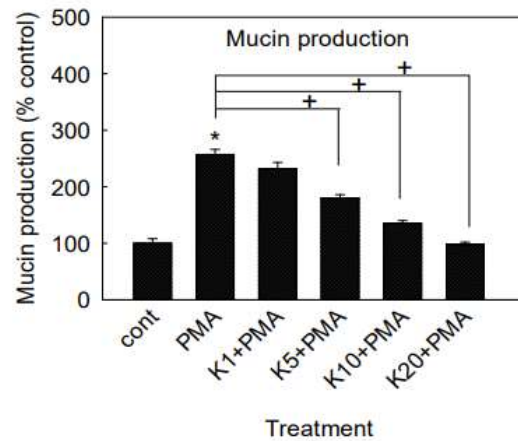
- 길경의 H₂O 추출물은 이산화황 흡입 노출에 의한 급성 기관지염 동물모델에서 호흡기 염증 상태에서의 점액 배출(분비)을 촉진하는 작용을 나타내었음
 - 따라서, 길경의 작용을 종합해보면
 - 1) 염증 상태에서 호흡기 뮤신의 생성 및 유전자 발현을 억제함으로써 더 이상의 점액 생성은 억제하지만
 - 2) 염증으로 인해 이미 생성되고 분비되어야 할 점액은 배출(분비)을 촉진시킴으로써
- 배농소증(排膿消腫)으로**
표현되는 한의학적 효능을 발현함을 의미함



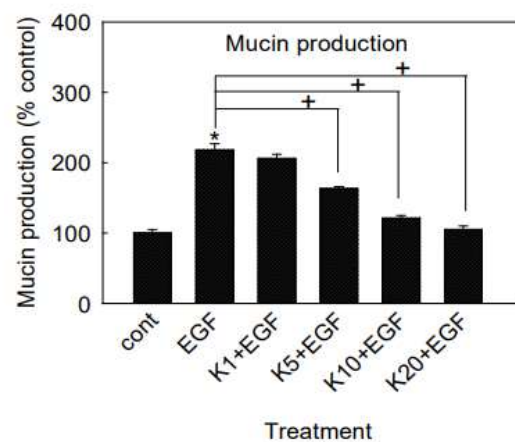
Effect of kaempferol on PMA- or EGF-induced MUC5AC mucin gene expression from NCI-H292 cells



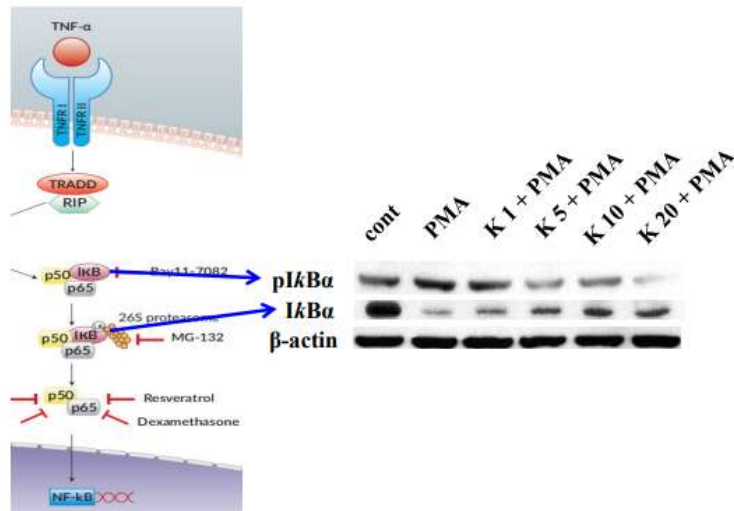
Effect of kaempferol on PMA-induced MUC5AC mucin production from NCI-H292 cells



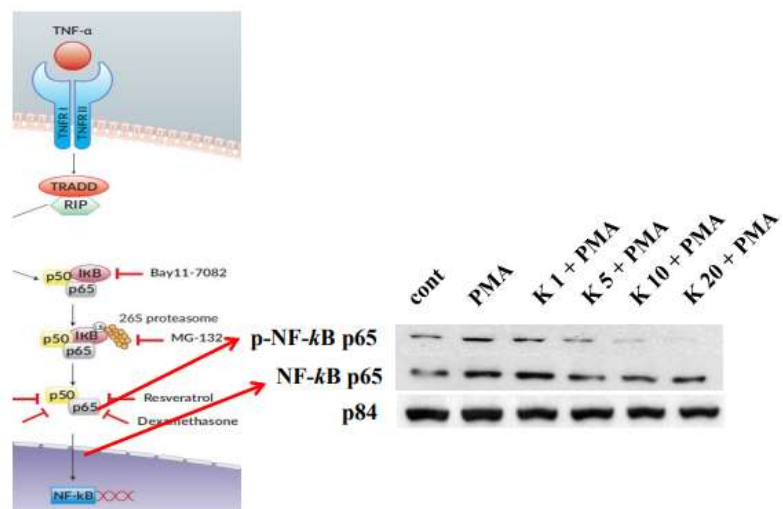
Effect of kaempferol on EGF-induced MUC5AC mucin production from NCI-H292 cells



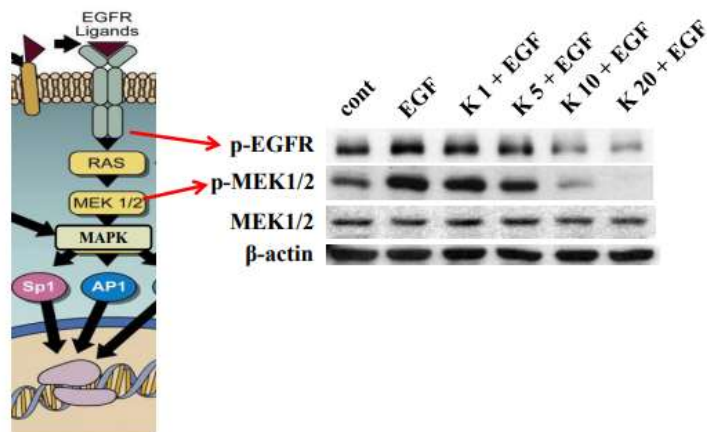
Effect of kaempferol on PMA-induced phosphorylation and degradation of I κ B α



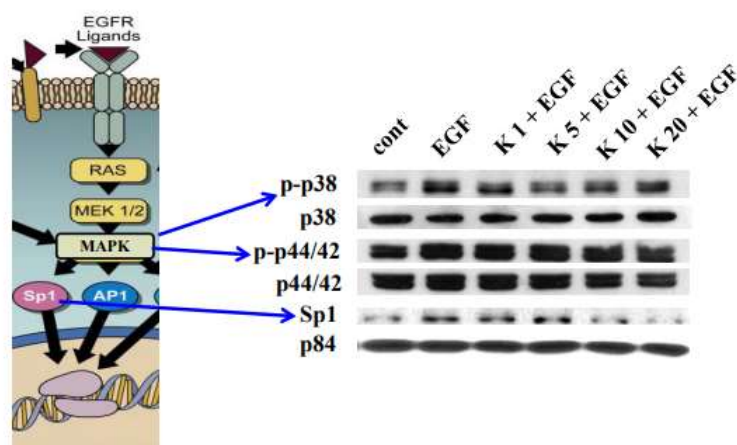
Effect of kaempferol on PMA-induced phosphorylation and nuclear translocation of NF- κ B p65



Effect of kaempferol on the phosphorylation of EGFR and the phosphorylation MEK1/2 in NCI-H292 cells



Effect of kaempferol on the phosphorylation of p38 and p44/42 and the nuclear expression of Sp1 in NCI-H292 cells



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	Choong Jae Lee Chungnam National University pharmacology		GET MY OWN PROFILE		TITLE		CITED BY		YEAR		TITLE		CITED BY		YEAR		
	Citations	1410	722	Effects of ursolic acid, baicalin and sulfur-containing compounds on mucin release from airway goblet cells					Carbonic anhydrase and Interferons inhibited mucin secretion from airway epithelial cells								
	h-index	22	16	CJ Lee, JH Seok, SM Han, JH Lee, JS Park, IC Seol, YH Kim					HJ Han, C Kim, HJ Lee, YS Kim, SS Kang, UK Seo, YH Kim, YC Park, ...								
	10-index	42	30	Plant medicine 70 (12), 1119-1122					Phytotherapy Research: An International Journal Devoted to Pharmacological ...								
	2 articles	2 articles	Effects of baicalin and wogonin on mucin release from cultured airway epithelial cells	33	2007	Effects of polycationic peptides on mucin release from airway goblet cells: relationship between polymer size and activity	28	2002									
not available	available	HJ Han, HJ Lee, YS Kim, SS Kang, JH Seol, JH Seok, UK Seo, CJ Lee			CJ Lee, SH Park, KH Hwang, KC Kim												
Based on funding mandates		Phytotherapy Research: An International Journal Devoted to Pharmacological ...			Information Research 51 (10), 402-404												
TITLE		CITED BY		YEAR		TITLE		CITED BY		YEAR		TITLE		CITED BY		YEAR	
Airway goblet cell mucin: its structure and regulation of secretion		184		1997		Apigenin regulates interleukin-1β-induced production of matrix metalloproteinase both in the knee joint of rat and in primary cultured articular chondrocytes		31		2016		Effect of alterations in extracellular nonpermeable on adrenoceptors: a microdialysis study in freely moving rats		28		1999	
KJ Kim, K McCracken, BC Lee, CY Shin, MJ Jo, CJ Lee, KH Jo						JS Park, DK Kim, HJ Shin, HJ Lee, HJ Jo, JH Jeong, YL Choi, CJ Lee, ...						DO Seo, CY Shin, CJ Lee, JH Doh, MSA Bae, PC Joo, KH Jo					
European Respiratory Journal 10 (11), 2644-2648						Biomolecules & Therapeutics 24 (2), 183						European Journal of Pharmacology 365 (1), 39-48					
Hesperidin suppressed proliferations of both human breast cancer and androgen-dependent prostate cancer cells		104		2010		Sildenafil regulates gene expression, production and secretion of mucin from cultured airway epithelial cells		31		2012		Luteolin inhibits the activity, secretion and gene expression of MMP-3 in cultured articular chondrocytes and production of MMP-3 in the rat knee		24		2014	
CJ Lee, JH Seok, SM Han, JH Lee, JS Park, IC Seol, YH Kim						HJ Kim, HJ Lee, SH Kim, MA Seok, YJ Lee, YS Kim, JH Hong, JH Seok, ...						Bj Kang, J Ryoo, CJ Lee, SC Hwang					
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Effects of baicalin, berberine, curcumin and hesperidin on mucin release from airway goblet cells		89		2003		Effects of the root of <i>Platycodon grandiflorum</i> on airway mucin hypersecretion in vivo and platycodon D3 and deapi-platycodon on production and secretion of airway mucin in vitro		28		2014		Vertecine, alvexidine and suchengbesine isolated from the bulbs of <i>Fritillaria thunbergii</i> Miq. inhibited the gene expression and production of MUC5AC mucin from human airway ...					
CJ Lee, JH Seok, SM Han, JH Lee, JS Park, IC Seol, YH Kim						J Ryoo, HJ Lee, SH Park, J Kim, D Lee, SH Lee, YS Kim, JH Hong, JH Seok, ...						EJ Kim, YP Yoon, KH Han, JH Kim, SY Min, HJ Lee, SH Lee, JH Hong, ...					
Plant medicine 69 (6), 525-528						Phytotherapy Research 21 (6), 529-533						Phytotherapy Research 23 (2), 85-104					
Inhibition of mucin release from airway goblet cells by polycationic peptides		77		1999		Inhibition of secretion, production and gene expression of mucin from cultured airway epithelial cells by puwadin		27		2011		Chondroprotective effects of wogonin in experimental models of osteoarthritis in vitro and in vivo		22		2015	
KH Jo, CJ Lee, CY Shin, MJ Jo, YC Kim						HJ Lee, SH Lee, MH Lee, JH Kim, OT Chung, JH Seok, CJ Lee						JS Park, HJ Lee, DY Lee, HJ Jo, JH Jeong, DH Kim, OC Nam, CJ Lee, ...					
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Effects of baicalin, curcumin and flavonoids on mucin release from cultured hamster tracheal surface epithelial cells		61		2004		Effects of <i>Morus alba</i> L. and natural products including morusin on in vivo secretion and in vitro production of airway MUC5AC mucin		28		2014		Molecular pathophysiology of ossification of the posterior longitudinal ligament (OPLL)		21		2019	
HJ Han, SH Lee, HJ Lee, HJ Seok, SM Han, JH Seok, CJ Lee						HJ Lee, J Ryoo, SH Park, SH Han, AR Kim, SH Lee, YS Kim, JO Kim, ...						DC Nam, HJ Lee, CJ Lee, SC Hwang					
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Genes and curcumin suppress epidermal growth factor-induced MUC5AC mucin production and gene expression from human airway epithelial cells		49		2009		Effects of luteone, luteol, and tanzanin derived from <i>Adenophora triphylla</i> on the gene expression and production of airway MUC5AC mucin		28		2016		Discur and methylprodioson isolated from the root of <i>Asparagus cochinchinensis</i> suppressed the gene expression and production of airway MUC5AC mucin induced by phorbol ester ...					
HJ Han, SH Lee, HJ Lee, HJ Seok, SM Han, JH Seok, CJ Lee						YP Yoon, HJ Lee, DY Lee, SH Lee, JH Hong, CJ Lee						HJ Lee, JS Park, YP Yoon, YJ Shin, SH Lee, YS Kim, JH Hong, KH Kim, ...					
Tuberculosis and respiratory diseases 79 (3), 200-208						Phytotherapy Research 23 (5), 588-593						Phytotherapy Research 23 (5), 588-593					
Luteolin inhibited the gene expression, production and secretion of MUC5AC mucin via regulation of nuclear factor kappa B signaling pathway in human airway epithelial cells		45		2010		Luteolin inhibited the gene expression, production and secretion of MUC5AC mucin via regulation of nuclear factor kappa B signaling pathway in human airway epithelial cells		28		2016		Effects of otopropogonin D and spicatoside A derived from <i>Liriodie</i> Tuber on secretion and production of mucin from airway epithelial cells		21		2014	
HJ Lee, HJ Seok, J Ryoo, YP Yoon, SH Park, CJ Lee						HJ Lee, HJ Seok, J Ryoo, YP Yoon, SH Park, CJ Lee						SH Park, HJ Lee, J Ryoo, KH Kim, SY Min, SH Lee, YS Kim, JH Hong, ...					
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TITLE		CITED BY		TITLE		CITED BY		TITLE		CITED BY		YEAR			
Effect of phorbol on TNF- α -induced MUC5AC mucin gene expression, production, degradation of I κ B and translocation of NF- κ B p65 in human airway epithelial cells		1		The Korean Journal of Physiology & Pharmacology 23 (5), 871-877				Inhibition of the expression of matrix metalloproteinases in articular chondrocytes by resveratrol through affecting nuclear factor-kappa B signaling pathway		8		2018			
J Ryoo, HJ Lee, SM Park, MA Shoker, JH Kim, JH Hong, JH Seok, CJ Lee				Effects of phorbol on the proteolytic activity, secretion and gene expression of MMP-3 in vitro and production of MMP-3 in vivo		12		DO Kang, HJ Lee, CJ Lee, JS Park							
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Phorbol ester or epidermal growth factor-induced MUC5AC mucin gene expression and production from airway epithelial cells are inhibited by apigenin and wogonin		18		Apigenin and wogonin regulate epidermal growth factor receptor signaling pathway involved in MUC5AC mucin gene expression and production from cultured airway epithelial cells		12		Effects of Sildenafil and Resveratrol on Degradation of $\alpha_1(I)$ and Translocation of NF- κ B Induced by Tumor Necrosis Factor- α in Cultured Airway Epithelial Cells		8		2014			
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Effect of oleic acid on the activity, secretion and gene expression of matrix metalloproteinase-3 in articular chondrocytes in vitro and the production of matrix		17		Cultural characteristics of oyster mushroom (<i>Pleurotus ostreatus</i>) on addition rate of <i>Cordarrina trisporioides</i>				Natural products as sources of novel drug candidates for the pharmacological management of osteoarthritis: A narrative review		8		2016			
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Suppressive effects of curcumin, glycyrrhizic acid and natural products derived from <i>Corydalis</i> on mucin production and gene expression from human airway epithelial cells		17		Oleic acid and Urolic acid Derived from <i>Corvus officinalis</i> (Seo, et al.) Suppress Epidermal Growth Factor- and Phorbol Ester-induced MUC5AC Mucin				Retain suppressed interleukin-1 β -induced gene expression, secretion and proteolytic activity of matrix metalloproteinase in cultured articular chondrocytes and production of ...		21		2017			
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Inhibition of TNF- α -induced MUC5AC mucin gene expression and production by wogonin through the inactivation of NF- κ B signaling in airway epithelial cells		17		Effects of poly-L-arginine on the mucin release from airway goblet cells of hamster and on the mucosubstances of airway goblet cells of rat		11		Investigation of bacteria in imported supplemental substrates for mushroom cultivation media		8		2018			
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Inhibition of airway MUC5AC mucin production and gene expression induced by epidermal growth factor or phorbol ester by glycyrrhizin and carboxymethylated chitosan		17		Detection of fungi in imported supplemental substrates for mushroom cultivation media				Isolation of the bacterium <i>Pseudomonas azotofixans</i> HCS as effective in antagonism of brown blotch disease caused by <i>Pseudomonas tolaasii</i>		8		2014			
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Apigenin inhibits tumor necrosis factor- α -induced production and gene expression of mucin through regulating nuclear factor-kappa B signaling pathway in airway epithelial cells		16		Effects of rosmarinic, luteolin and methyl iodide on secretion, production and gene expression of MUC5AC mucin from airway epithelial cells		10		Occurrence of bacterial soft rot of <i>Pleurotus ostreatus</i> caused by <i>Burkholderia gladioli</i> var. <i>agrariorum</i> in Korea		8		2015			
HJ Seok, MA Shoker, HJ Lee, J Ryoo, CJ Lee				YP Yoon, J Ryoo, SM Park, HJ Lee, S Lee, JH Seok, JH Hong, ...				HJ Lee, HS Yoon, CS Chung, JC Chung, JH Moon, JS Bae, ...							
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Resveratrol inhibits mucin gene expression, production and secretion from airway epithelial cells		16		Effect of chrysin on gene expression and production of MUC5AC mucin from cultured airway epithelial cells		10		Anti-stress effect of Arctostaphylos juice in SD rats and ICR mice		8		2004			
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Tuesilagine suppressed the production and gene expression of MUC5AC mucin via regulating nuclear factor-kappa B signaling pathway in airway epithelial cells		16		Effect of chrysin on gene expression and production of MUC5AC mucin from cultured airway epithelial cells		10		Effect of platycodon D on airway MUC5AC mucin production and gene expression induced by growth factor and proinflammatory factor		7		2010			
BO Choi, Y Kim, YP Yoon, HJ Lee, CJ Lee				HD Shin, HJ Lee, MA Shoker, CH Park, J Ryoo, JH Kim, JH Seok, ...				HJ Lee, SY Lee, BK Jeon, JH Yoon, YS Kim, MH Lee, CJ Lee							
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Tuesilagine suppressed the production and gene expression of MUC5AC mucin via regulating nuclear factor-kappa B signaling pathway in airway epithelial cells		16		Effect of chrysin on gene expression and production of MUC5AC mucin from cultured airway epithelial cells		10		Marketability of Agaricus bisporus mushrooms influenced by controlled atmosphere storage and shelf temperature conditions		7		2009			
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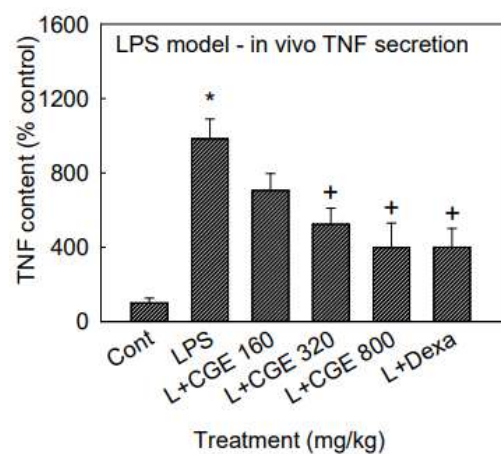
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H J Lee, C J Lee Natural Product Sciences 23 (3), 231-237 Effects of Homogenized Acid and Natural Products Derived from Pinellia ternata on Secretion, Production and Gene Expression of MUC5AC Mucin from Cultured Airway Epithelial Cells H J Lee, C J Lee Natural Product Sciences 23 (1), 29-34 Flavonoids Regulate Airway MUC5AC Mucin Expression through Modulation of Multiple Signaling Pathways A Sider, C Sider, A Sider, M A Sider, H J Lee, C J Lee Research & Reviews: A Journal of Pharmacology 7 (3), 21-33 Effects of Caffeic Acid, Myricetin and Rosmarinic Acid on the Gene Expression and Production of Airway MUC5AC Mucin H J Lee, H J Lee, J H Hong, C J Lee Natural Product Sciences 22 (4), 275-281 Two Flavonoids Derived from Scutellaria Radix Suppressed TNF- α Induced MUC5AC Mucin Gene Expression and Production via Inactivation of NF- κ B Pathway in Human Airway C J Lee, M A Sider, H J Lee, J H Hong, C J Lee C75: A STICKY SITUATION: MUCIN BIOLOGY, 44702-44792 Two Flavonoids Derived from Scutellaria Radix Suppressed Tnf- α Induced Muc5ac Mucin Gene Expression And Production Via Inactivation Of Nf- κ B Pathway In Human Airway C J Lee, M A Sider, H J Lee, J H Hong, C J Lee AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE 187 Effect of Chrysin on Tumor Necrosis Factor- α Induced Mucin Production, Mucin Gene Expression and Translocation of Nuclear Factor- κ B in Cultured Airway H O Hui, H J Lee, M A Sider, J H Hong, O T Chung, J H Seok, ... YAKCHEOK 18 (3), 188-193 Effects of Curcumin and Genistein on Phorbol Ester or Tumor Necrosis Factor- α Induced Mucin Production from Human Airway Epithelial Cells S Y Lee, H J Lee, J H Kim, B H Jeon, J O Kim, C J Lee Tuberculosis and Respiratory Diseases 70 (3), 219-223 Regulation of Tumor Necrosis Factor- α Induced Airway Mucin Production and Gene Expression by Carbenoxolone, Flunixin, and Salbutamol H J Lee, S Y Lee, B H Jeon, J H Kim, M H Lee, J O Kim, C J Lee Tuberculosis and Respiratory Diseases 69 (5), 345-353 https://scholar.google.com/citations?user=FT1NC&hl=ko&as_sides=	8		Effects of natural products on proliferation of human breast cancer cells induced by estrogen or PhIP, a heterocyclic amine compound derived from cooked meat C J Lee, M H Lee, H J Lee, S Y Lee, J H Seok, M A Sider, L Wilson Cancer Research 70 (5 Supplement), 772-772 Suppression of Induced Mucin Production from Human Airway Epithelial Cells by Coumestrol and Indomethacin J H Lee, H G Kim, B H Jeon, C J Lee YAKCHEOK 94 (3), 415-421 Effects of Postharvest Heat Exposure and MAP on Quality Maintenance of Oyster Mushroom Y H Park, C J Lee, C S June Biomolecules & Therapeutics 18 (2), 125-129 Effects of Phorbol Ester, G-6978, Ro-31-8220 and Rotenone on Basal Mucin Release from Airway Goblet Cells H J Lee, H J Lee, J H Seok, U S Lee, C J Lee Biomolecules & Therapeutics 13 (4), 257-265 Effects of Daidzein, Acetaminophen, Nimesulide and Acetylsalicylic Acid on Mucin Release from Cultured Hamster Tracheal Surface Epithelial Cells H J Lee, H J Lee, C S Yoon, S Y Lee, J H Seok, C J Lee Biomolecules & Therapeutics 13 (4), 248-252 Reliability analyses of the GDI α nerve-fiber analyzer Y P Yoon, H J Lee, S Y Lee, J H Hong, C J Lee, P M Kim Philippine Journal of Ophthalmology 19 (3), 215-217 Effect of Erythromycin on Basal and Stimulated Mucin Release from Cultured Hamster Tracheal Surface Epithelial Cells J S Park, J H Seok, O M Hui, J H Lee, K A Park, C J Lee Biomolecules & Therapeutics 12 (2), 87-91 Effects of Proinflammatory Cytokines and Natural Products on Mucin Release from Cultured Hamster Tracheal Surface Epithelial Cells J S Park, H G Kim, J H Seok, O M Hui, J S Park, U S Lee, C J Lee The Korean Journal of Physiology and Pharmacology 9 (6), 323-328 Consistent and Specific Suppression of Mucin Release from Cultured Hamster Tracheal Surface Epithelial Cells by Poly-L-Lysine C J Lee, J H Lee, J H Seok, O M Hui, J S Park, S H Bae, H S Kang, S C Park The Korean Journal of Physiology and Pharmacology 7 (3), 143-147 Effect of polymer of lysine on the mucin release from primary cultured hamster tracheal surface epithelial cells C J Lee, S Kim, H H Hong Journal of Dental hygiene science 2 (1), 25-29 https://scholar.google.com/citations?user=FT1NC&hl=ko&as_sides=	3		1	2008	1	2008		2021		2020		2020		2019		2017	
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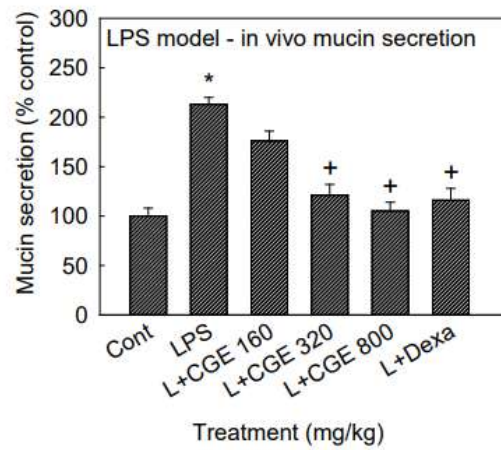
호흡기 염증성 질환의 조절을 위하여 임상에서
사용 중인 기존의 천연물신약/생약제제들



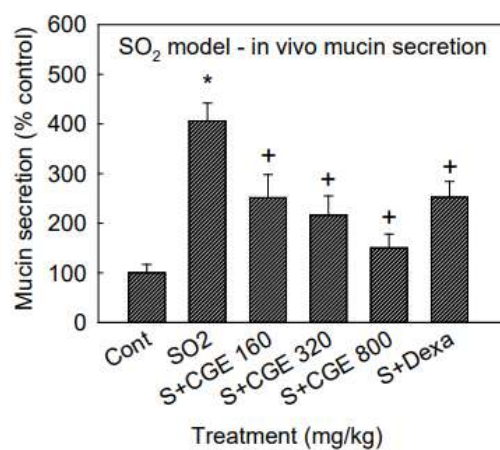
CGE inhibited LPS-induced TNF- α secretion in
bronchoalveolar lavage fluid (BALF)



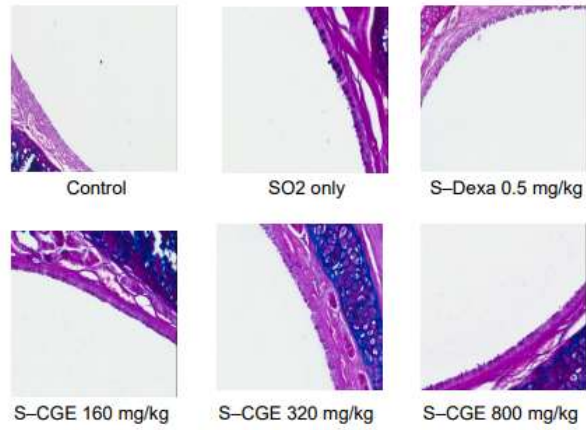
CGE inhibited LPS-induced mucin secretion in bronchoalveolar lavage fluid (BALF)



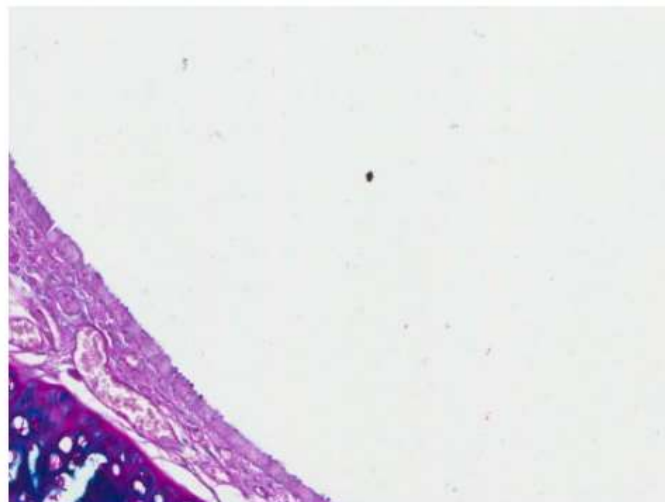
CGE inhibited SO₂-induced mucin secretion in bronchoalveolar lavage fluid (BALF)



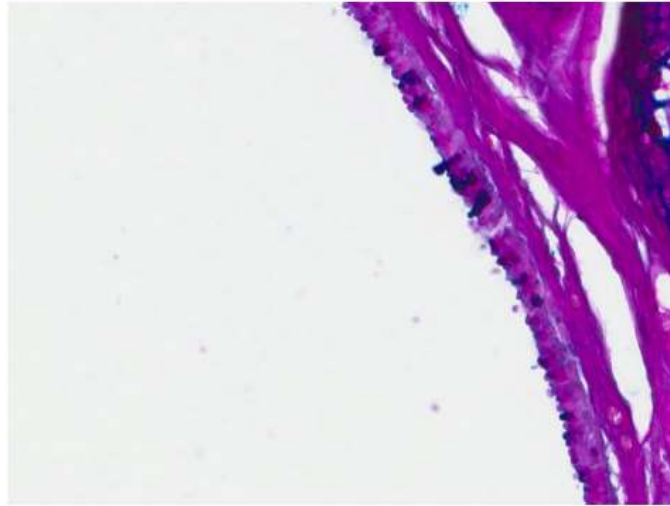
**CGE inhibited SO₂-induced increase of
mucosubstances in goblet cells of tracheal
tissues**



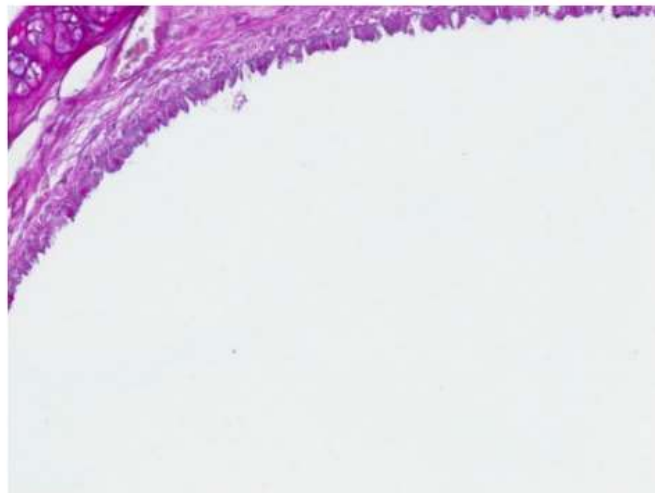
Control



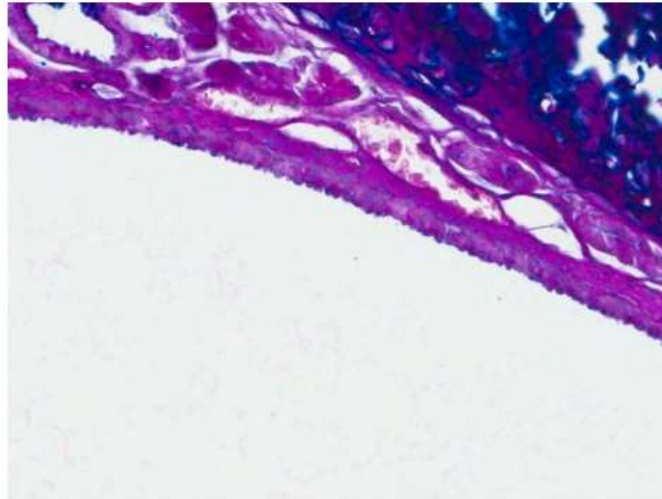
SO2 only



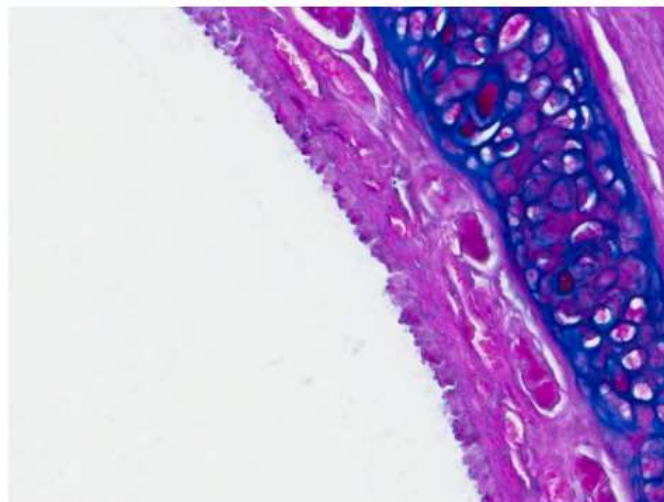
S-Dexa 0.5 mg/kg



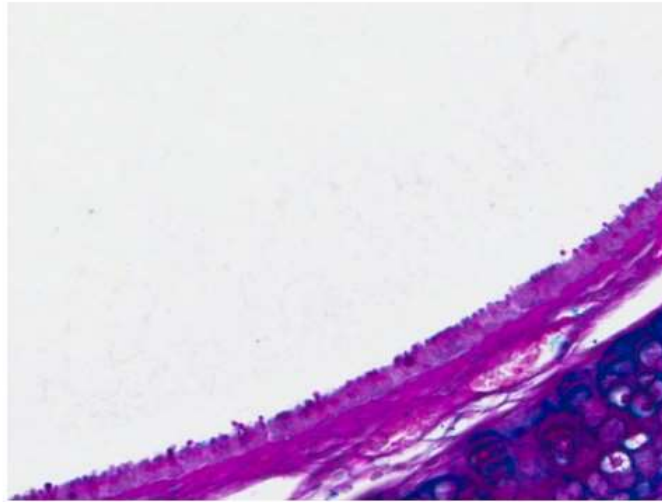
S-CGE 160 mg/kg



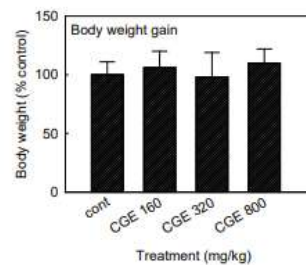
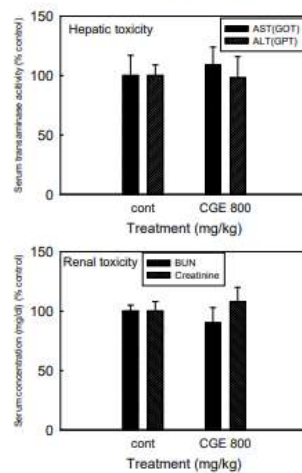
S-CGE 320 mg/kg



S-CGE 800 mg/kg



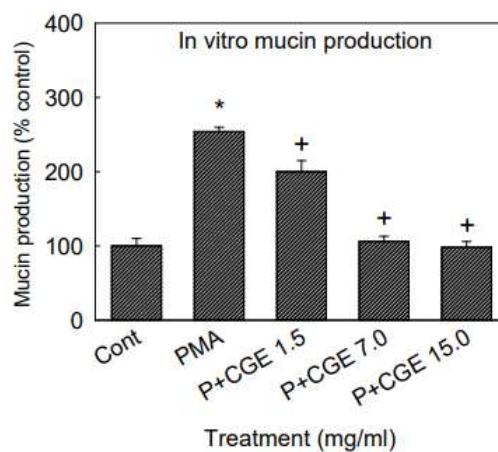
CGE did not show toxicities on liver and kidney and not affect body weight gain in experimental animals

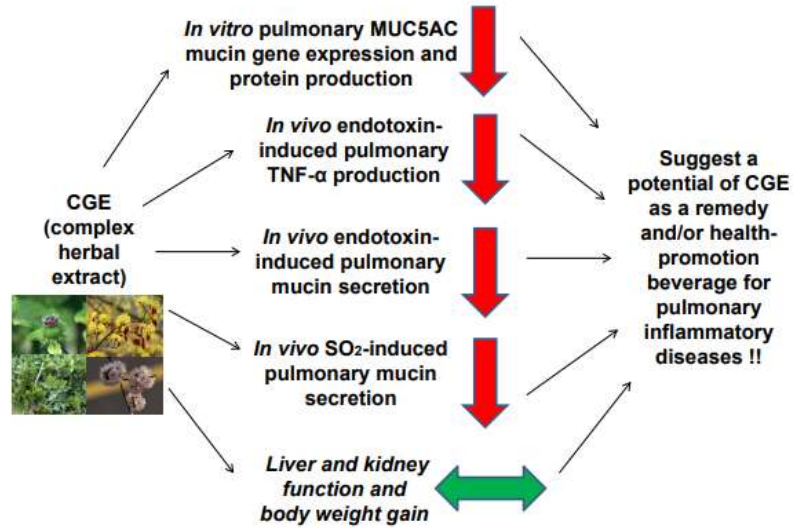


CGE suppressed PMA- induced MUC5AC mucin gene expression from NCI-H292 cells



CGE inhibited PMA-induced MUC5AC mucin production from NCI-H292 cells





**특발성 폐섬유증 동물모델에
대한 천연물 신약 후보 조성물
Complex P의 기초적 약리작용
탐색 연구**

**IPF 조절 천연물신약 후보 복합조성물
Complex P의 약효 검증
및 작용기전 규명**

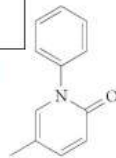
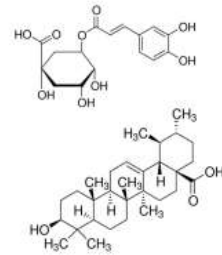
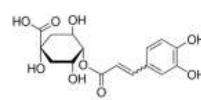
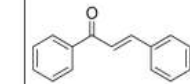
Complex P란?

- 경험적으로 호흡기 염증성 질환에 사용되어 왔으며, 생약 자체 혹은 그 주성분들이 항염증, 항산화, 항암 등 다양한 생리활성을 발현하는 것으로 보고된 생약들의 복합조성물
- IPF 동물 실험모델에서의
각 생체 지표에 대한 효과는 ?

Pirfenidone의 화학 구조와 유사한 구조의 천연물 발굴을 통한 IPF 조절 신약의 개발 가능성



IPF의 조절을 위하여 임상에서
사용 중인 Pirfenidone
(피레스파 정)



Pirfenidone
5-Methyl-1-phenylpyridin-2-one;
 $M_r = 185.2$; $C_{12}H_{11}NO$

Nature Reviews | Drug Discovery

**항염증성 생약군으로부터
류마티스성 관절염/골관절염
개선(조절)용 천연물 신약 혹은
건강기능식품 개발**

‘건기식 상품’으로서의 JGB의 경쟁력

1. 구성 원료생약의 수급 난이도/가격 경쟁력 :
 - 1) 수급성은 전반적으로 양호함.
 - 2) 원료생약 3종 공히 대한약전에 수재된 생약인 동시에 건기식 원료로서의 요건에 전혀 문제가 없으며, 가격 또한 타 생약군과 비교 시 비교적 저가에 속함.
2. JGB248은 류마티스성 관절염 치료 및 치료보조제로서, 항염증/진통 효과 평가 결과로만 본다면, 기존에 출시된 SK제약의 SKI306X(상품명 JOINS)와 거의 동등한 효능 및 효력을 나타냄.

건기식 상품으로서의 JGB의 경쟁력

3. 그러나, JGB 구성 3종 생약은 동양의학의 약물학에 해당하는 ‘본초학’에 명기된 효능 분류 체계 상,
 - 1) 이기약 (스트레스 해소, 소화기능 촉진)
 - 2) 청열해독약 (항염증, 해열, 항산화 작용)
 - 3) 신온해표약 (진통 및 해열, 발한 작용)등에 각각 속하므로,
4. 천화분 (청열사화약), 하고초(청열사화약), 위령선(거풍습약) 등으로 구성된 SKI306X(상품명 JOINS)보다도

실제 임상에서 환자에게 적용시 진통, 항염, 면역조절 작용이 훨씬 더 탁월할 가능성이 높음.

수종의 생약으로부터 가축사료 첨가용 항생제 대체물질 개발 및 실용화

 특 허 증 CERTIFICATE OF PATENT		
특 허 제 10-1012672 호 (PATENT NUMBER)	출원번호 (APPLICATION NUMBER) 제 2007-0121223 호	출원일 (FILING DATE) 2007년 11월 27일
	등록일 (REGISTRATION DATE) 2011년 01월 27일	
발명의명칭 (TITLE OF THE INVENTION) 황련, 금은파 및 상엽의 혼합 추출물을 포함하는 항생효과를 갖는 사료첨가제와 그의 제조방법		
특허권자 (PATENTEE) 충남대학교산학협력단(160171-0*****) 대전광역시 유성구 공동 220번지 충남대학교		
발명자 (INVENTOR) 등록사람관에 기재		
위의 발명은 「특허법」에 의하여 특허등록원부에 등록되었음을 증명합니다.		

Lessons from Anecdotes of Ancestors : 실험모델에서 약효발현 여부의 결정 요인

치효산 가미방, 청금강화탕, 소청룡탕,
과루지실탕, 취연탕, 통규탕 가미, 청폐탕,
형방패독산, 필용방 감길탕, 청화보음탕,
정천탕, 선방패독탕, 배농산급탕, 은교산...

가미 사물탕, 가미 이중탕, 금수육군전,
가미 육군자탕, 가미 신기탕, 팔미환료,
청상보하탕, 맥문동탕, 이음전, 생맥산,
육미환료, 가미 보중익기탕.....

Lessons from Anecdotes of Ancestors : 노인의 기침과 수양성 객담 배출

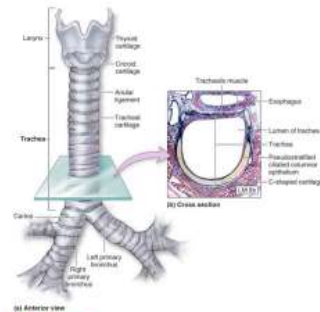
노인의 기침과 수양성 객담의 조절은, 호흡기 질환에 직접 작용하는
항생제, 점액용해제, 거담제 등 치료약물에 잘 반응하지 않는
경우가 많다.

→이 경우에 한방 약물인 부자를 병용투여하면, 수양성 객담과
기침이 현저히 줄어드는 현상을 임상에서 관찰할 수 있다.



이러한 현상은,

1. 君火之官인 心臟,
 2. 主血統割하는 장기인 脾臟,
 3. 主水의 장기인 腎臟
- 등의 기능저하로,

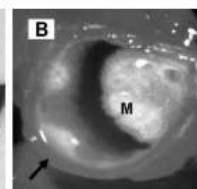
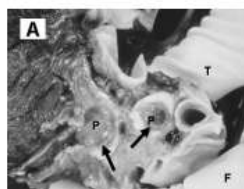
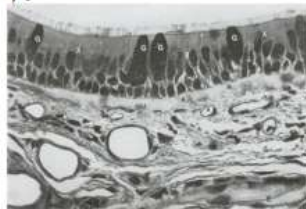


- (1) 혈액순환의 효율이 저하되며,
 - (2) 혈액 내의 수분이 기관지 조직 중으로 삼출되고,
 - (3) 이러한 異常 분포된 체내 수분이 원활히 제거되지 않으면,
- 그 결과 수양성 객담이 많아지게 되고, 기관지 조직에의 울혈 및 부종의 증가로 기침이 심해지는 것으로 이해될 수 있다.

이 때, 心, 脾, 腎 三陽虛를 補할 수 있는 한방 약물인 附子를 병용투여하면,

→ 기관지 조직에 이상 분포된 수분을 제거하여 신장을 통해 배설하게 하고,

→ 따라서, 수양성 객담 및 기침이 현저히 줄어드는 것으로 해석할 수 있다.



Conclusion

Discovery of candidates
for novel drug
from natural products:
mining gold
from tales of ancestors

→ Patience, sincerity and idea
are needed !!

Acknowledgement

식품의약품 안전처, 한국연구재단, 농림수산물식품부
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Mira Choi, M.S., Dong Joon Kim, Lab technician
Min Jeong Hong, Lab technician, Cheol Su Kim, M.S.



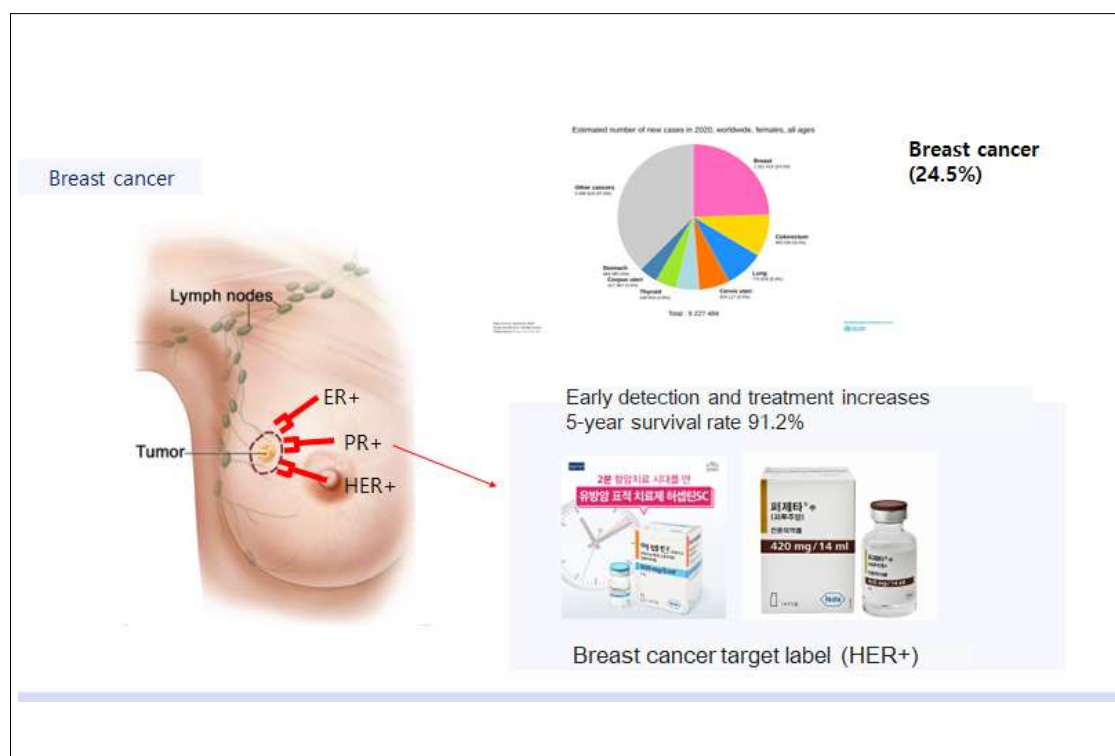
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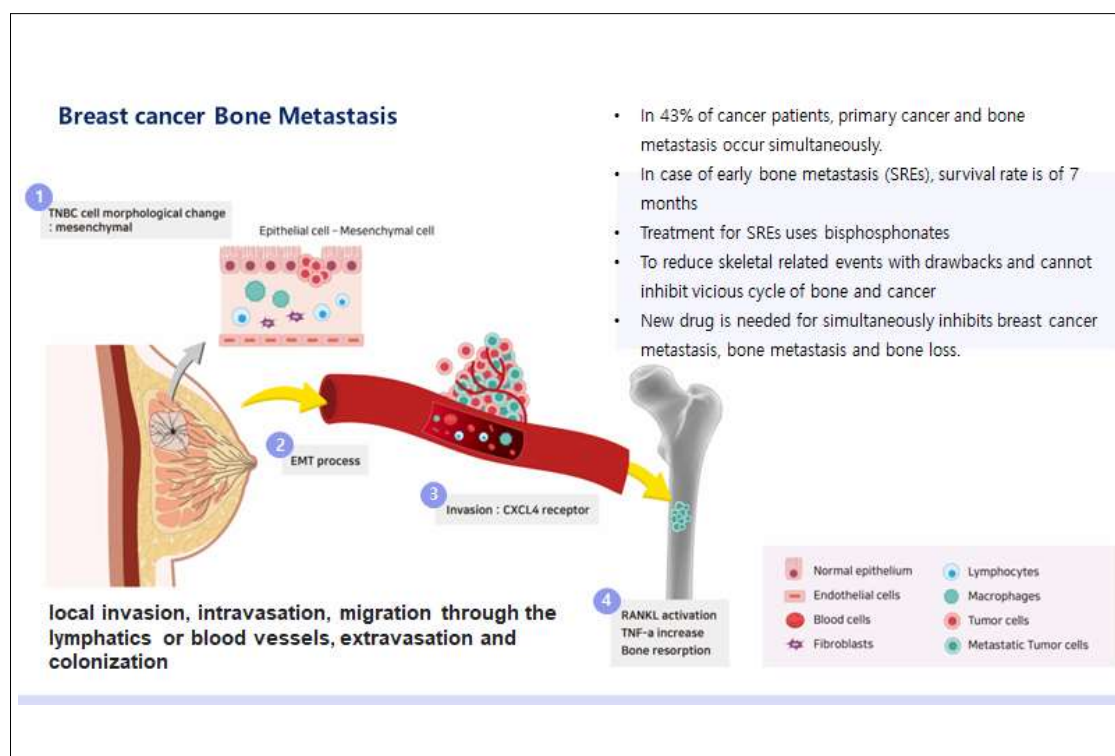
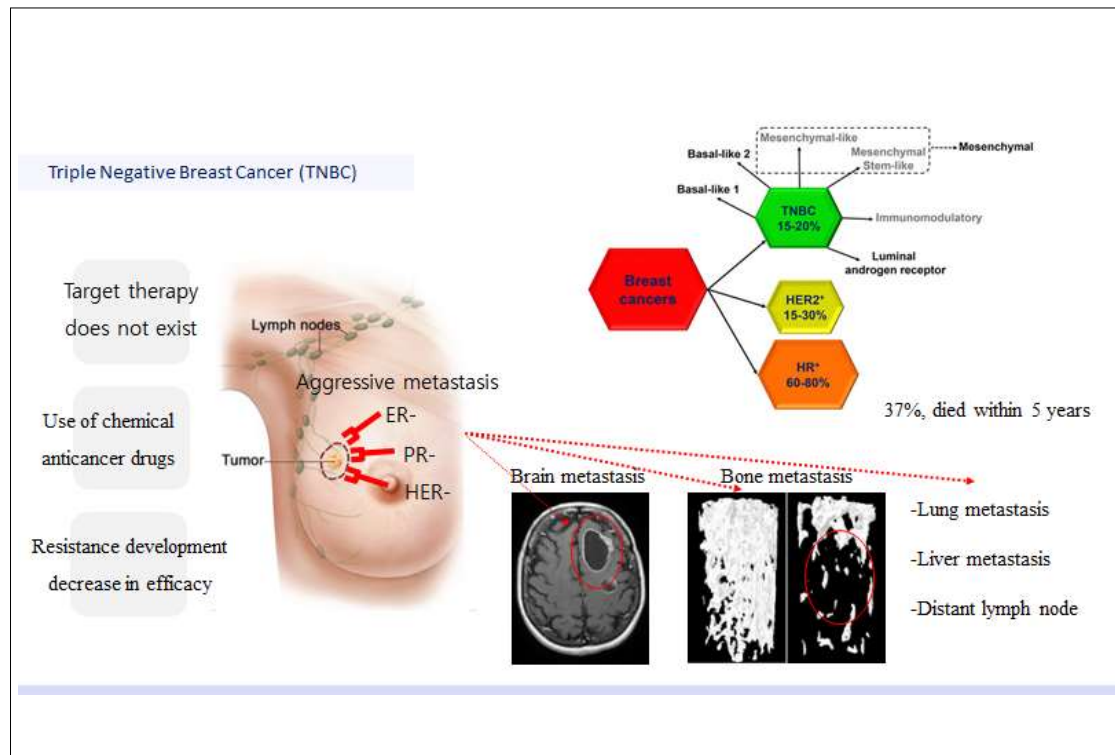
**약물학 전공
대학원생 발표 세미나**

사로즈 스텔스타 학생

Kalkitoxin thiol amide inhibits distant breast cancer metastasis

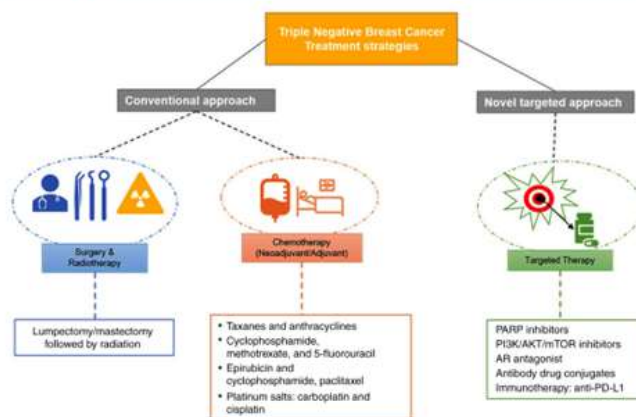
Shrestha Saroj Kumar
201955153
Department of Dental Science
Jeonbuk National University
지도교수: 소윤조



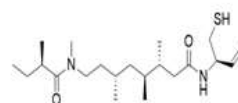


TNBC Chemotherapy resistance inhibition strategy

- There is no treatment of TNBC currently
- Chemotherapy under clinical trials and targeted anticancer drugs are being developed as a combination therapy method
- There is limitation for treating cancer, like intracellular toxicity of anticancer drug and development of resistance to targeted anticancer drugs, so a strategy need to avoid resistance.



- Kalkitoxin thiol amide (KTA) is derived originally from the cyanobacterium *Moorena producens*, found growing attached to coral reefs.



Molecular structure of Kalkitoxin thiol amide

2022.09.28.

**약물학 전공
대학원생 발표 세미나**

올라 하산 학생

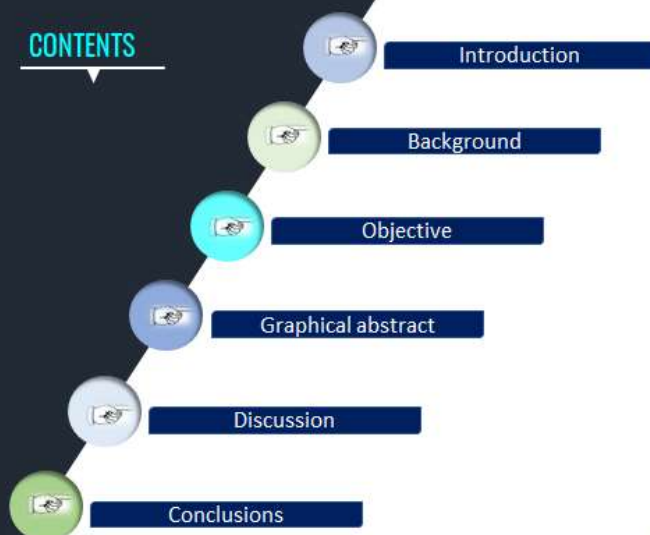
TMBIM6 regulates ER stress and inflammation in refractory asthma



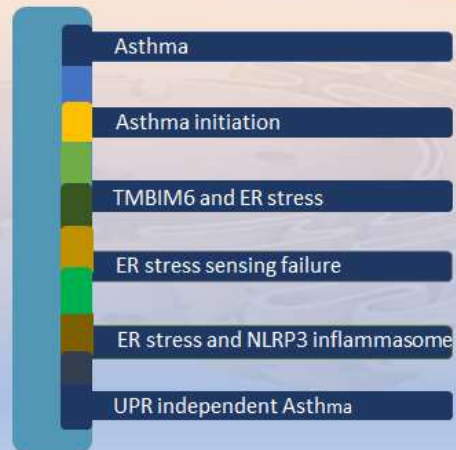
Presented by - ULLAH AHSAN
(Supervisor; Prof. Chae Han Jung)

PhD student
Student ID: 202255413
School of Pharmacy
Department of Pharmacology
Jeonbuk National University Graduate School

CONTENTS

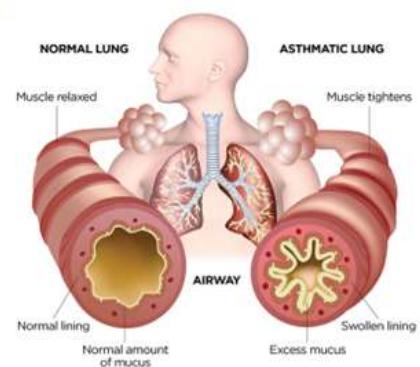


Introduction



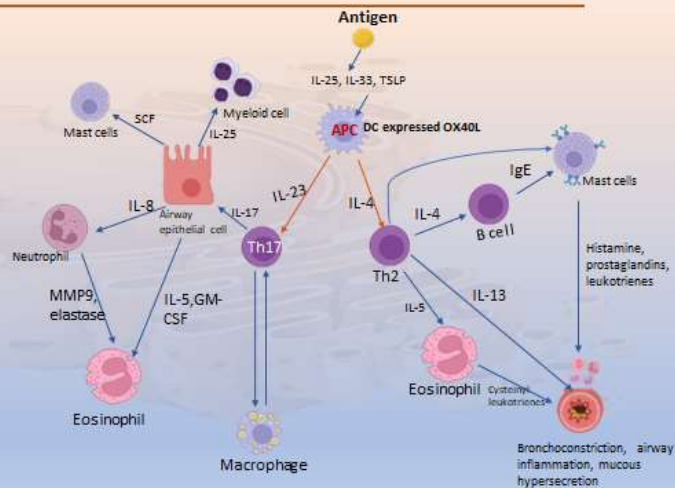
Asthma

- Asthma is a heterogenous, chronic airway inflammatory disease with different clinical phenotypes as well as a range of endotypes.
- Asthma is considered as one of the top five respiratory disease in the world and it affects 334 million people globally.
- The main manifestations of asthma are dyspnea, wheezing, chest tightness and cough and is characterized by airways inflammation, mucus metaplasia which impacts lung structure and function.
- Although the term "severe asthma" remains vague associated with Th2 and Th17, called refractory asthma that do not respond to currently employed treatments.
- Despite significant scientific advances, a mechanistic understanding of the pathogenesis of asthma remains challenging.



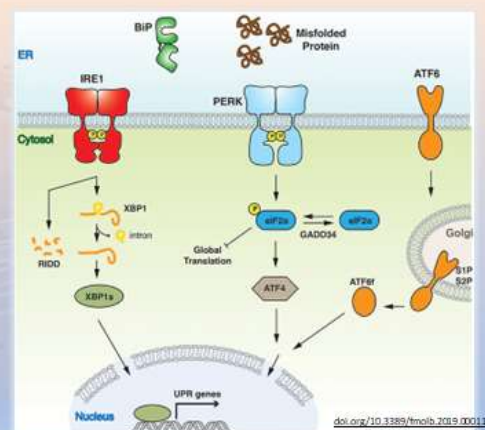
Asthma initiation

- On the cellular level, asthma can be roughly divided into Th2 inflammation-related and non-Th2 inflammation-related asthma.
- The inflammation associated with Th2-type asthma is mainly eosinophilic inflammation and non-Th2 type is neutrophilic inflammation.
- Upon exogenous allergens exposure or activation ILC2; Th2 cells are activated to produce related Th2 cytokines.
- Non-Th2 inflammation is mediated predominantly by Th1 and Th17 pathways.



TMBIM6 and ER Stress

- TMBIM6 is a pH-dependent, voltage-gated calcium channel like protein which is expressed in the ER.
- The ER is an essential largest intracellular organelle mainly responsible for folding and modifying of newly synthesized proteins.
- ER stress involves accumulation of unfolded or misfolded proteins due to disruption of homeostasis in the ER function triggers the ER stress response, called UPR.
- High amplitude of UPR signaling causes the response to become maladaptive. In mammals, three distinct ER stress sensors exist: IRE1, PERK, and ATF6.
- TMBIM6 suppressed the ER stress as a stress regulator.
- A cell may activate over 400 UPR target genes involved in responding to ER stress, such as chaperone proteins, ribosome biogenesis genes, ERAD effectors, and genes to expand the ER lumen.

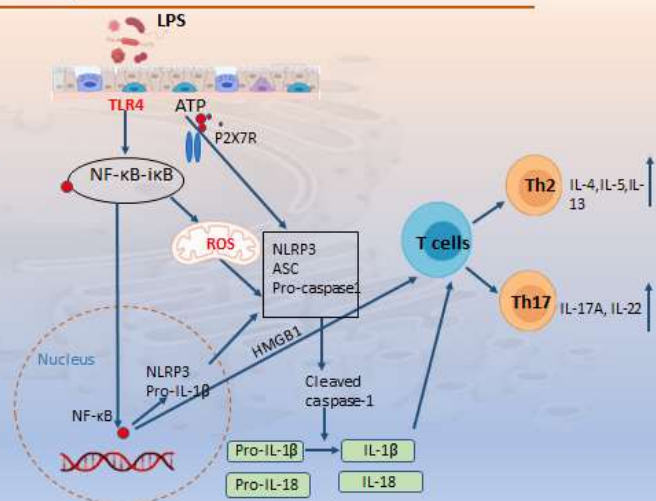


ER stress sensing failure: Involvement of chaperones

- TM6IM6 knockout or deficiency enhances impaired ER stress response and eventually causes to ER stress response failure during asthmatic condition declining chaperone systems of the ER.
- For example, both BiP ATPase activity and PDI enzymatic function have been shown to be significantly decreased in severe asthma.
- The accumulation of HMWC PDI which compromises protein folding and lead to the formation of insoluble multiprotein complexes, eventually alter the protein folding capacity in TM6IM6 knockout mice.
- This is possibly due to an increased rate of oxidation of chaperone (**protein quality control machinery**) in severe asthma leading to structural changes and consequently decreased enzymatic functions.
- Thus ER stress and the failure to correctly fold proteins are associated with loss of protein function.
- Further ERAD impairment induces proteostatic dysfunction essentially leads to a vicious cycle of increasing ER stress, protein accumulation, and stress response impairment.

ER stress and NLRP3 inflammasome

- Both ER stress and inflammation are defensive responses triggered by stressful stimuli, but can become destructive when sustained.
- ER stress can regulate the NLRP3 inflammasome in many diseases and related mechanism including asthma.
- The NLRP3 inflammasome is macromolecular complex that can trigger inflammation, and its activation is induced by a variety of signals.
- The assembly of NLRP3 inflammasome consists of the NLRP3 receptor, the adaptor protein ASC, and caspase-1 that acts as an effector protein.
- Mature IL-1 β is a potent proinflammatory mediator in many immune reactions.



UPR independent Asthma

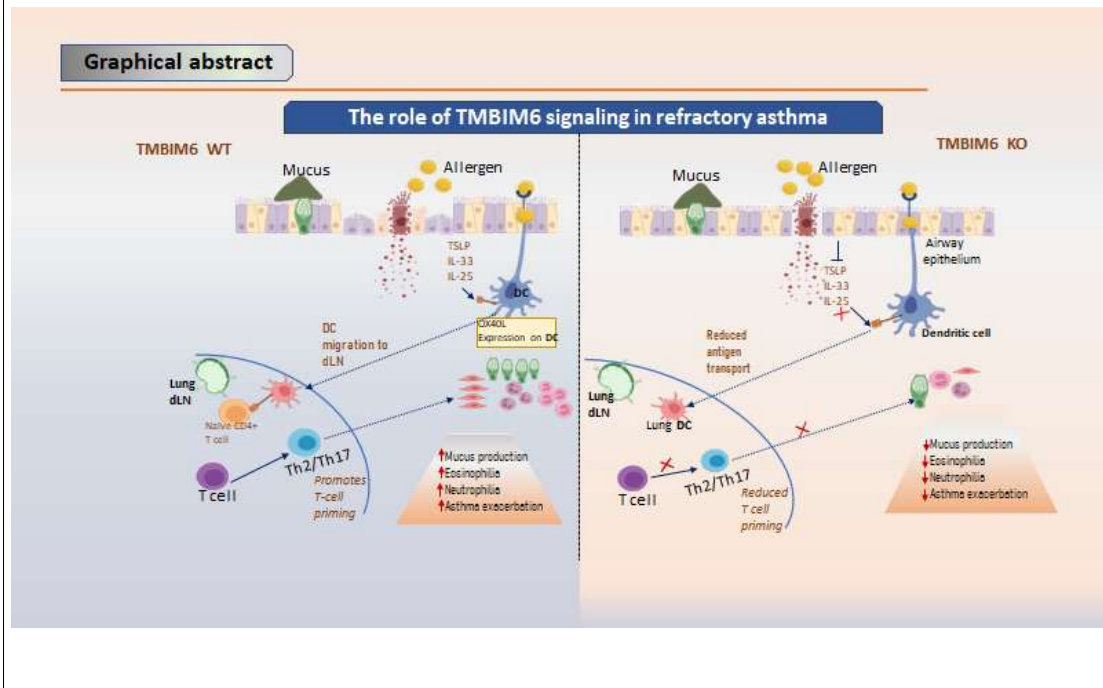
- Emerging evidence delineating the potential functions of ER stress in asthma development through critical roles of UPR as well as distinct from the UPR pathways in asthma.
- Here, we show that ER stress causes activation of the NLRP3 inflammasome, with subsequent release of the pro-inflammatory cytokine interleukin-1 β but is independent of the classical unfolded protein response (UPR).
- We thus propose that the NLRP3 inflammasome senses and responds to ER stress downstream of a previously uncharacterized ER stress response signaling pathway distinct from the UPR, providing mechanistic insight to the link between ER stress and chronic inflammatory diseases.
- The precise signaling pathway involved remains elusive, similar to other NLRP3 activators described to date.

Background and Objective

Background: Inflammasome activation with the production of IL-1 β received substantial attention recently in inflammatory diseases. However, the role of inflammasome in the pathogenesis of asthma is not clear. Using a OVA/LPS model of allergic lung inflammation, we investigated the role of NLRP3 inflammasome and related it to IL-1 β signaling pathway. This study investigated the previously unknown regulatory mechanism of TM6IM6 during asthmatic condition. Wild-type (WT) and the TM6IM6 knockout mice were used to demonstrate the effects of refractory asthma to identify pathogenic mechanisms and investigate new treatment approaches.

Objectives: As the mechanism by which ER stress triggers inflammation remains poorly understood. Here, we sought to examine the chaperone activity to investigate the roles and therapeutic targeting of the NLRP3 inflammasome as a potential sensor of ER stress.

Graphical abstract



Discussion

Since there are multiple links between ER stress and inflammation, it is not implausible that these links may also be related to asthma. Intriguingly, the role of TMBIM6 regulates ER stress and inflammation and ER stress mediated inflammasome activation has not attracted a great deal of attention.

We demonstrated that deficiency of TMBIM6 causing ER stress response failure which causes impairs sXBP1, accumulation of HMWC of PDI, reduced ER chaperones activity, and ERAD dysfunction. Here, we show that ER stress induced NLRP3 inflammasome, with subsequent release of the pro-inflammatory cytokine interleukin-1 β in TMBIM6 WT than knockout mice which is result of decay of IL-25 (data not shown)

Excessive post translational oxidation of PDI or dysfunctional PDI chaperone activity alter the folding capacitance of glycoprotein or newly synthesized protein

In the present study, we also demonstrated that TMBIM6, potent controller of Epithelial derived cytokine. Our findings suggest that TMBIM6 may play a role in the innate immune response maintaining the proper folding capability by PDI; is independent of adaptive immunity.

Our work supports the notion that the structural changes and impairment of enzymatic activity of PDI itself, unable to facilitates disulfide (-S-S-) bond formation on glycoproteins being processed and secreted by the ER in TMBIM6 knockout condition.

Conclusions

NLRP3 inflammasome response to IL-1 β production is critical for the induction of a Th2 and Th17 inflammatory allergic response in refractory asthma and are potential therapeutic targets in this disease.



THANK YOU
for your kind attention

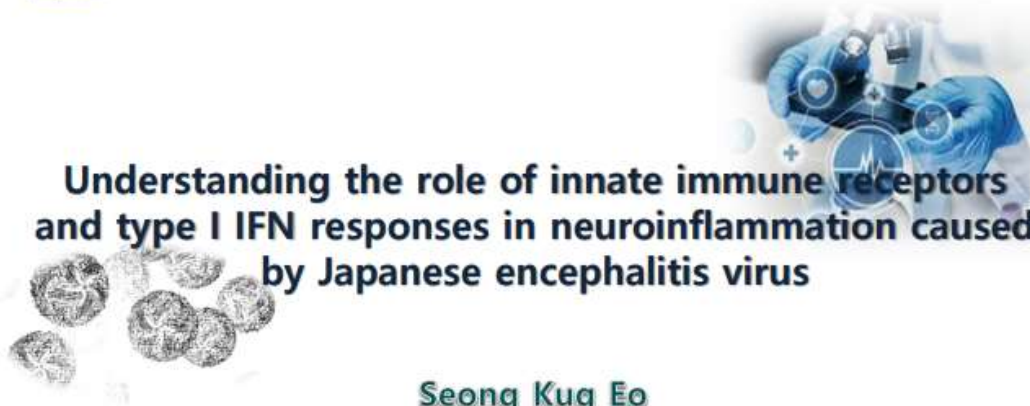


2022.10.05.

우수연구자 초청 세미나

인수공연구소

어성국 교수님



Understanding the role of innate immune receptors and type I IFN responses in neuroinflammation caused by Japanese encephalitis virus

Seong Kug Eo

Viral Immunology Lab

CHONBUK NATIONAL UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

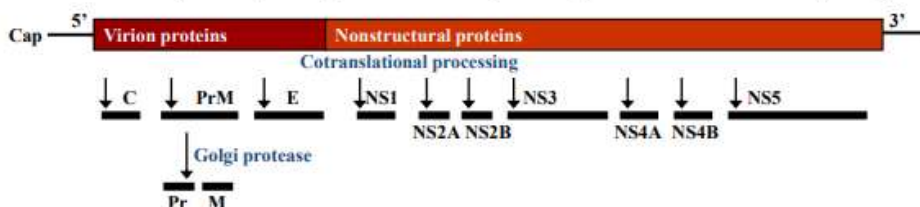


Japanese encephalitis virus (JEV, 일본뇌염바이러스)

Family *Flaviviridae* Genus *Flavivirus*

Genus *Flavivirus*:

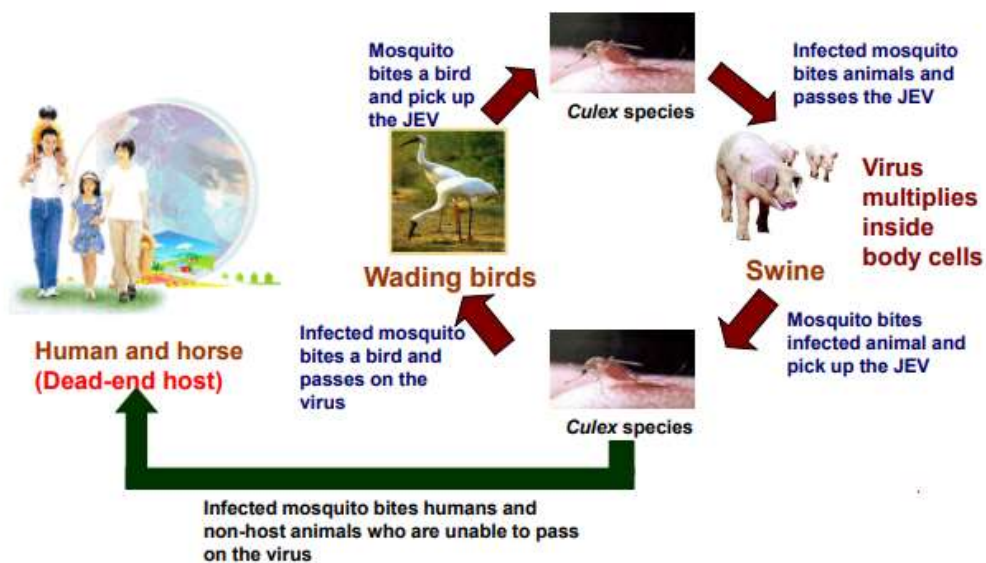
1. Positive-sense, single-stranded RNA virus (10.6-10.9 kb)
2. Transmitted to vertebrates by infected mosquitoes
(*arthropod-borne viruses*, *arbovirus*)
3. Producing disease in human beings and animals (*zoonotic disease*)
4. Japanese encephalitis virus (JEV), West Nile virus (WNV),
Dengue virus (DenV), St. Louis encephalitis virus (SLEV),
Murray Valley encephalitis virus (MVEV), Yellow fever virus (YFV)



Representative flaviviruses that cause diseases in human and animals

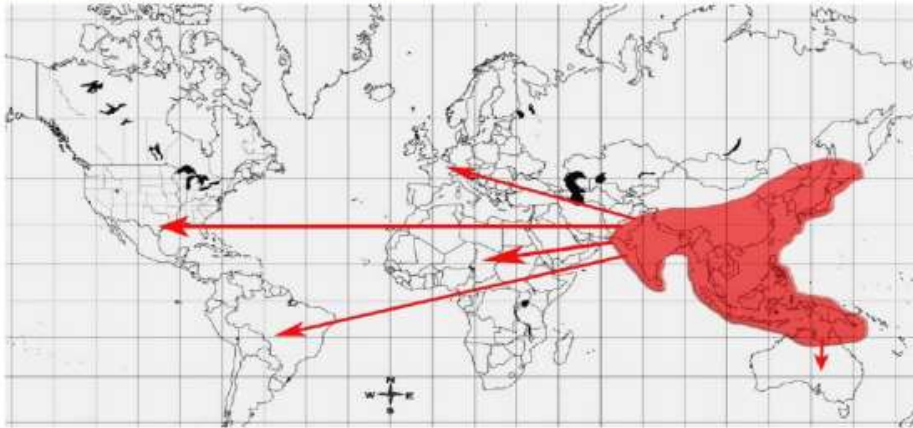
Virus	Host of concern (Reservoir host)	Disease in human (or animals)	Geographical distribution	Cases per year
Japanese encephalitis virus (JEV)	Swine, human (birds)	Encephalitis (Abortion, Neonatal disease)	Asia (eastern and southern Asia)	Approx. 72,000 cases/yr (25-30% neuropsychiatric sequela)
Zika virus (ZV)	Human (human and monkey)	Fever with rash, Headache, Microcephaly during pregnancy	Asia, Africa, Oceania, US, Southern America	2015, 0.4-1.3 million in Brazil 2016, 1,526 cases in US 19 cases in Korea up to 2017
West Nile virus (WNV)	Human (birds)	Fever with rash, rarely arthralgia or encephalitis	Mediterranean, Europe, US, Asia	2010, 1,021 cases; 2012, 5,674 cases; 2014, 2205 cases; 2016, 2038 in USA (approx. 1% serious manifestation)
Dengue virus 1,2,3,4	Human (human and monkey)	Hemorrhage Fever and Shock Syndrome(DHF/DSS) [Increasing Variants causing Dengue Encephalitis with intracerebral hemorrhage]	Tropical world	3.9 billion at infection risk (2013) 50-100 million case(febrile illness) 500,000 case (DHF/DSS)

Transmission Cycle of JEV



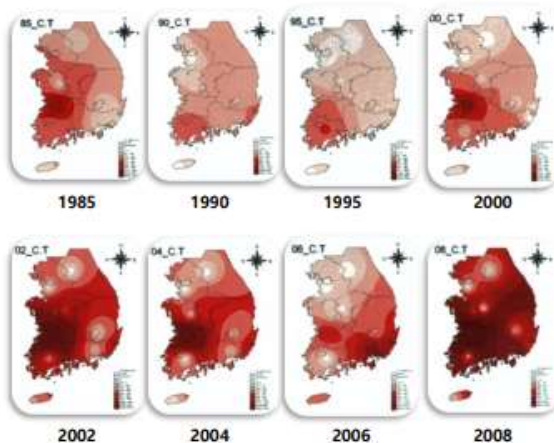
Epidemiology of Japanese encephalitis virus

The global distribution and spread of Japanese encephalitis virus by global warming and the change of ecosystem

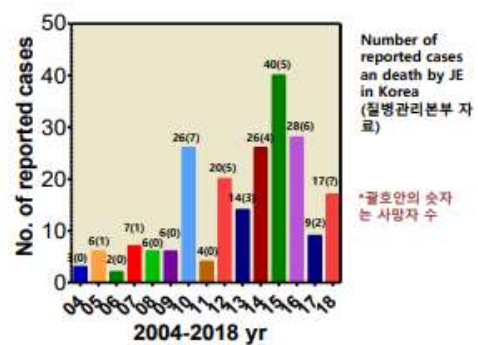


(Plos Neglected Tropical Pathogen, 2009)

지구온난화에 의하여 모기 밀집도의 증가와 일본뇌염 환자의 급격한 증가



Geographical distribution of *Cx. Tritaeniorhynchus* in Korea from 1985 to 2008 (Master Thesis, 2011)



Number of reported cases and death by JE in Korea (질병관리본부 자료)

*괄호안의 숫자는 사망자 수

Clinical Signs Human

- ◇ Incubation period: 6 to 8 days
- ◇ Most asymptomatic or mild signs
- ◇ Children and Elderly
 - At highest risk for severe disease
 - Elderly: High case fatality rate (30%)
- ◇ Approx. 72,000 cases annually
 - About 5-30% fatality rate
 - Serious neurologic sequela: 33-50%
- ◇ 30-40 case/year in Korea
 - Gradually increase risk by global warming

Vaccination

- Live attenuated vaccine: used in equine and swine, successful for reducing incidence
- Inactivated vaccine (JE-VAX): used for humans Japan, Korea, Taiwan, India, Thailand, Used for endemic or epidemic areas



(The Lancet, 2008)

Clinical Signs Swine

- Incubation period not known
- Exposure early in pregnancy more harmful
- Birth of stillborn or mummified fetuses
- Piglets: Neurological signs, death
- Boars: Infertility, swollen testicles



Previous Study

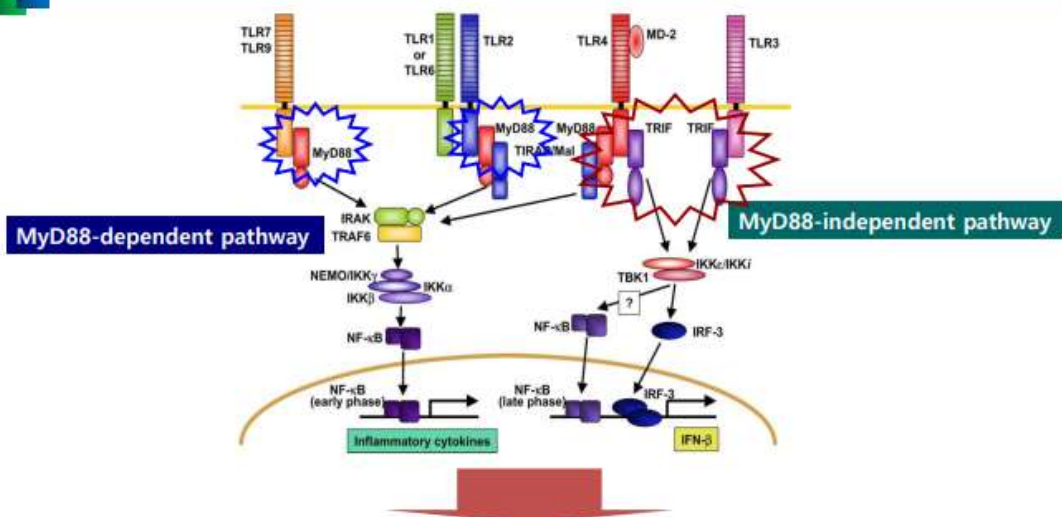
I

Functional modulation of dendritic cells and macrophages by Japanese encephalitis virus through MyD88 adaptor molecule-dependent and independent pathway *J. Immunol.* 2009

II

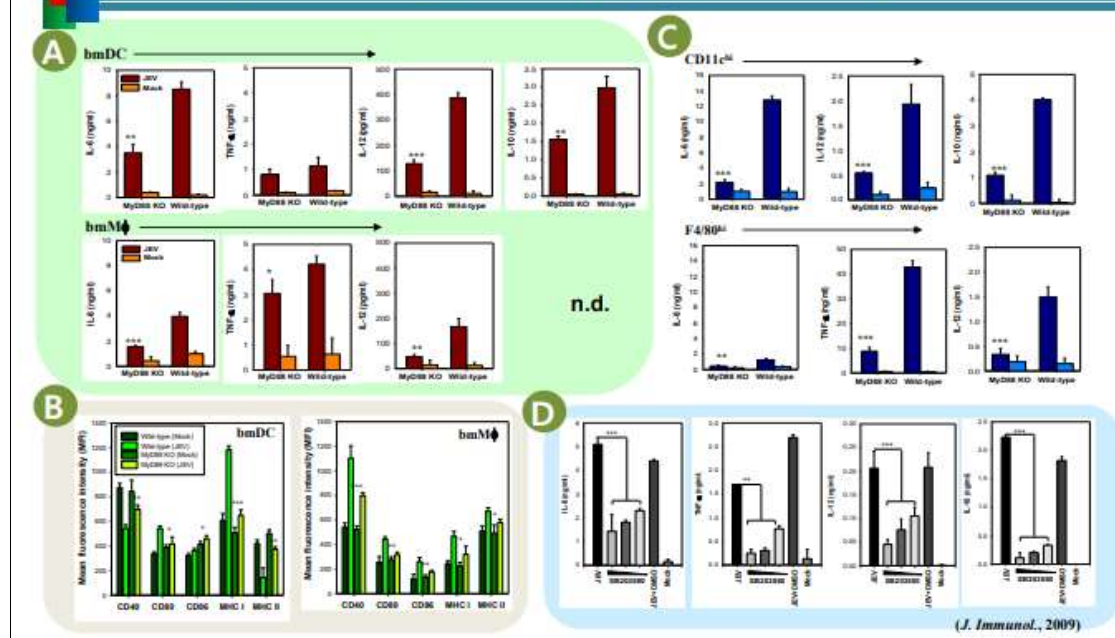
Multifront assault on antigen presentation by Japanese encephalitis virus subverts CD8+ T cell responses *J. Immunol.* 2010

Toll-like receptor (TLR) signaling

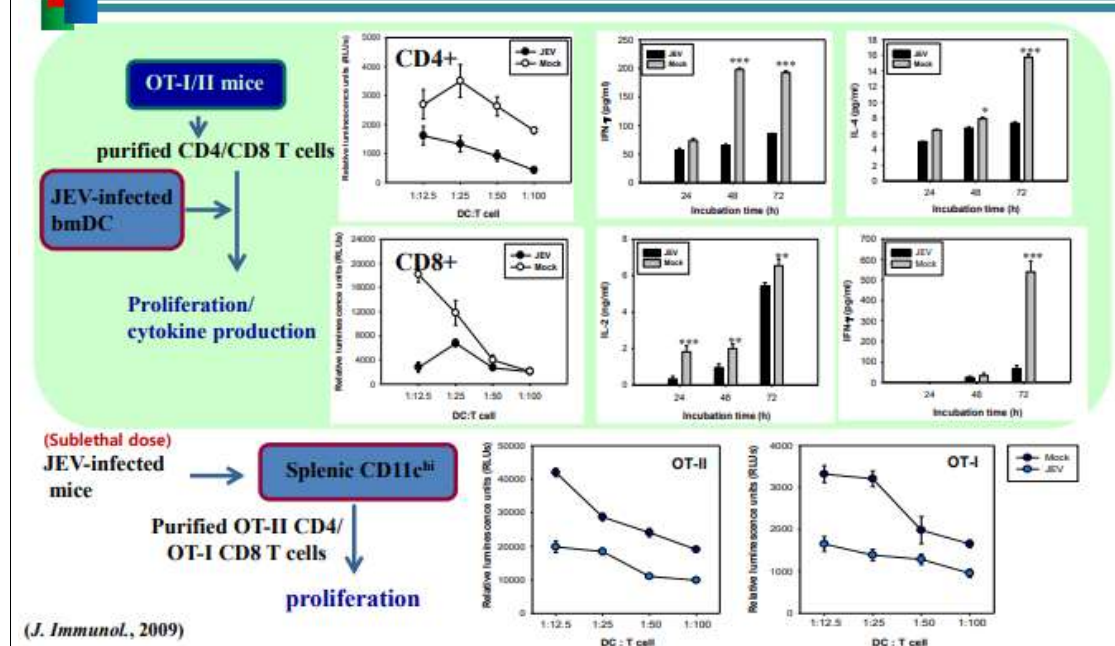


Used MyD88-deficient mice to investigate the role of TLRs in modulating the function of DCs and macrophages by JEV infection

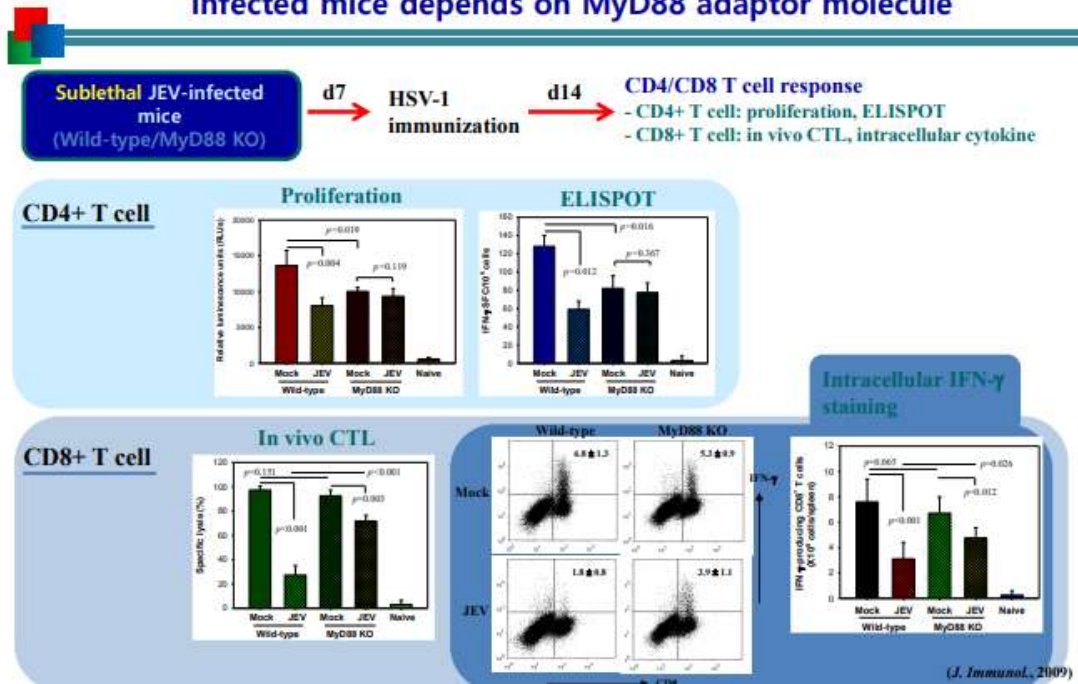
The production of cytokine from JEV-infected DCs partially depends on MyD88 adaptor molecule and p38 MAPK



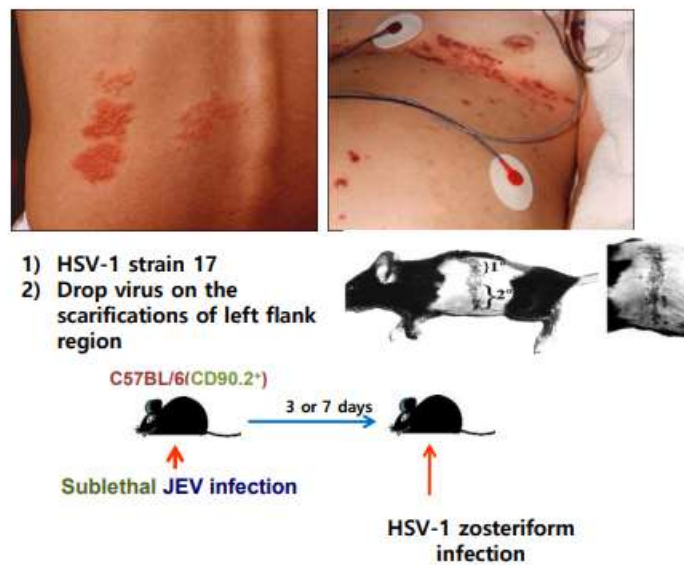
Poor capacity of JEV-infected DCs to prime antigen-specific CD4⁺ and CD8⁺ T cells



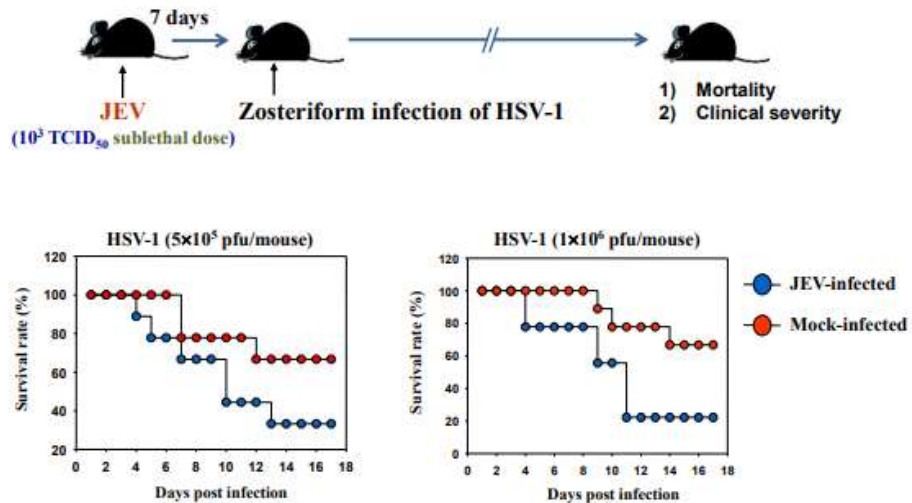
Reduced induction of CD4⁺ and CD8⁺ T cell responses in JEV-infected mice depends on MyD88 adaptor molecule



Zosteriform infection model of Herpes virus

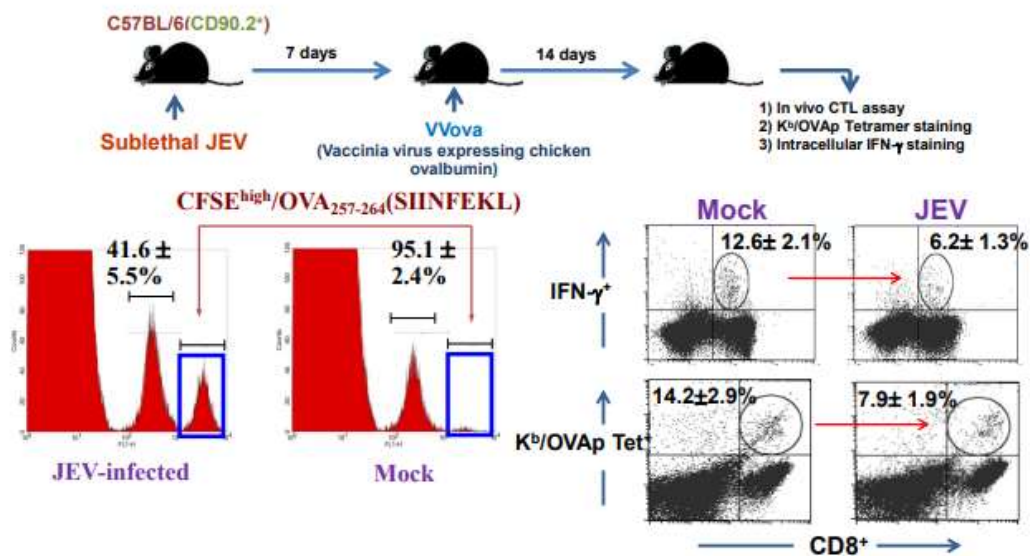


Increased mortality of JEV-infected mice against zosteriform infection of herpes simplex virus



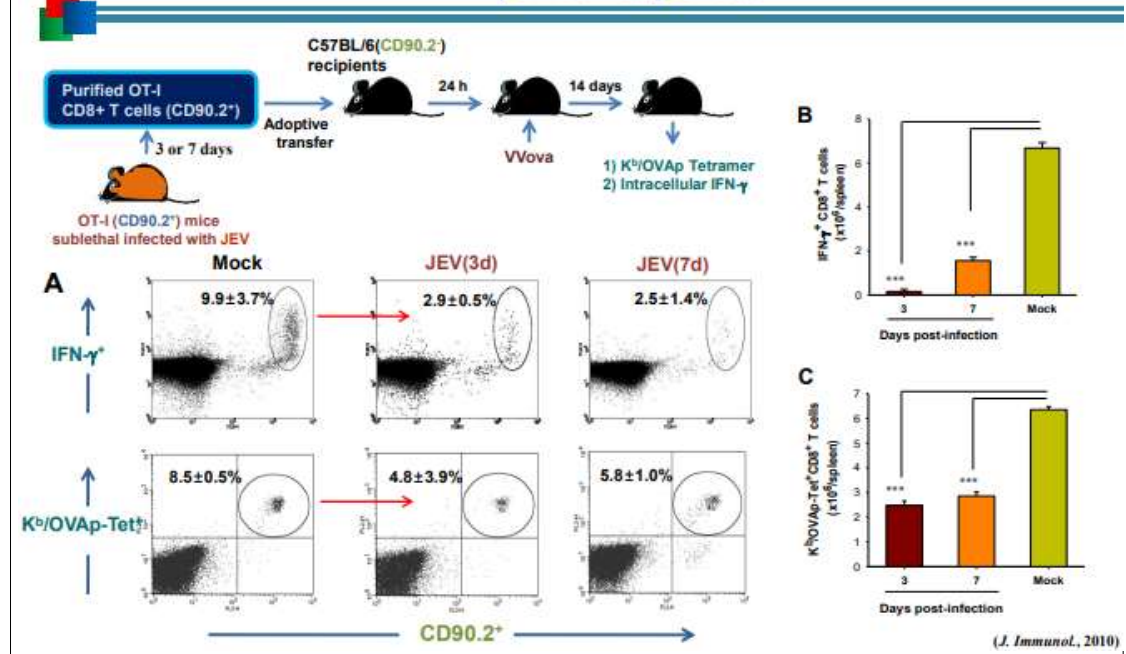
(J. Immunol., 2010)

JEV-infected mice show subversion of antigen-specific CD8⁺ T cells

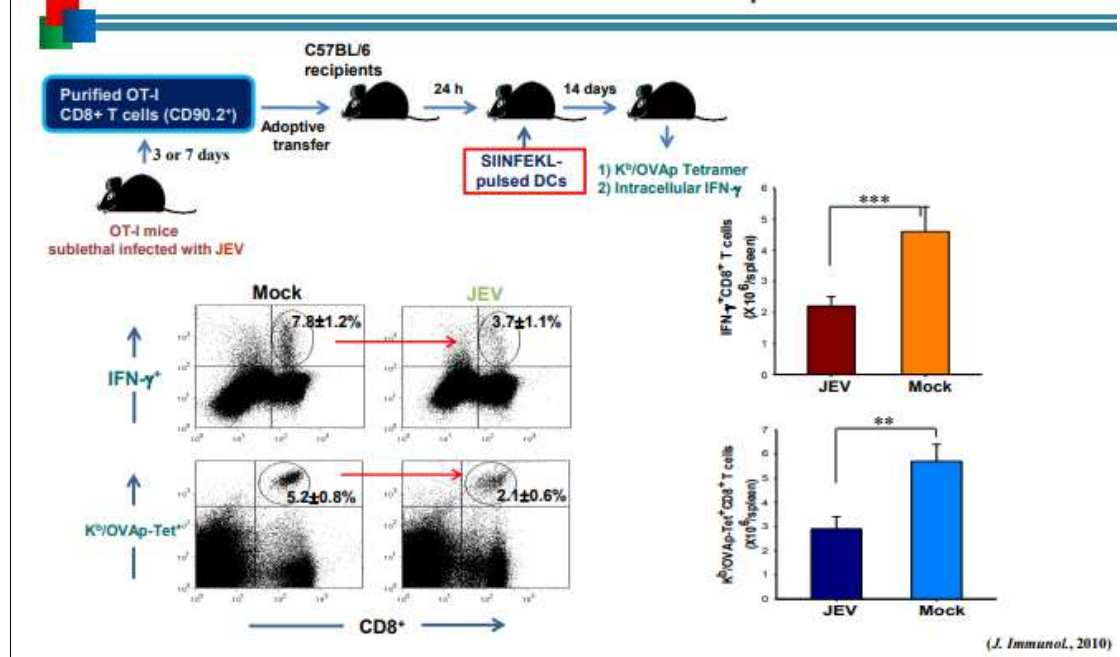


(J. Immunol., 2010)

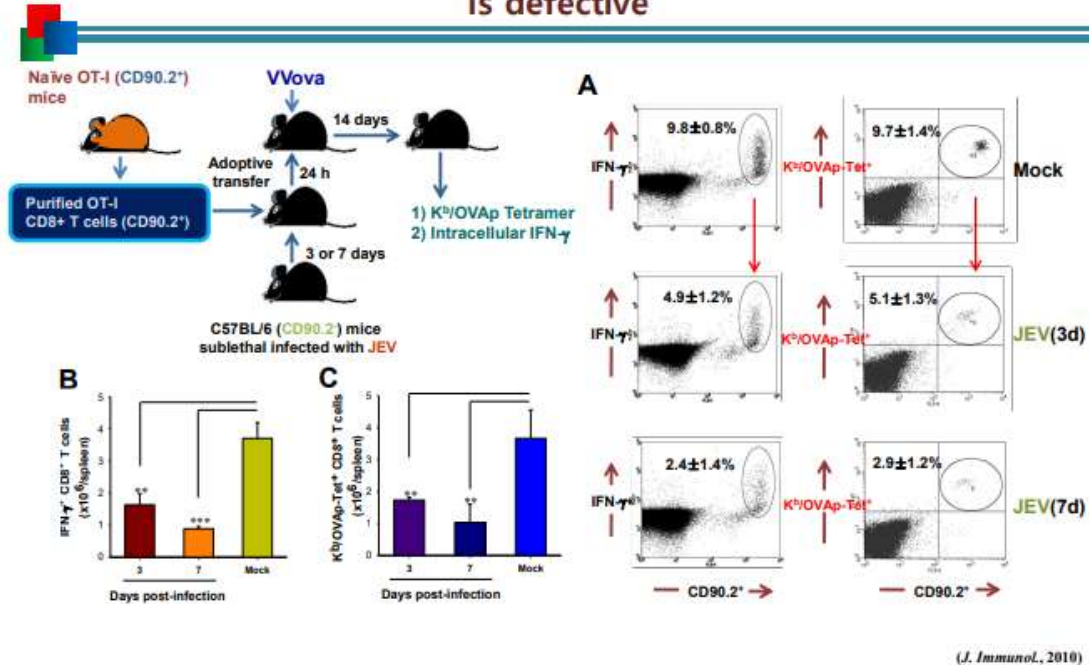
CD8⁺ T cells obtained from JEV-infected mice show impaired responses



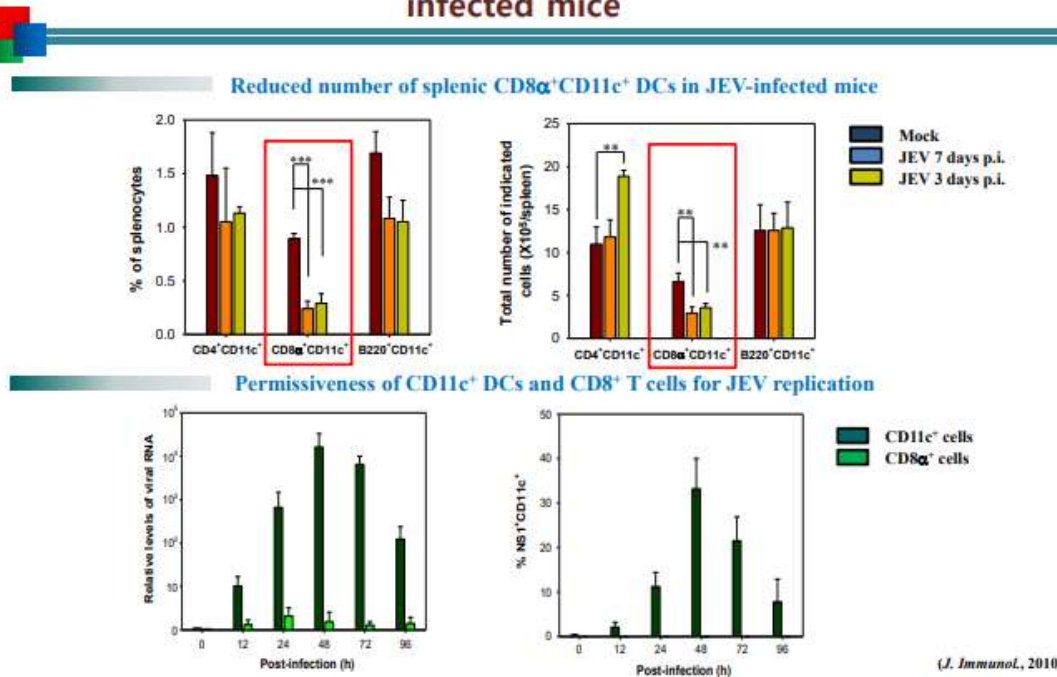
Impaired responses of CD8⁺ T cells purified from JEV-infected mice after immunization with SIINFEKL-pulsed DCs

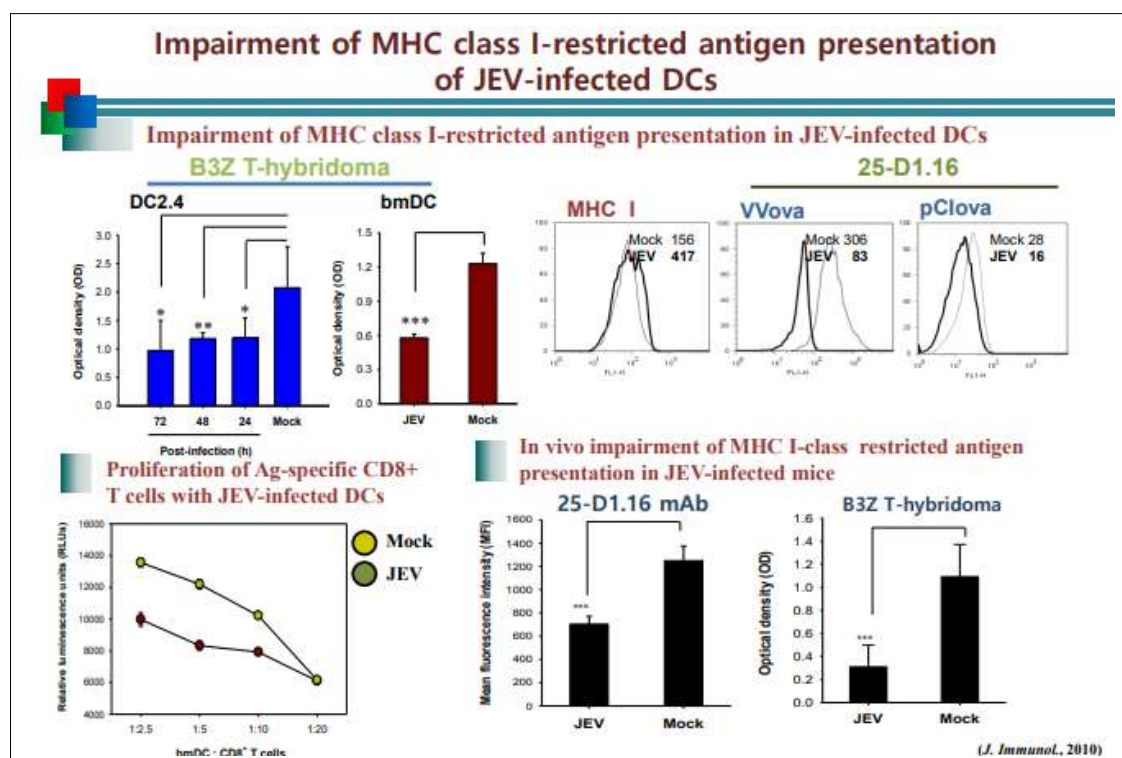
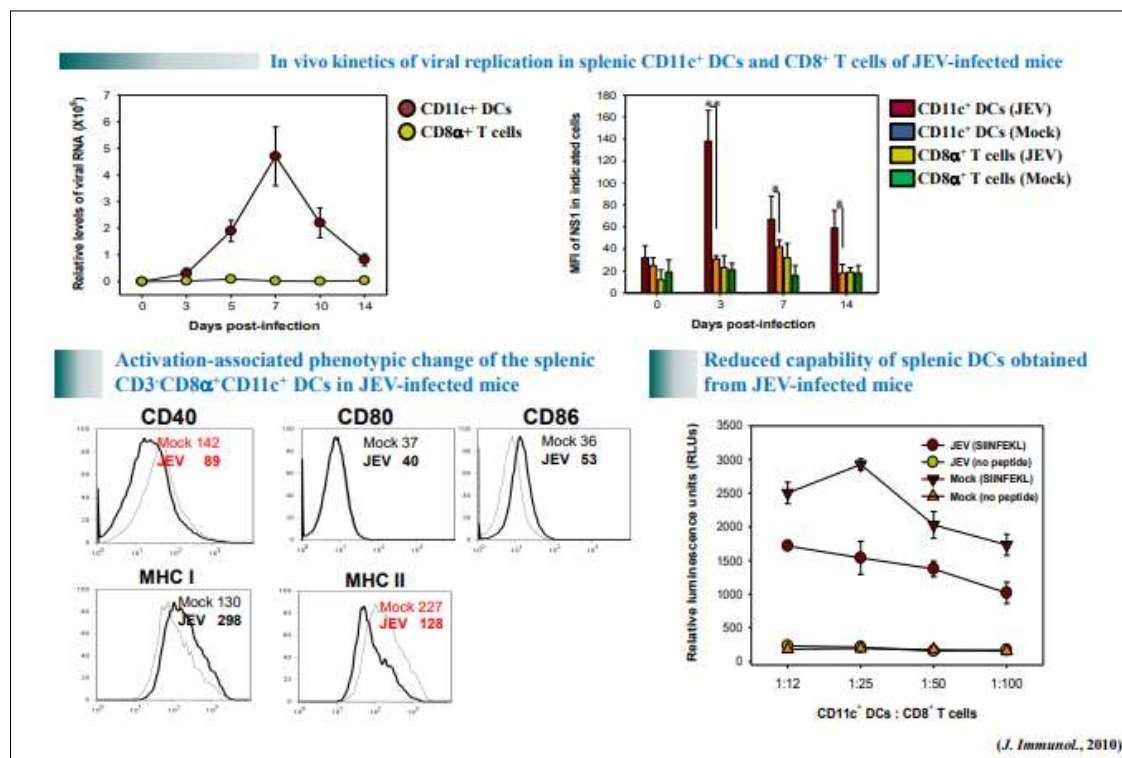


The antigen-presenting machinery of JEV-infected mice is defective



Skewed proportion of splenic DC subtypes in JEV-infected mice





Conclusions I

- DCs and macrophages are permissible for viral replication of JEV in different efficiency
- The differential modulation of cytokine production in JEV-infected DCs and macrophages (no production IL-10 in JEV-infected macrophages)
- Differential phenotypic modulation of JEV-infected DCs and macrophages (Reduction of CD40 and MHC II in DCs)
- The cytokine production and phenotypic alteration JEV-infected is partially dependent on MyD88 and p38 MAPK molecules (MyD88-dependent and – independent pathway)
- Moreover, JEV infection induces the reduced CD4⁺ and CD8⁺ T cell responses depending on MyD88 molecules

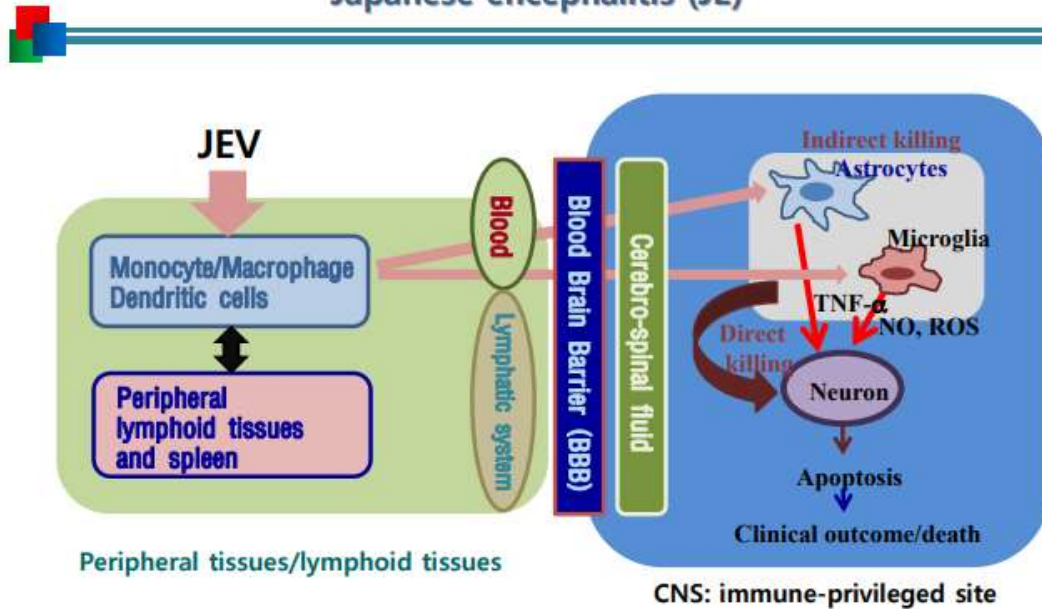
Functional modulation of dendritic cells and macrophages by Japanese encephalitis virus through MyD88 adaptor molecule-dependent and independent pathway (*J. Immunol.* 2009)

Conclusions II

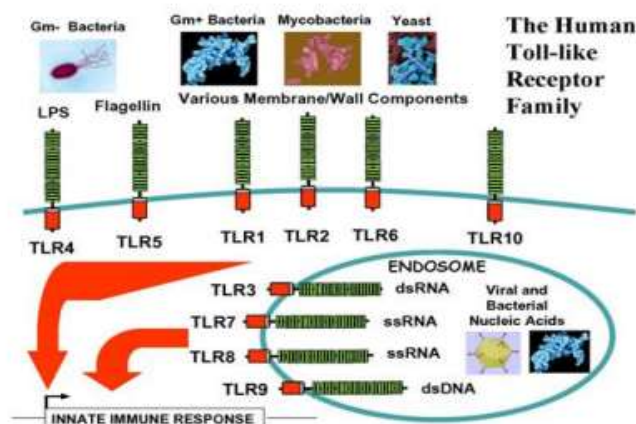
- Subverted responses of antigen-specific CD8⁺ T cells in JEV-infected animals by showing in vivo CTL assay, tetramer and intracellular IFN- γ staining
- CD8⁺ T cells purified from JEV-infected mice show impaired responses
- The antigen-presenting machinery in JEV-infected mice is defective as shown in adoptive transfer of naïve CD8⁺ T cells obtained from OT-I mice
- Skewed proportion of splenic DC subtypes in JEV-infected animals. Specifically JEV infection induces reduction of CD8 α ⁺CD11c⁺ DC number.
- Further, JEV infection shows impaired presentation of MHC class I-restricted antigen in CD8 α ⁺CD11c⁺ DCs.

Multifront assault on antigen presentation by Japanese encephalitis virus subverts CD8⁺ T cell responses (*J. Immunol.* 2010)

The immunopathological progression of Japanese encephalitis (JE)



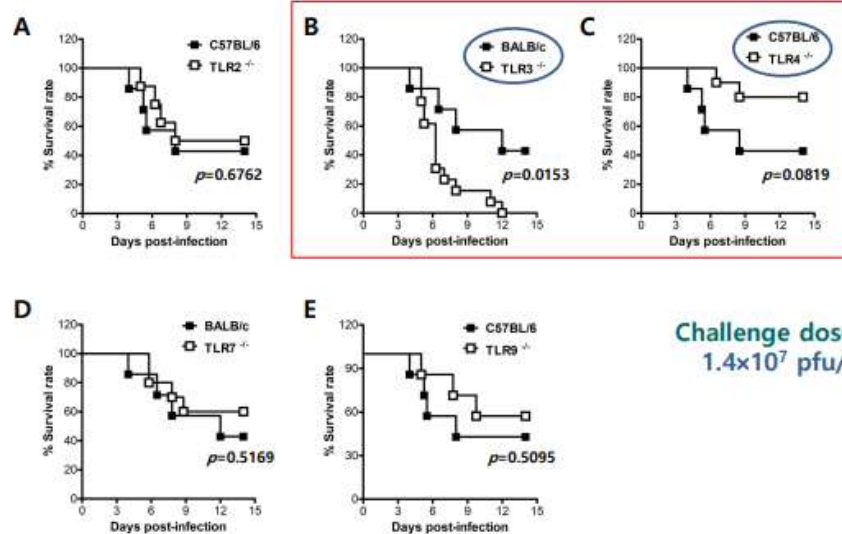
TLRs recognize highly conserved structural motifs known as **pathogen-associated microbial patterns (PAMPs)**, which are exclusively expressed by microbial pathogens, or danger-associated molecular patterns (DAMPs) that are endogenous molecules released from necrotic or dying cells.



The role of innate immune receptors in the progression of JE

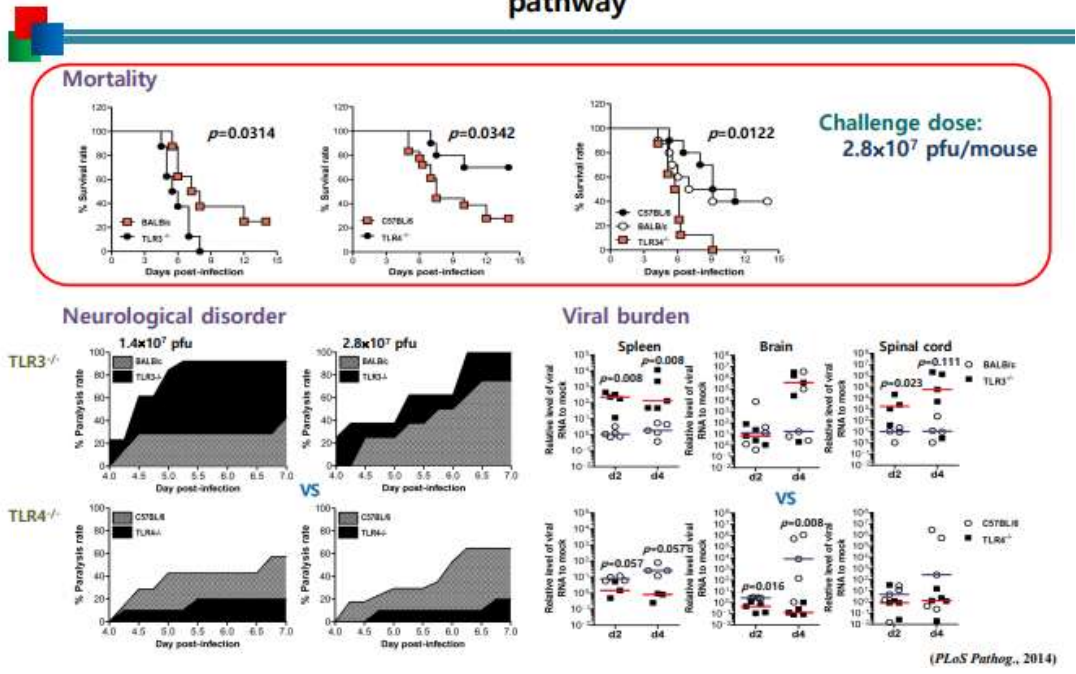
What is the role of Toll-like receptors (TLRs) in the progression of JE following lethal infection with JEV?

Susceptibility of TLR2^{-/-}, TLR3^{-/-}, TLR4^{-/-}, TLR7^{-/-}, TLR9^{-/-} mice against encephalitis caused by lethal JEV infection

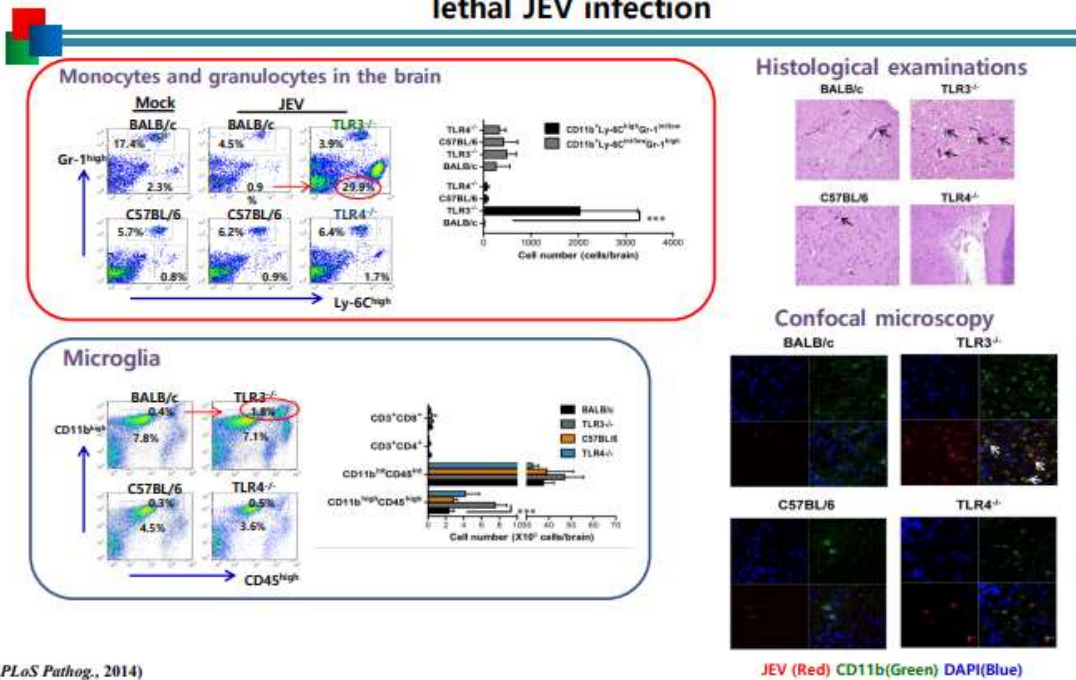


(PLoS Pathog., 2014)

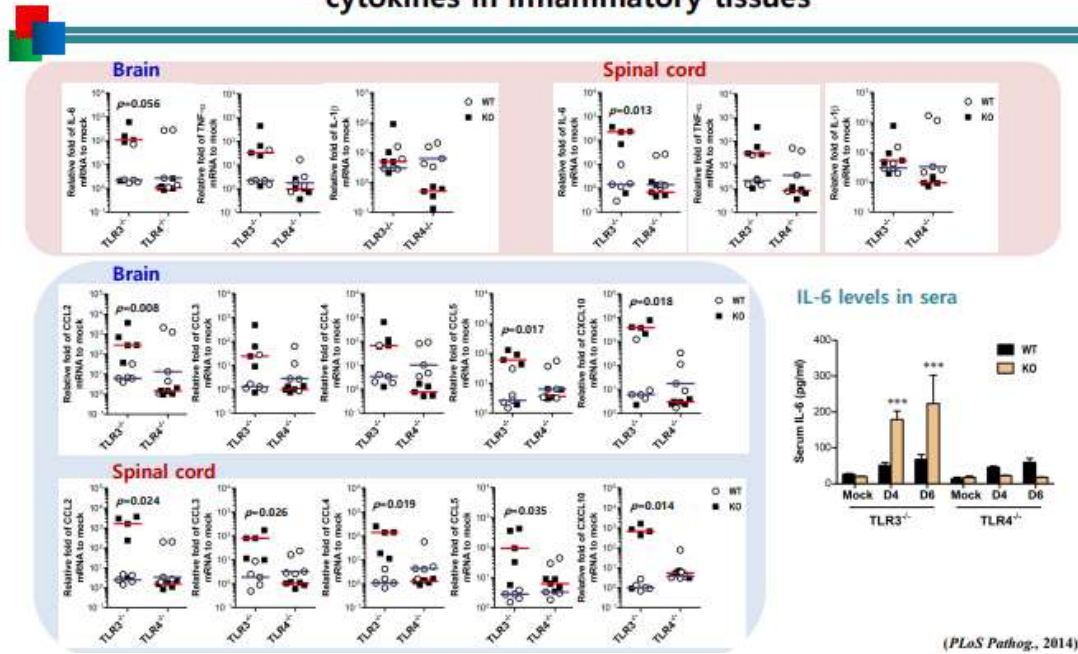
Contrasting regulation of JE by triggering TLR3 and TLR4 signal pathway



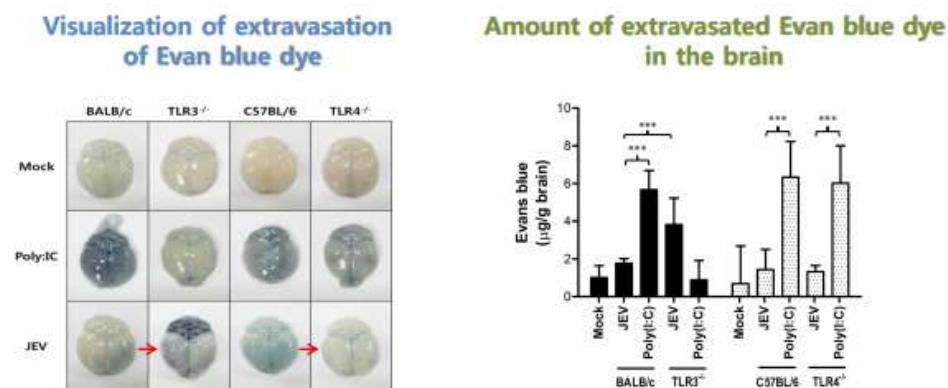
Enhanced inflammation of the CNS in TLR3^{-/-} mice following lethal JEV infection



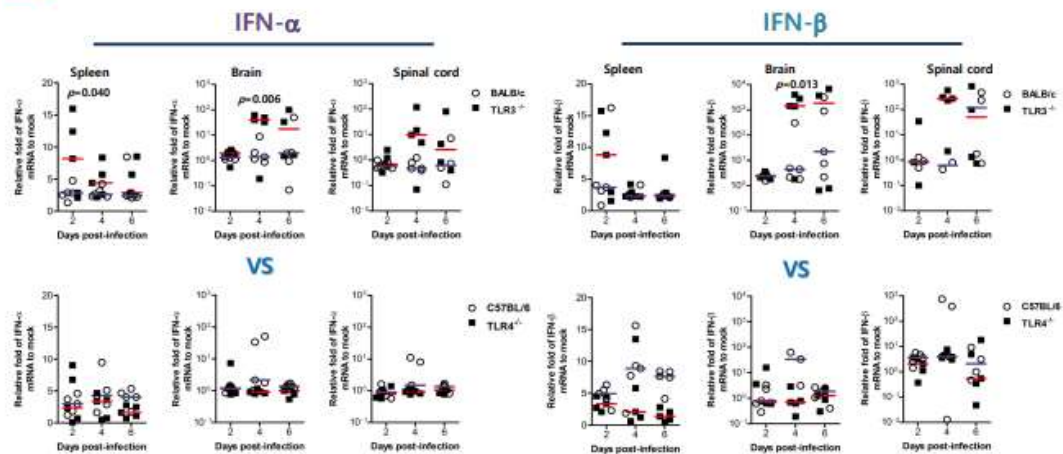
The ablation of TLR3 induces huge production of pro-inflammatory cytokines in inflammatory tissues



BBB permeability is increased after lethal JEV infection in TLR3 $^{-/-}$ but not TLR4 $^{-/-}$ mice



Localized type I IFN expression in extraneural tissue and the CNS of TLR3^{-/-} and TLR4^{-/-} mice following JEV infection



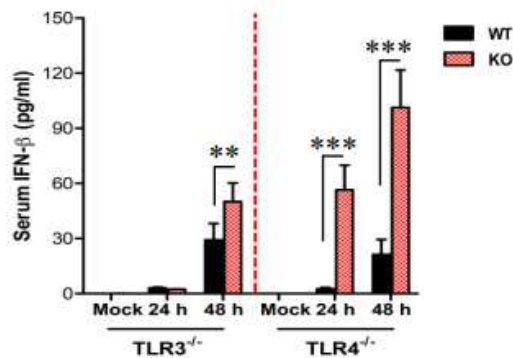
(PLoS Pathog., 2014)

Question

What is the mechanisms underlying contrasting susceptibility of TLR3 or TLR4-ablated host to JE ?

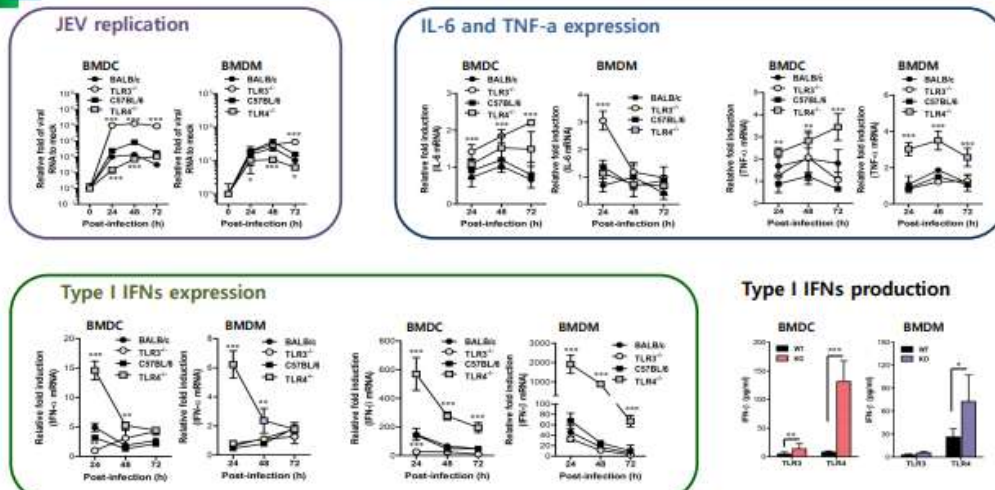
How dose ablation of TLR4 enhance the resistance to JE ?

Early and enhanced production of systemic type I IFN in TLR4^{-/-} mice following JEV infection



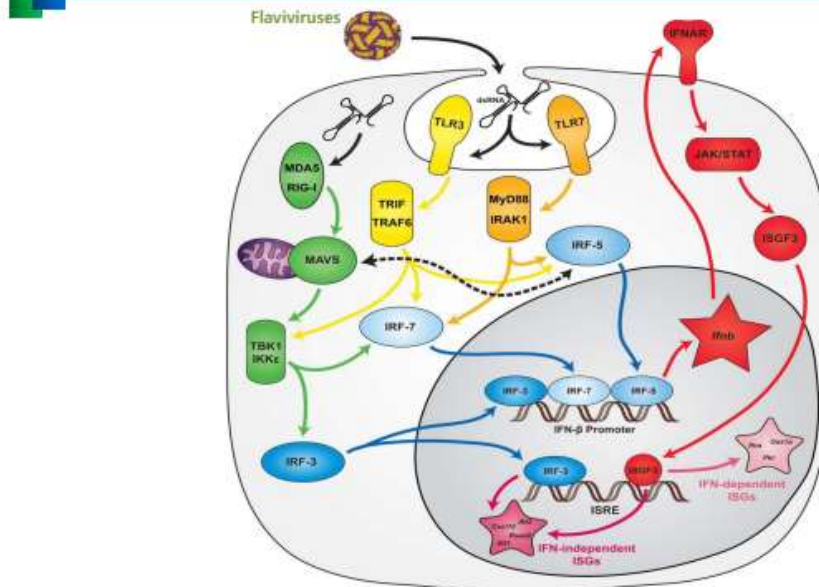
(PLoS Pathog., 2014)

Enhanced virus control and type I IFN responses in myeloid cells derived from TLR4^{-/-} mice after JEV infection



(PLoS Pathog., 2014)

Mode of Type I IFN and ISG induction in mDCs following flaviviral infection

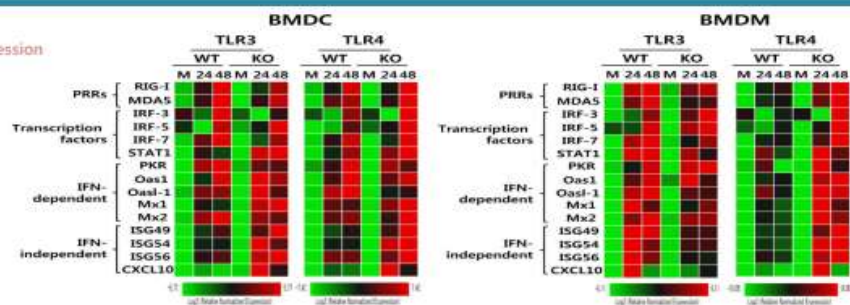


Myeloid Dendritic cells (mDCs)

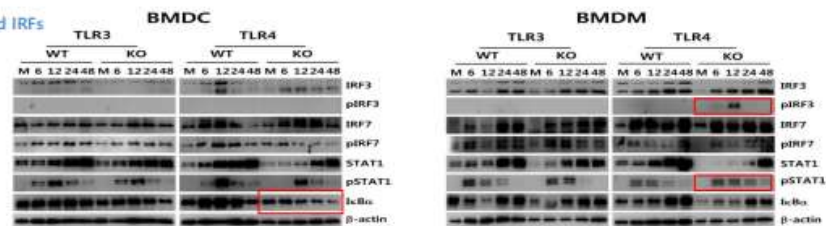
(PLoS Pathog., 2013)

Enhanced induction of type I IFNs and ISGs in the absence of TLR4 is associated with alternative IRF3 phosphorylation and IκBα degradation in myeloid-derived DCs and macrophages

Heatmap for ISG expression

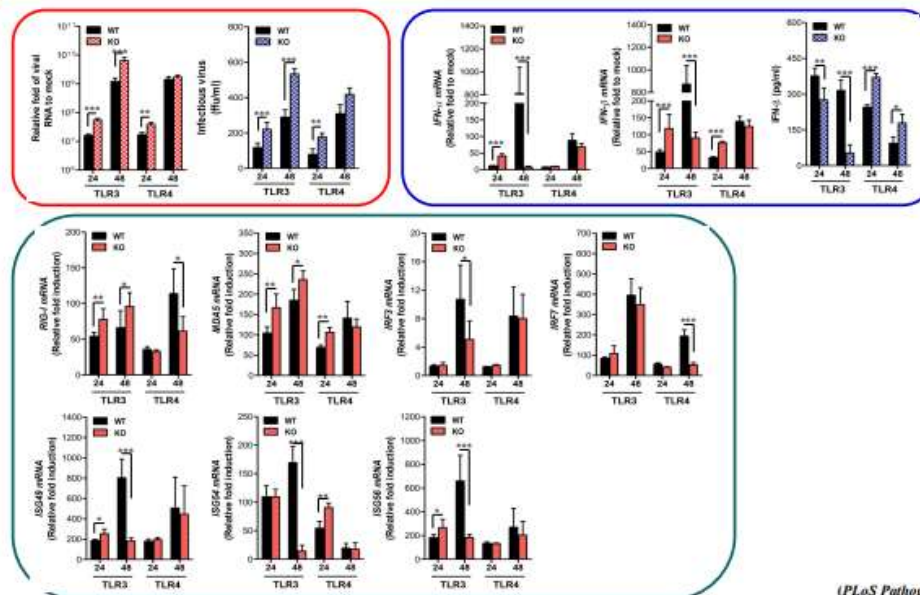


Western blot for STATs and IRFs



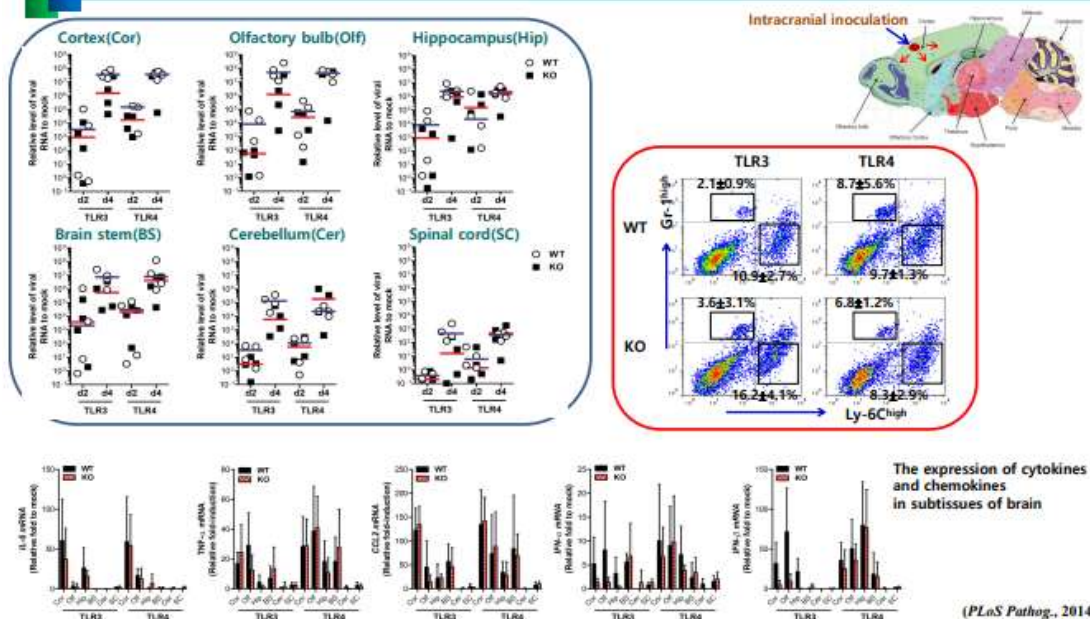
(PLoS Pathog., 2014)

Induction of type I IFN and ISGs in primary cortical neurons derived from TLR3 and 4-deficient mice after JEV infection



(PLoS Pathog., 2014)

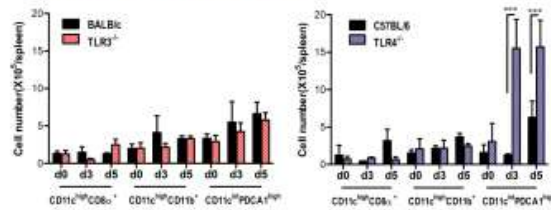
The spread of JEV in the brain of TLR3^{-/-} and TLR4^{-/-} mice after intracranial inoculation



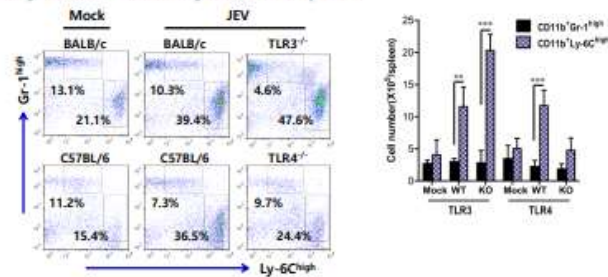
(PLoS Pathog., 2014)

Alteration of myeloid-derived and immune cell subsets in lymphoid tissues of TLR3^{-/-} and TLR4^{-/-} mice during JE progression

Subpopulation of DCs in the spleen



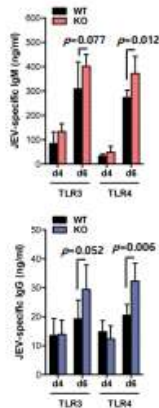
Monocytes and Granulocytes in the spleen



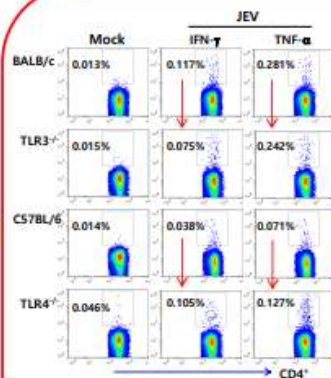
(PLoS Pathog., 2014)

TLR4 is dispensable to induce adequate adaptive immune responses specific for JEV antigen

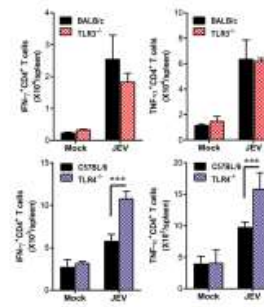
JEV-specific Abs



JEV Ag-specific CD4⁺ T cells



C

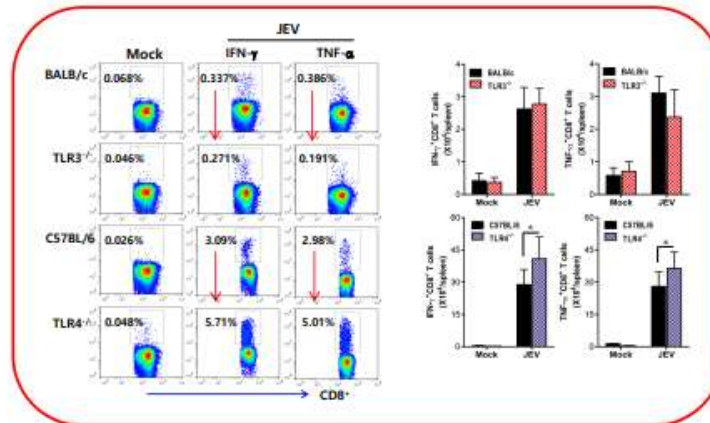


(PLoS Pathog., 2014)

TLR4 is dispensable to induce CD8⁺ T cell-responses specific for JEV antigen



JEV Ag-specific CD8⁺ T cells

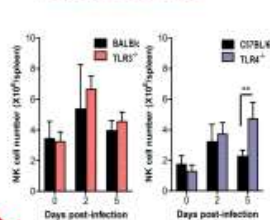


(PLoS Pathog., 2014)

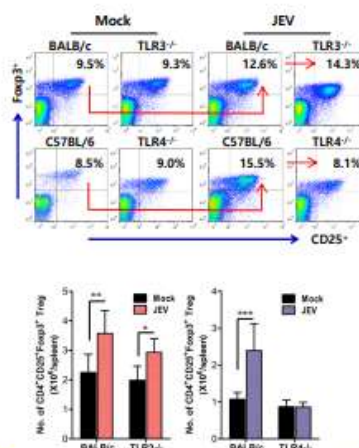
Alteration of myeloid-derived and immune cell subsets in lymphoid tissues of TLR3^{-/-} and TLR4^{-/-} mice during JE progression



NK cell number



CD4⁺Foxp3⁺ Tregs



(PLoS Pathog., 2014)

- A balanced triggering of TLR signal array by viral components during JE progression could be responsible for determining disease outcome through regulating negative and positive factors

LETTER

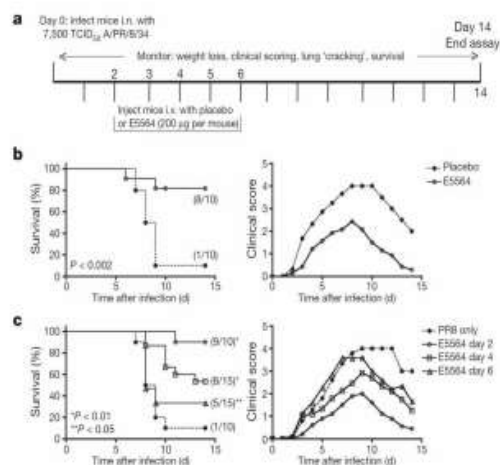
Carl van Dine¹, Wendy LaF², Alan J. Lind¹, Michael Jardi³, Pragnan Maiti¹, Anthony M. Polner⁴, Christopher L. Day⁵,
Suzie W. Miller⁶, Patricia L. Krasner⁷, David W. Sims⁸, Nathan S. Jones⁹, Robert S. Jones¹⁰, Daniel F. Bragstad¹¹

There is a pressing need to develop alternatives to annual influenza

cytotoxic and antiviral agent ribonuclease for stimulating influenza infection. Previous studies reported that acute lung injury caused by influenza virus infection was associated with increased levels of proinflammatory cytokines [10]. However, we have observed altered phospholipid that directly promotes Toll-like receptor (TLR)-dependent inflammation. Subsequently, we reported that TLR-1 intake is highly intrinsically to influenza-induced lethality, and proposed that therapeutic antagonism of TLR1 might provide robust protection against influenza-induced acute lung injury. Here we report that therapeutic administration of fibrinase-like venom (FLV) significantly reduces mortality in mice infected with influenza A virus. Influenza-infected animals induced lethality in mice, as well as lung pathology, clinical symptoms, cytokine and oxidized phospholipid expression, and decreases viral titers. CD14 and TLR2 are also required for Fibrinase-mediated protection, and CD14 directly binds fibrinogen and inhibits ligand binding to MD2. Thus, fibrinase blockade of TLR1 may represent a novel approach to reduce morbidity and mortality associated with influenza and possibly other viral infections.

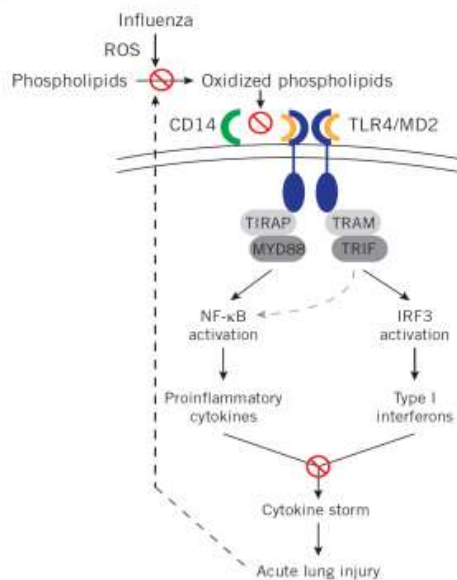


Eritoran



(Nature, 2013)

Proposed Mechanism of Eritoran



TLR4 antagonist: Eritoran

Eritoran is considered to block TLR4 activation of oxidized phospholipids produced by influenza infection (Nature, 2013)

(Nature, 2013)

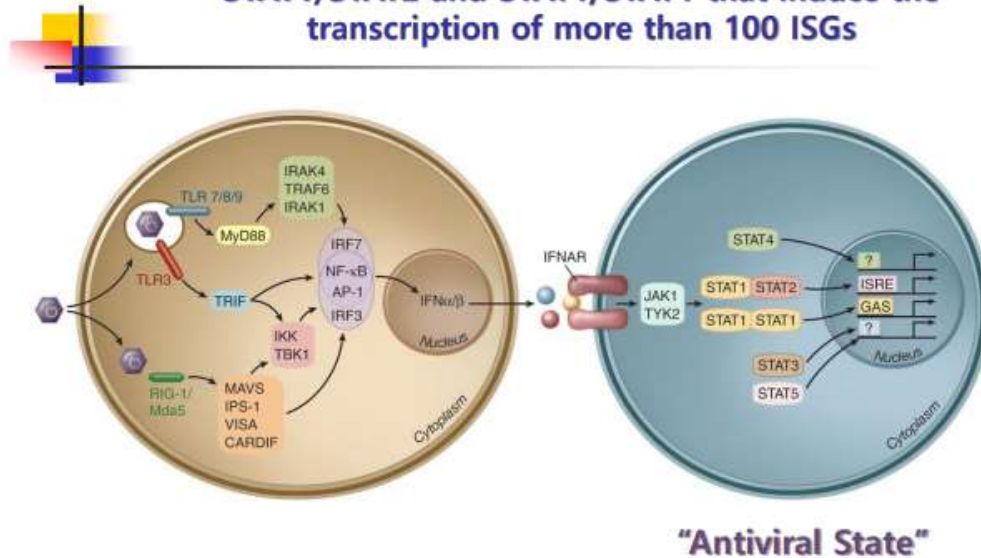
The role of type I IFNs in the progression of JE



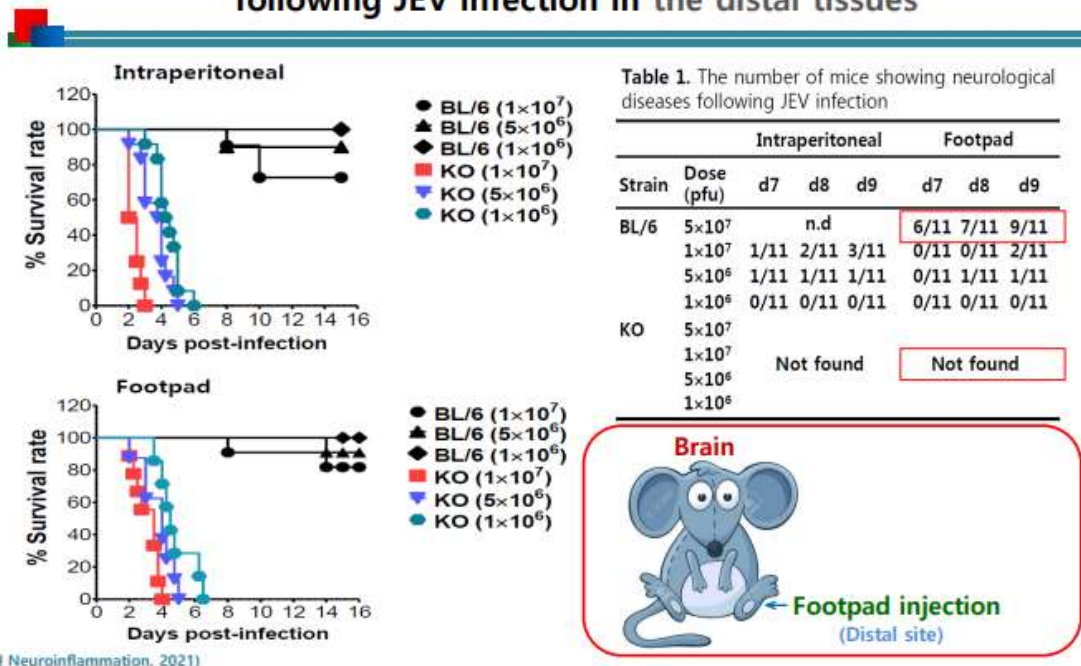
The role of type I IFNs (IFN-I) in the progression of JE has not been acknowledged

Type I IFN signaling limits hemorrhage-like diseases by infection with Japanese encephalitis virus through modulating a prerequisite infection of CD11b⁺Ly-6C⁺ monocytes

IFN-I leads to "antiviral state" through activation of STAT1/STAT2 and STAT1/STAT1 that induce the transcription of more than 100 ISGs



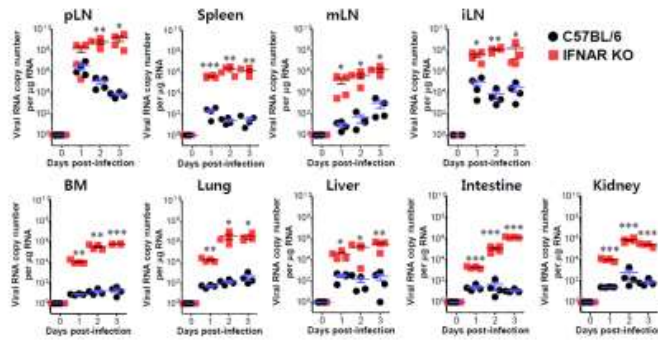
IFN-I signal is required for CNS-neuroinflammation following JEV infection in the distal tissues



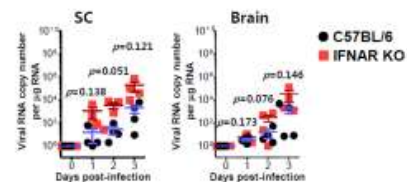
IFN-I signal limits viral dissemination in the host body, but dose not significantly affect CNS-invasion following distal inoculation of JEV



Extra-neural tissues



CNS tissues

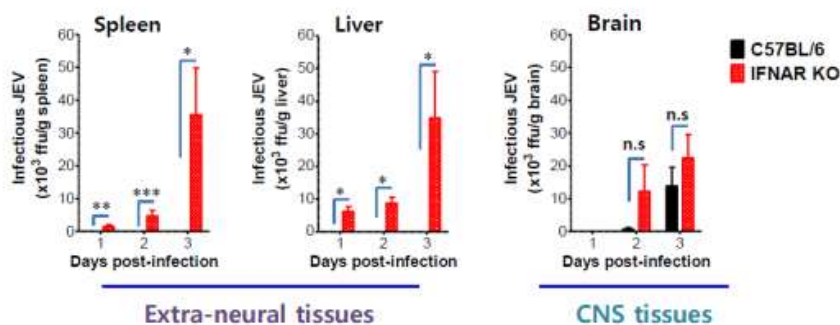


(J Neuroinflammation, 2021)

IFN-I signal limits viral dissemination in the host body, but dose not significantly affect CNS-invasion following distal inoculation of JEV

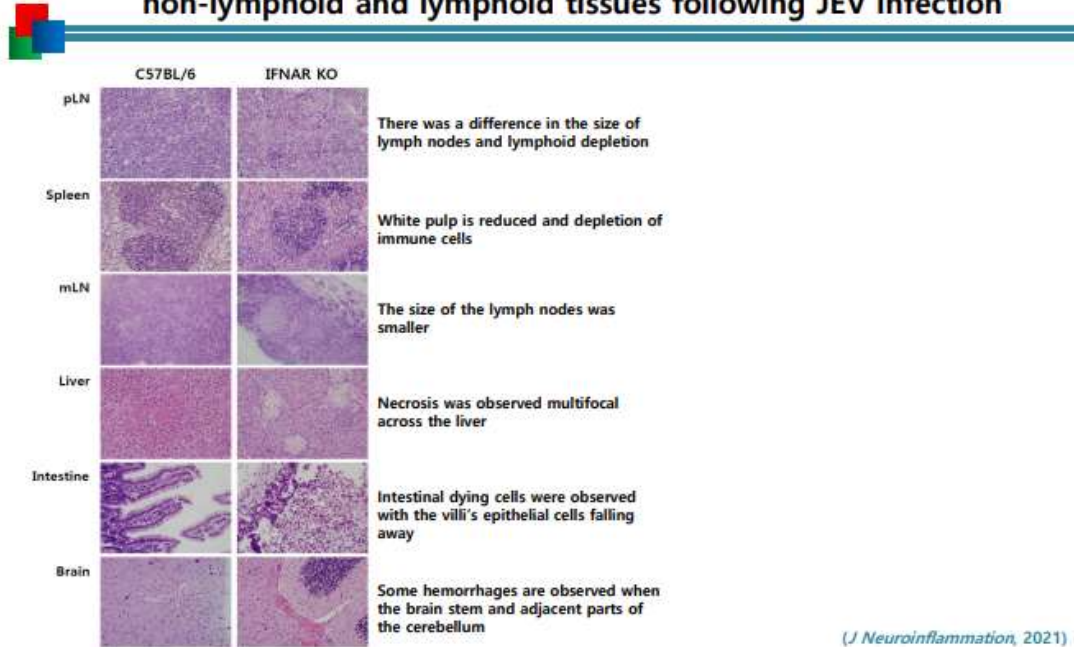


Infectious virus (Focus-forming assay)

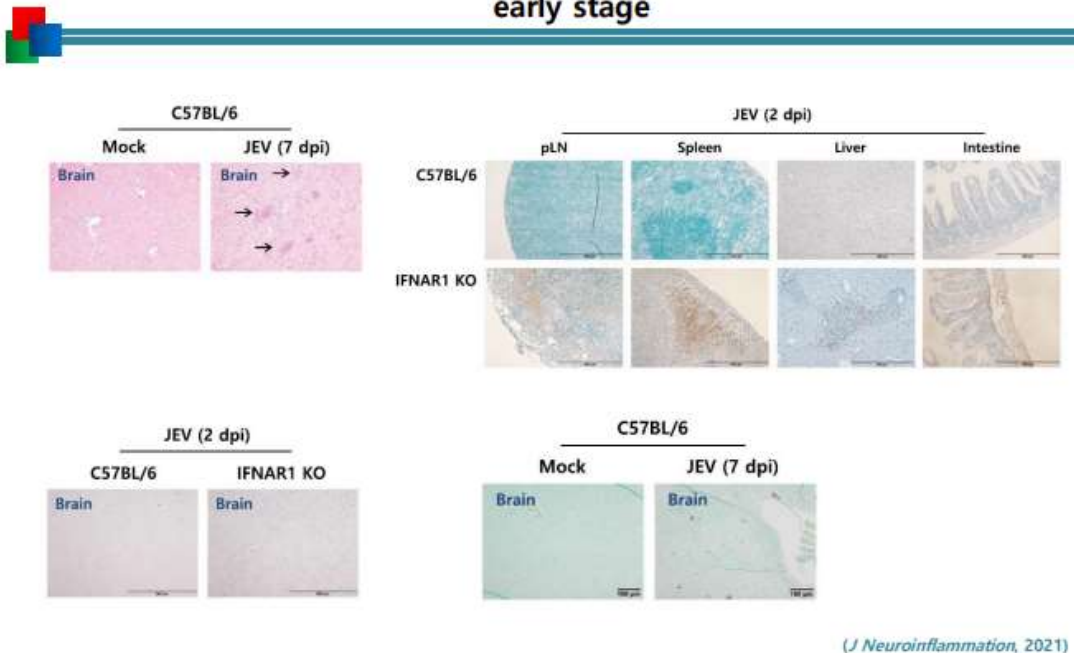


(J Neuroinflammation, 2021)

Ablation of IFN-I signal results in pathological processes in various non-lymphoid and lymphoid tissues following JEV infection

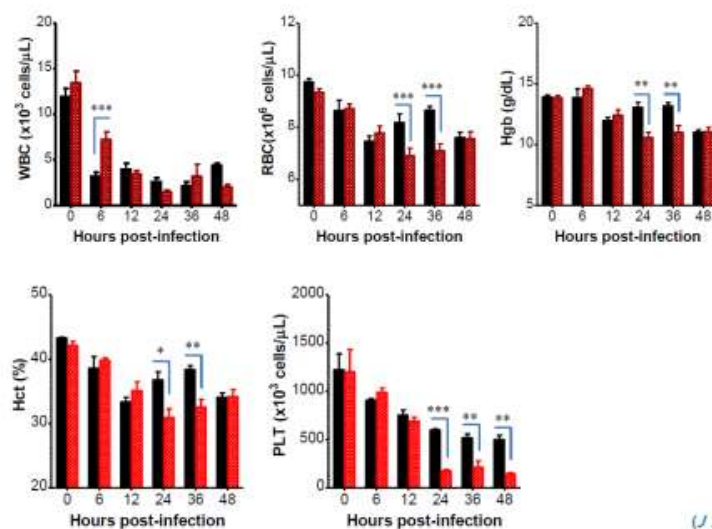


IFN-I signal limits the dissemination of JEV in CNS tissues at the early stage



IFN-I signal restricts viral hemorrhage-like diseases following JEV infection

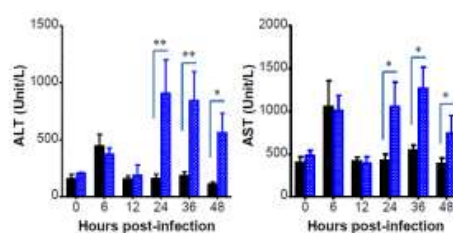
Complete Blood Cell (CBC) Count



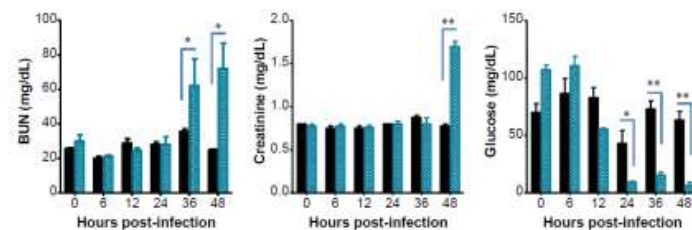
(J Neuroinflammation, 2021)

Ablation of IFN-I signal results in functional injury of liver and kidney following JEV inoculation in the distal site

Liver function

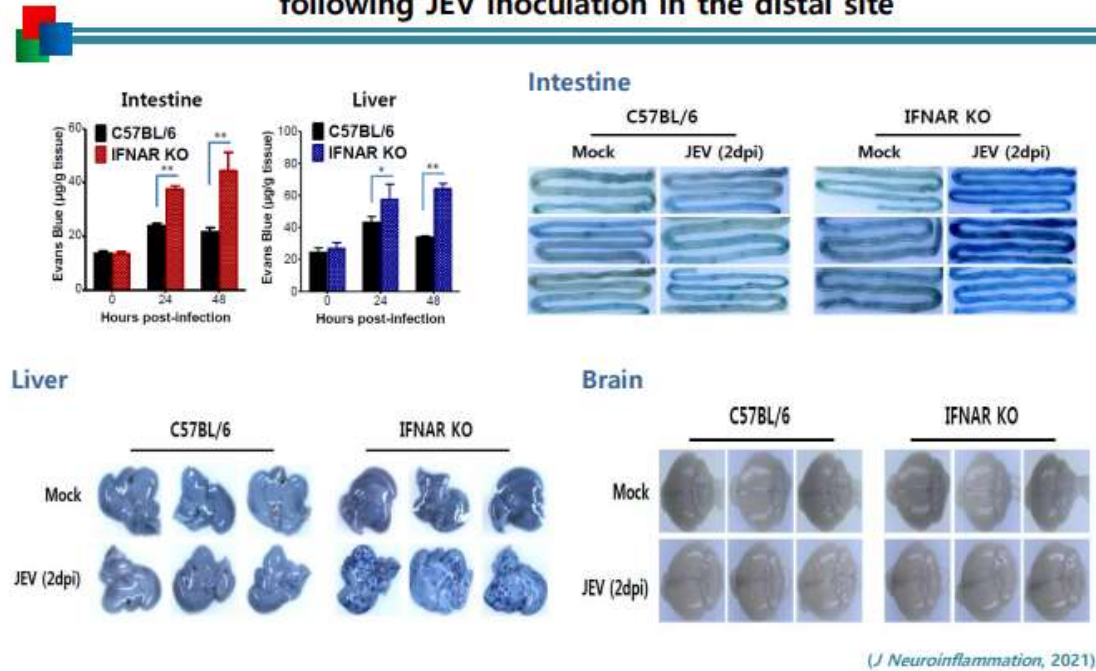


Kidney function

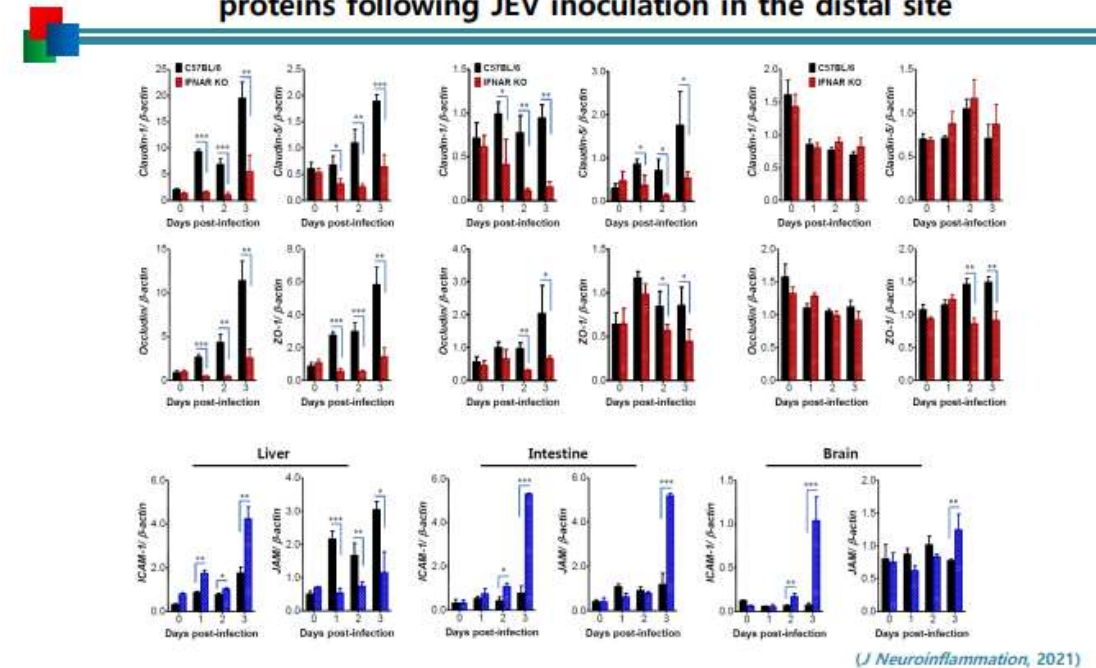


(J Neuroinflammation, 2021)

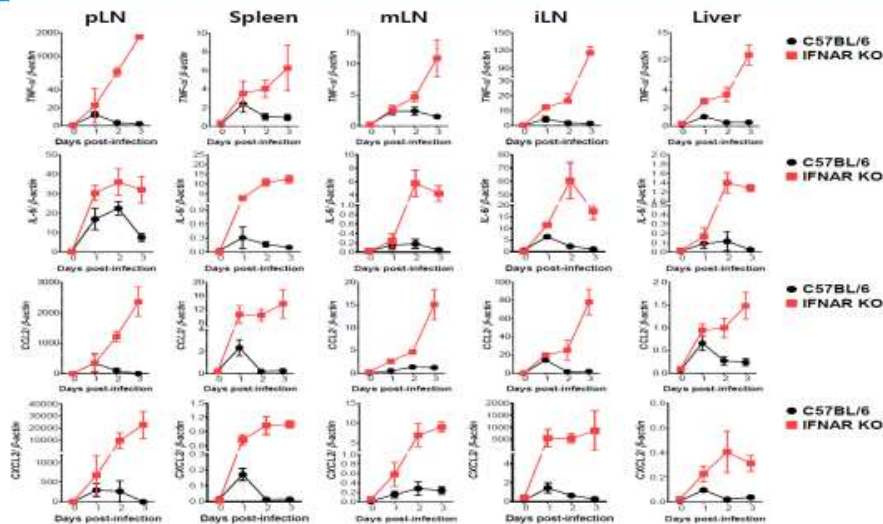
IFN-I signal restricts vascular leakage in the major organs following JEV inoculation in the distal site



Ablation of IFN-I signal reduces the expression of Tight Junction proteins following JEV inoculation in the distal site

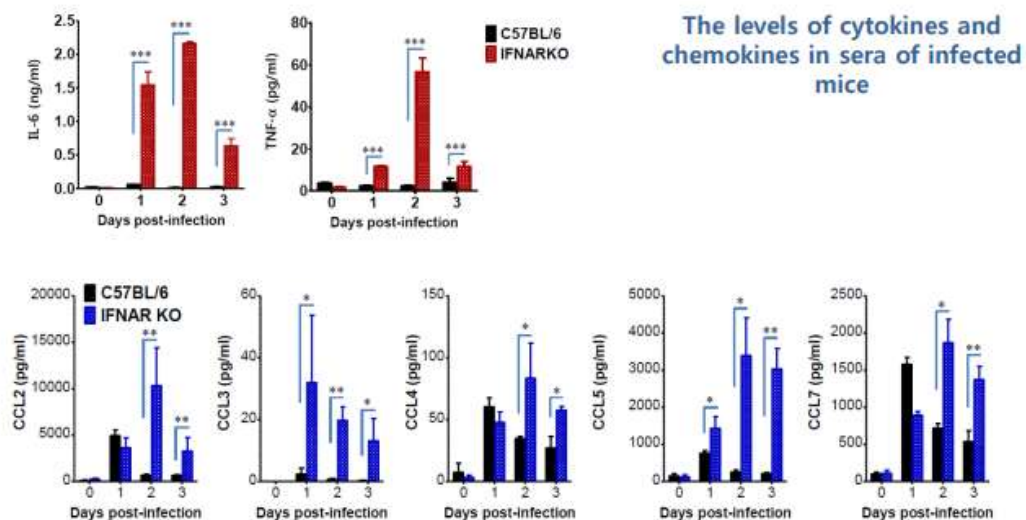


Ablation of IFN-I signal enhances the expression of pro-inflammatory cytokines in the peripheral tissues following JEV inoculation in the distal site



(J Neuroinflammation, 2021)

Ablation of IFN-I signal induces "cytokine storm" following JEV inoculation in the distal site



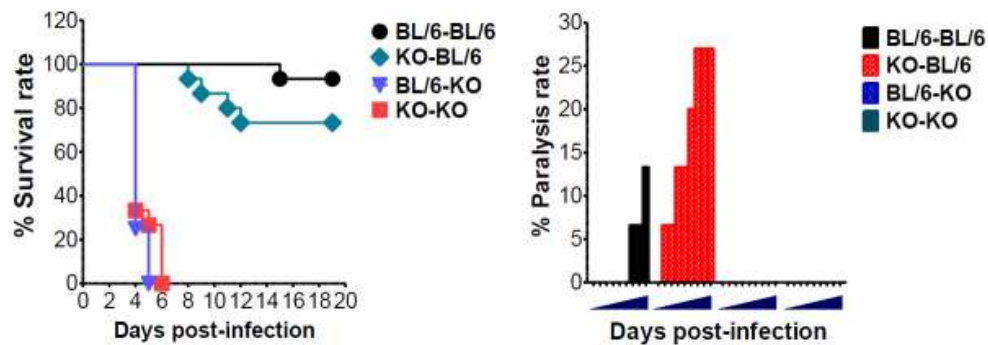
The levels of cytokines and chemokines in sera of infected mice

(J Neuroinflammation, 2021)

Intrinsic IFN-I signal in resident cells plays a dominant role in limitation of viral hemorrhage-like diseases following JEV inoculation in the distal site



BM chimera experiment

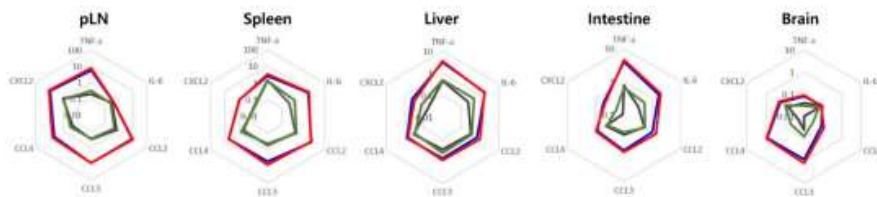


(J Neuroinflammation, 2021)

Intrinsic IFN-I signal in resident cells limits cytokine storm for preventing viral hemorrhage-like diseases following JEV inoculation in the distal site



Inflammatory cytokine mRNA

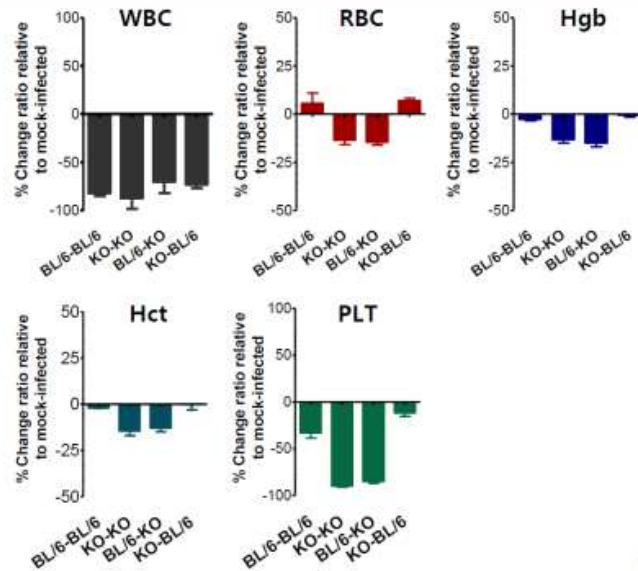


Serum levels of inflammatory cytokines



(J Neuroinflammation, 2021)

Intrinsic IFN-I signal in resident cells play a dominant role in limitation of viral hemorrhage-like diseases following JEV inoculation in the distal site

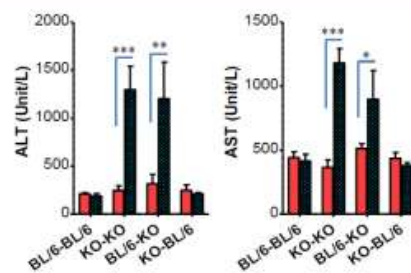


(J Neuroinflammation, 2021)

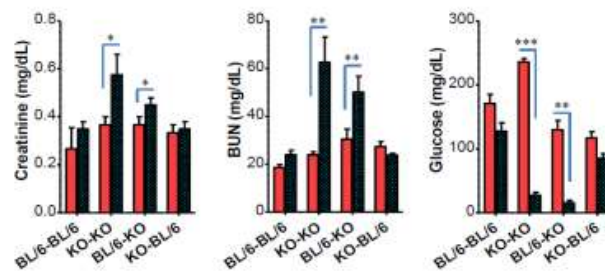
Intrinsic IFN-I signal in resident cells play a dominant role in limitation of liver and kidney injury following JEV inoculation in the distal site



Liver function



Kidney function

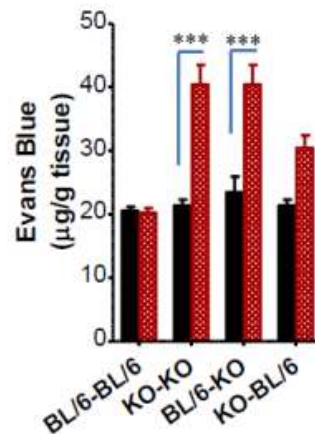


(J Neuroinflammation, 2021)

Intrinsic IFN-I signal in resident cells play a dominant role in limitation of vascular leakages following JEV inoculation in the distal site

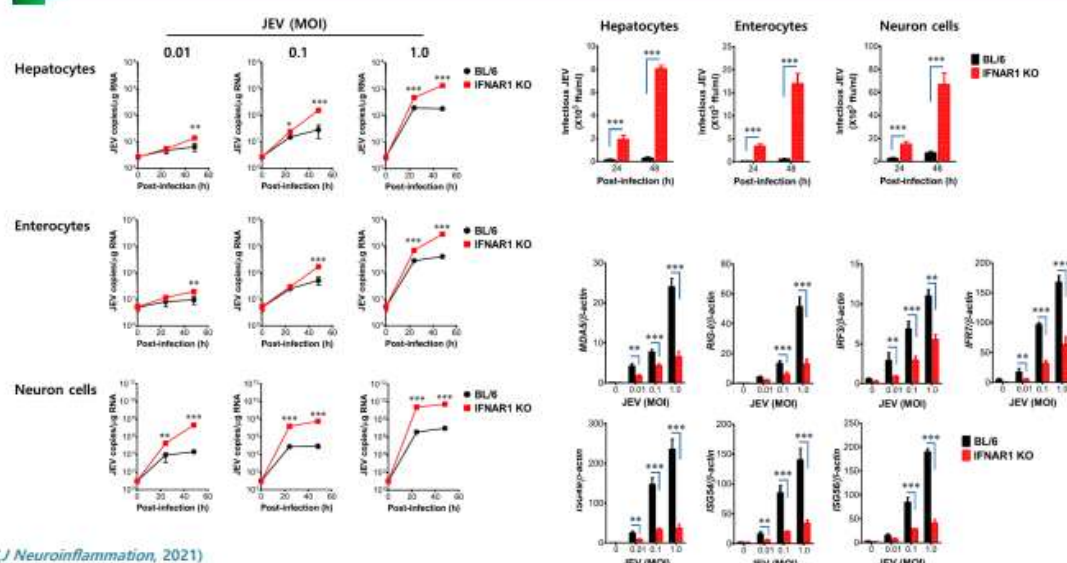


The amount of extravasated Evans blue dye in the intestine tissues



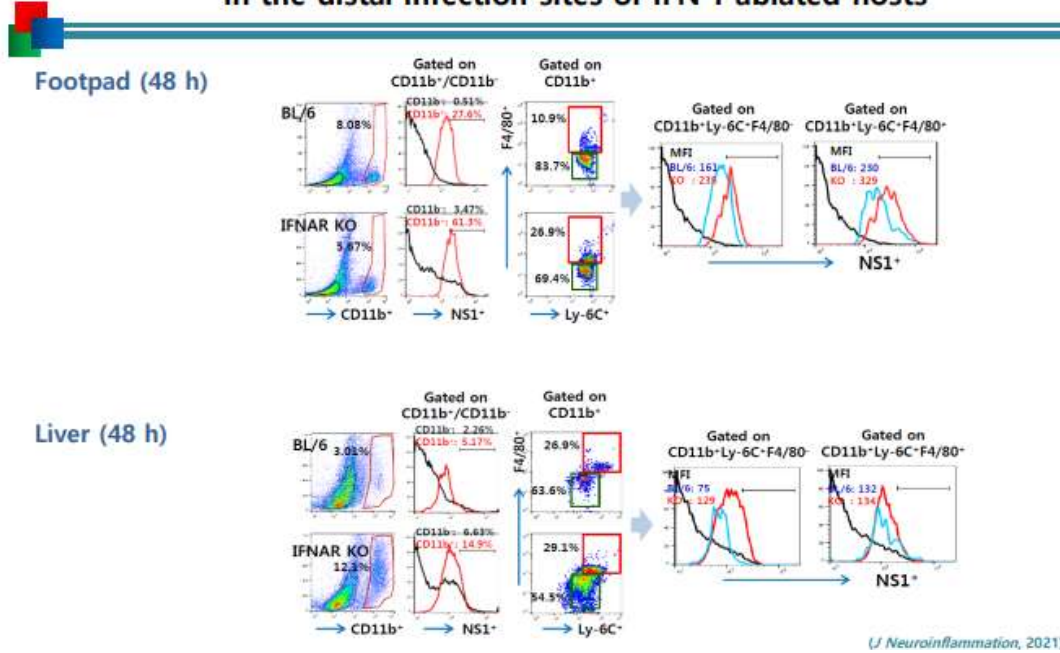
(J Neuroinflammation, 2021)

IFN-I signal-ablated resident cells show impaired IFN-I innate responses

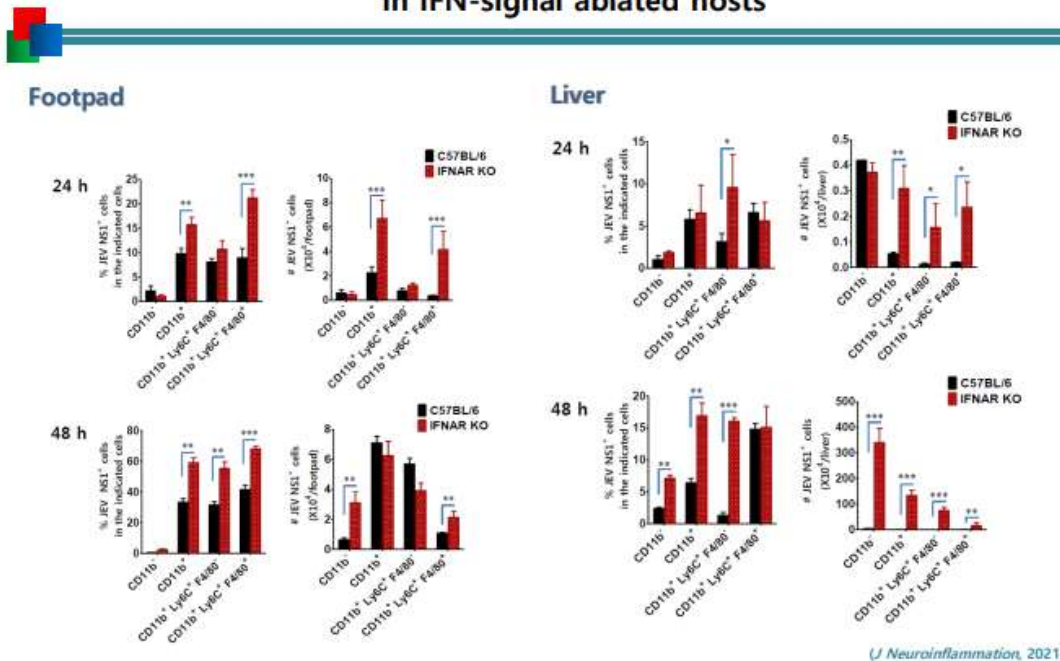


(J Neuroinflammation, 2021)

Early and higher infection of CD11b⁺Ly-6C⁺ monocytes in the distal infection sites of IFN-I-ablated hosts

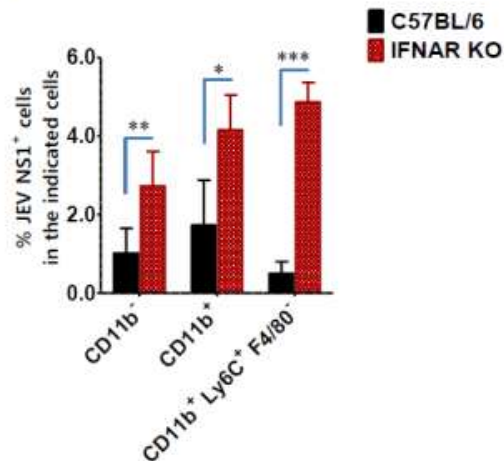


Pre-requisite infection of CD11b⁺Ly-6C⁺ monocytes with JEV in IFN-signal ablated hosts



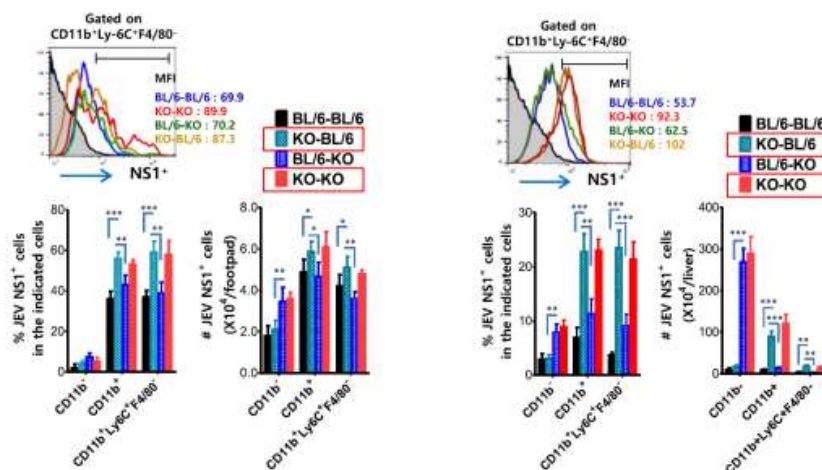
Pre-requisite infection of CD11b⁺Ly-6C⁺ monocytes with JEV in IFN-signal ablated hosts

Blood (24 h)



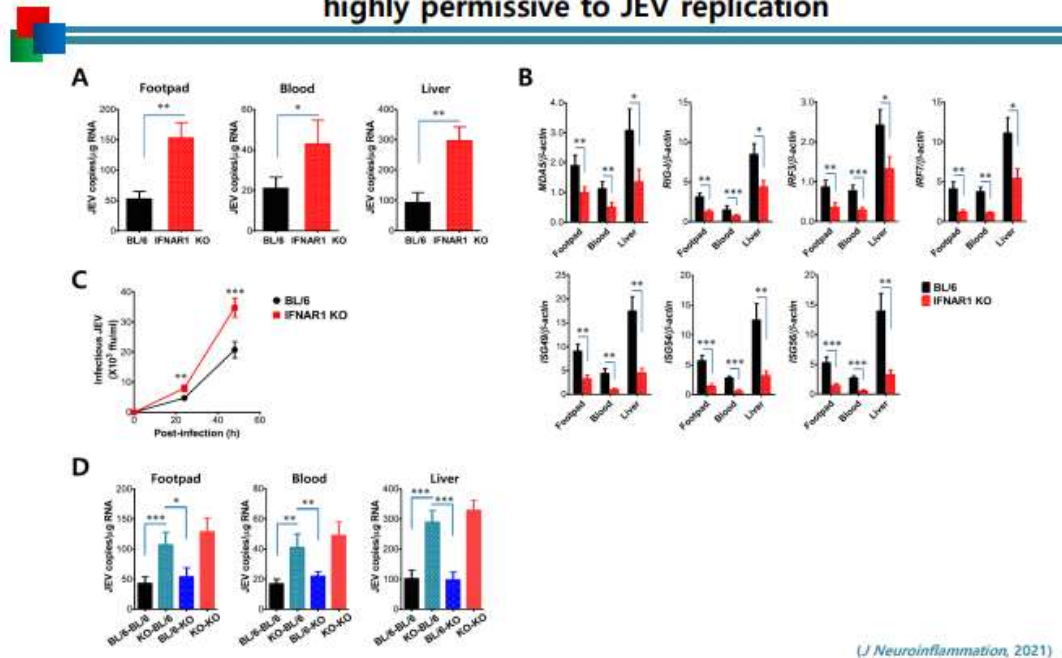
(J Neuroinflammation, 2021)

Involvement of CD11b⁺Ly-6C⁺ monocytes in quick dissemination in IFN-I signal-incompetent mice using BM chimeric models

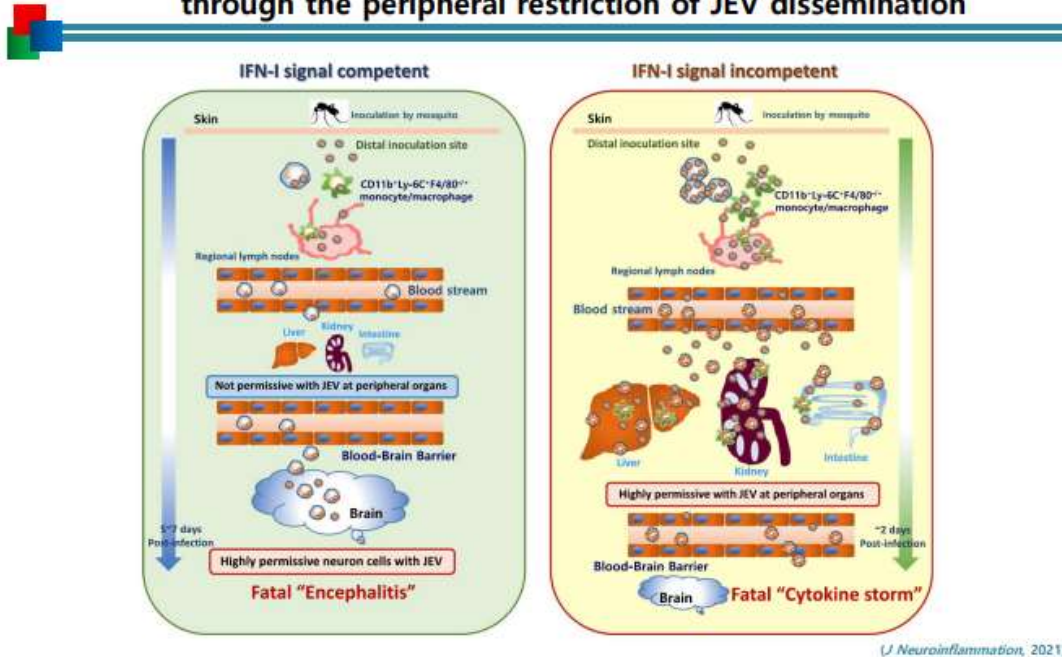


(J Neuroinflammation, 2021)

Circulating IFN-I signal-deficient CD11b⁺Ly-6C⁺ monocytes are highly permissive to JEV replication



Proposed model for IFN-I role in inducing CNS neuroinflammation through the peripheral restriction of JEV dissemination





Conclusions II

- IFN-I signal is required for CNS-neuroinflammation following JEV infection in the distal tissues
- IFN-I signal limits viral dissemination in the host body, but does not significantly affect CNS-invasion following distal inoculation of JEV
- IFN-I signal restricts viral hemorrhage-like diseases following JEV infection
- IFN-I signal restricts vascular leakage in the major organs following JEV inoculation in the distal site
- Ablation of IFN-I signal induces "cytokine storm" following JEV inoculation in the distal site
- Intrinsic IFN-I signal in resident cells plays a dominant role in limitation of viral hemorrhage-like diseases following JEV inoculation in the distal site
- Early and higher infection of CD11b⁺Ly-6C⁺ monocytes in the distal infection sites of IFN-I-ablated hosts

IFN-I signaling limits hemorrhage-like diseases by infection with Japanese encephalitis virus through modulating a prerequisite infection of CD11b⁺Ly-6C⁺ monocytes



The role of type I IFNs in the progression of JE



Vet School
CBNU

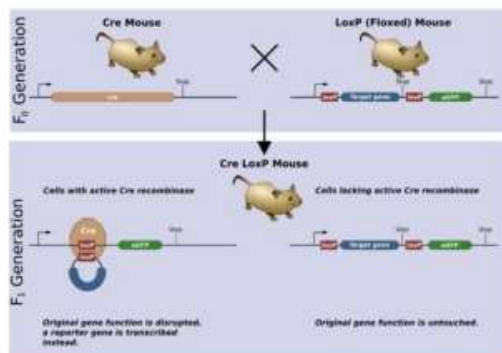
What is next?

Deficient IFN Signaling by Myeloid Cells Leads to MAVS-Dependent Virus-Induced Sepsis



Amelia K. Pinto¹, Hilario J. Ramos², Xiaobo Wu¹, Shilpa Aggarwal^{2†}, Bimmi Shrestha¹, Matthew Gorman³, Kristin Y. Kim⁴, Mehul S. Suthar⁴, John P. Atkinson^{1,5}, Michael Gale Jr², Michael S. Diamond^{1,3,5}

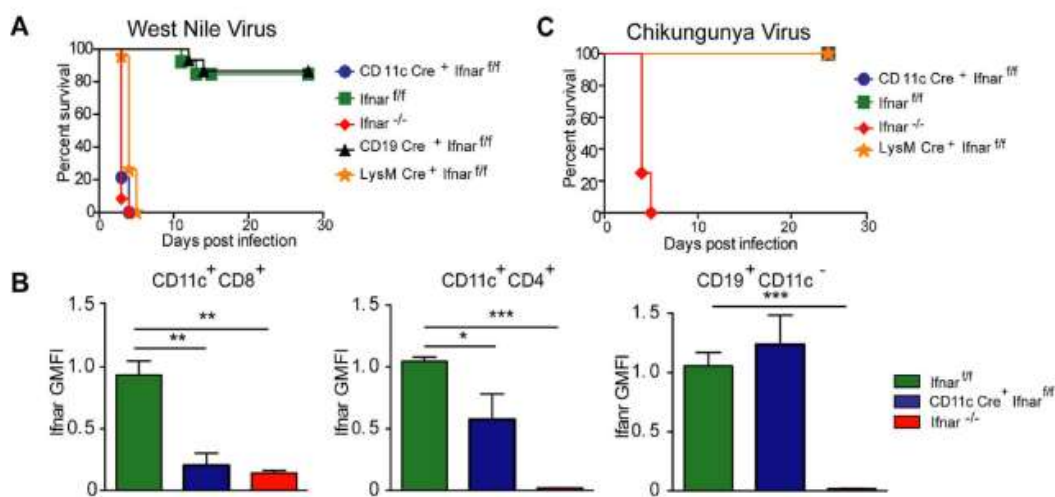
¹ Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, United States of America, ² Department of Immunology, University of Washington School of Medicine, Seattle, Washington, United States of America, ³ Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, United States of America, ⁴ Department of Pediatrics, Emory Vaccine Center, Children's Healthcare of Atlanta, Emory University, Atlanta, Georgia, United States of America, ⁵ Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, United States of America



Washington University School of Medicine in St. Louis

JOHN T. MILLIKEN DEPARTMENT OF INTERNAL MEDICINE

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Jackson Laboratory (USA)
Dr. Bum Seok Kim (Pathology Lab. CBNU)
Dr. Koanhoi Kim (Pusan Natl. Univ.)
Eisai Pharmaceutical Company (USA)

*Thank you for
your attention!*

Flaviviral Immunology Lab



Vet. School
CBNU

2022.10.12.

**병태생리학 전공
대학원생 발표 세미나**

이용균 학생



Limonium tetragonum extracts enhance
endurance capacities in mice
마우스에서 갯질경 추출물의 지구력 증진 효과

Yong Gyun Lee

School of Pharmacy,
Jeonbuk National University



Contents

-Introduction

Skeletal muscle
Exercise
Mitochondrial biogenesis
Limonium tetragonum

-Method

Treadmill exercise protocol

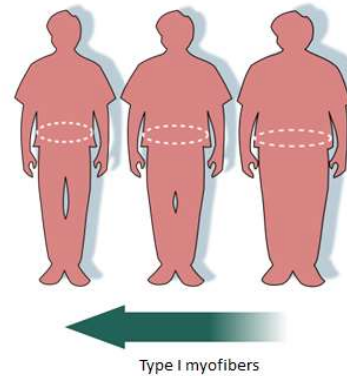
-Result

-Conclusion



Skeletal muscle

- Skeletal muscle is considered to be a major tissue for the disposal of glucose and free fatty acids and as such plays a critical role in the regulation of whole-body glucose metabolism and energy homeostasis.
- Skeletal muscle consists of two different type of myofibers.
- A reduction in the proportion of type I myofiber, which is mitochondria rich, was observed in obesity, and the proportion of type I myofiber positively correlates with overall metabolic health.



3



Slow-twitch fiber vs Fast-twitch fiber

Muscle fiber type

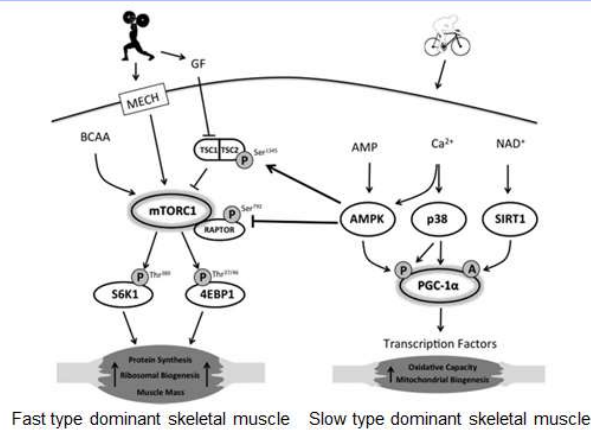
	Type I Slow oxidative	Type IIa Fast oxidative glycolytic	Type IIb Fast glycolytic
Fiber & motor unit size	Small	Intermediate	Large
Mitochondria and capillaries	Many	Many	Few
Myoglobin content	High (red fibers)	High (red fibers)	Low (white fibers)
Glycogen content	Low	Intermediate	High
Main ATP source	Oxidative phosphorylation	Oxidative phosphorylation	Anaerobic glycolysis
Glycolytic enzyme activity	Low	Intermediate	High
Rate of fatigue	Slow	Intermediate	Fast
Myosin ATPase activity	Low	High	High
Contractile speed	Slow	Fast	Fast
Examples of major locations	Postural muscles of back	Major muscles of legs	Extraocular eye muscles

From Google image

4



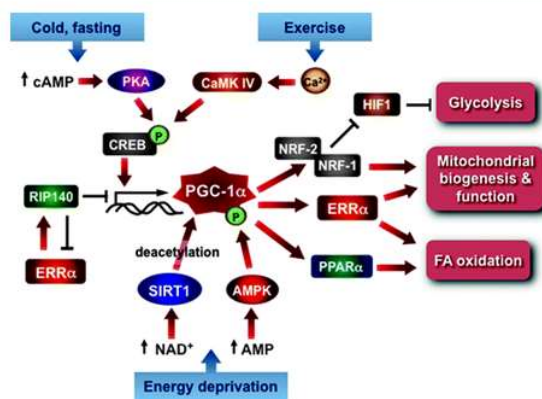
Resistance exercise vs Endurance exercise



From Google image



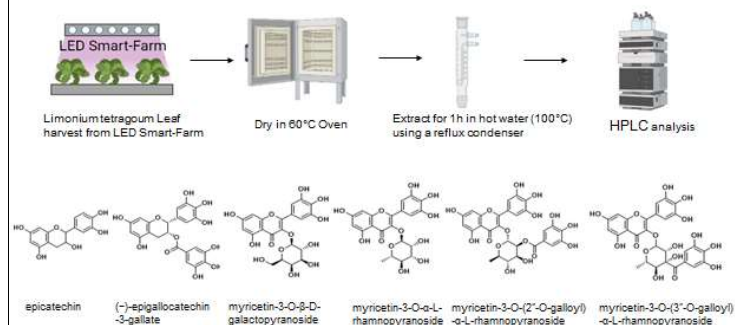
Mitochondrial biogenesis



From Google image



Limonium Tetragonum Extract (LTE)



- *Limonium tetragonum* is halophyte that can be easily found in the west coast of Korea.
- *Limonium tetragonum* already was studied for beneficial effect on anti-oxidative properties, high-fat-induced obesity, alcohol-induced liver damage, and Liver fibrosis.
- Several compound in *Limonium tetragonum* has positive effect on skeletal muscle.
- We hypothesized that *Limonium tetragonum* may be able to increase endurance exercise performance.

7

2022.10.12.

**병태생리학 전공
대학원생 발표 세미나**

임유진 학생

Determination of NDMA Impurity in Zidovudine by LC/MS

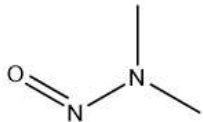
JEONBUK NATIONAL UNIVERSITY

약학대학 약품분석실험실 임유진

목차

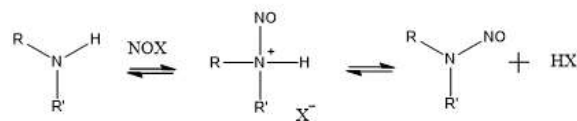
1. NDMA
2. NDMA 이슈 현황
3. Zidovudine
4. 연구 목적
5. 분석법 개발
6. 결론

1. NDMA (*N*-nitrosodimethylamine)

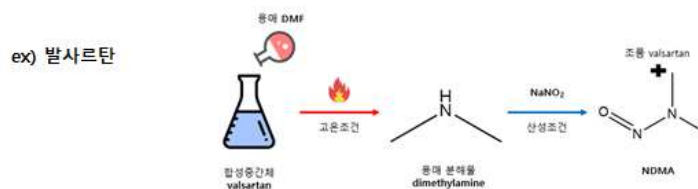
	Molecular formula : C ₂ H ₆ N ₂ O
	Molecular weight : 74.083 g/mol
	LogP : -0.57
	pKa : 3.52
	Solubility : 290mg/mL

- WHO 산하의 국제 암 연구소 분류 상 2A군(인간에게 발암물질로 작용할 가능성이 있는 물질)에 해당
 → 인체 발암 가능성은 충분한 증거가 없지만, 동물실험에서 증거가 발견된 물질
- 1일 최대 허용량 : 96ng/day (EMA)
 → 사람이 평생 노출되어도 (약 70년) 발암 위험성이 자연 발생률보다 더 증가하지 않는 수준

1. NDMA - 생성과정

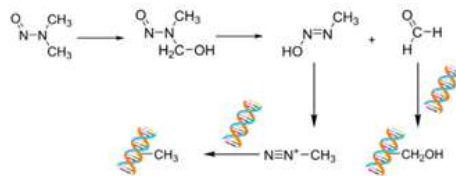


의약품에서 NDMA의 오염은 전구체 아민류가 산성 조건에서 질소산화물이 함께 존재할 경우 발생 가능



용매로 사용된 DMF의 고온 공정 중 분해물인 디메틸아민이 아질산염과 산성조건에서 반응하여 NDMA 생성

1. NDMA - 유전독성 및 발암성



- NDMA는 대사되어 aldehyde와 alkylidiazohydroxide로 분해되며 이는 연속적으로 alkylidiazonium 이온을 생성
 - Alkylidiazonium은 강력한 알킬화 친전자체로 단백질 및 DNA를 포함하는 세포 내 거대분자와 반응해 부가를 형성
 - DNA의 친핵성 부위에서 알킬화는 잠재적인 돌연변이 및 발암성을 갖는 화학적으로 안정한 부가물을 생성
- 대사 산물로 formaldehyde는 WHO 기준에 의해 인간에게 발암성으로 분류

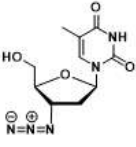
2. NDMA 이슈 현황

발암물질 NDMA 검출 의약품들		
물질명	치료질환	검출일시
발사르탄	고혈압	2018년 8월
라니티딘	위궤양	2019년 9월
니자티딘	위궤양	2019년 11월
메트포르민	당뇨	2019년 12월

자료: 업계종합

- NDMA 물질에 대한 정확한 정량분석이 필수적
- 현재 FDA, EMA를 필두로 LC-MS/MS, GC-MS/MS 등의 기기를 이용한 NDMA 분석 조건을 확립하고 정보를 공개
- 관련 당국의 지침에 따라 원료 및 완제 의약품에 대한 위험평가를 실시하여 보고 및 허가 변경
- NDMA 발생 가능성이 있는 의약품에 대한 선제적인 파악 및 분석 방법 개발의 필요성 대두

3. Zidovudine

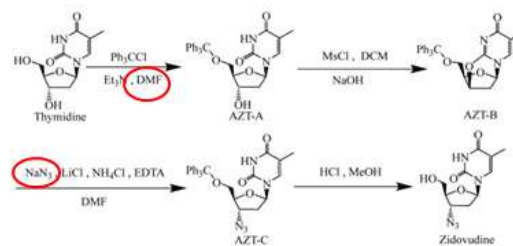
	Molecular formula : $C_{10}H_{13}N_3O_4$
	Molecular weight : 267.241 g/mol
	LogP : -0.1
	pKa : 9.96
	Solubility : 16.3mg/mL in water



- 분류 : HIV (인체 면역 결핍 바이러스) 치료제
- 특징 : 단독 혹은 다른 항레트로바이러스제와 병용으로 HIV 감염증 치료
- 용법, 용량 : 1일 2회 1정씩 경구 투여 (1일 총 투여량은 600mg)
- 니트로사민류 불순물 잠정관리기준 : NDMA 0.16 ppm
- 현재 국내에서는 *캄비비어* 1건의 의약품이 판매

Zidovudine 300mg + Lamivudine 150mg 혼합제제

4. 연구 목적



- Zidovudine 합성 과정 중 dimethylformamine (DMF)의 사용과 sodium azide에서 아질산염 이온과 함께 NDMA 생성 가능성 존재
- Zidovudine을 NDMA 생성 위험 약물로 선정
- Zidovudine 원료 의약품과 완제 의약품에 적용 가능한 NDMA 분석방법 개발

5. 분석법 개발



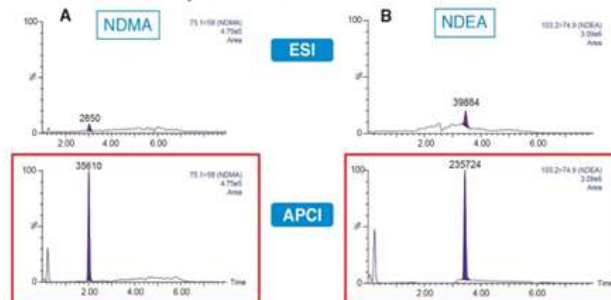
HPLC : SHIMADZU Nexera X3

Mass spectrometer : Sciex triple quad 5500+

Drug	Zidovudine
일일 최대 투여량	600mg/day
NDMA	
일일 섭취허용량	96 ng/day
NDMA	
잠정관리기준	0.16 ng/mg
시료량	600 mg
용매 부피	50 mL
NDMA	
최대 허용 잔류량	96ng / 50mL
NDMA 한계농도	1.92 ng/mL

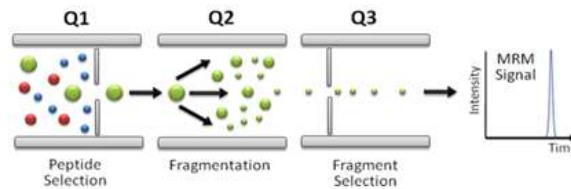
ESI vs APCI sensitivity

APCI 10X > sensitivity vs. ESI Probe



니트로사민류의 경우 ESI와 APCI의 감도는 10배 차이

MRM (Multiple Reaction Monitoring)

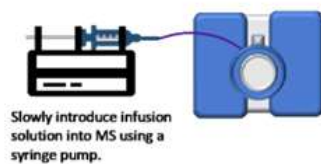


- **Q1**
Ion source 에서 형성된 이온들 중 특징적인 이온 (Precursor ion)을 선택적으로 통과시킨다.
- **Q2**
Precursor ion을 가스와 충돌시켜 조각이온을 얻는다.
- **Q3**
깨져서 생성된 조각이온들 중 특징적인 이온 (Product ion)을 검출부로 전달하여 크로마토그램을 얻는다.

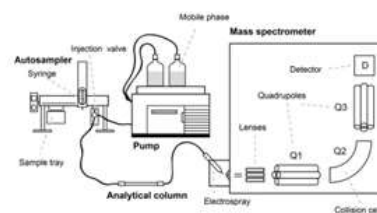
****NDMA라는 특정한 화합물에서 고감도의 크로마토그램을 얻어야 하기 때문에 MRM 사용**

실험과정

1) Standard infusion



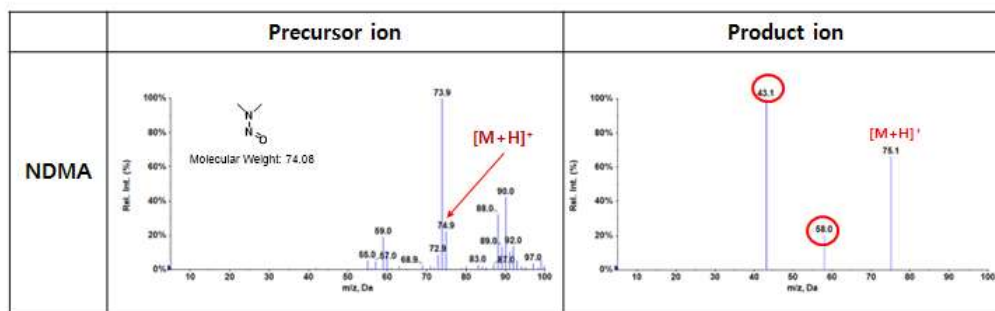
2) LC-MS/MS 분석법 최적화



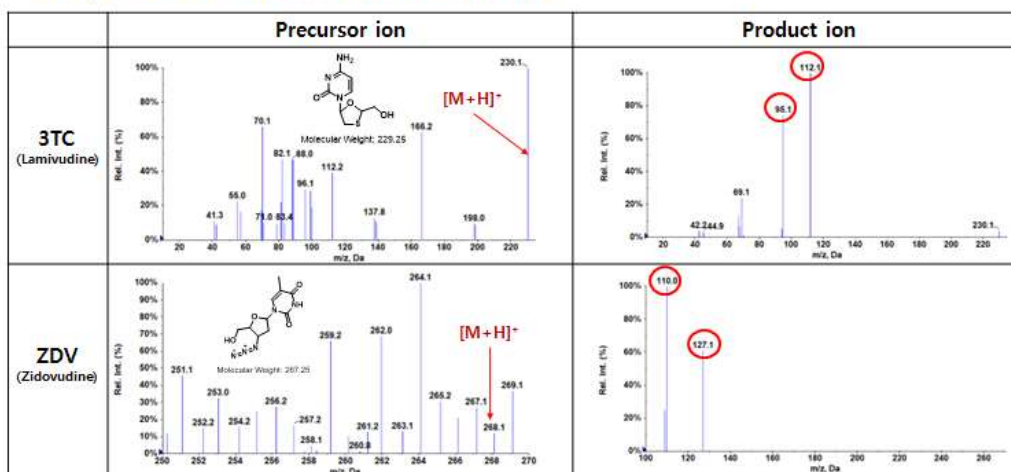
3) Validation

4) 실제 샘플 (원료, 완제의약품)에 적용

MS spectra of NDMA



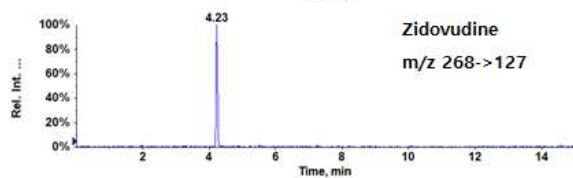
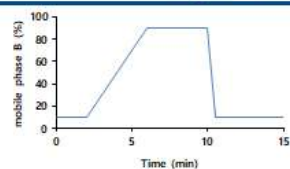
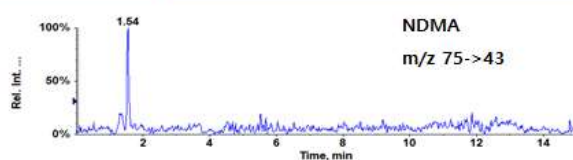
MS spectra of Zidovudine and Lamivudine



MS condition

	MRM Condition			
	m/z	DP (v)	CE(V)	CXP (V)
NDMA	75 -> 43 (정량)	80	23	12
	75 -> 58 (정성)	80	17	16
Zidovudine	268 -> 127	46	31	14
	268 -> 110	46	40	12
Lamivudine	230 -> 112	11	20	12
	230 -> 95	11	50	12

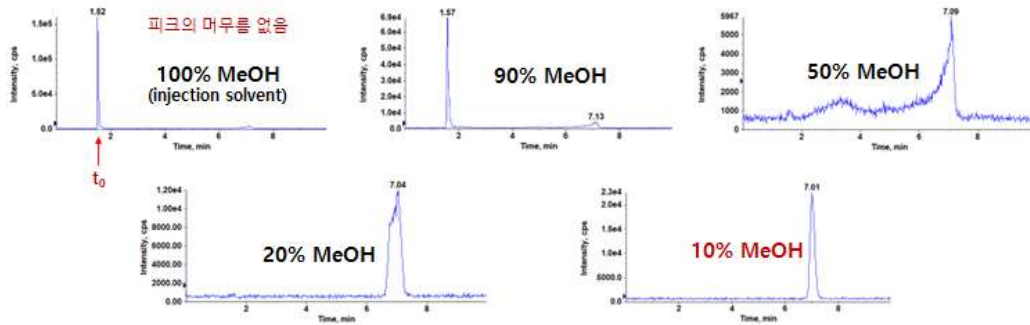
- DP : declustering potential
- CE : Collision energy
- CXP : Collision cell exit potential



• Column: Phenomenex Kinetex F5 (100 × 3.0 mm, 2.6 μm)

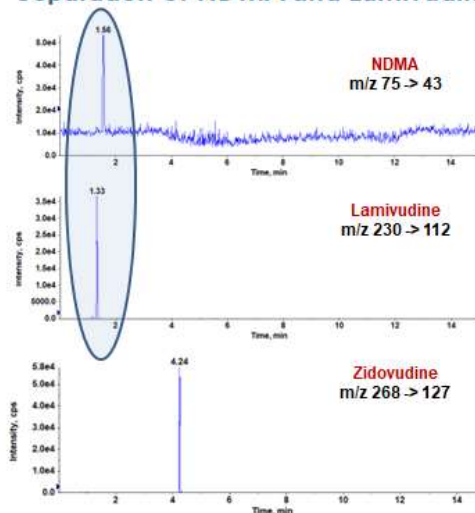


Effect of injection solvent



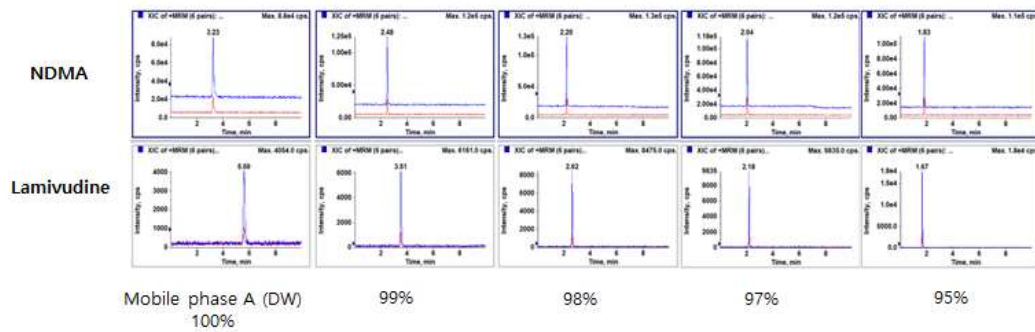
- 이동상: 초기 10% ACN으로 시작
- NDMA와 지도부딘은 inj. Sol.에 영향받지 않음 (peak 형성 잘됨, 머무를 일정함)
- 주입시료 중 유기용매 (MeOH) 비율이 10% 이하에서 Lamivudine의 peak 형성 및 머무를 가능

Separation of NDMA and Lamivudine



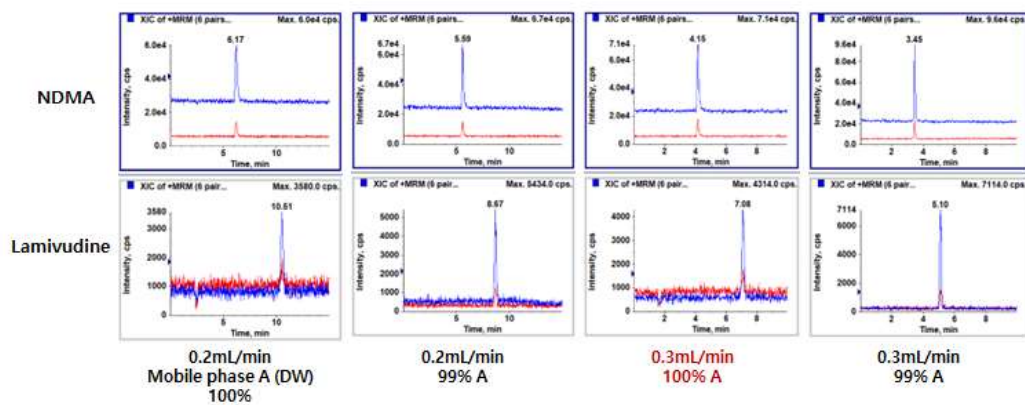
- 초기 10% B 용매로 시작하는 조건에서는 라미부딘-NDMA-지도부딘 순서로 용출되며, NDMA와 라미부딘 0.2분 차이로 용출
- 검액(검비버어정; 지도부딘 300mg + 라미부딘 150mg) 분석시 고농도의 라미부딘 peak가 NDMA와 overlap 될 것이 예상됨
- divert valve 를 사용하여 라미부딘이 주입되지 않도록 하기 위한 두 물질 사이의 충분한 RT 차이가 확보되지 않았음
- $\Rightarrow$ 두 물질의 분리가 필요 (1분 이상 차이 확보 필요)
- $\Rightarrow$ 라미부딘이 NDMA보다 뒤에 용출되는 조건 탐색

Separation of NDMA and Lamivudine



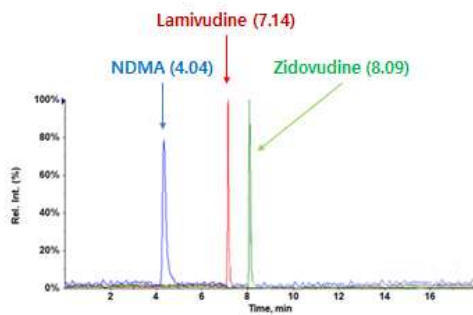
- Mobile phase B: ACN, flow rate: 0.4mL/min, column temp.: 40 °C
- 이동상 B (ACN) 비율이 5% 이상에서는 Lamivudine 이 먼저 용출
- 이동상 B (ACN) 비율이 2% 이하에서 NDMA 와 Lamivudine 분리 가능
- => 초기 100% or 99% A 용매로 시작하는 조건 설정

Flow test (Mobile phase B : Acetonitrile)



- NDMA 용출 후 라미부딘이 MS로 주입되지 않도록 하기 위하여 divert valve 사용
- => 두 물질 사이의 충분한 RT 차이를 확보하기 위하여 0.3 mL/min 설정

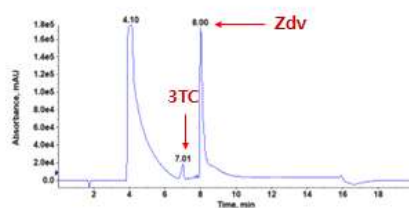
Chromatography & MS condition



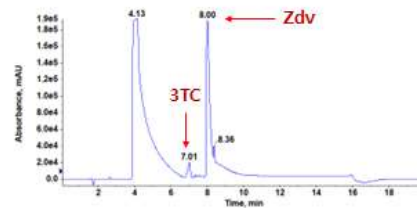
NDMA + Lamivudine + Zidovudine
Standard mixture

Liquid Chromatography Condition				
Instrument	SHIMADZU Nexera X3			
Column	Kinetex xB 2.6µm #5 100Å (100 × 3.0mm)			
Column temp.	30°C			
Mobile phase A	0.1% Formic acid in Water			
Mobile phase B	0.1% Formic acid in Acetonitrile			
Gradient	Time (min)	Solvent A(%)	Solvent B(%)	
	0	100	0	
	4	100	0	
	10	10	90	
	14	10	90	
	14.5	100	0	
Acquisition time	3.5.5 min			
	Injection volume	10µL		
Flow rate	0.3mL/min			
Mass Spectrometer Condition				
Instrument	AB SCIEX QTRAP 5500+			
Ionization mode	APCI (+) MRM			
APCI gas temp.	300°C			
APCI gas 1	30 psi			
APCI Corona (NC)	3 uA			
Curtain gas (CUR)	25 psi			
Collision gas (CAD)	N ₂			
MRM Condition				
	m/z	DP (V)	CE(V)	CXP (V)
NDMA	75 → 43 (당량)	80	23	12
	75 → 58 (결정)	80	17	16
NDMA-d6	80.9 → 46	56	23	10
Zidovudine	268 → 127	46	31	14
	268 → 110	46	40	12
Lamivudine	230 → 112	11	20	12
	230 → 95	11	50	12

LC-UV Chromatogram of Combivir tablet



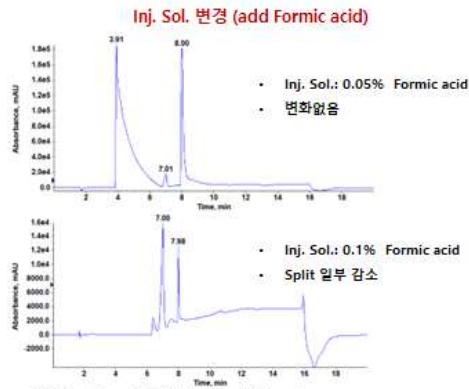
Combivir tablet
(Zdv 600mg+3TC 300mg/50mL)



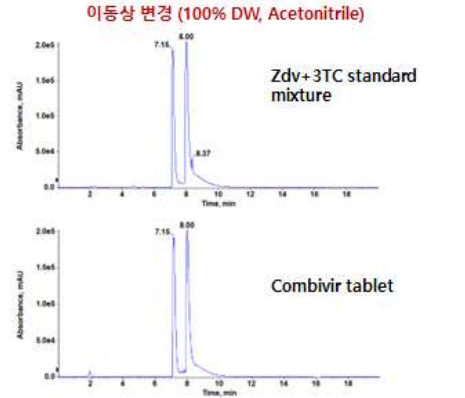
Zdv + 3TC standard mixture
(Zdv 12mg+3TC 6mg/mL)

- 고농도의 지도부딘, 라미부딘 성분이 주입되었을 시 tail peak의 NDMA에 대한 영향을 LC-UV 로 확인
- 앞서 설정한 분석조건하에서 검액(완제의약품) 주입시 NDMA 위치에서 거대 peak 검출 -> 첨가제 의심
- 지도부딘과 라미부딘 표준품을 완제의약품의 비율과 동일하게 제조한 혼합물에서도 피크 검출 -> 첨가제가 아닌 고농도의 물질 때문인 것으로 추정

Optimization of chromatographic condition



- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: 0.1% formic acid in Acetonitrile
- 앞서 설정한 0.1% FA 포함 이동상 사용시, inj.sol.의 pH는 검액 중 라미부딘 성분의 분리에 영향을 줌. 지도부딘은 영향없음.
- inj.sol.의 pH 조절만으로 라미부딘의 완벽한 분리는 어려움



표준액 및 검액조제 방법

(가) 표준원액 및 내부표준액의 조제

NDMA 표준원액은 NDMA 표준물질을 메탄올에 녹여 농도가 1000ng/mL가 되도록 하여 표준원액으로 하였다.

NDMA-d6의 표준원액은 NDMA-d6 표준물질을 메탄올에 녹여 농도가 1000ng/mL가 되도록 하여 표준원액으로 하였다.

(나) 표준액의 조제

표준원액, 내부표준액을 정해진 검량선의 농도에 해당하는 양을 취하여 10mL 용량의 부피플라스크에 넣고 증류수로 표선을 맞추어 표준액으로 조제하였다. 조제한 표준액을 autosampler vial에 넣어 최종시료로 하였다.

(다) 검액의 조제

1. 원료의약품

지도부딘 약 600mg을 정밀하게 달아 50mL용량의 부피플라스크에 옮겨 담은 후 증류수를 넣어 충분히 흔들어서 섞고 표준액과 내부표준액을 첨가한 후 증류수로 표선을 맞추었다.

필요시 7500rpm에서 10분 이상 원심분리 과정을 거친 후 상층액을 취하여 0.2 um 멤브레인필터 (PTFE)로 여과한 여액을 검액으로 한다.

2. 완제의약품-정제

의약품 20정 이상을 가지고 그 질량을 정밀하게 달아 가루로 한다.

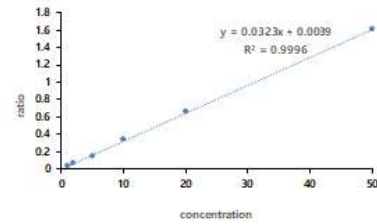
지도부딘으로서 약 600mg에 해당하는 양을 정밀하게 달아 50mL 용량의 부피플라스크에 옮겨 담은 후 증류수를 넣어 충분히 흔들어서 섞고 표준액과 내부표준액을 넣어 다시 증류수로 표선을 맞춘다.

필요시 7500rpm에서 10분 이상 원심분리 과정을 거친 후 상층액을 취하여 0.2um 멤브레인필터(PTFE)로 여과한 여액을 검액으로 한다.

Validation

시험항목	시험 방법	시험 기준
특이성 (Specificity)	함시험액, 표본액 및 길액의 측정	표본물질에 인접한 피크가 있을 때, 분리도는 1.5 이상
직선성 (Linearity)	LOQ를 포함한 최소 5개 이상 농도를 사용해 회귀분석을 작성 (n=3)	결정계수 ≥ 0.998
범위 (Range)	LOQ ~ LOQ의 50배 이상의 범위 설정	-
정밀성 (Precision)	검정액을 사용하여 길액선 범위내 3개 농도 (저농도, 중간농도, 고농도)에서 반복 측정 - 반복성: 농도별 3회 분석 - 실험실내: 농도별 3회 분석 - 실험실간: 농도별 3회 분석	정밀성: RSD가 20%
정확성 (Accuracy)	검정액을 사용하여 길액선 범위내 3개 농도 (저농도, 중간농도, 고농도)에서 반복 측정 - 일간: 농도별 3회 분석 - 일내: 농도별 3회 분석	정확성: 80 ~ 120 %
검출한계 (Limited of detection)	LOD (n=3) 측정	S/N ≥ 3
정량한계 (Limited of quantitation)	LOQ (n=3) 측정	S/N ≥ 10
완건성 (Robustness)	유량 및 컬럼 온도 등을 변경하여 측정	검정액의 피크 머무름시간, 피크 넓이의 변동을 측정
시스템적합성 (System suitability)	(1) LOQ 감도 확인 (2) 직선성 (3) 6회 반복 측정	(1) S/N ≥ 10 (2) 결정계수 ≥ 0.998 (3) 재현성 피크 머무름시간, 면적, RSDs $\leq 5\%$

*Calibration range: 1ng/mL ~ 50ng/mL



Validation 및 data processing 완료

Validation

-정확성 (Accuracy)

종류	이론값(ng/mL)	평균측정값 (ng/mL)	표준편차 (ng/mL)	정확성 (%)
원료의약품	3	3.00	0.13	99.90
	20	20.23	0.12	101.12
	40	40.20	0.08	100.41
원제의약품	3	3.10	0.02	103.54
	20	20.17	0.82	100.99
	40	40.63	1.33	101.54

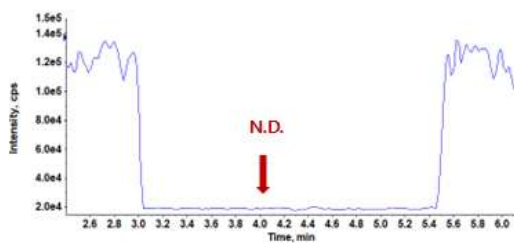
-정밀성 (Precision)

종류	이론값(ng/mL)	평균측정값 (ng/mL)	표준편차 (ng/mL)	정밀성 RSD (%)
원료의약품	3	2.94	0.07	2.42
	20	20.2	0.16	0.81
	40	39.83	0.45	1.13
원제의약품	3	3.00	0.08	2.58
	20	20.40	0.16	0.80
	40	40.80	0.57	1.40

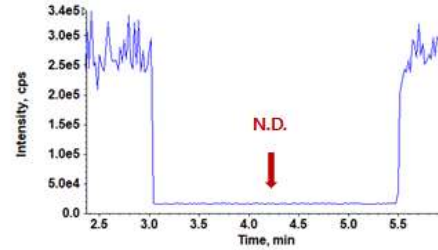
-완건성 (Robustness)

변경항목	변경값	NDMA (LQC 10 ng/ml)			
		평균측정값 (ng/mL)	변동률 (%)	정확성 (%)	변동률 (%)
유량 (mL/min)	0.3 (기준)	10.1	5.0	101.13	3.7
	0.27	10.50		104.89	
컬럼 온도 온도 (°C)	30 (기준)	10.1	0.9	101.13	0.9
	27	10.19		102.04	

Real sample assay



- 검액(Combivir tablet)에서 NDMA 검출 안됨



- 원료의약품 (API)에서 NDMA 검출 안됨

6. 결론

- Zidovudine의 1일 최대 투여량을 고려한 NDMA 허용량인 1.96ng/mL보다 낮은 0.3 ng/mL 부터 검출할 수 있는 고감도의 분석법을 개발
- 개발된 분석법은 validation 항목을 모두 통과하여 타당한 분석 방법인 것을 검증함
- Zidovudine 원료의약품과 완제의약품에 적용해 NDMA 가 미검출 된 것을 확인
- 물 기반의 시료 전처리 과정으로 경제적인 분석법
- 이 연구는 Zidovudine 기반의 의약품에 대한 NDMA 관련 품질관리에 유용할 것으로 생각됨

감사합니다

JEONBUK NATIONAL UNIVERSITY

2022.10.26.

우수연구자 초청 세미나

가천대 약대

서승용 교수님

2022.10.26 JBNU



Chemical Proteomics to Reveal Novel Therapeutic Targets and Drug Discovery for the Treatment of Ocular Neovascular Diseases

**College of Pharmacy, Gachon University
Seung-Yong Seo, Ph.D.**



CONTENTS

- Phenotypic-based Approaches
- Chemical Proteomics
- Wet Age-related Macular Degeneration
- Synthesis of Naturally Occurring
Homoisoflavonoids

Where Do Recent Small Molecule Clinical Development Candidates Come From?

Lead generation strategies:



Target classes:

- Kinases (30%)
- Other enzymes (23%)
- GPCR's (17%)
- Epigenetic (9%)

Disease areas:

- Oncology (10%)
- CNS/Pain (18%)
- Infection (14%)
- Metabolic (12%)

- Known compounds (43%)
- Random high throughput screening (29%)
- Focused screening
- Structure-based drug design (SBDD)
- Fragment-based lead generation (FBLG or FBDD)
- DNA-encoded library screening (DEL)

J. Med. Chem. **2018**, *61*, 9442-9468

- increase in molecular weight ($\Delta MW = +85$)
- no change in lipophilicity ($\Delta clogP = -0.2$)
- >50% structurally very different and more complex.

3

Phenotypic and Target-based Approaches



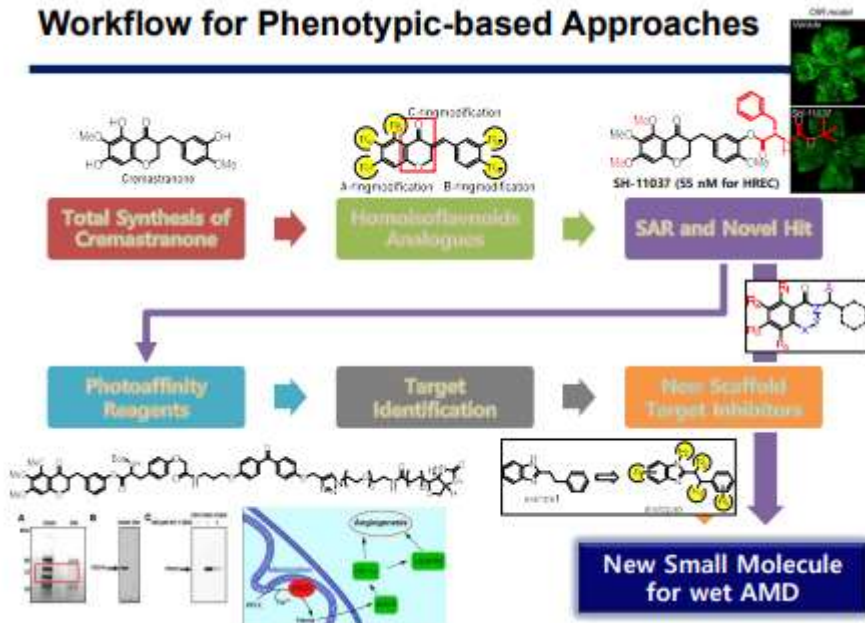
Drug Discov Today: Technol. **2017**

Modern drug discovery uses both phenotypic and target-based approaches

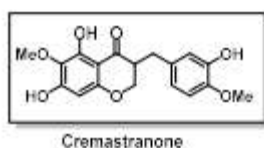
The feedback between their assays improves the disease relevance of phenotypic assays, increases the quality (selectivity) of new drugs, and contributes to our knowledge of disease mechanisms.

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Workflow for Phenotypic-based Approaches



Antiangiogenic Homoisoflavanoids



Cremastranone



Muscari armeniacum



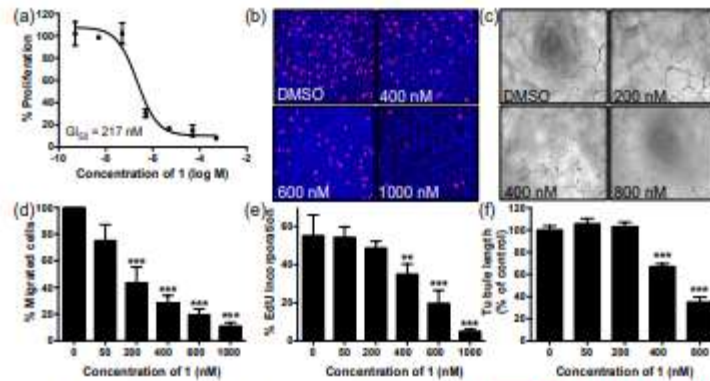
Cremastra appendiculata



Chionodoxa luciliae

- Isolated from the plants *Muscari armeniacum*, *Chionodoxa luciliae*, *Scilla natalensis*, *Merwillia plumbea*, and *Cremastra appendiculata* (D. Don)
- Anti-inflammatory, anti-oxidative, anti-bacterial, anti-cancer, anti-histaminic, anti-viral, and anti-phosphodiesterase activities
- A potent inhibitor of the proliferation of human umbilical vein endothelial cells (HUVECs). Inhibitor of vascular tube formation and new vessel growth induced by basic fibroblast growth factor
- Antiangiogenic activities in the laser-induced choroidal neovascularization and oxygen induced retinopathy mouse models, used for testing novel therapies for neovascular age-related macular degeneration (wet AMD) and retinopathy of prematurity (ROP), respectively

Biological Activities of Cremastranone



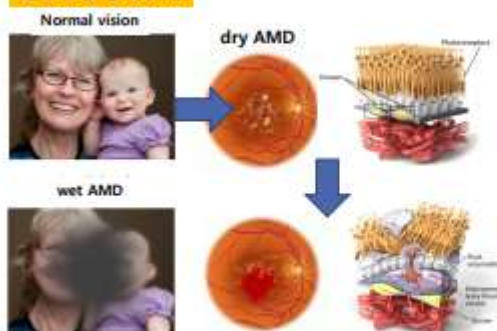
Synthetic cremastranone (I) blocks in vitro angiogenesis of human retinal microvascular endothelial cells (HRECs) in a dose-dependent manner.

(a) The effect of I on proliferation of HRECs was tested using an alamar blue fluorescence assay. (b, e) In an EdU incorporation assay, I blocked DNA synthesis as evidenced by a decrease in incorporation of EdU (pink) into the nuclei of HRECs (nuclei, blue). (c, f) Tube formation by HRECs on Matrigel was blocked by I; the extent of tube formation was measured as tubule length. (d) Migration of HRECs in a scratch-wound assay was inhibited by I.

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습성 노인성 황반변성 (Wet age-related macular degeneration)

황반변성 병적상태

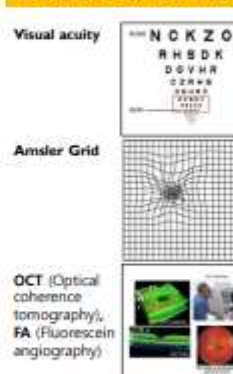


망막혈관 질환

Retinal neovascularization

- 습성 황반변성 (wet/neovascular AMD)
- 당뇨병성 망막병증 (Diabetic retinopathy, DR)
- 미숙아 망막병증 (Retinopathy of prematurity, ROP)
- 황반부종 (Macular edema)

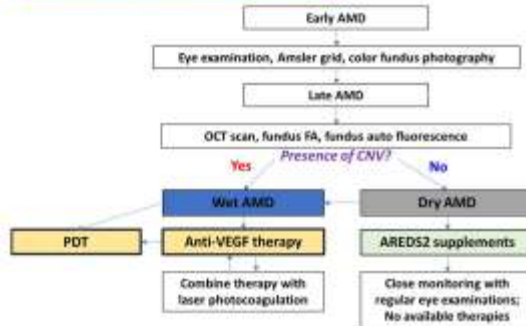
황반변성 진단, 치료효과 평가



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습성 노인성 황반변성: 치료제 현황

Current AMD Clinical Treatment



망막혈관 질환 치료제

Treatment of neovascularization in the eye

- *Photocoagulation
- *Photodynamic therapy (PDT)
- *Antiangiogenic biologics

Anti-VEGF Biologics

- *Ranibizumab (Lucentis, Fab)
- *Aflibercept (Eylea, VEGF trap)
- *Bevacizumab (Avastin, mAb)
- *Pegaptanib (Macugen, aptamer)



Intravitreal injection (IVI) 12

wet AMD 저분자 치료제 개발 현황

name	company	clinical stage	target	structure	name	company	clinical stage	target	structure
Voriconazole (V-82)	Tyngene/Beta	Phase 2	CSF1R /PDGFR /VEGFR		BI-14907	Boehringer Ingelheim	Phase 2	unidentified	structure undisclosed
Pegcetacoplan (APL-2)	Apellis	Phase 2	complement C3	PEGylated cyclic peptide	PAM-9000	Grybug Vasc Inc	Phase 2	VEGFR	
Acrizantide (UNA-518)	Alcon	Phase 2	VEGFA		44-1327	Chenode Biomedical	Phase 2	LRXK	structure undisclosed
AKST-4298 (ALS-4298)	Boehringer Ingelheim; Alkermes	Phase 2	CCL11 /CCR3		BG-7921	Roche	Phase 1	unidentified	structure undisclosed
AR-13503 (Metasorb metabolite)	Aerie Pharmaceuticals	Phase 2	PKC /ROCK1 /ROCK2		OTX-TX1 (Axitinib intravitreal implant)	Ocular Therapeutics	Phase 1	VEGF /PDGF	
BI-144807	Boehringer Ingelheim	Phase 2	unidentified	structure undisclosed	Sputumex Lactate	Genvec (Origeneas) OHR	Phase II (Diabetic maculopathy)	Calmodulin modulator /FGFR	

References: 1. Cortellis, 2. Pharmacodia

시장분석 및 개발현황: Medical Unmet Need

• VEGF inhibitor (Lucentis®, Eylea® or Avastin®)

>50% do not achieve significant vision gain

2/3 will continue to have fluid at the back

25% will have further vision loss at 12 months

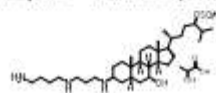
Ocular and systemic side effects

High price and monthly injection

**Critical need for
novel targets and treatments**

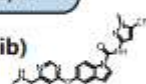
• Squalamine lactate (Evison®)

"...Squalamine Eye Drops failed to reduce the average number of Lucentis injections required by the trial participants." –VisionWare, (2017.01.23)



- All target same pathway
- Non-responsive or refractory
- No approved small molecule therapies

• LHA510 (Acrizanib)



"Topical VEGF receptor inhibitor, LHA510, did not demonstrate efficacy in a Proof-of-Concept study in patients with neovascular age-related macular degeneration" –IVOS, (2018.07)

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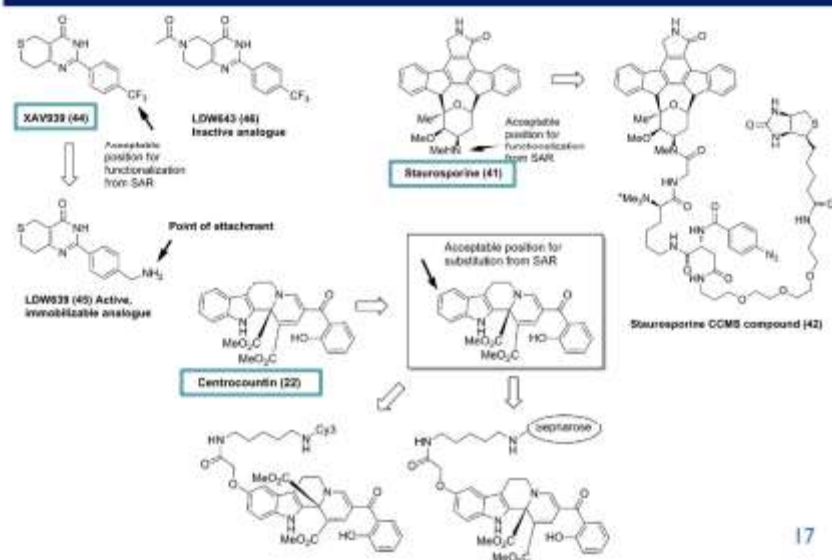
Natural Product Target Identification Examples

Table 1. Some classic compound-target pairs identified by affinity chromatography.

Compound	Target	References
Norepinephrine	β -Adrenergic receptor	Lefkowitz, Haber, and O'Hara (1972)
FK506	FKBP12	Harding, Galat, Uehling, and Schreiber (1989)
Geldanamycin	HSP90	Whitesell, Minnaugh, De Costa, Myers, and Neckers (1994)
Fumagillin	Methionine aminopeptidase 2	Sin et al. (1997)
Parthenolide	I κ B kinase β	Kwok, Koh, Ndubuisi, Eloffson, and Crews (2001)
Pateamine A	Eukaryotic initiation factor 4A	Low et al. (2007)
Thalidomide	Cereblon	Ito et al. (2010)

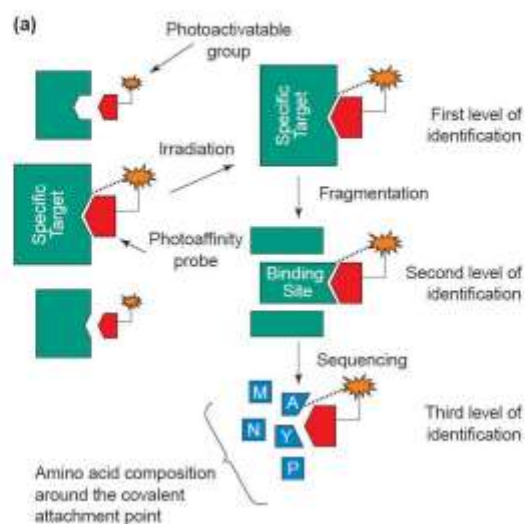
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Chemistry for Target Identification



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Photoaffinity Probe for Chemical Proteomics



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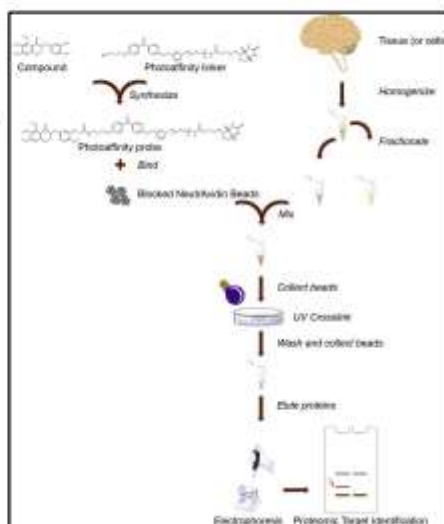
Protocols for Chemical Proteomics

CHAPTER SIXTEEN

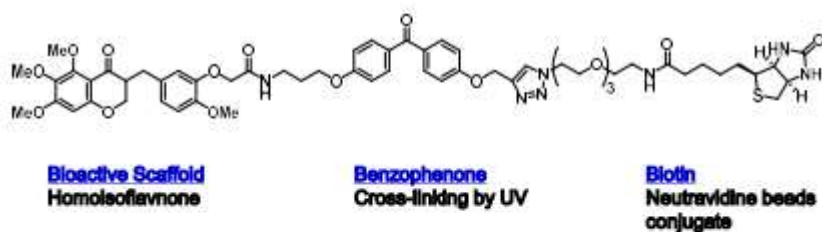
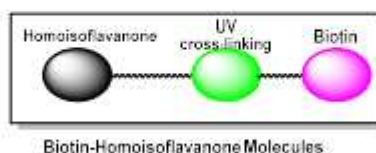
Small molecule target identification using photo-affinity chromatography

Seung-Yong Seo¹, Timothy W. Conson^{2*}
¹College of Pharmacy, Gachon University, Incheon, South Korea
²Yale University School of Medicine, Indianapolis, IN, United States
 *Corresponding author: e-mail address: tconson@iuh.edu

Methods in Enzymology **2019**, 622, 347-374



Photoaffinity Probes for Target Identification

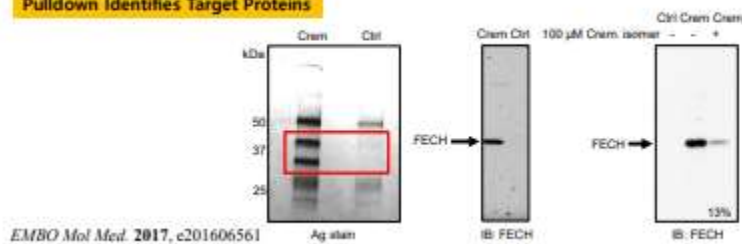


Target Identification by Photoaffinity Probes (1)

Homoisoflavonoid-base Chemical Probes



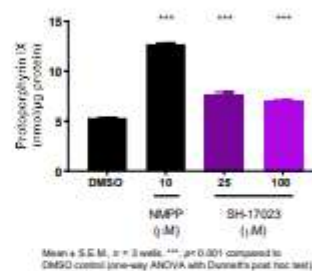
Pulldown Identifies Target Proteins



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Target 1: Mitochondrial Heme Synthesis Enzymes

Ferrochelatase (FECH)



- FECH: mitochondrial inner-membrane protein
- Final, rate-limiting step in heme biosynthesis
- No documented role in angiogenesis

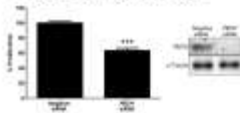
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FECH, In-vitro Proof of Concept

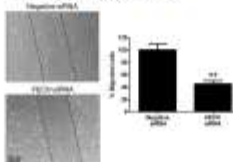
Essential Protein for Angiogenesis *in vitro*

FECH KD Inhibits

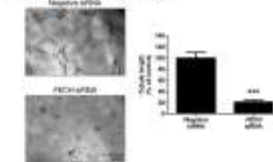
•HREC Proliferation



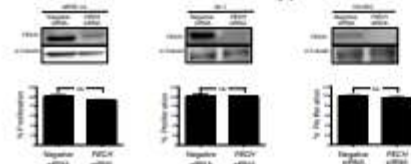
•HREC Migration



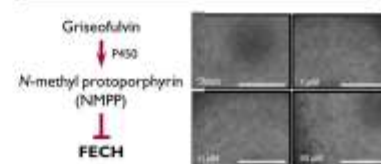
•Tube Formation



•Little Effect on Other Cell Types



FECH Inhibitors Block Tube Formation

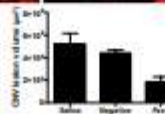


EMBO Mol Med. 2017, e201606561

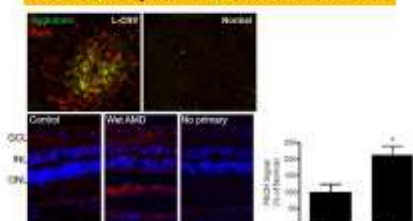
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FECH, In-vivo Proof of Concept, MoA

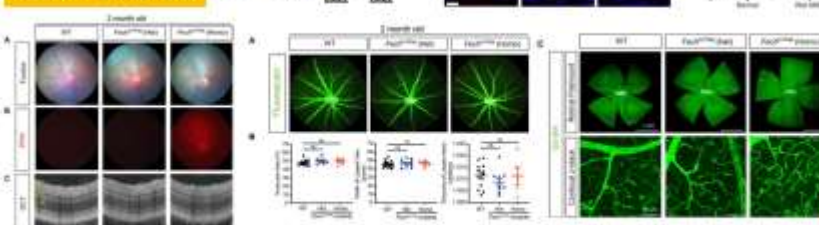
Fech Required for Neovascularization



FECH Overexpressed in Neovascularization



Fech^{+/Par} mutant mice



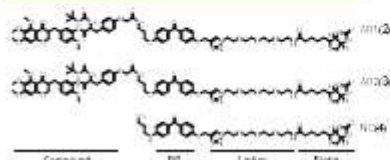
EMBO Mol Med. 2017, e201606561

Invest Ophthalmol vis Sci 2021, 62(2), 36

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Target 2: Epoxygenated Fatty Acids Metabolism

Homoisoflavonoid-base Chemical Probes

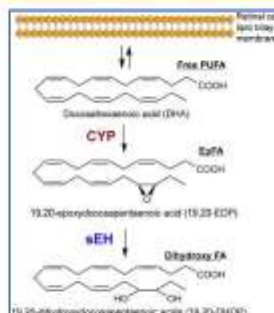


Pulldown Identifies Target Proteins



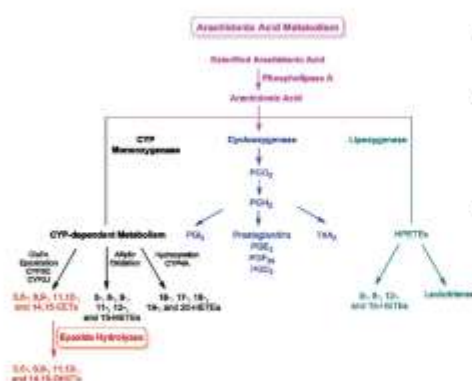
ACS Chem. Biol. 2018, 13, 45–52.

Soluble epoxide hydrolase (sEH)



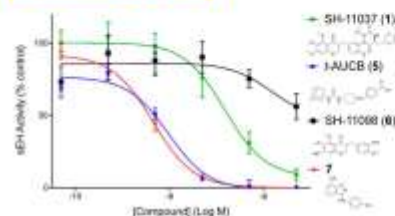
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PUFA metabolism by the CYP-sEH Pathway

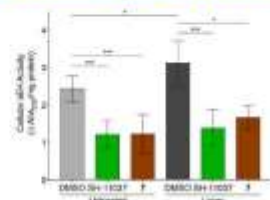


sEH, Proof of Concept

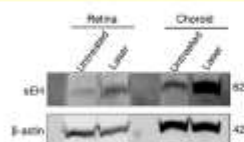
sEH enzymatic activity



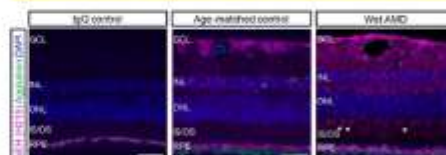
sEH activity, up-regulated in L-CNV eye tissue



Immunoblot of sEH in L-CNV mouse



Central retinal sections from eyes of wet AMD patient

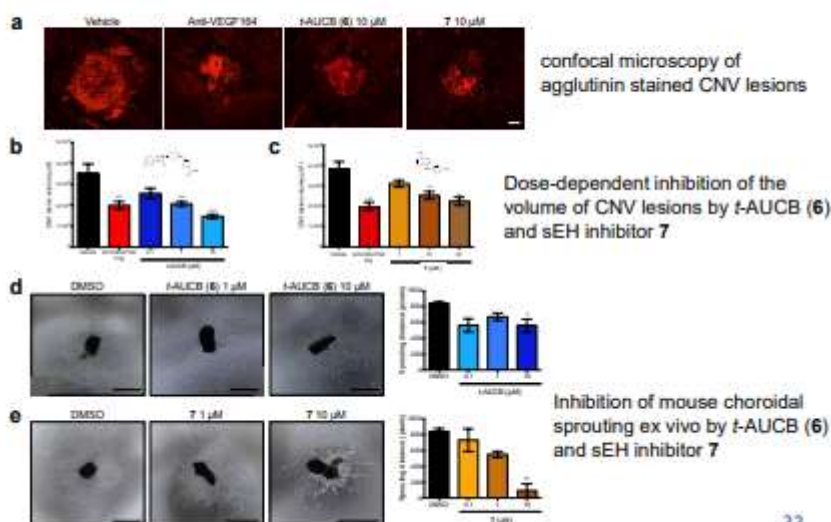


ACS Chem. Biol. 2018, 13, 45–52.

GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; IS/OS, photoreceptor inner segments/outer segments; RPE, retinal pigment epithelium

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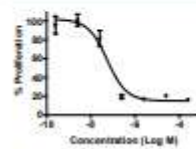
sEH, Proof of Concept



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선도물질 도출연구: 망막혈관 생성 억제 In-vitro

선도물질 1 (GI_{50} = 55 nM for HREC)

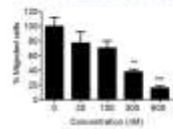
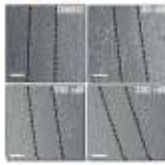


Cell type	유도물질1	유도물질2	선도물질1
HREC	43	0.217	0.035
HUVEC	16	0.377	0.75
Y79	87	9.8	12
92-1	8	47	>100
ARPE-19	>100	>100	>100

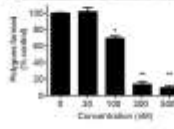
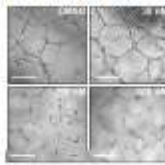
HREC: Human retinal microvascular endothelial cell

- HREC proliferation: **14-fold** over HUVECs, **218-fold** over Y79, and **>1000-fold** over 92-1
- **Cytostatic effects** on HRECs rather than general cytotoxicity.

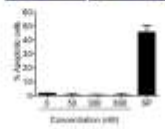
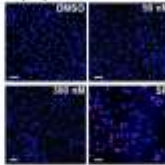
Migration



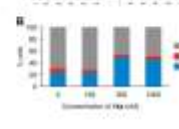
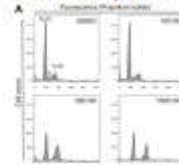
Tube formation



Does not induce apoptosis



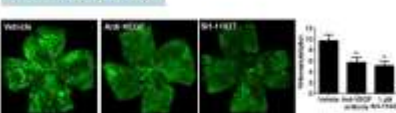
Arrests HRECs at the G2/M phase



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선도물질 도출연구: 망막혈관 생성 억제 In-vivo

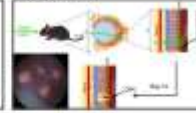
OIR in vivo model



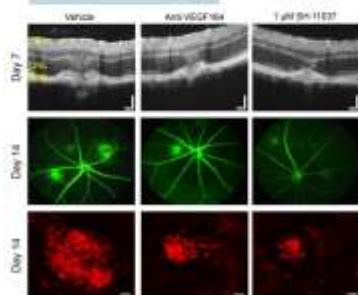
OIR model



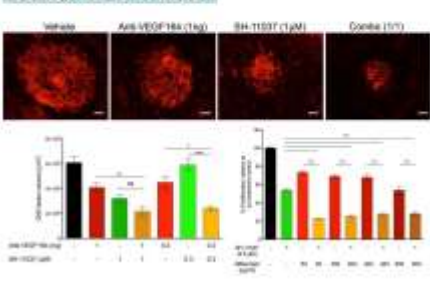
L-CNV model



L-CNV in vivo model



Synergy with anti-VEGF

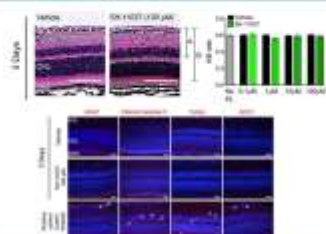


OIR: Oxygen-induced retinopathy
L-CNV: laser-induced choroidal neovascularization

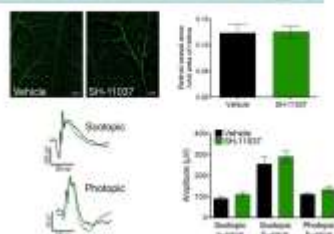
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선도물질 도출연구: Ocular Tox, DMPK profile

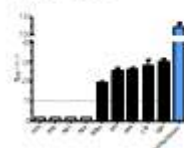
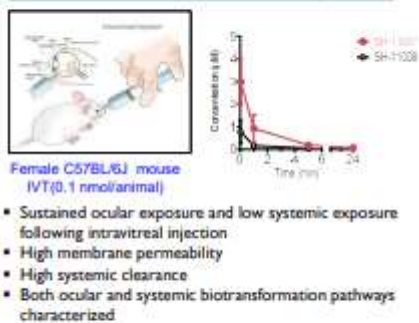
Not cause acute and chronic ocular toxic



Not affect retinal function and vessels

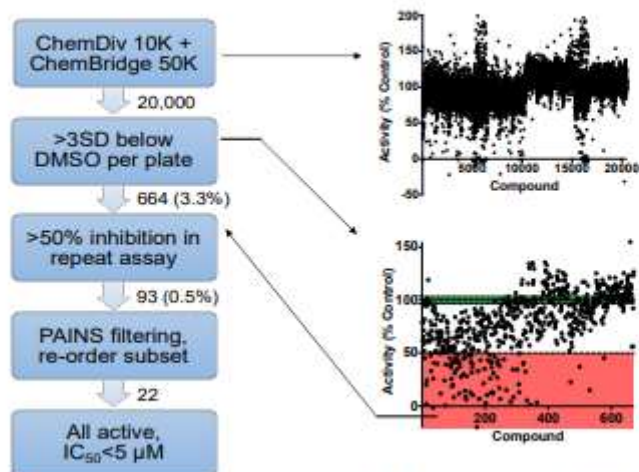


Mouse ocular PK after Intravitreal Injection



43

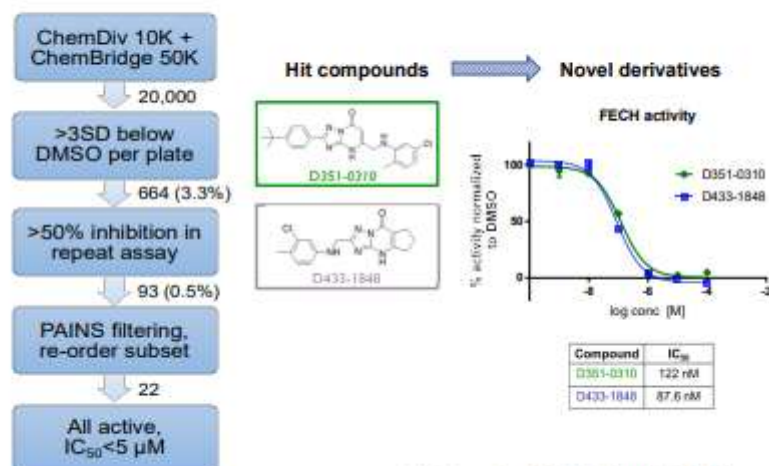
Workflow for High-throughput FECH Screen



Cell Chem. Biol. 2022, 29, 1010-1023

53

Potent FECH Inhibitors Identified



Cell Chem. Biol. 2022, 29, 1010-1023

54

Homoisoflavonoids (HIFs)

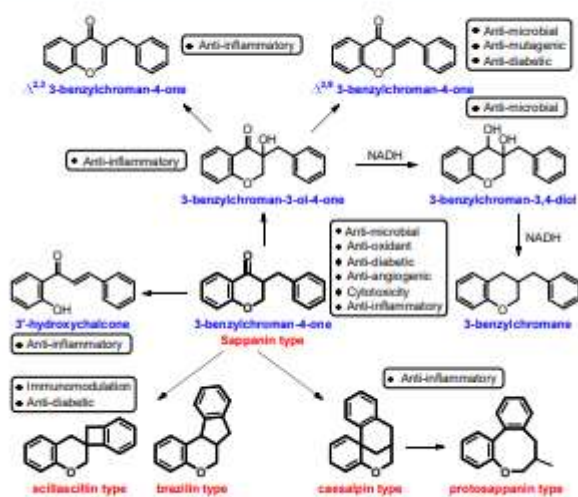


- A special subclass of flavonoids which contain one additional carbon atom
- Up to now, about 240 natural occurring homoisoflavonoids have been reported
- **Occurrence:** HIFs are rarely found in nature, mainly existing in *Fabaceae* and *Asparagaceae* families and being less common in *Polygonaceae*, *Portulacaceae*, *Orchidaceae*, and *Gentianaceae* families.
- **Bioactivities:** anti-microbial, anti-mutagenic, anti-oxidant, immunomodulatory, anti-diabetic, cytotoxic, anti-angiogenic, vasorelaxant, and anti-inflammatory effects.

Nat. Prod. Rep., 2013, 30, 1165-1210
 Planta Medica, 2014, 80, 1053-1066

60

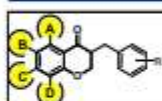
Biosynthetic Pathway of Homoisoflavonoids



Planta Medica, 2014, 80, 1053-1066

61

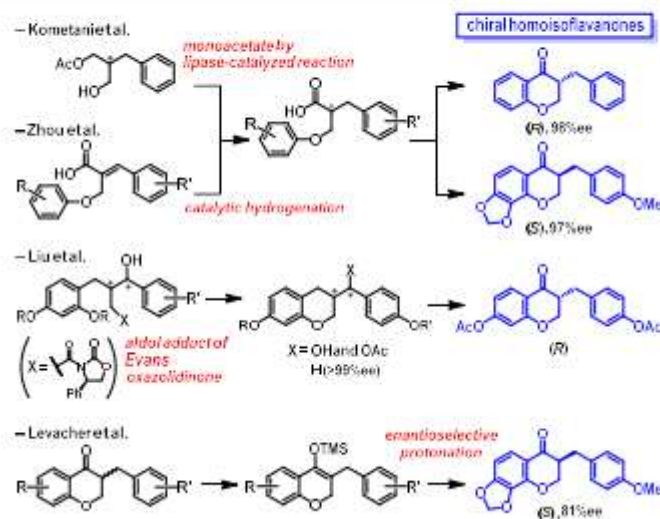
Natural Product Homoisoflavonoids



1차원						
2차원						
3차원						
4차원						

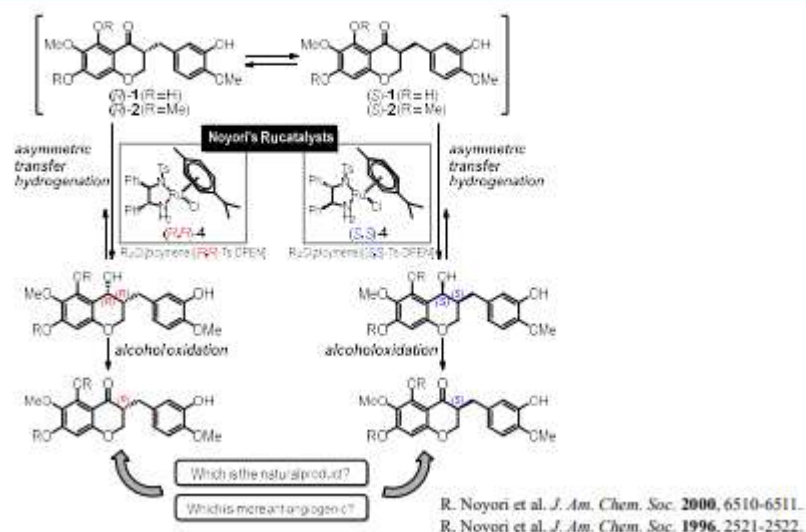
62

Previous Asymmetric Synthesis

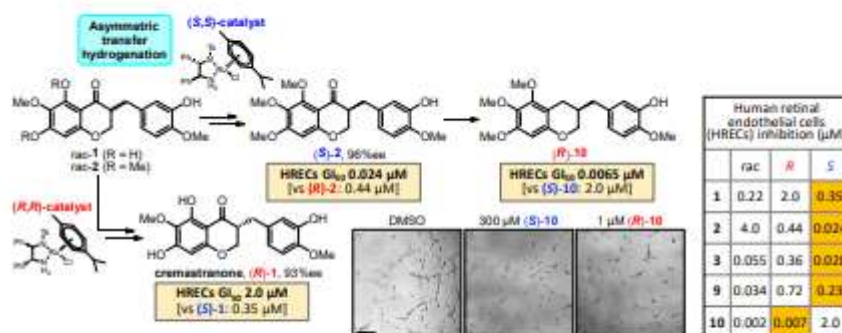


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Our Asymmetric Synthetic Plan by ATH



Bioactivity of Chiral Homoisoflavanones

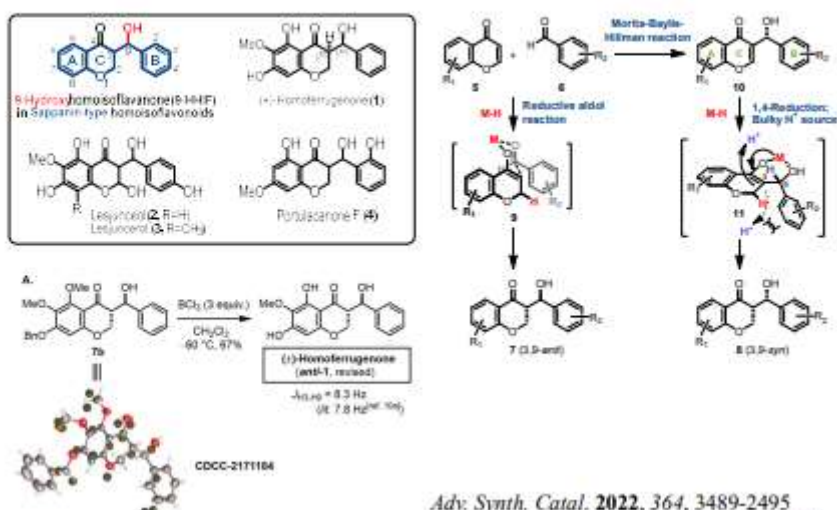


Naturally occurring cremastranone $\rightarrow$ (R)-configuration
More potent anti-proliferative activity $\rightarrow$ (S)-configuration

M. Heo et al. *J. Org. Chem.* **2019**, *84*, 9995-10011

65

9-Hydroxyhomoisoflavanones, Homoferrugenone and Portulacanonone F



72

Summary

- Phenotypic-based drug discovery
- Synthesis of antiangiogenic homoisoflavonoids and their photoaffinity probes for target identification of ocular neovascularization
- Homoisoflavonoid-binding proteins by chemical proteomics
 - Ferrochelatase (FECH)
 - Soluble epoxide hydrolase (sEH)
- NP-based drug discovery and preclinical development for the treatment of wet AMD and dry AMD
- Asymmetric synthesis and structural determination of various homoisoflavonoids

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2022.11.02.

**약품분석학 전공
대학원생 발표 세미나**

이호중 학생

울금 정향 복합추출물의 기능성 원료 표준화

JEONBUK NATIONAL UNIVERSITY

전북대학교 약학대학 약품분석학 이호중

목차

연구 배경

연구 목적

연구 결과

활용방안

울금과 정향 소개



울금(*Curcuma longa* L.)



정향(*Syzygium aromaticum*)

2.5~4.5cm	길이	1.5~2cm
덩이 줄기	형태	꽃봉오리
회황색	색	적홍갈색
매운맛	맛	매운맛
차가운 성질	성질	따뜻한 성질
국내 등 아시아에서 재배	재배	인도네시아

연구 배경

울금

- 커큐민 성분은 항산화, 항염, 면역력 개선 및 염증 제거에 효능이 있음
- 전립선 질환에 대한 예방, 치료 또는 개선 효과가 있음

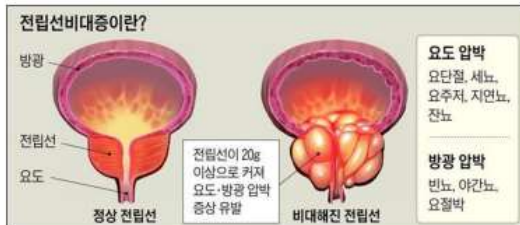
정향

- 항균, 항산화, 항스트레스 같은 효과가 있음
- 정향의 추출물의 전립선 특이항원 억제 효과에 의해 각종 전립선 질환에 대한 예방, 치료 효과가 있음

울금 정향 복합 추출물

- 서로 상반 관계의 약재이지만 전립선 질환에 효과적임
- 전립선 비대증과 관련된 인자를 효과적으로 억제하는 효능이 있음

전립선 비대증이란



- 노화, 비만, 흡연, 음주, 유전적 요인
- 테스토스테론 → 5알파 환원효소 → 디하이드로테스토스테론(DHT)

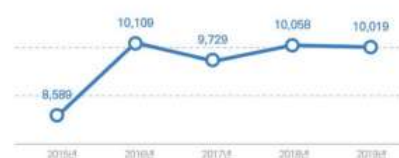
전립선 비대증 환자 추이

[자료: 대한비뇨기과학회]



전립선비대증 수술 환자수 추이

단위: 명, 자료: 건강보험심사평가원



전립선 비대증 치료제 개발&시장 현황



전립선비대증 치료제 개발 현황

제약사	제품명	성분	단계
종근당	CKD-397		3상
일동제약	Double Tab	탐스로신/타다라필	개발 중단
영진약품	YBH-1603		개발 중단
일동제약	TS tab	탐스로신/솔리테나신	3상 완료
제일약품	JLP-1207		3상 완료
동국제약	DKF-313	두타스테리드/타다라필	3상 진입
유유제약	YY-201		1상

전립선 비대증 치료 약물(전문의약품)



5알파환원효소 억제제(전문의약품)		
의약품명	프로스카	아보다트
성분명	피나스테리드	두타스테리드
효과	5알파환원효소 1형 70% 억제, 전립선 크기 감소	5알파환원효소 1형, 2형 90% 억제, 전립선 크기 감소
부작용	성기능 장애, 발진, 두드러기, 두통, 위장장애	발기부진, 성욕감소, 사정장애
용법·용량	1일 1회 경구 투여 5mg	1일 1회 경구 투여 0.5mg



알파차단제(전문의약품)			
의약품명	하루날디 0.2mg	카두라엑스엘 4mg	자트랄엑스엘 10mg
성분명	탐스로신	독사조신	알루조신
효과	베노장에	베노장에	베노장에
부작용	두통, 어지러움, 기저성저혈압	어지러움, 두통, 소화불량, 피로, 복통	어지러움, 두통, 발진, 복통, 기관지염
용법·용량	1일 1회 식후 경구 투여 0.2mg	1일 1회 식후 경구 투여 4mg	1일 1회 식후 경구 투여 10mg

전립선 비대증 치료 약물(일반의약품&건강기능식품)



	일반의약품	
의약품명	카리토프텐	유린탄캡슐
성분명	쿠쿠르비트종자유엑스	글루탐산, 알라닌, 글리신
효과	빈뇨, 잔뇨 등 증상 개선	야뇨증 개선, 빈뇨 증상 완화
부작용	속쓰림, 위통, 구토	위통, 식욕부진, 두통
용법·용량	1회 1캡슐(500mg) 1일 2회 경구 투여	1회 2캡슐(820mg) 1일 3회 경구 투여



	건강기능식품	
건강기능식품명	쏘팔메토	전립선 사군자
성분명	로르산	사군자추출분말
효과	전립선 건강 기능 유지, 빈뇨, 잔뇨 개선	전립선 크기 감소, 빈뇨, 잔뇨 개선
부작용	속쓰림, 위통, 구토	설사, 메스꺼움, 소화불량
용법·용량	1회 1캡슐(115mg) 1일 1회 경구 투여	1회 2캡슐(2000mg) 1일 1회 경구 투여

연구 목적

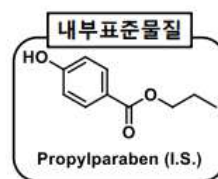
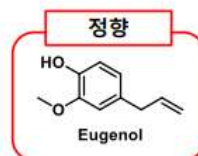
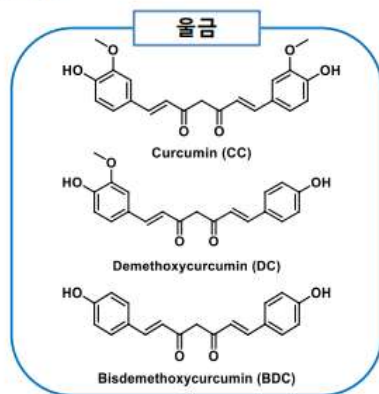
울금과 정향의 복합추출물 표준화

1. 기능성 원료 개발을 위한 울금과 정향의 복합추출물 제조 공정 표준화

2. 울금과 정향의 복합추출물에 대한 최적의 추출조건을 개발

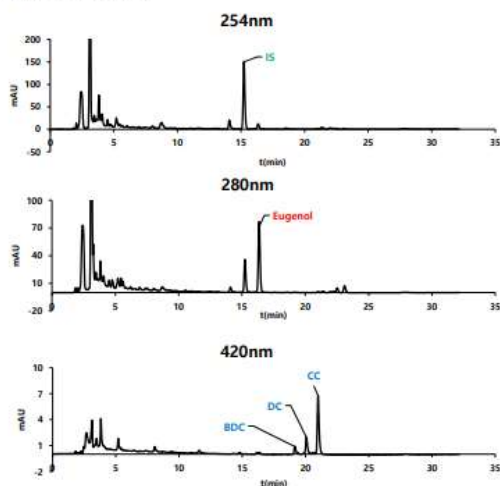
3. 기능성 원료 표준화를 위한 지표성분 선정 및 HPLC 동시 분석법 개발 및 검증

지표성분 선정



원재료의 생산에서부터 제조과정 전반에 걸쳐 기술과 정보를 관리하기 위해 지표성분 선정필요

HPLC Condition

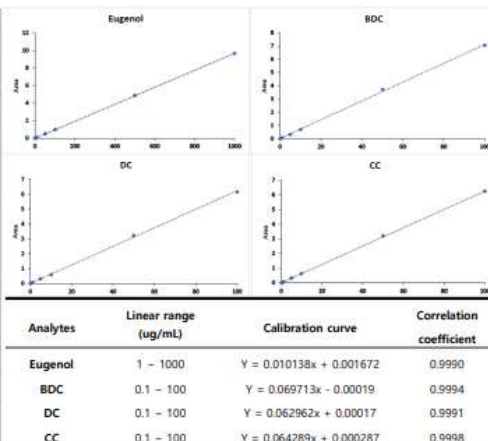


Parameter	Condition
Mobile phase	A : 0.1% Acetic acid in water B : 0.1% Acetic acid in Acetonitrile
Diluent	Mobile phase
HPLC	Agilent 1260 Infinity II - DAD
Column	phenomenex Luna C18, 250 x 4.6mm, 5 μm
Column temperature	40 °C
Wavelength	420 nm (BDC, DC, CC) 280 nm (Eugenol) 254 nm (IS)
Injection volume	10 μL
Flow rate	1.0 mL/min
Run time	50 min

Time(min)	Solvent A (%) ^a	Solvent B (%) ^b
0	70	30
30	30	70
40	30	70
42	70	30
50	70	30

Validation

시험항목	시험방법	시험기준
특이성 (Specificity)	공시험액, 표준액 및 검액의 측정	분석물질에 인접한 피크가 있을 때 분리도는 1.5 이상
직선성 (Linearity)	LOQ를 포함한 최소 5개 이상 농도를 사용해 검량선을 작성 (n=3)	결정계수 ≥ 0.999
범위 (Range)	LOQ ~ LOQ의 50배 이상의 범위 설정	
정밀성 (Precision)	검액을 사용하여 검량선 범위내 4개 농도 위치정량한계, 저농도, 중간농도, 고농도)에서 반복측정 - 시험 내 : 농도별 5회 분석 - 시험 간 : 농도별 3회 분석	정밀성 : RSD가 20%
정확성 (Accuracy)	검액을 사용하여 검량선 범위내 4개 농도 위치정량한계, 저농도, 중간농도, 고농도)에서 반복측정 - 시험 내 : 농도별 5회 분석 - 시험 간 : 농도별 3회 분석	정확성 : 80 ~ 120%
안정성 (Stability)	저농도, 고농도 3회 반복 분석	평균값이 이론값의 15% 이내
정량한계 (Limited of quantitation)	LOQ (n=3) 측정	S/N ≥ 22 (Eugenol) S/N ≥ 14 (BDC, DC, CC)
회수율 (Recovery)	세 가지 농도 (저농도, 중간농도, 고농도)에서 3회 반복 측정	회수율 100%가 될 필요없지만 정량성과 재현성이 있어야 함
희석타당성 (Dilution integrity)	최고정량한계 농도를 초과하는 시료를 검체로 희석하여 각 희석배수마다 5번 반복 분석	정확성, 정밀성 15% 이내



Validation

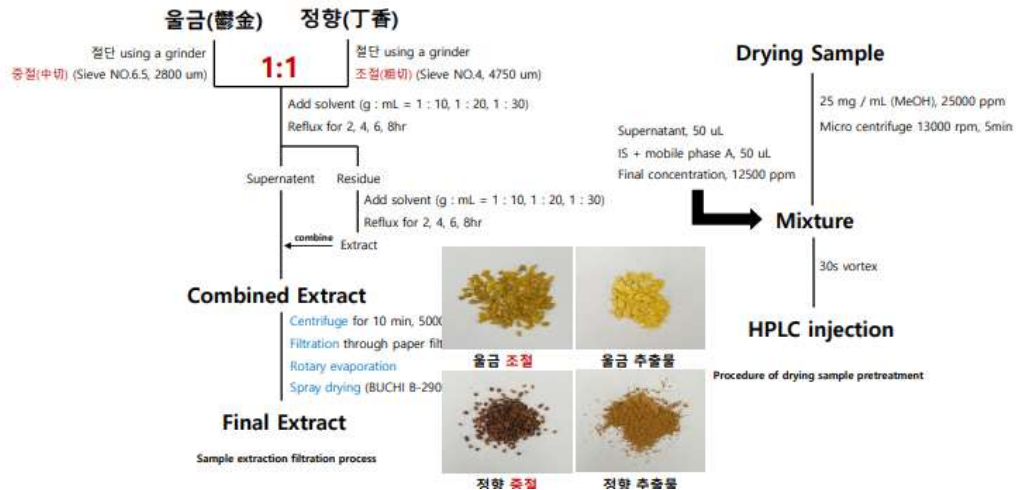
정확성&정밀성(precision & accuracy)

Analytes	retention time (min)	concentration (ug/mL)	within - run (n=5)				between - run (n=5)			
			mean (ug/mL)	SD	RSD (%)	recovery (%)	mean (ug/mL)	SD	RSD (%)	recovery (%)
Eugenol	16.434	1	0.96	0.018	1.82	96.27	0.98	0.012	1.27	97.70
		3	3.15	0.057	1.81	105.08	3.12	0.099	3.18	103.86
		45	45.20	0.379	0.84	100.45	45.31	0.910	2.01	100.69
		750	740.16	27.348	3.69	98.69	734.20	16.063	2.19	97.89
BDC	19.222	0.1	0.10	0.002	2.06	97.67	0.10	0.004	4.32	99.56
		0.3	0.30	0.006	1.99	100.48	0.30	0.013	4.34	99.81
		4.5	4.42	0.067	1.51	98.14	4.39	0.149	3.39	97.58
		75	76.01	2.993	3.94	101.35	77.42	2.039	2.63	103.23
DC	20.146	0.1	0.10	0.003	3.30	96.98	0.10	0.004	3.69	96.83
		0.3	0.31	0.010	3.15	101.72	0.30	0.015	5.14	99.28
		4.5	4.4	0.045	1.02	98.44	4.35	0.170	3.90	98.62
		75	74.95	2.999	4.00	99.93	75.10	2.204	2.93	100.14
CC	21.069	0.1	0.10	0.002	1.59	96.83	0.10	0.005	5.39	95.74
		0.3	0.31	0.008	2.63	101.89	0.30	0.014	4.87	98.58
		4.5	4.45	0.047	1.06	98.87	4.36	0.165	3.80	98.81
		75	74.94	2.951	3.94	99.92	74.40	2.089	2.81	99.21

회수율(recovery)

Analytes	Spiked amount (ug/mL)	Measured amount (ug/mL, n=5)	SD	RSD (%)	Recovery (%)
Eugenol	50	52.86	2.10	3.98	105.73
	300	309.89	18.71	6.04	103.30
	750	777.69	49.30	6.34	103.69
BDC	0.3	0.31	0.00	1.15	103.94
	4.5	4.60	0.16	3.40	102.18
	75	76.87	4.44	5.77	102.49
DC	0.3	0.30	0.01	3.13	100.39
	4.5	4.44	0.16	3.54	98.72
	75	74.39	4.32	5.81	99.19
CC	0.3	0.32	0.03	8.73	105.86
	4.5	4.49	0.21	4.63	99.85
	75	73.59	4.28	5.81	98.12

추출물 제조&전처리 과정

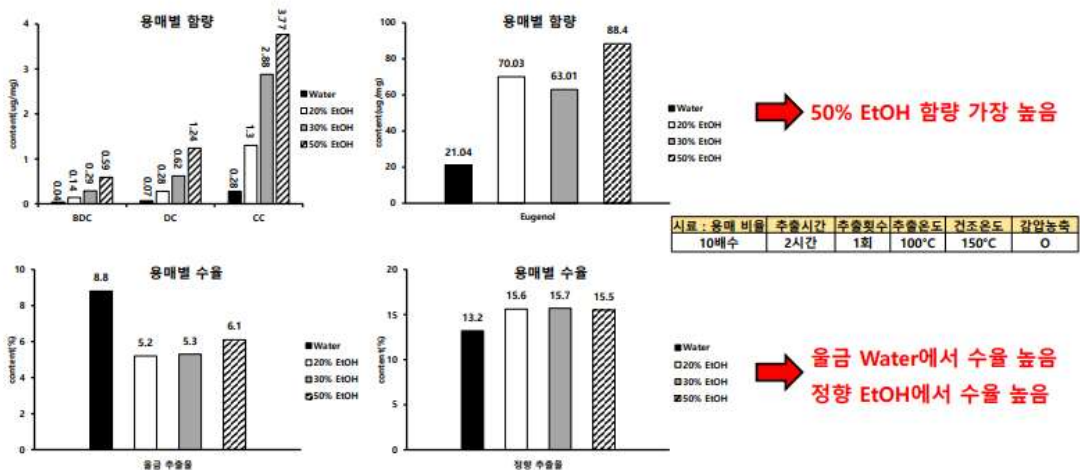


건조 비교

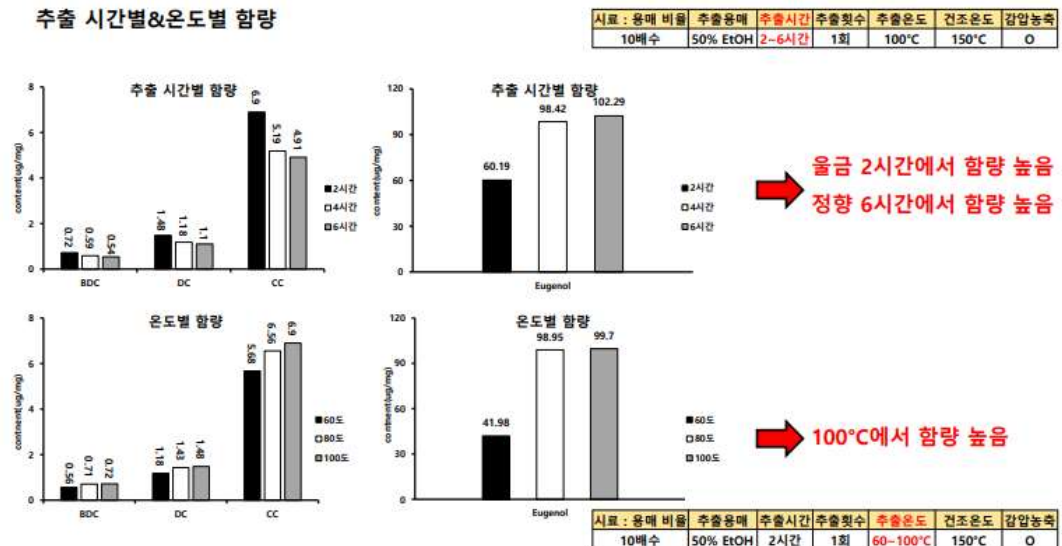


종류	급속분무건조	동결진공건조
원리	미립화된 액체를 120°C 내외의 공기와 접촉하여 순간 건조하는 원리	영하 10°C~30°C의 저온에서 동결시킨 후 진공 상태로 수분을 승화시켜 건조하는 원리
장점	고열에 건조하여 생산 속도가 빠르고 생산비용이 낮음 대량생산에 많이 사용 다른 건조에 비해 비교적 열에 약한 식품 생산에 적합	수분에 불안정하고 열에 극히 민감한 원료 건조 시 적합 열에 의한 영양소 파괴가 전혀 없음 복원성, 향기성분유지에 최적
단점	열에 아주 민감한 제품은 안됨 분무건조기 벽에 일부 원료가 붙어 고열에 계속 노출	생산 시간이 오래 걸림 장비가 고가이며 에너지 비용이 높음 건조 비용이 높음

용매별 함량&수율

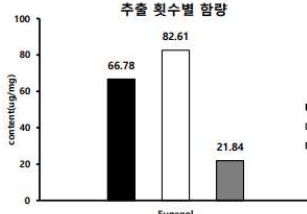
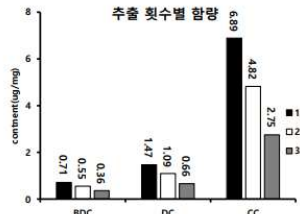


추출 시간별&온도별 함량

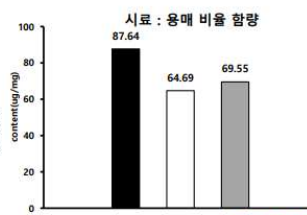
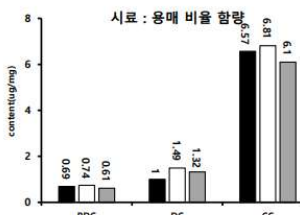


추출 횟수별&시료 : 용매 비율 함량

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10배수	50% EtOH	2시간	1~3회	100°C	150°C	O



→ 올금 1회 추출에서 함량 높음
정향 2회 추출에서 함량 높음

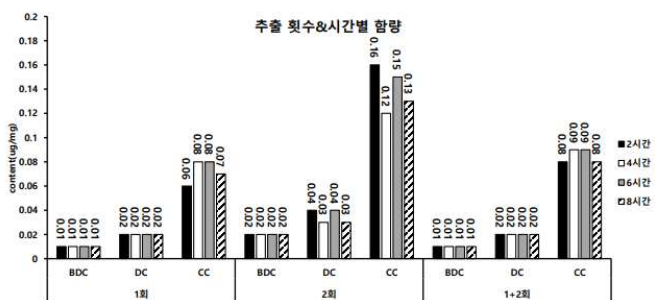
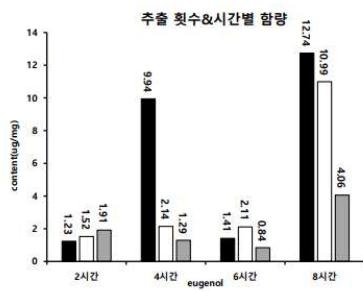


→ 올금 20배수에서 함량 높음
정향 10배수에서 함량 높음

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10~30배수	50% EtOH	2시간	1회	100°C	150°C	O

복합 추출물 추출 횟수& 시간별 함량

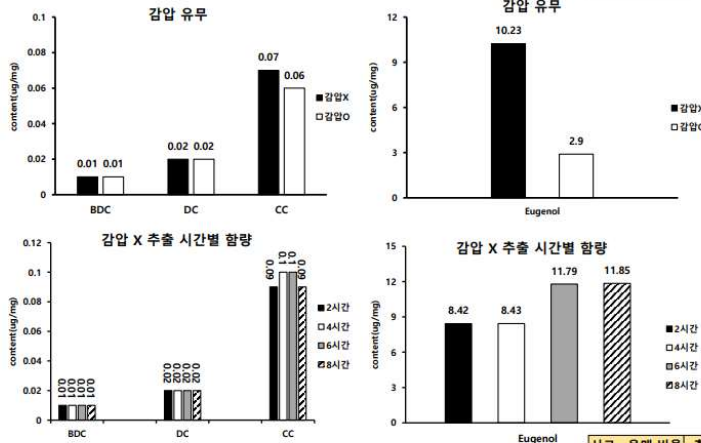
시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10배수	Water	2~6시간	1~2회	100°C	150°C	O



→ 올금 2회 추출, 2시간에서 함량 높음
정향 1회 추출, 8시간에서 함량 높음

복합 추출물 감압 유무

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도
10배수	Water	2시간	1회	100°C	150°C

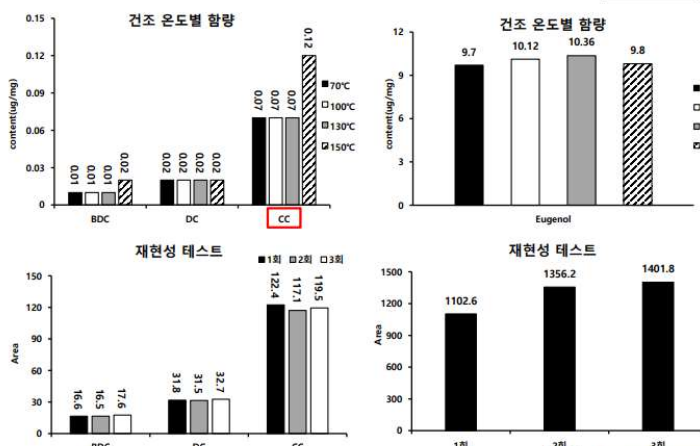


→ 올금 2~8시간 큰 차이 없음
정향 6, 8시간 큰 차이 없음

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10배수	Water	2~8시간	1회	100°C	150°C	X

복합 추출물 건조 온도별 함량&재현성 테스트

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10배수	Water	2시간	1+2회 합	100°C	70~150°C	X

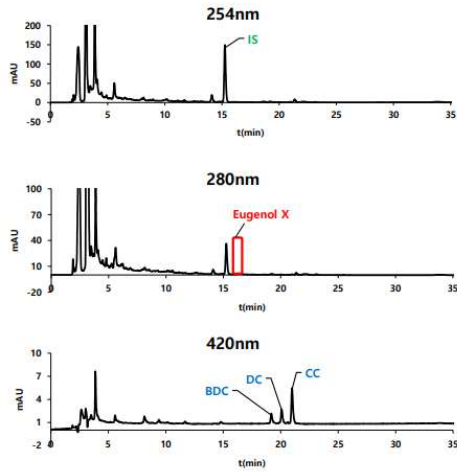


→ CC 성분만 150°C에서 높게 나옴
정향은 큰 차이 없음

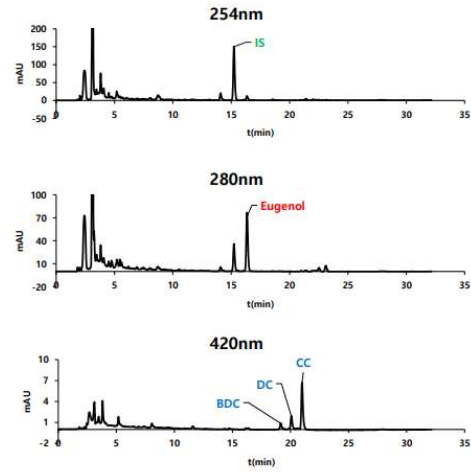
→ 모두 재현성 있음

시료 : 용매 비율	추출용매	추출시간	추출횟수	추출온도	건조온도	감압농축
10배수	Water	2시간	1+2회 합	100°C	150°C	X

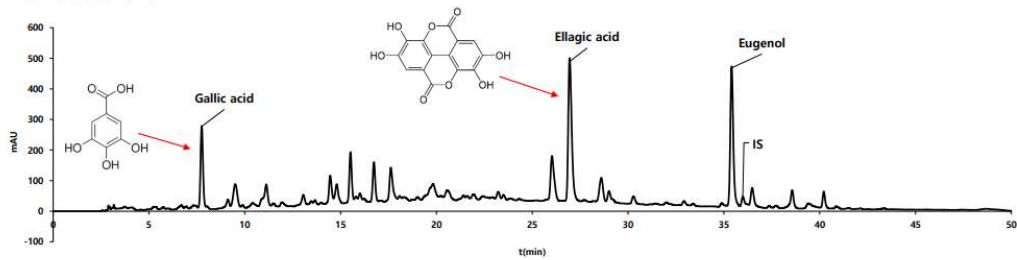
대량생산 건조시료(감압 O)



실험실 건조시료(감압 X)



추가 실험계획



- 정향의 새로운 지표성분 선정을 위한 분석법 개발 진행 중
- 개발된 분석법 검증을 위한 밸리데이션 진행 예정
- 개발된 분석법을 이용해 새로운 지표성분 함량 측정 및 추출 조건 보완 진행 예정
- 데이터 처리 및 보고서 작성 진행 예정

활용방안

- 개발된 분석법을 적용해 기능성 원료 표준화를 위한 안정성 과학적 근거 자료로 활용 가능
- 최적화된 추출 조건을 통해 추출법의 효율성 경제성 비교를 위한 자료로 활용 가능
- 시료 함량 분석 및 원료의 품질평가 자료로 활용 가능

감사합니다

JEONBUK NATIONAL UNIVERSITY

2022.11.02.

**약품분석학 전공
대학원생 발표 세미나**

민대홍 학생

HPLC를 이용한 대마 종 14종 칸나비노이드의 동시분석

JEONBUK NATIONAL UNIVERSITY

전북대학교 약학대학 약용분석실험실 민대홍



대마란?

- 대마초에는 3종의 인정된 종과 400가지 이상의 생리 활성 성분이 있음.

-> 대마 3종 Cannabis sativa (S), Cannabis indica (I), Cannabis ruderalis (R)

-> 칸나비노이드, 폴리페놀, 플라보노이드, 테르펜, 테르페노이드 등

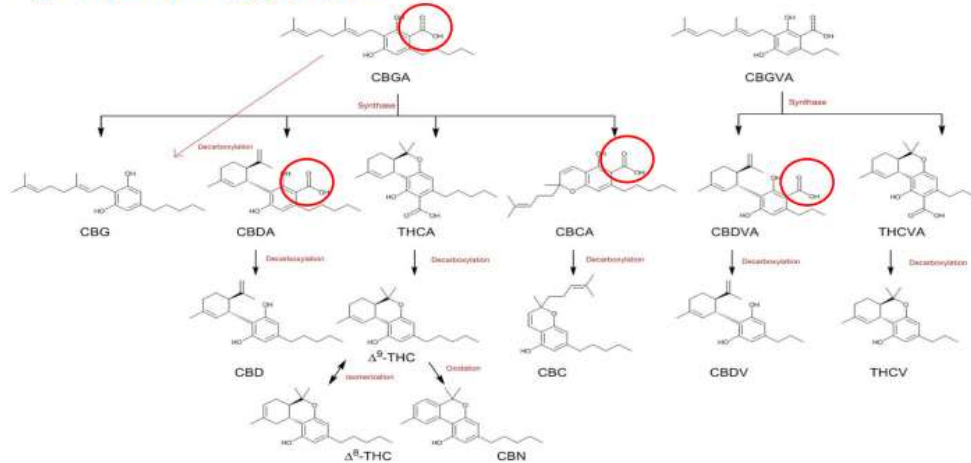
- 치료활성 평가를 위해 연구되는 칸나비노이드는 4가지가 있음.

-> 중성 칸나비노이드 Tetrahydrocannabinol (THC), cannabidiol (CBD)

-> 산성 칸나비노이드 Tetrahydrocannabinolic acid (THCA), Cannabidiolic acid (CBDA)



칸나비노이드 생합성 경로



대마 연구의 현황

- 과거 대마초는 수 세기 동안 마약류에 포함된 향정신성을 지닌 식물로 알려져 있음

-> 그러나 현대에 들어 대마초에 관한 연구들과 대마초 성분 CBD, THC를 이용한 의약품들이 많이 나오고 있음

- 미국의 경우 2018년 농업 개선법을 통해 0.3% 미만의 THC를 함유한 대마를 농산물로 법제화

-> 0.3% 이하의 대마는 더 이상 마약류로 분류되지 않고 농작물로 인식

- FDA는 2018년 6월 처음으로 CBD성분을 이용한 뇌전증치료제인 에피디올렉스의 사용을 승인

-> 하지만 CBD의 안전성 평가에 대한 이해가 아직 제한적이어서 의약품 이외에 식품 등에 대해서는 허가에 신중한 입장.

대마성분을 이용한 의약품



Epidiolex

(뇌전증 자가 치료제)
성분 : cannabidiol (CBD)
투여형태 : 경구캡슐, 주사제



Sativex

(다발성경화증 완화제)
성분 : Nabiximols
천연물 THC(2.7mg), CBD(2.5mg)
투여형태 : 경구스프레이



Canemes

(암환자 진통제)
성분 : nabilone
(합성 Tetrahydrocannabinol, THC)
투여형태 : 경구캡슐

우리나라 대마 정책현황

식약처, '식의약 규제혁신 100대 과제' 발표

- 국민안전·산업발전 지원 위한 현행 제강령 규제혁신 추진 -

◀ 분야별 규제혁신과제 현황 ▶

개	산업발전 지원	국민안전 지원	산업발전 지원	국민안전 지원
100건	19건	45건	13건	23건



48	안정·안전 부담 개선	자가치료용 대마 성분 의약품 휴대 출입국 허용	기초 대마의 항암·진통·항염·항산화 등 다양한 자가치료 목적으로 국내에서 식약처원의 승인을 받은 경우에만 휴대할 수 있는 등 개선 대마 성분 의약품의 자가 치료용으로 국내에서 휴대·반입할 수 있는 승인 대상으로 추가 개선 외국산 대마 성분 의약품의 휴대·반입 허용	대마류 관리에 관련 법률 시행령 개정 (23.6)
7	신산업 지원	대마 성분 의약품 제조·수입 허용	기초 중추·학술연구지원적 의료목적만 허용 개선 대마의 의료 목적 사용 목적의 효능과 부작용을 고려한 의료 개선 의료목적 허용 범위 확대 개선 대마 성분 의약품의 국내 제조·수입 허용 개선 외국산 대마 성분 의약품의 휴대·반입 허용 및 국내 의료산업 경쟁력 강화에 기여	대마류 관리에 관련 법률 개정 (24.1)

• 현재 우리나라는 cannabidiol (CBD) 성분을 추출하고 원료 의약품 생산, 수출을 위한 작업 착수.

• 최근 22. 08. 20 식물의약품안전처 식의약 규제혁신 100대 과제 발표

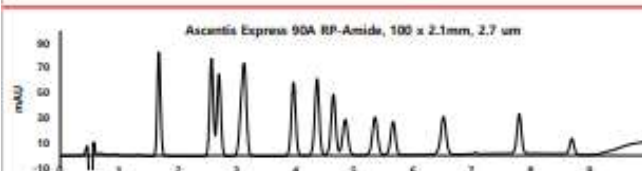
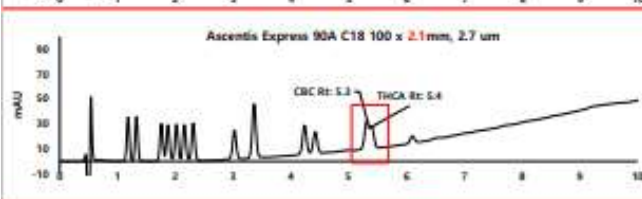
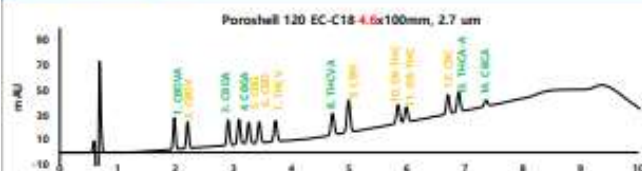
-> 자가치료용 대마 성분 의약품 휴대 출입국 허용

-> 대마 성분 의약품 제조 및 수입 허용

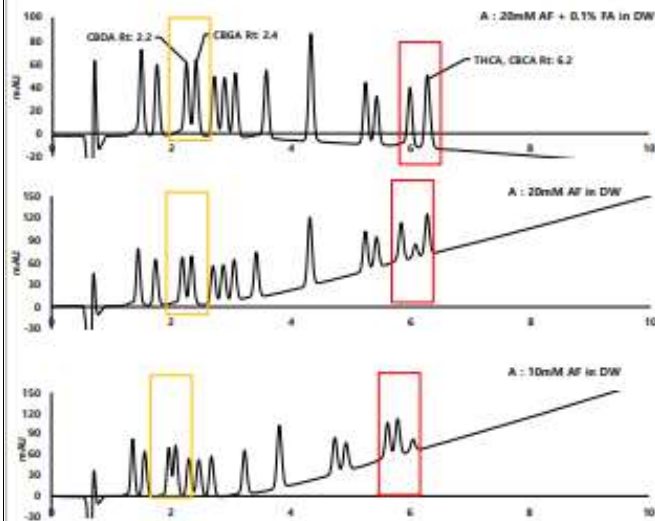
연구 목표

- 대마초 성분 중 14종 칸나비노이드를 지표성분 선정.
-> 주로 알려진 중성 칸나비노이드 8종, 산성 칸나비노이드 6종
- 다성분 동시분석을 위한 액체크로마토그래피 조건 최적화하여 분석법 검증.
-> 분석법 검증 가이드라인 항목에 따라 평가 및 검증
- 대마 중 생리활성성분의 함량을 분석하여 품질관리 및 의료용 용도로 사용할 수 있는 기초자료 제공.

Column 차이



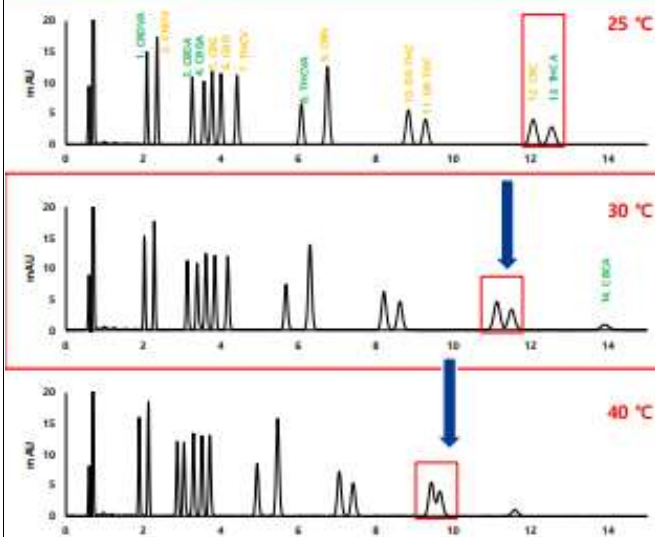
- Poroshell 120 EC-C18
-> 빠른 유속 때문에 감도 불리
 - Express 90A C18
-> 분석시간 빠름 CBC, THCA 분리 안됨
 - Express 90A RP-Amide
-> 전체적인 분석시간 증가
- ※ Express 90A C18 CBC, THCA를 분리하기 위해 추가로 이동상 조성 테스트 필요하다고 판단



이동상에 따른 변화

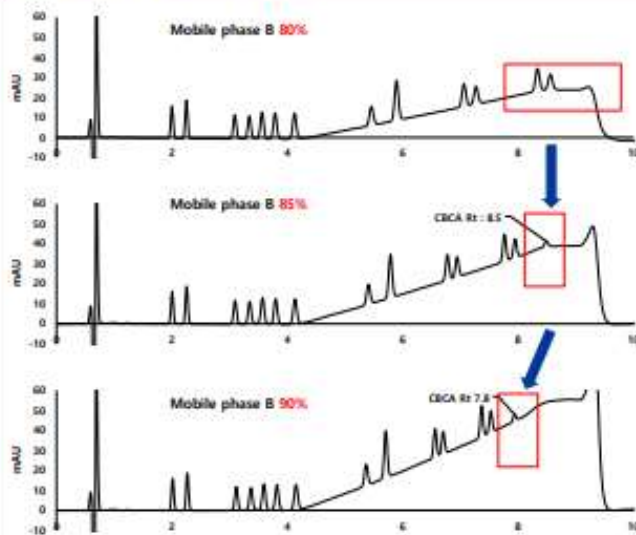
- **20mM AF + 0.1% FA in DW**
-> THCA, CBCA 합쳐지며 CBDA, CBGA 또한 분리도가 떨어짐
- **20mM AF in DW**
-> CBC, CBCA, THCA 분리 확인, CBDA, CBGA 분리도 확보 못함
- **10mM AF in DW**
-> CBC, THCA, CBCA 분리 되었지만 CBDA, CBGA 불용

※ CBDA, CBGA 분리를 더 시도하기 위해 기울기를 이용하여 시도하였으나 더 이상 분리 안됨 따라서 기존 Porashell EC-120 C18 컬럼으로 선택함



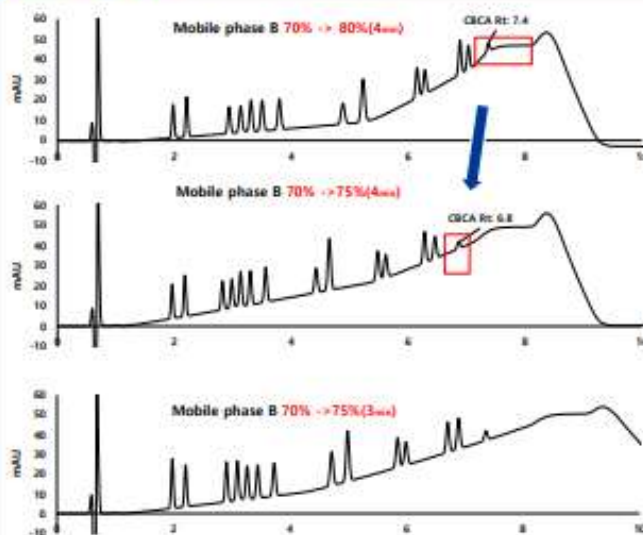
컬럼 온도

- **25 °C**
-> 전체적인 용출이 늦게 용출되며 CBCA 용출 안됨.
 - **30 °C**
-> 25도에 비해 빠른 용출을 볼 수 있었음.
-> 14분 정도에 작은 피크 CBCA가 용출되었음
 - **40 °C**
-> 용출이 빨라지며 CBC, THCA가 겹치며 분리되지 않음
- ※ 용출시간이 조금 더 빠르며 CBC, THCA가 겹치지 않고 분리도를 확보할 수 있는 **30°C** 온도 선택



Gradient Elution 변화

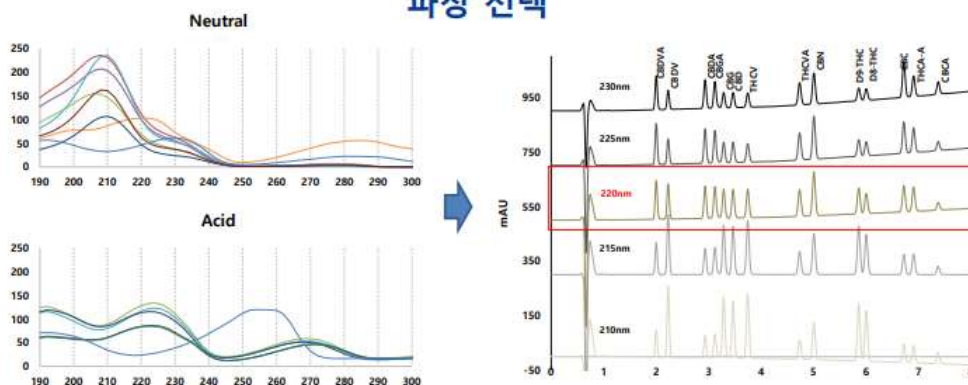
- 80%
-> 마지막 물질 CBA 용출 안됨.
 - 85%
-> CBA 용출 확인, 분석시간 단축 필요
 - 90%
-> 모든 물질 8분 이내로 용출하여 분석시간 단축
- ※ 90% 까지 유기용매 비율을 올려도 겹침 없이 분리가 가능하였고, 분석시간이 짧으면 경제적, 효율적이라 판단하여 유기용매를 높이는 것으로 선택



Gradient Elution 변화

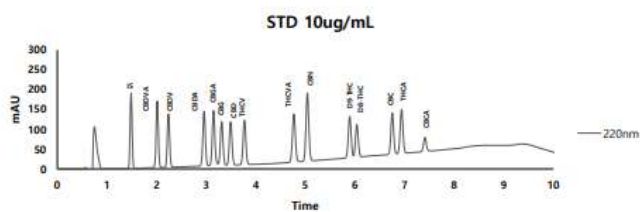
- 70% -> 80%(4min)
-> CBA 물질을 적분할 때 재현성 확보 불가.
 - 70% -> 75%(4min)
-> 전체적인 베이스라인 안정화 필요
 - 70% -> 75%(3min)
-> 모든 물질 8분 이내로 용출하여 분석시간 단축
- ※ 전체적인 베이스라인 안정화와 정량을 위한 적분 고려하여 70%→ 75%(3)으로 한 마지막 그림으로 조건 선택

파장 선택



- DAD를 이용하여 190~400 nm 파장을 동시에 검출하여 14종 지표성분에 대한 최대흡수파장을 파악.
- > 지표성분 14종에 대한 최대흡수파장을 파악하여 한 번에 정량.
- > 지표성분을 제외한 다른 성분에 대한 간섭을 최소화 하는 검출파장을 검토하여 선택.

분석법



Parameter	Condition
Mobile phase	A : 0.1% Formic acid in DW B : 0.1% Formic acid in ACN
Diluent	Mobile phase A
HPLC	Agilent 1260 Infinity II
Column	Poroshell 120 EC-C18 2.7um. 4.6x100mm
Column temperature	30 °C
Wavelength	220nm (13 cannabinoids) 254nm (CBCA)
Injection volume	15 µL
Flow rate	1.5 mL/min
Run time	13 min
Time (min)	Mobile Phase A (%) Mobile Phase B (%)
0	30 70
3	25 75
7	10 90
8	10 90
10	30 70
13	30 70

분석법 검증

Range 0.2 ~ 100 ug/mL

시험항목	시험 방법	시험 기준
특이성 (Specificity)	공시험예, 표준액 및 검액의 측정	분석물질에 인접한 피크가 있을 때 분리도는 1.5 이상
직선성 (Linearity)	LOQ를 포함한 최소 5개 이상 농도를 사용해 검량선을 작성 (n=3)	결정계수 ≥ 0.996
범위 (Range)	LOQ ~ LOQ의 50배 이상의 범위 설정	-
정밀성 (Precision)	검액을 사용하여 검량선 범위내 4개 농도 (최저정량한계, 저농도, 중간농도, 고농도)에서 반복측정 - 시험 내 : 농도별 5회 분석 - 시험 간 : 농도별 3회 분석	정밀성 : RSD가 20%
정확성 (Accuracy)	검액을 사용하여 검량선 범위내 4개 농도 (최저정량한계, 저농도, 중간농도, 고농도)에서 반복측정 - 시험 내 : 농도별 5회 분석 - 시험 간 : 농도별 3회 분석	정확성 : 80 ~ 120 %
안정성 (Stability)	저농도, 고농도 3회 반복 분석	평균값이 이론값의 15% 이내
정량한계 (Limited of quantitation)	LOQ (n=3) 측정	S/N ≥ 10
회수율 (Recovery)	세 가지 농도 (저농도, 중간농도, 고농도)에서 3회 반복 측정	회수율100%가 될 필요 없지만 일관성과 재현성이 있어야함
희석타당성 (Dilution integrity)	최고정량한계 농도를 초과하는 시료를 경제로 희석하여 각 희석배수마다 5번 반복 분석	정확성, 정밀성 15% 이내

No.	components	Regression equation	R ²
1	CBDVA	$y=0.0996x+0.0043$	0.9967
2	CBDV	$y=0.0927x+0.0041$	0.9982
3	CBD	$y=0.0784x+0.0024$	0.9975
4	CBGA	$y=0.0815x+0.0028$	0.9980
5	CBG	$y=0.1298x+0.0030$	0.9981
6	CBD	$y=0.0616x+0.0006$	0.9985
7	THCV	$y=0.0836x+0.013$	0.9986
8	THCVA	$y=0.0832x+0.0043$	0.9976
9	CBN	$y=0.0964x+0.0045$	0.9979
10	d9-THC	$y=0.0781x+0.0025$	0.9981
11	d8-THC	$y=0.0950x+0.0021$	0.9986
12	CBC	$y=0.0754x+0.0026$	0.9981
13	THCA	$y=0.0764x+0.0017$	0.9979
14	CBGA 254nm	$y=0.1310x+0.0039$	0.9987

R² ≥ 0.996

Pre - Acc

일 내
RSD(%): 1.7~6.1
Accuracy(%): 88.3 ~ 92.1

일 간
RSD(%): 4.3 ~ 8.9
Accuracy(%): 90.77 ~ 102.4

Standard	Retention time (min)	concentration (µg/mL)	within - run (n=5)				between - run (n=5)				Standard	Retention time (min)	concentration (µg/mL)	within - run (n=5)				between - run (n=5)				
			mean	SD	RSD(%)	Accuracy (%)	mean	SD	RSD(%)	Accuracy (%)				mean	SD	RSD(%)	Accuracy (%)	mean	SD	RSD(%)	Accuracy (%)	
1 CBDVA	2	0.2	0.18	0.01	6.13	89.32	0.18	0.01	6.98	90.77	8	THCVA	4.7	0.2	0.19	0.01	4.41	95.57	0.19	0.01	6.25	94.03
		0.6	0.55	0.02	3.99	92.16	0.6	0.05	8.07	99.58			0.6	0.56	0.02	3.36	92.9	0.61	0.05	8.16	101.31	
		6	5.45	0.13	2.35	90.82	5.77	0.35	5.98	96.25			6	5.42	0.12	2.25	90.28	5.73	0.33	5.71	95.49	
		75	66.92	1.16	1.73	89.23	75.05	5.59	7.45	100.06			75	67.58	1.15	1.71	90.11	76.17	5.57	7.31	101.56	
		0.2	0.18	0.01	4.17	91.82	0.18	0.01	6.49	90.56			0.2	0.19	0.01	4.39	95.38	0.19	0.01	7.18	92.63	
2 CBDV	2.2	0.6	0.56	0.02	3.32	92.8	0.61	0.05	8.36	101.45	9	CBN	5	0.2	0.16	0.01	5.04	90.18	0.16	0.01	4.83	91.82
		6	5.49	0.13	2.29	91.5	5.80	0.37	6.34	97.28			6	5.45	0.12	2.28	90.76	5.78	0.35	6.01	96.42	
		75	66.28	1.15	1.74	88.37	75.4	5.75	7.63	99.53			75	67.1	1.14	1.7	89.47	75.64	5.68	7.5	100.86	
		0.2	0.19	0.01	4.54	94.12	0.19	0.01	4.93	95.94			0.2	0.16	0.01	5.04	90.18	0.16	0.01	4.83	91.82	
		0.6	0.56	0.02	2.79	92.65	0.61	0.05	8.17	101.5			0.6	0.55	0.02	3.23	91.68	0.61	0.05	8.93	101.47	
3 CBDA	2.9	6	5.46	0.12	2.27	90.98	5.78	0.32	5.64	96.07	10	d9-THC	5.9	6	5.43	0.12	2.22	90.51	5.8	0.36	6.2	96.59
		75	66.89	1.18	1.73	89.18	75.49	5.72	7.58	100.65			75	68.21	1.14	1.72	88.21	75.13	6.1	8.11	100.18	
		0.2	0.17	0.01	3.19	86.39	0.18	0.01	5.53	88.13			0.2	0.19	0.01	5.16	94.27	0.19	0.01	3.93	95.73	
		0.6	0.55	0.02	3.83	91.11	0.59	0.05	8.74	98.82			0.6	0.56	0.02	3.14	93.15	0.61	0.05	8.57	101.17	
		6	5.41	0.13	2.32	90.09	5.76	0.34	5.98	95.99			6	5.42	0.13	2.33	90.35	5.77	0.34	5.95	96.19	
4 CBGA	3.1	75	66.49	1.18	1.74	88.65	75.37	5.71	7.56	100.75	11	d8-THC	6	75	66.66	1.13	1.7	88.88	75.77	6.12	6.08	101.02
		0.2	0.19	0.01	5.3	95.08	0.19	0.01	4.83	94.64			0.2	0.2	0.01	4.55	100.51	0.18	0.01	7.71	91.9	
		0.6	0.56	0.02	2.81	93.41	0.61	0.05	8.94	101.54			0.6	0.58	0.02	3.23	96.02	0.6	0.05	7.74	100.75	
		6	5.48	0.12	2.25	91.28	5.79	0.37	6.37	96.55			6	5.4	0.12	2.21	89.99	5.72	0.34	5.92	95.34	
		75	66.41	1.15	1.74	88.55	74.92	6.23	8.21	99.89			75	67.6	1.16	1.72	90.14	76.07	6.33	7	101.42	
5 CBG	3.3	0.2	0.19	0.01	4.05	95.33	0.19	0.01	5.47	92.87	12	CBC	6.7	0.2	0.19	0.01	4.87	96.07	0.19	0.01	6.28	94.37
		0.6	0.56	0.02	2.82	93.45	0.61	0.05	8.74	101.79			0.6	0.56	0.02	3.17	93.39	0.6	0.05	8.66	100.47	
		6	5.46	0.12	2.26	90.98	5.79	0.34	5.84	96.55			6	5.39	0.12	2.23	89.8	5.71	0.33	5.69	95.19	
		75	66.37	1.12	1.7	88.76	75.35	5.85	7.76	100.47			75	67.67	1.18	1.74	90.23	76.13	6.02	6.61	101.51	
		0.2	0.19	0.01	5.06	92.71	0.19	0.01	5.56	93.04			0.2	0.19	0.01	4.45	94.54	0.19	0.01	6.06	93.28	
7 THCV	3.7	0.6	0.56	0.02	2.88	92.75	0.61	0.06	8.97	100.47	14	CBGA	7.4	0.6	0.55	0.02	2.89	91.79	0.6	0.05	8.1	100.1
		6	5.47	0.12	2.26	91.09	5.8	0.35	6.94	96.72			6	5.39	0.12	2.24	89.85	5.72	0.33	5.83	95.29	
		75	66.46	1.14	1.71	88.62	75.11	5.86	7.8	100.14			75	68.04	1.18	1.74	90.72	76.71	5.79	7.55	102.28	

Sample extraction

Drying sample

Sample mix grinding 50mg
Add solvent 5mL
Solvent % : 100, 70, 50, 30
Room temperature
Extract time : 60min
Vortex 30s
Centrifugal separator 15000rpm, 5min

Supernatant

Dilution 1/20 MeOH

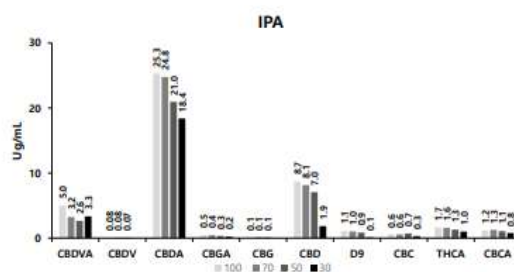
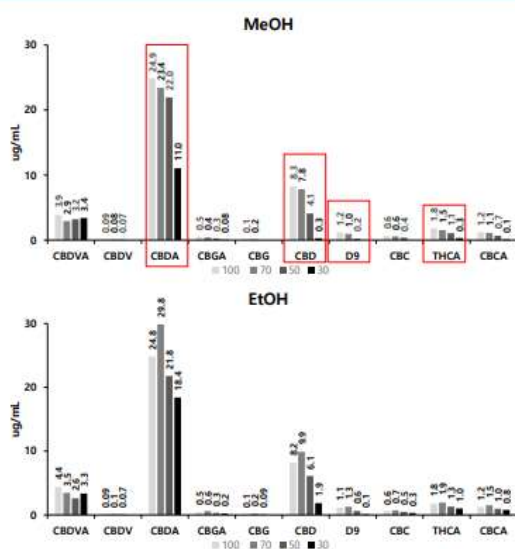
HPLC Inj

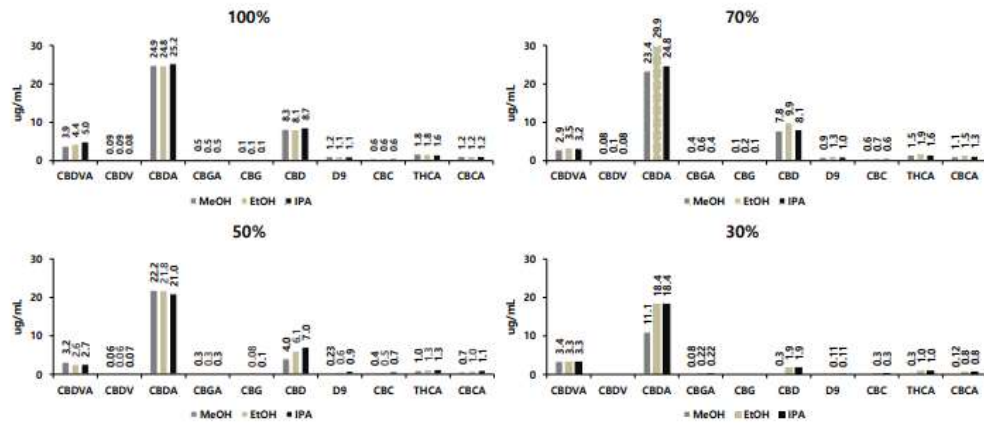


용매에 따른 추출비교

- 유기용매 비율에 따라 점차 추출물이 떨어지는 것을 볼 수 있음.

※ 에탄올 70%에서 추출이 많이 될 수 있는 것을 보았지만 데이터 오류로 판단 되어 추가 실험 예정에 있음.



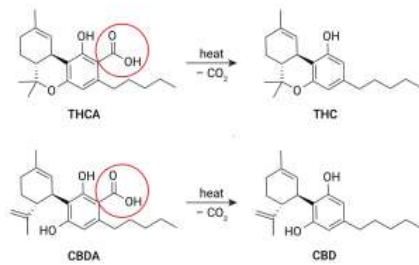


- 유기용매 30%일 때 추출율이 떨어짐.
- ※ 유기용매 비율이 100% 일 때 추출율이 제일 좋은 것을 알 수 있었음

결론

- 14종 칸나비노이드에 대한 동시분석법 개발 및 검증.
 - > 다양한 조건을 테스트 하여 분석법 최적화.
 - > 가이드라인 항목 중 특이성, 직선성, 정확성, 정밀성, 안정성, 회수율 등을 평가하여 분석법 검증.
 - > 검증된 분석법을 적용하여 대마 함량 분석을 통해 14종 물질에 대한 품질관리.
- 대마 시료를 용매에 따른 추출비교.
 - > 용매에 따른 추출비교를 통해 추출율이 좋은 용매, 비율을 찾고있음

추가 실험 계획



추가 추출 실험 계획

- 시료대비 용매 비율, 추출시간 테스트 예정
-> 10mg/2mL, 20mg/mL 등 시료대비 용매 비율 실험 예정
-> 20, 30, 60, 90 (min) 등 추출시간 실험 예정
- 추출 온도 변화에 따른 산성 칸나비노이드의 함량 변화 측정
-> 40 °C, 60 °C 온도 테스트

감사합니다

JEONBUK NATIONAL UNIVERSITY

2022.11.09.

우수연구자 초청 세미나

가천대 약대

이후근 교수님



Automated High-Throughput Sample Preparations In Clinical Proteomic Analysis

Hookeun Lee, Ph.D.
College of Pharmacy
Gachon University

**THE MAIN DIFFERENCE BETWEEN
GENOMICS AND PROTEOMICS:
THE SAME GENOME**

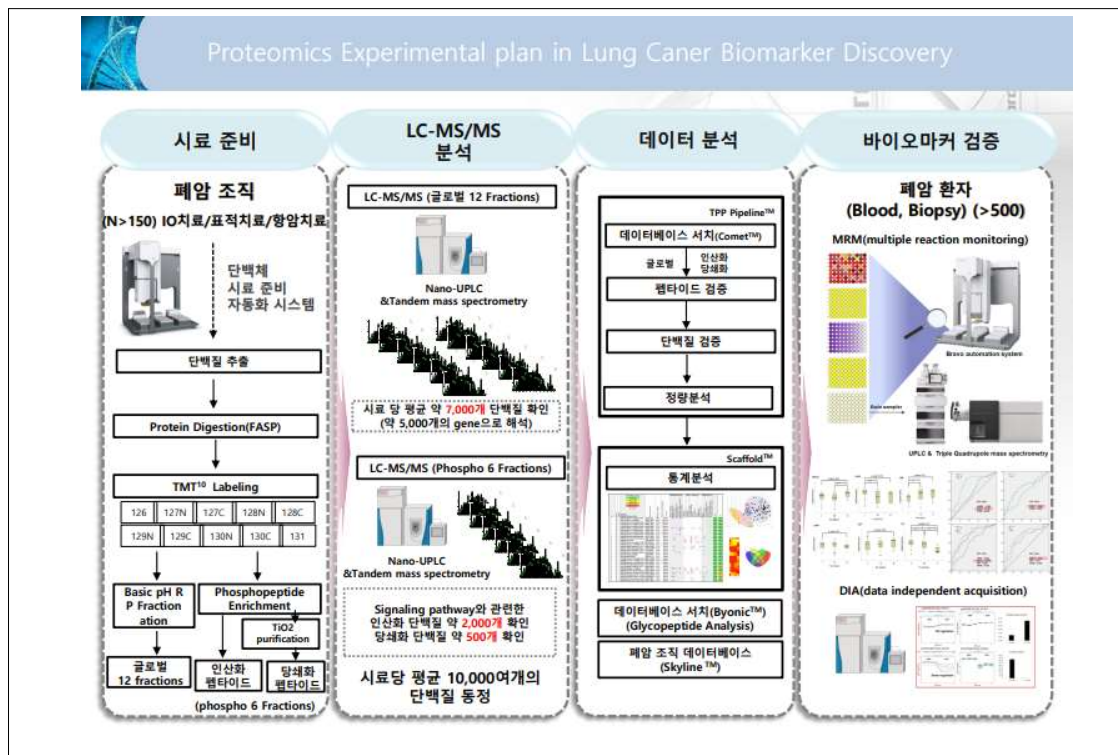
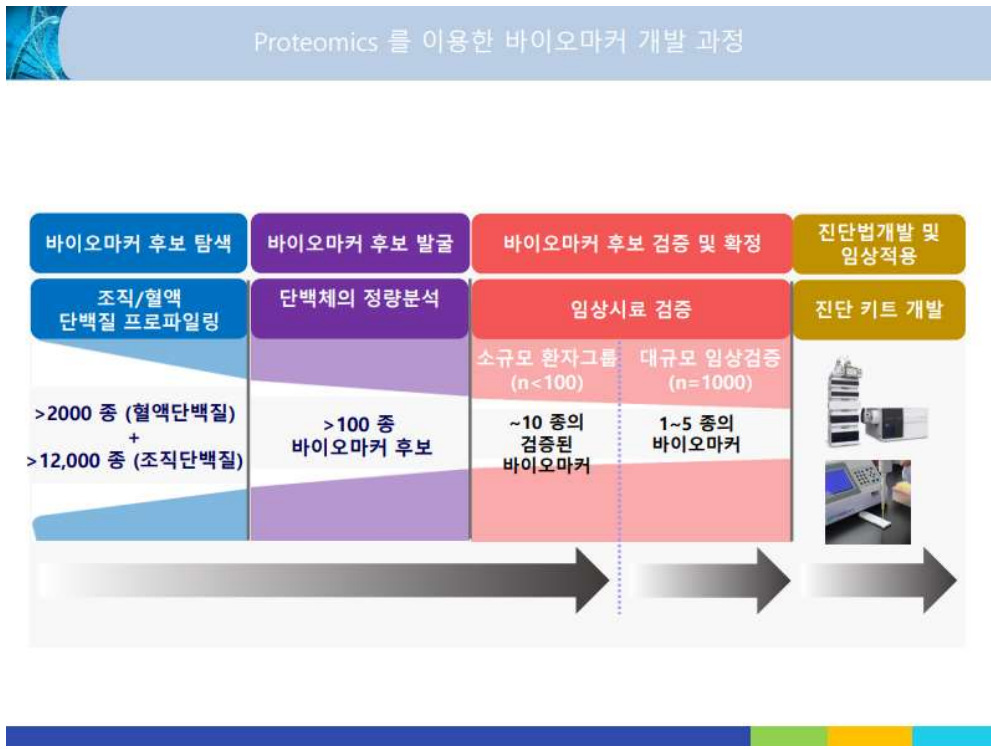


CATERPILLAR



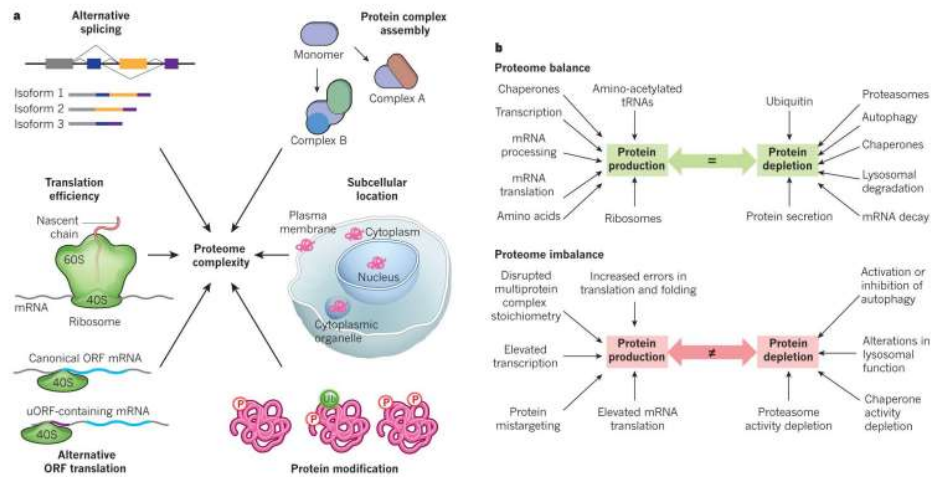
BUTTERFLY

THE DIFFERENT PROTEOME





PROTEIN COMPLEXITY IN A CELL

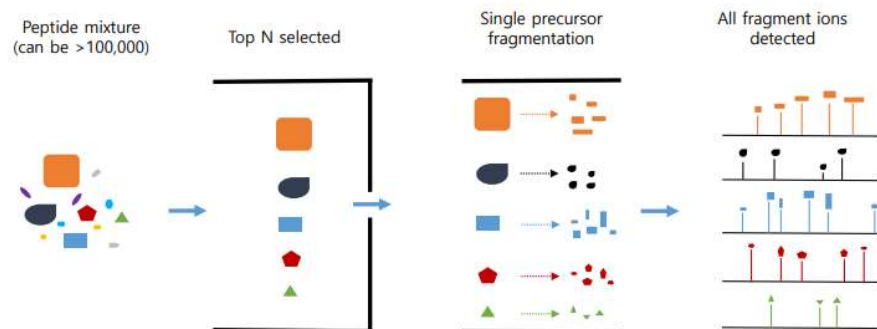


Harper and Bennett, Nature, 2016 (10.1038/nature19947)



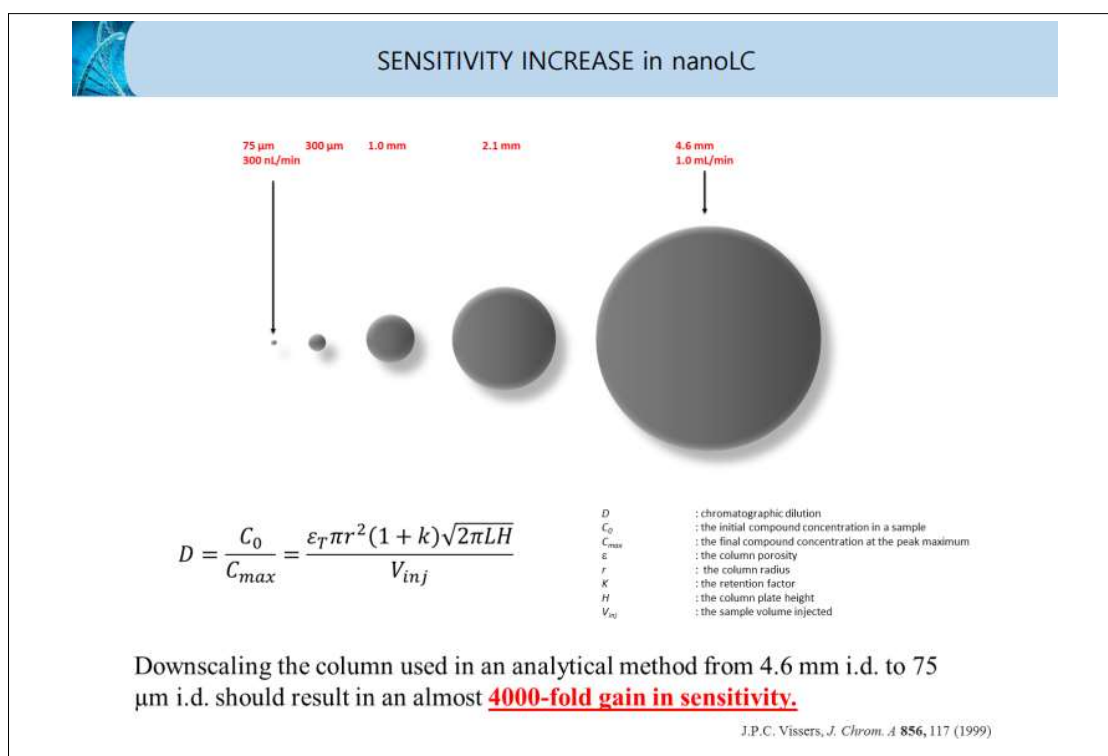
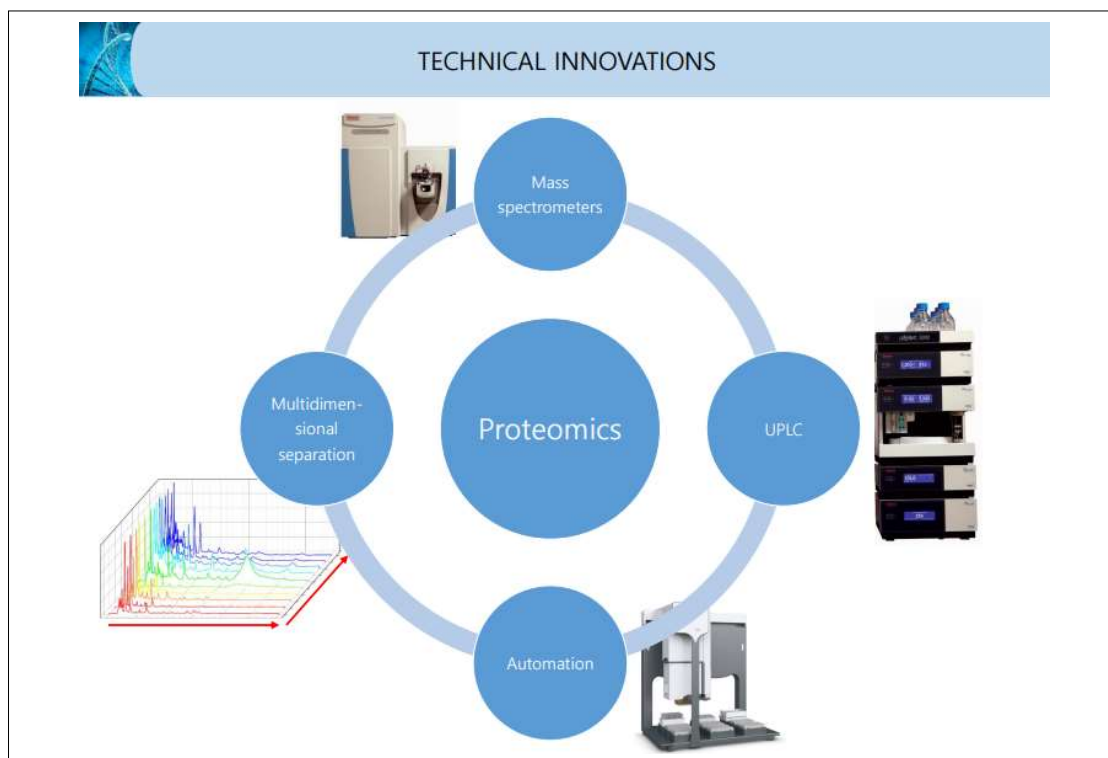
UNDERSAMPLING IN DDA MODE

Data-dependent acquisition (DDA) mode



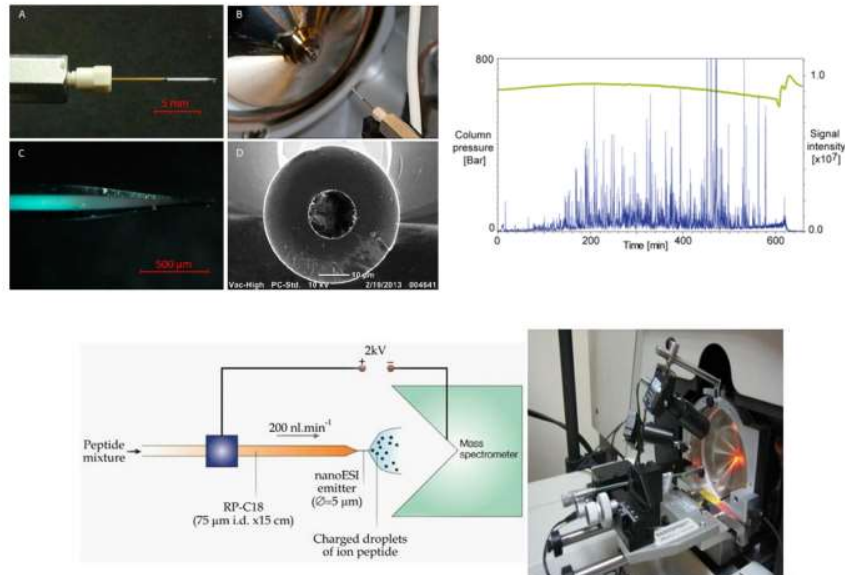
Undersampling: DDA proteomics unable to identify all peptides due to a large dynamic range of peptides. Only top N peptides are selected.



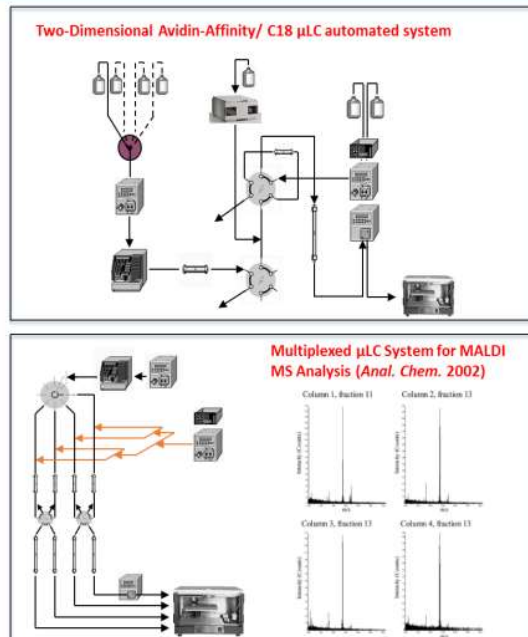
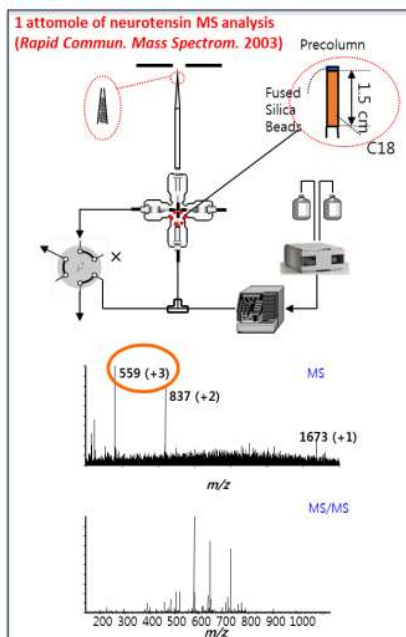




Microcapillary (Nano) LC column and Mass Spectrometer



DEVELOPMENTS OF nanoLCs FOR PROTEOMIC ANALYSIS





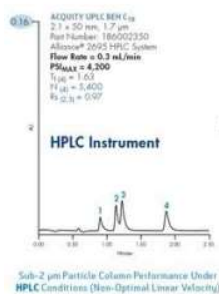
NANO-UPLC

HPLC

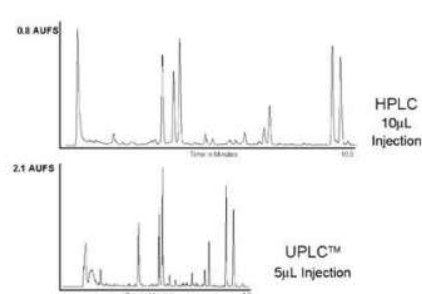
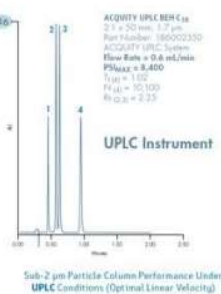
Pressure < 6000 psi
Particle size 3-5 μm
Column inner diameter 3-10 mm

UPLC

Pressure $\uparrow$ (15,000 psi)
Particle size $\downarrow$ (<2 μm)
Column inner diameter $\downarrow$ (0.75-1.8 mm)
Run time $\downarrow$
Sensitivity $\uparrow$
Sample throughput $\uparrow$



Same Column



https://www.waters.com/waters/ko_KR/Improving-Productivity-with-UPLC-Technology/nav.htm?locale=ko_KR&cid=134804757

<https://www.americanlaboratory.com/914-Application-Notes/19157-New-Frontiers-in-Chromatography/>



DEVELOPMENTS OF NOVEL MASS SPECTROMETERS

ThermoFisher Scientific

2003

2005

2006

2011



Finnigan™ LTQ FT

- Mass accuracy: 2 ppm
- Resolution: 100,000 at m/z 400
- Scan speed: 1s/scan

LTQ Orbitrap

Orbitrap Elite

Q Exactive series



	Q Exactive Focus	Q Exactive	Q Exactive Plus	Q Exactive HF-X	Q Exactive HF	Q Exactive UHMR
Resolution	70,000	140,000	140,000 ~280,000	240,000 at m/z 200	240,000 at m/z 200	200,000 at m/z 400
Mass range	50-3,000	50-8,000				350-80,000
Scan speed		12 Hz		40 Hz	18 Hz	12 Hz
Multiplexing	3	10		20		NA
Scan function	SIM	SIM, PRM		FS, AIF, SIM, PRM, DIA, ddHCD		FS, AIF, MS/MS, PseudoMS ³

Finnigan™ LTQ FT Ultra

- Mass accuracy: 1.2 ppm



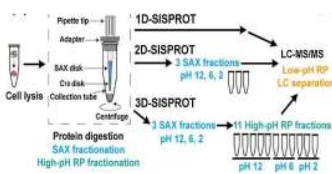
MULTIDIMENSIONAL SEPARATION

MD separation: combination of different separation strategies to increase in-depth proteome analysis

MD separation

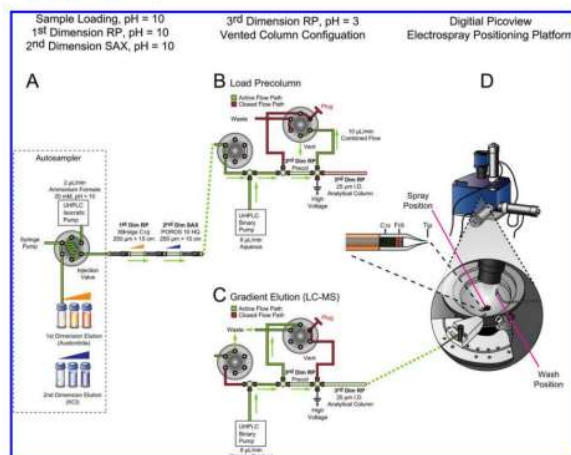
- Online system
- Offline system
- Combined offline-online system

3D-SISPROT spin tip allows 3D peptide fractionation



Chen et al., J. Chromatogr. A, 2017 (10.1016/j.chroma.2017.01.033)

An online 3D-LC system



Zhou et al., Anal. Chem., 2011 (10.1021/ac200639v)



AUTOMATION IN HIGH-THROUGHPUT SAMPLING

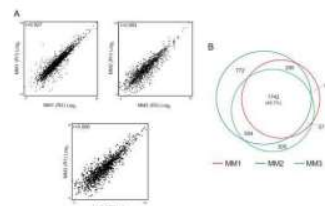
Automate affinity purification, digestion, cleanup, phosphopeptide enrichment in one system



Agilent AssayMAP
Bravo Platform



Increase recovery rate and reproducibility



Murillo et al., Plos One, 2018 (10.1371/journal.pone.0208562)

Handle up to 96 samples simultaneously

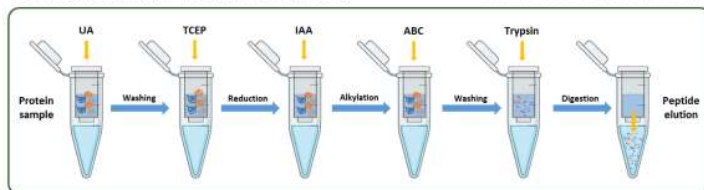


Reduce handling time

Protocol

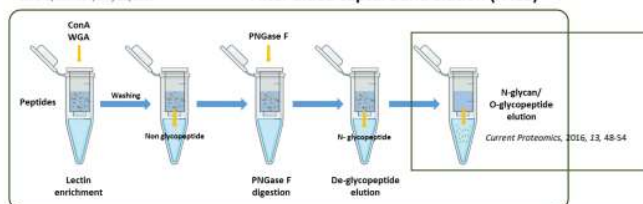
Filter aided sample preparation (FASP) NATURE METHODS | VOL.6 NO.5 | MAY 2009

NATURE METHODS | VOL.6 NO.5 | MAY 2009



Cell 141, 897–907, May 28, 2010

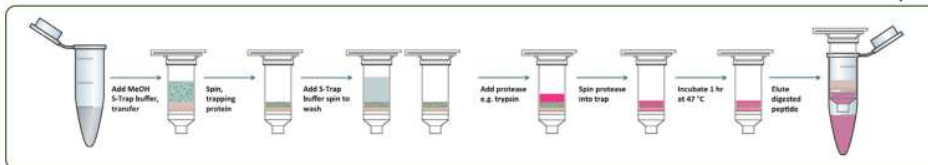
Filter aided capture and elution (FACE)



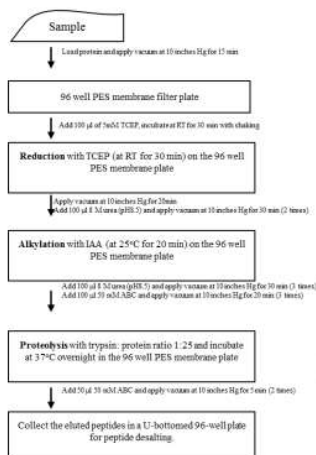
Current Proteomics, 2016, 13, 48-54

S-Trap™ midi spin columns protocol

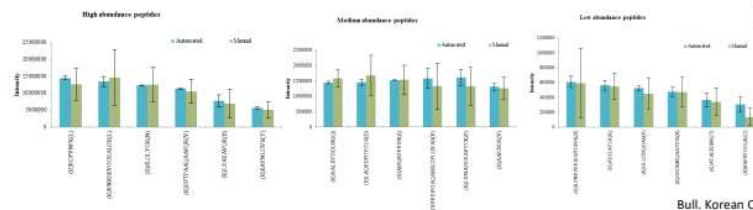
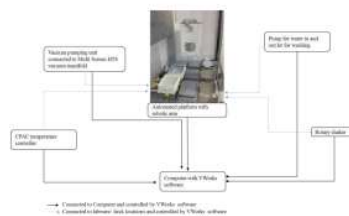
S-trap



FASP (Filter aided sample preparation) Automation

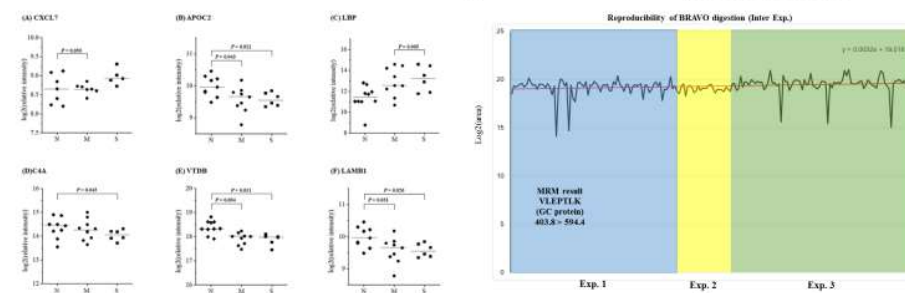
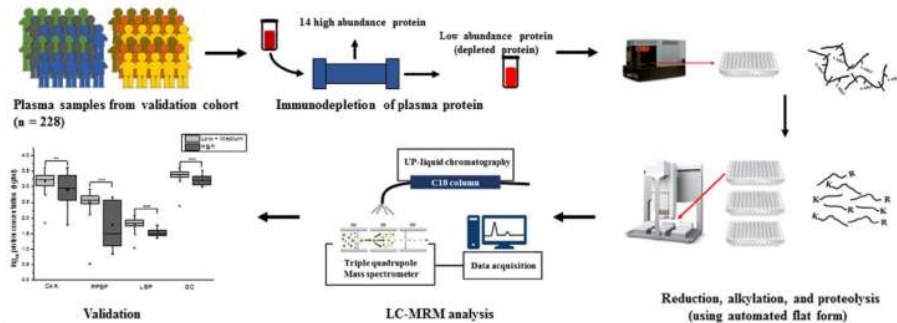


Bravo Automation System



Bull. Korean Chem. Soc. 2015, Vol. 36, 1791–1798

FASP (Filter aided sample preparation) Automation -Application



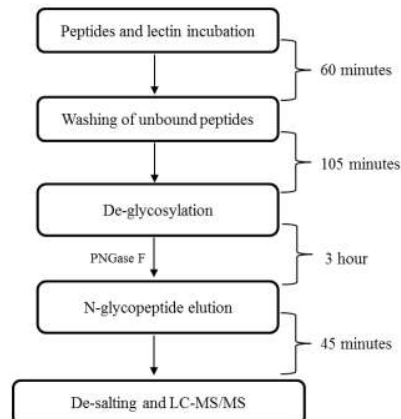
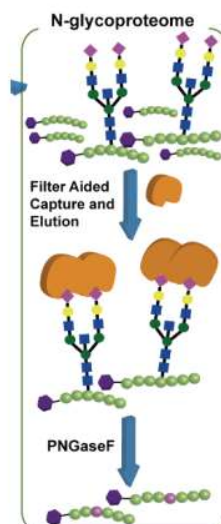
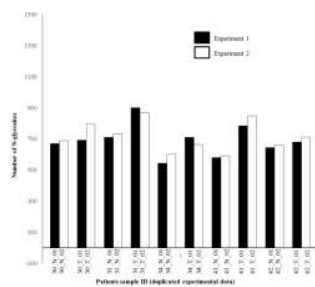
Patent 10-2016-0130041

BMJ Open Diab Res Care 2020;8:e001152

FACE(filter aided capture & elution) Automation



* Automated Liquid Handling Platform

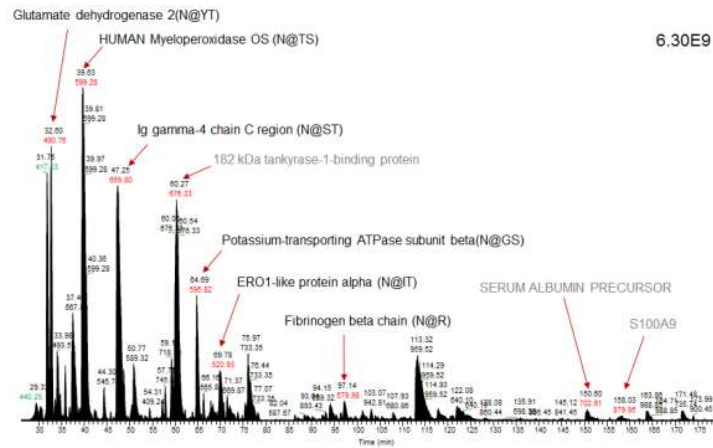


* Automated sample preparation protocol for high-throughput N-glycopeptide enrichment

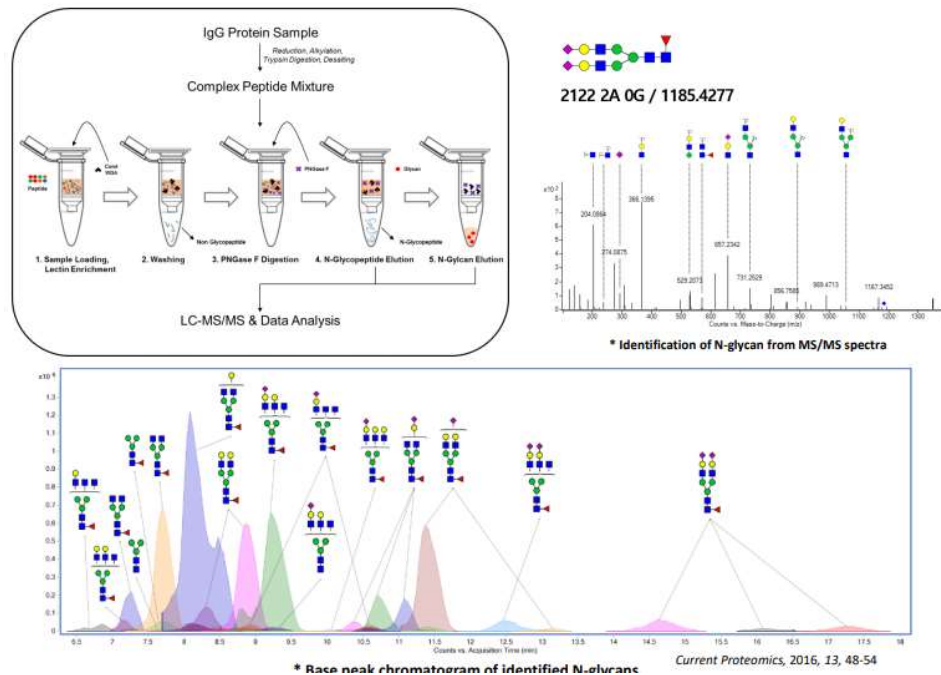
Current Proteomics, 2016, 13, 55-60

Scientific Report 5, 18189, 2015

LC-MS/MS analysis of N-linked glycoproteome



N-Glycan Capturing Method Automation

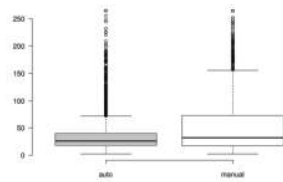
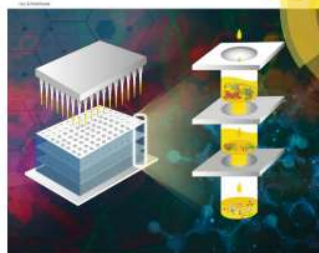


MS/MS Spectra of Captured N-Glycans

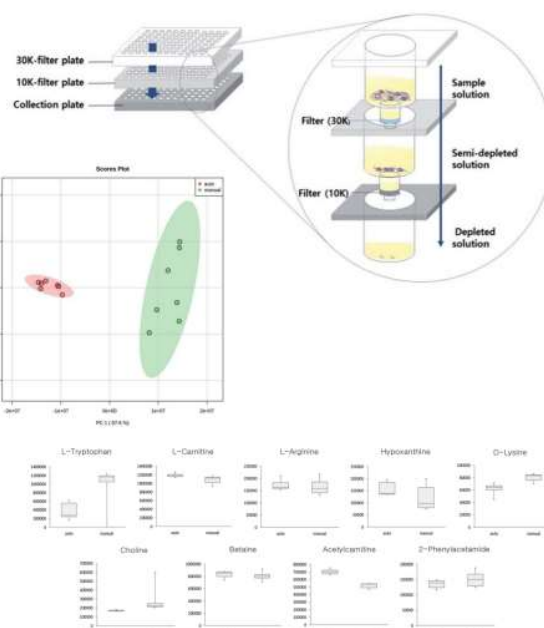


Double-Filtration for Serum Metabolite LC-MS Analysis

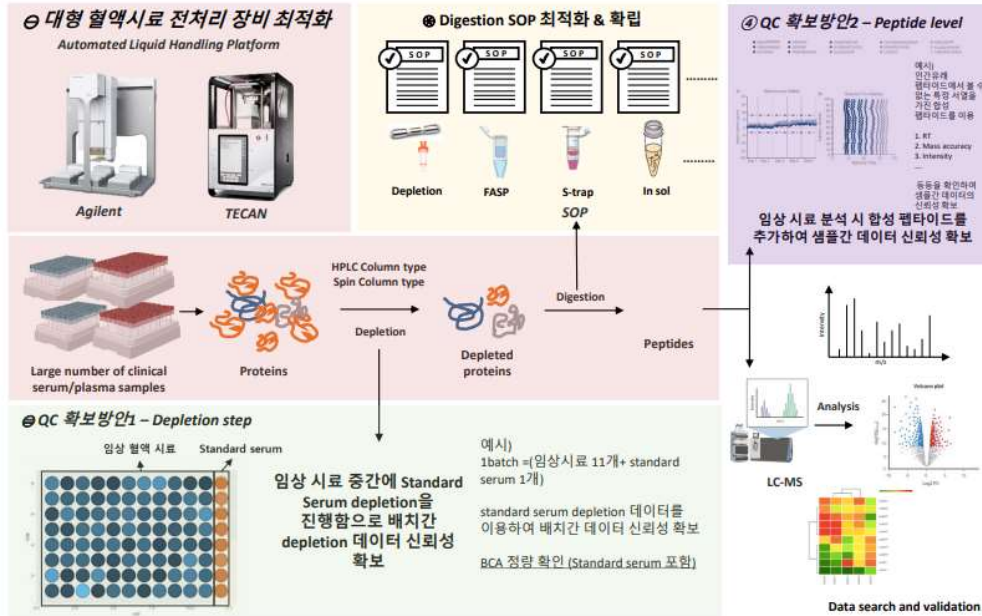
Analytical Methods



Anal. Methods, 2019, 11, 4060–4065



High-throughput Platforms in Proteomic Analysis (S-trap, FASP, In-sol Digestion)



Quantitative proteomic analysis using ICAT reagent

Proteomic analysis of Interferon response in human liver cells

Abstract

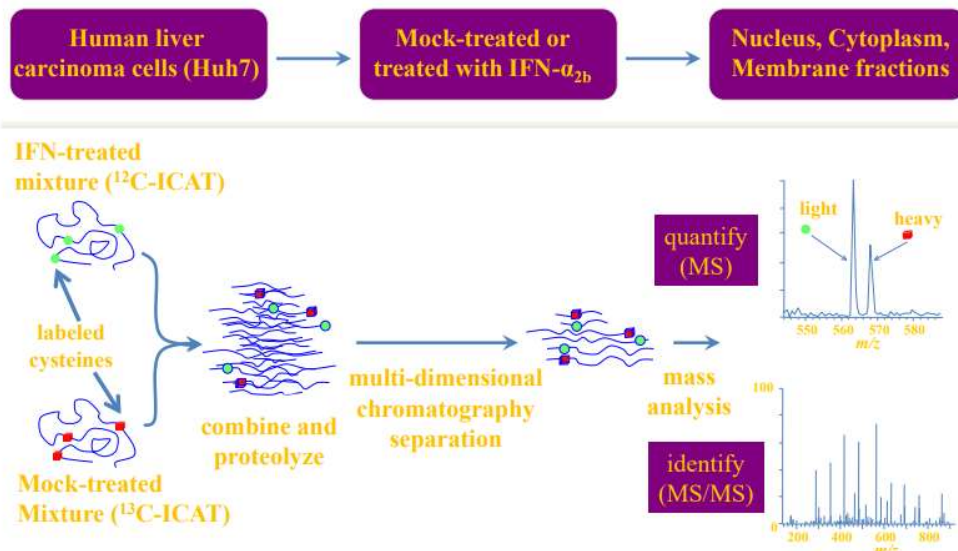
Background: Interferons (IFNs) play a critical role in the host antiviral defense and are an essential component of current therapies against hepatitis C virus (HCV), a major cause of liver disease worldwide. To examine liver-specific responses to IFN and begin to elucidate the mechanisms of IFN inhibition of virus replication, we performed a global quantitative proteomic analysis in a human hepatoma cell line (Huh7) in the presence and absence of IFN treatment using the isotope-coded affinity tag (ICAT) method and tandem mass spectrometry (MS/MS).

Results: In three subcellular fractions from the Huh7 cells treated with IFN (400 IU/ml, 16 h) or mock-treated, we identified more than 1,364 proteins at a threshold that corresponds to less than 5% false-positive error rate. Among these, 54 were induced by IFN and 24 were repressed by more than two-fold, respectively. These IFN-regulated proteins represented multiple cellular functions including antiviral defense, immune response, cell metabolism, signal transduction, cell growth and cellular organization. To analyze this proteomics dataset, we utilized several systems-biology data-mining tools, including Gene Ontology via the GoMiner program and the Cytoscape bioinformatics platform.

Conclusions: Integration of the quantitative proteomics with global protein interaction data using the Cytoscape platform led to the identification of several novel and liver-specific key regulatory components of the IFN response, which may be important in regulating the interplay between HCV, interferon and the host response to virus infection.

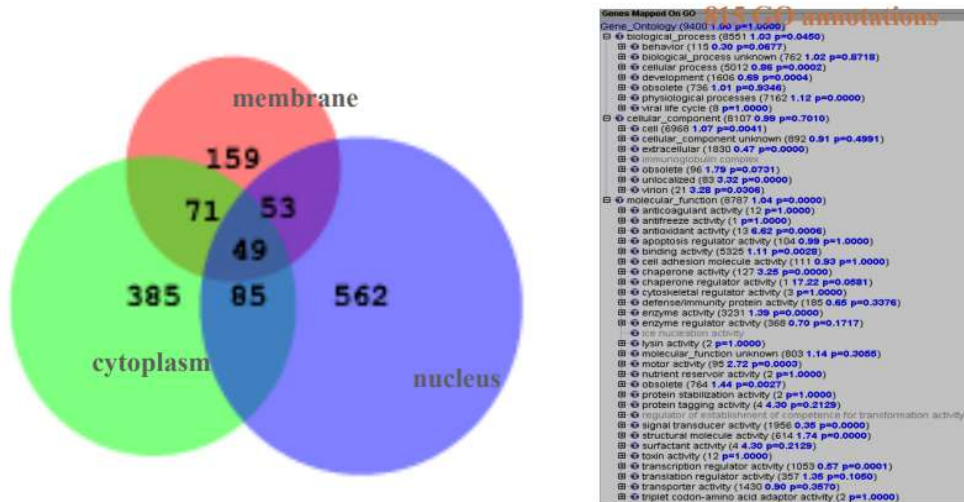
Yan, W. et al, Genome Biology 2004, 5, r54

ICAT quantitative analysis



Protein identification of the Huh7 cells

Total 1364 proteins at $P \geq 0.4$ (95 % confidence level) 815 GO annotations



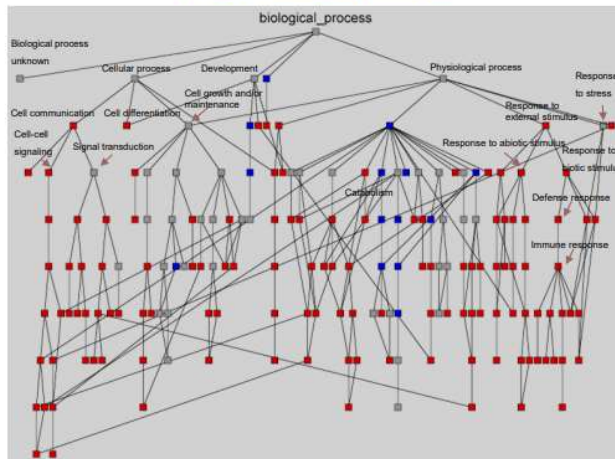
IFN regulated Proteins

- 54 IFN-induced proteins (> 2-fold abundance change)
 - 15 previously reported proteins
 - 39 novel IFN-induced proteins
- 24 IFN-repressed proteins
 - have not been studied thoroughly in the field

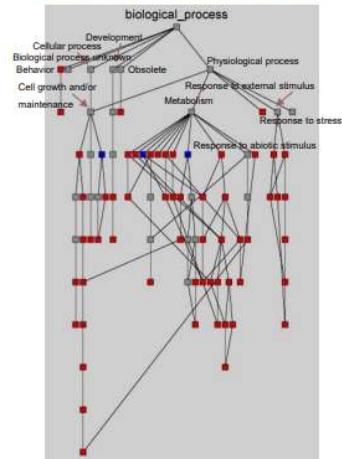
Distribution of IFN-regulated proteins

generated by GoMiner Program (Zeeberg, B. R. et al, *Genome Biol.* 2003, 4, R28.)

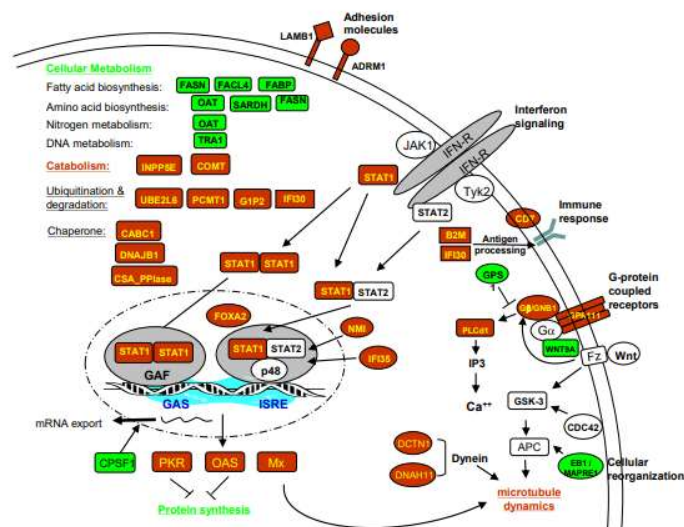
54 IFN-induced proteins



24 IFN-repressed proteins



Summary of Cellular Pathways of IFN-regulated proteins



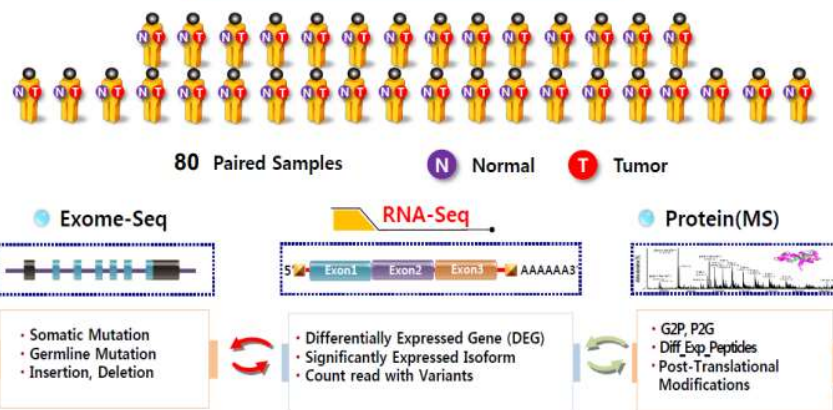


Proteogenomic analysis of gastric cancer tissue



Proteogenomic Analysis

Early-Onset Diffuse Gastric Cancer (EOGC)



Mun et al., *Cancer Cell* (2019)



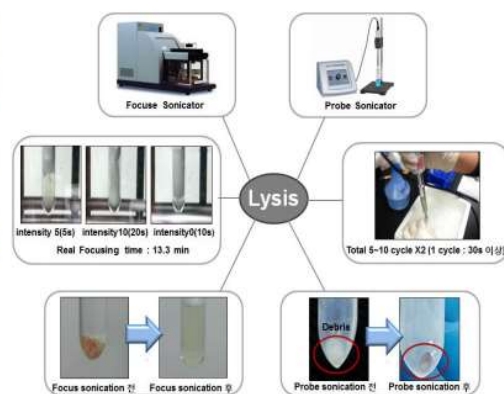
Sample – 76 normal & cancer tissue



Sample preparation – Tissue pulverization & Protein lysis



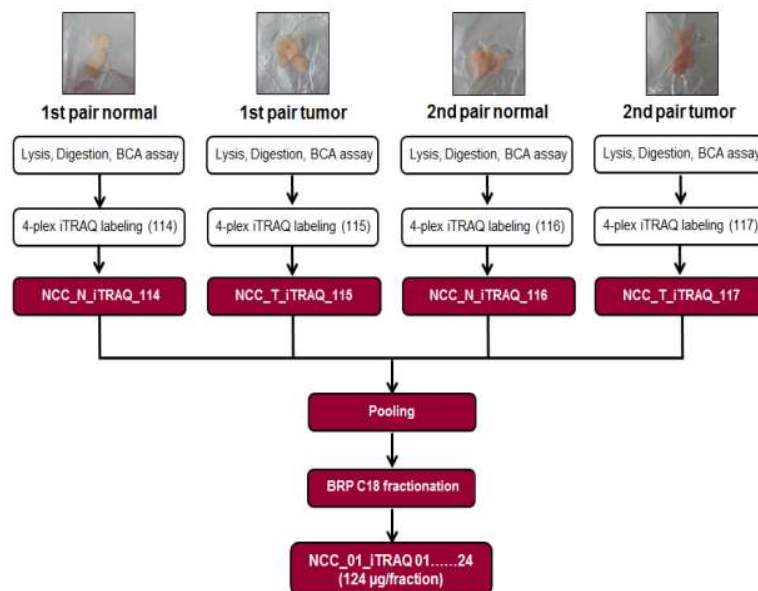
Tissue pulverization



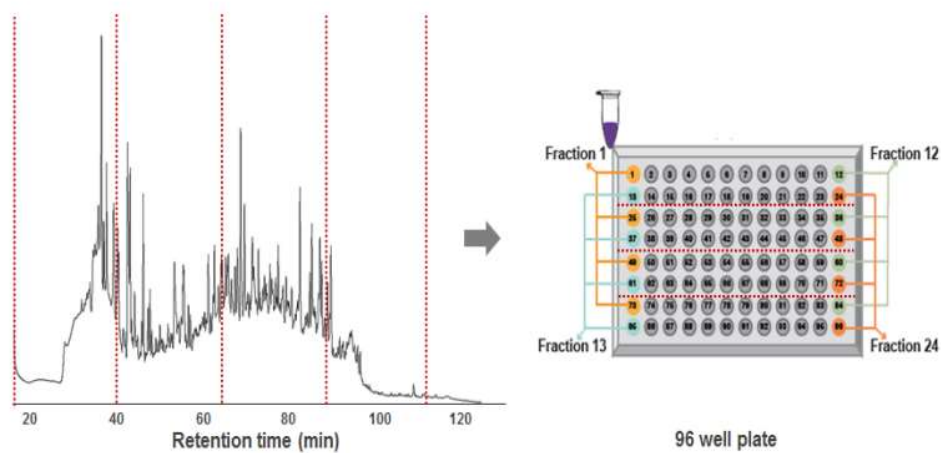
Protein lysis



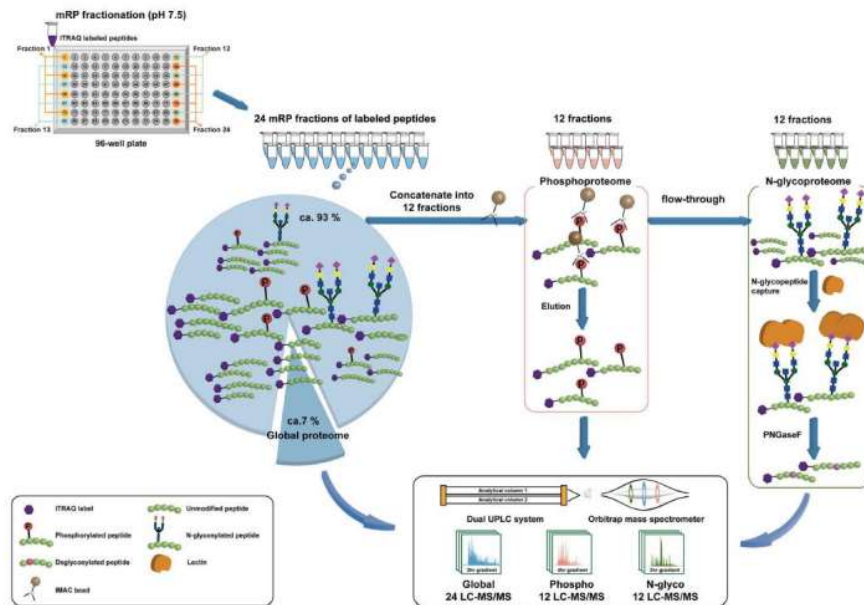
Method - Global proteome



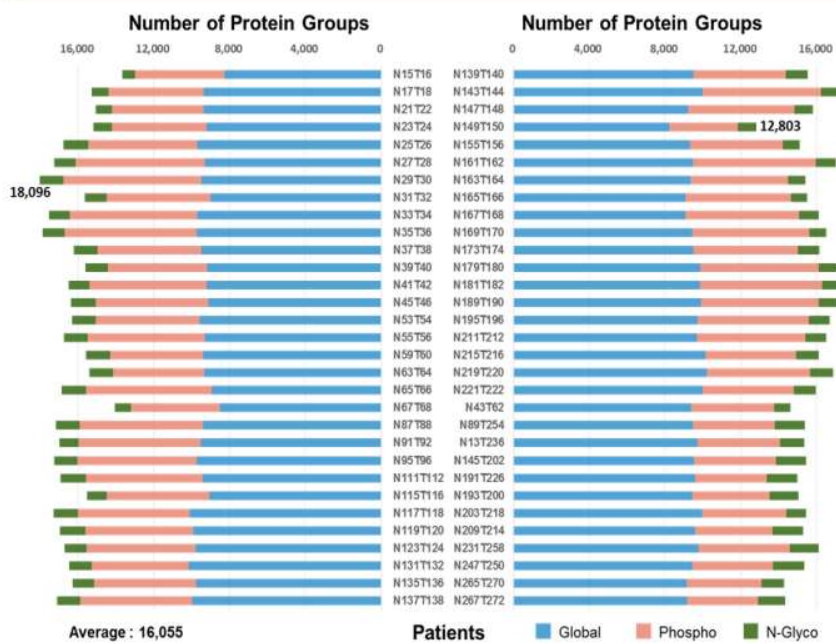
Method - Global proteome (Basic pH RP fractionation)



Method - Overall workflow in the SEPG method for integrated profiling

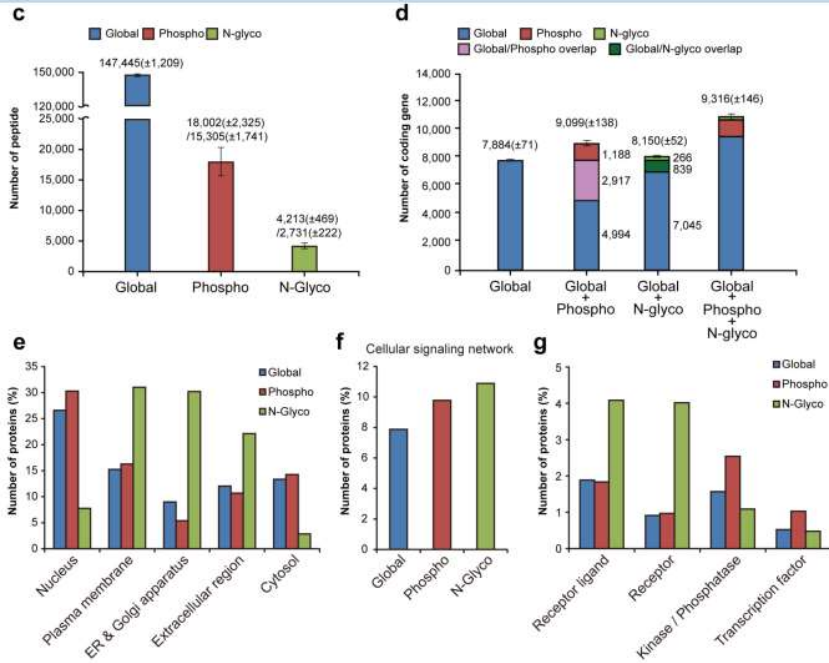


Result - Protein Identification of whole proteome

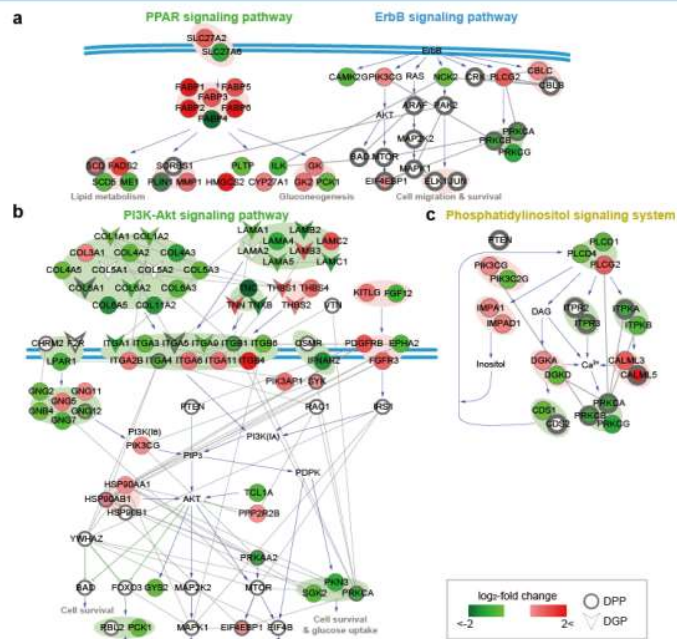




Integrated analysis of global proteome, phosphoproteome, and glycoproteome



Integrated analysis of global proteome, phosphoproteome, and glycoproteome



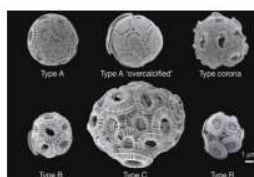


Proteomic Profiling of *Emiliania huxleyi* Using a Three-Dimensional Separation Method



Emiliania huxleyi

- A member of the coccolithophores group (calcareous alga)
- Unicellular marine phytoplankton with unique morphology
- Uptake CO₂ by photosynthesis and calcification to form coccoliths
- Play an essential role in global carbon and sulfur cycling, and contribute to marine cloud formation and climate regulation
- Widely distributed throughout the oceans
- Genome sequence database constructed in 2013 (30,569 protein-coding genes)
- Studied in various fields (medicine, material science, biogeography, geology, ecophysiology, and paleo climatology)
- Lack of proteomics data



Read et al. *Nature* 499 (2013)



<https://www.nsf.gov/>

Journal of Microbiology, Biotechnology, Vol. 24, No. 1, 2014
doi:10.1007/s10291-013-0300-0
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zDNA Microarray as a Tool for Identification of Biomimetic Proteins in the Coccolithophore *Emiliania huxleyi* (Haptophyta)

Fahad Qasbi¹, Robert M. Brown², Xianli Zhang¹, Thomas M. Kallunki¹,
Michael A. Finkel¹, David Chavira¹, and Remy A. Rose¹

¹Department of Biological Sciences and ²Department of Computer Science,
California State University-Fullerton, Fullerton, CA 92681-0001, USA

Received 12 January 2014; accepted 12 February 2014

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Light-Dependent Transcriptional Regulation of Genes of Biogeochemical Interest in the Diploid and Haploid Life Cycle Stages of *Emiliania huxleyi*

Yuping Wu¹, Xianli Zhang¹, and Remy A. Rose¹

¹Department of Biological Sciences, California State University-Fullerton, Fullerton, CA 92681-0001, USA

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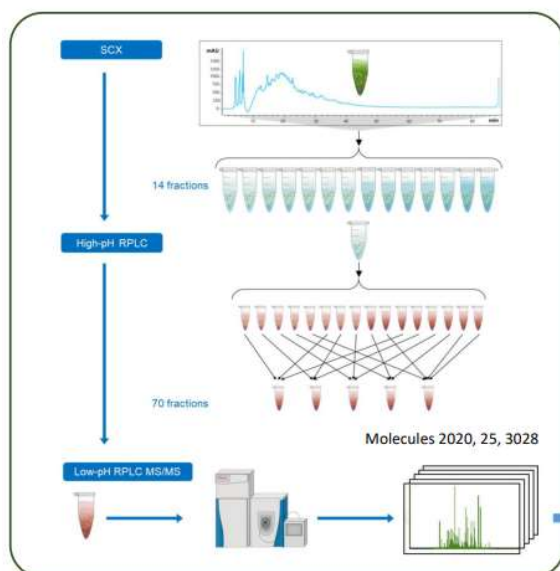
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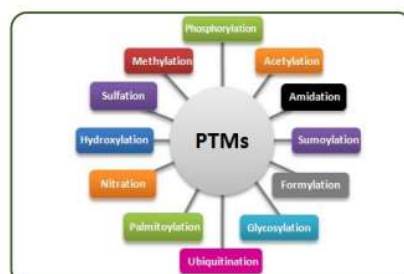
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Proteomic Profiling of *Emiliania huxleyi* (3D, PTM)

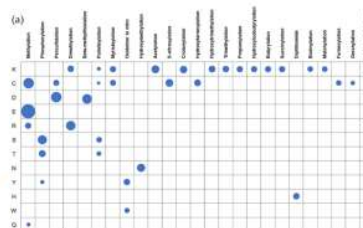
3D-liquid chromatography (LC)



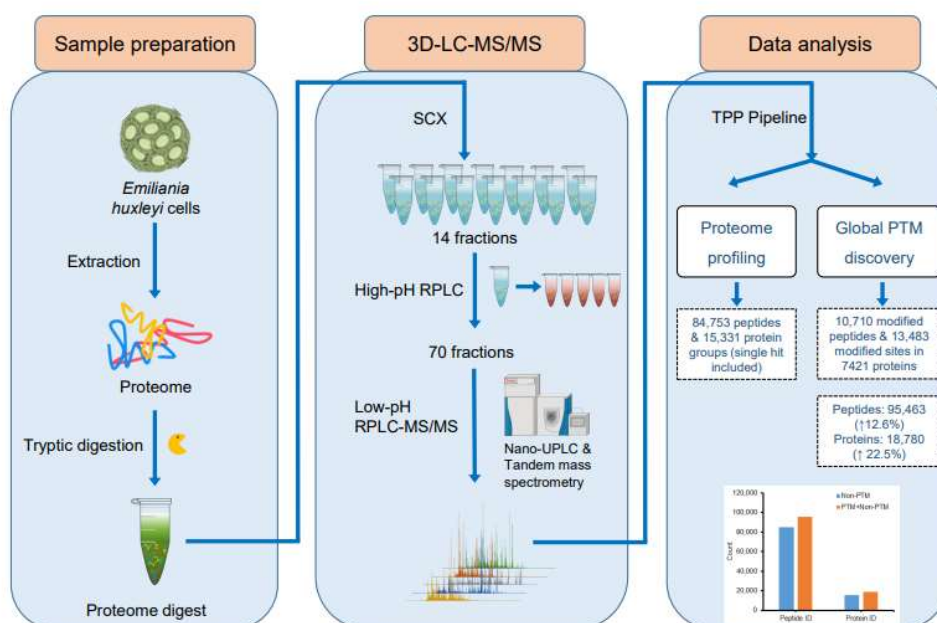
Post translational modification (PTM)



Molecules 2021, 26, 2027



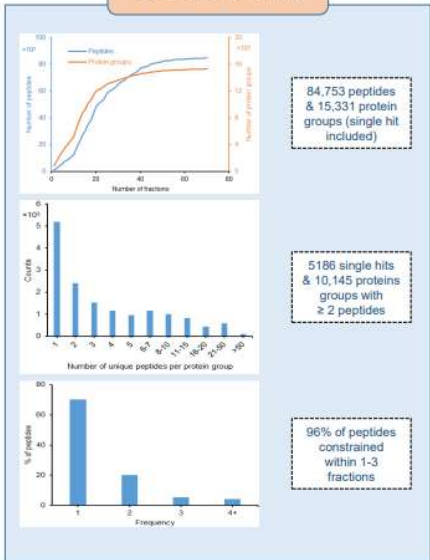
3D-LC WORKFLOW FOR PROTEOMIC PROFILING AND PTM DISCOVERY



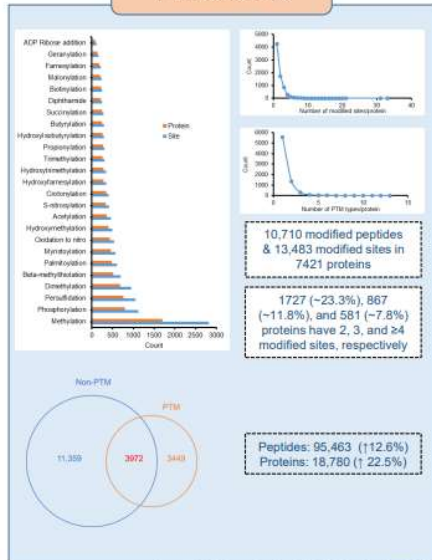
Lee et al., *Molecules*, 2020 (10.3390/molecules25133028)
Lee et al., *Molecules*, 2021 (10.3390/molecules26072027)

POWER OF 3D-LC IN PROTEOMIC PROFILING AND PTM DISCOVERY

Proteome profiling



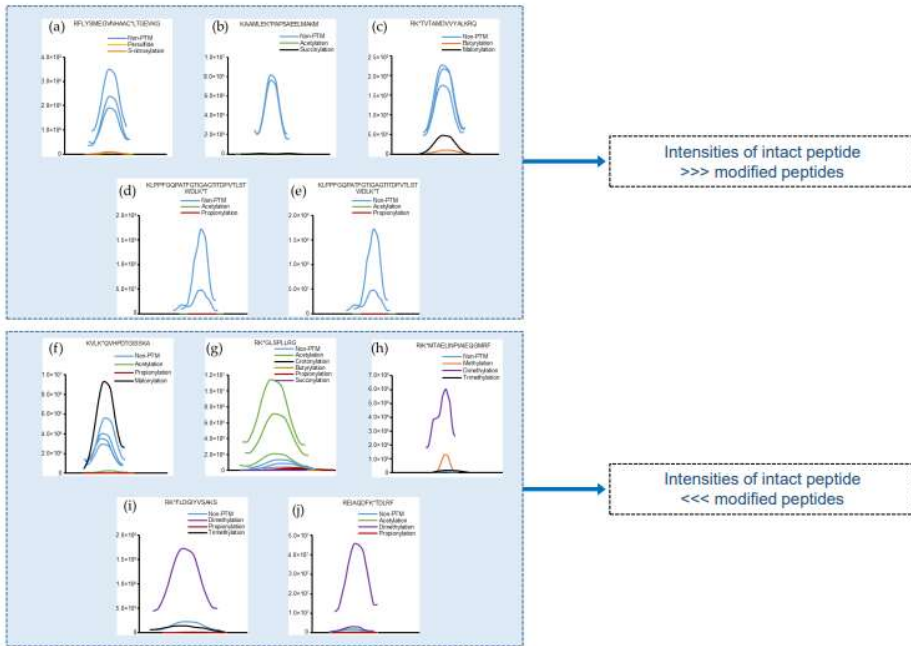
PTM discovery



Lee et al., *Molecules*, 2020, 10.3390/molecules25133028

Lee et al., *Molecules*, 2021, 10.3390/molecules26072027

EFFECTS OF PTM SEARCH ON QUANTITATIVE ANALYSIS



Lee et. al., Molecules, 2021 (10.3390/molecules26072027)

תודה
Dankie Gracias
Спасибо
Köszönjük
Grazie Dziękujemy
Ďakujeme Vielen Dank Paldies
Kiitos Tänname teid 谢谢
Thank You Tak
感謝您 Obrigado Teşekkür Ederiz
Σας Ευχαριστούμ 감사합니다
Bedankt Děkujeme vám
ありがとうございます
Tack

2022.11.16.

생화학 전공
대학원생 발표 세미나

조혜진 학생

Role of hepatic FPN in systemic energy metabolism

202155105 약학과 조혜진(박사과정)

*School of Pharmacy,
Jeonbuk National University*

Metabolic syndrome

Metabolic Syndrome

defined as 3 or more of the following

- Abdominal obesity



- Elevated plasma triglycerides



- Low plasma HDL cholesterol



- Elevated blood pressure



- Elevated fasting blood glucose

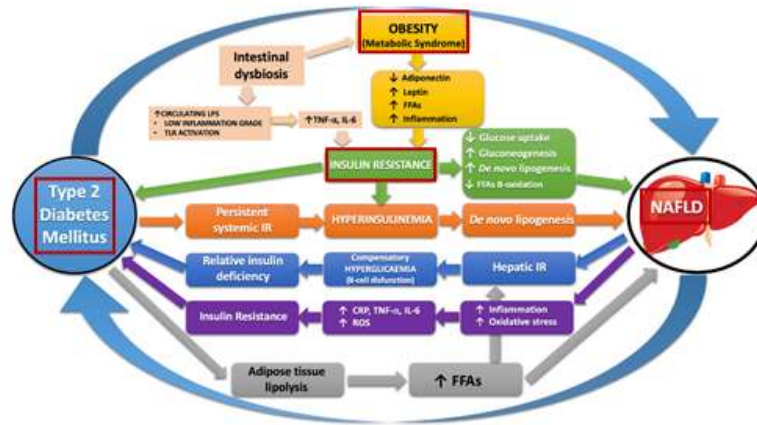


Diseases Associated with Metabolic Syndrome

- Type 2 Diabetes
- Hypertension
- Non-alcoholic Fatty Liver Disease (NAFLD)
- Cardiovascular Events

(Felix F. Lillich et al., *Front. Pharmacol.*, 2021)

Pathophysiological mechanisms of NAFLD and T2DM



(Carlo Acerno et al., *Exploration of Medicine*, 2020)

Iron accumulation in NAFLD and metabolic syndrome

The dysmetabolic *iron overload* syndrome(DIOS) is now a frequent finding in the general population, as is detected in about 1/3 of patients with nonalcoholic fatty liver disease (NAFLD) and the metabolic syndrome.

Review

Iron in fatty liver and in the metabolic syndrome: A promising therapeutic target

Paola Dongiovanni, Anna Ludovica Fracanzani, Silvia Fargion, Luca Valentini*

Department of Internal Medicine, Centro Medico Mondeluzio del Fegato, Università degli Studi di Milano, and Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinica, Milan, Italy

Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women

Chul-Hee Kim¹, Hong-Kyu Kim^{2,*}, Sung Jin Bae³, Joong-Yeol Park⁴, Ki-Jup Lee⁵

¹Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul National University Medical Research Center, Seoul 151-747, South Korea; ²Seoul National University, Seoul 151-747, South Korea; ³Seoul National University, Seoul 151-747, South Korea; ⁴Seoul National University, Seoul 151-747, South Korea; ⁵Seoul National University, Seoul 151-747, South Korea

➡ Iron overload ? ➡ Metabolic syndrome

Prevalence of Body Iron Excess in the Metabolic Syndrome

Corrado Bazzani, et al.

European Journal of Endocrinology (2005) 154 333-345

Association between serum ferritin and the insulin resistance syndrome in a representative population

C. E. Winkle, B. Huettner, L. C. Bollerstein, J. Schömerich, K. D. Polzella¹ and C. Hübner

Department of Internal Medicine I, University of Regensburg, 93042 Regensburg, Germany and ²Department of Internal Medicine III, Hospital Tübingen, Tübingen, Germany

LIVER, PANCREAS, AND BILIARY TRACT

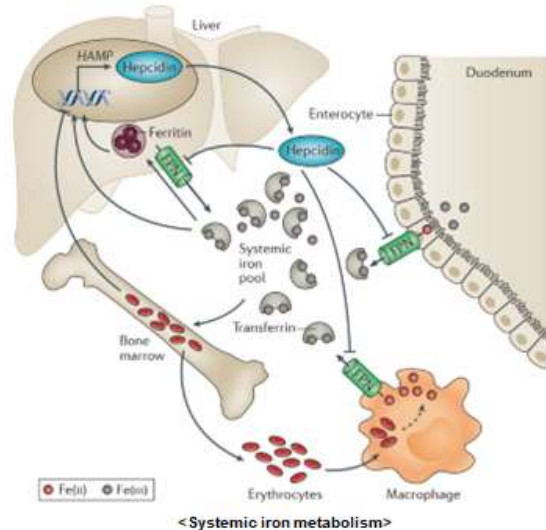
Insulin Resistance-Associated Hepatic Iron Overload

MOHAMED EL-SAYED, et al.

Insulin Sensitivity and Liver Fat: Role of Iron Load

Michael Haug, et al.

Iron metabolism



(Bart J. Crielard et al., nature reviews drug discovery, 2017)

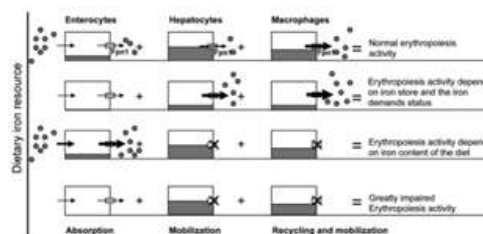
Ferroportin (FPN)

- The only known *iron exporter*.
- Transmembrane protein that *transports iron from the inside of a cell to the outside of the cell*.
- High levels of ferroportin are found in *enterocytes in the duodenum (to transport absorbed iron)*, in *hepatocytes (to transport stored iron)* and in *macrophages (to transport recycled iron)*, which together control systemic iron levels.

➡ Role of hepatic FPN in Systemic energy metabolism?

Ferroportin1 in Hepatocytes and Macrophages Is Required for the Efficient Mobilization of Body Iron Stores in Mice

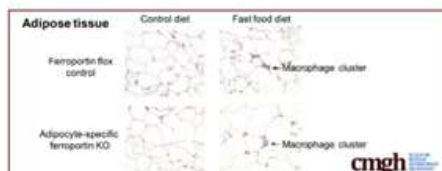
Zhaohua Zhang, Fan Zhang, Xie Gao, Peng An, Yusheng Tao, and Fuli Wang



ORIGINAL RESEARCH

Ferroportin Expression in Adipocytes Does Not Contribute to Iron Homeostasis or Metabolic Responses to a High Calorie Diet

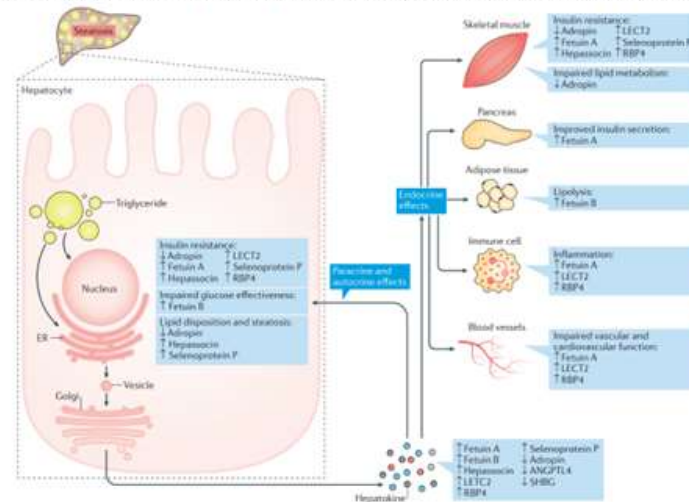
Laurence Bellon,^{1,2,3,4} Lesley Anne Jackson,^{1,2} Kim Briddle,^{1,2} Erica Secor,^{4,5} Daniel Wallace,^{1,2} Nehreen Santhipura,^{1,2} Janka Reiling,^{1,2,3} Gregory Miller,^{1,2} Salvatore Mangiacca,⁶ Sofianos Andrikopoulos,⁶ V. Nathan Subramanian,^{1,2,3} and Daniel O'Leary^{1,2,3}



➔ Mechanism by which hepatic FPN can communicate with other organs?

Hepatokine

- Proteins that are secreted by hepatocytes
- Nonalcoholic fatty liver disease, in particular, is associated with altered production of hepatokines



(R. Meex et al., *Nature Reviews Endocrinology*, 2017)

감사합니다

JEONBUK NATIONAL UNIVERSITY

2022.11.16.

**생화학 전공
대학원생 발표 세미나**

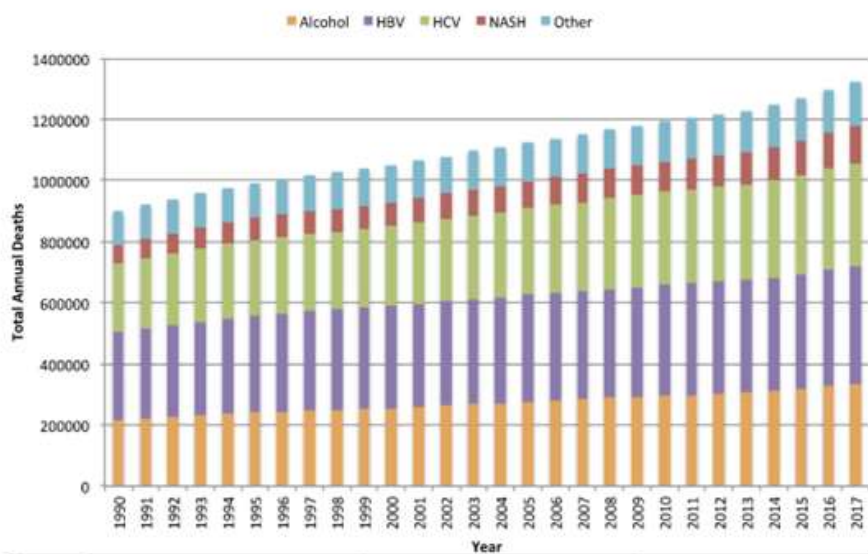
김길환 학생

Role of PAK4 in HSC activation and liver fibrosis

School of Pharmacy

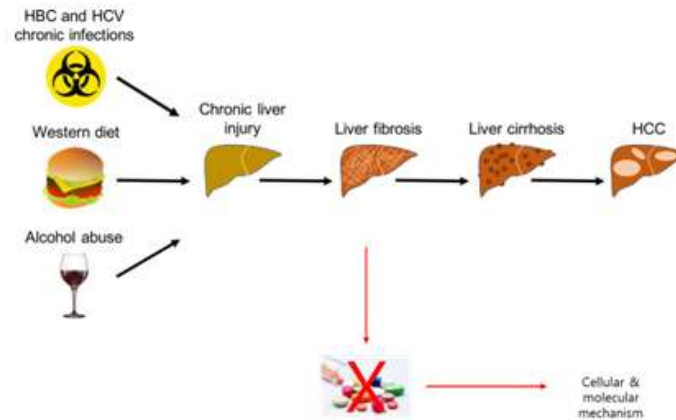
김 길환(석박통합)

Liver fibrosis patient in global



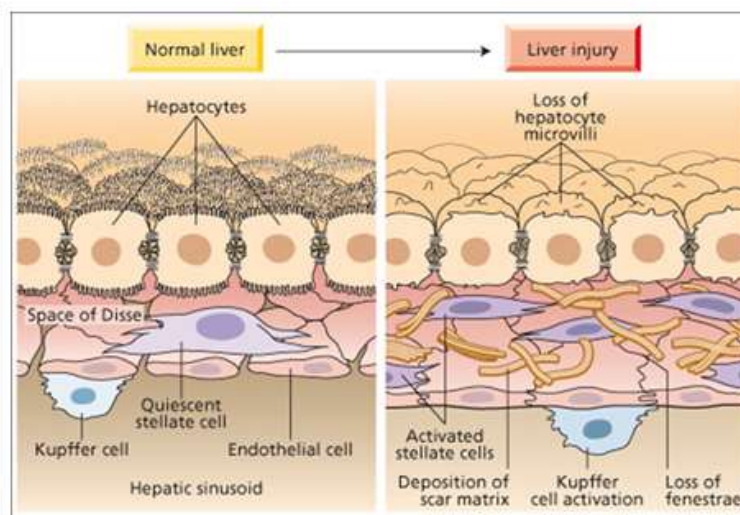
Shantanu Cheemerla ClinCal Liver Disease, vol 17, nO 5, MaY 2021

Liver disease



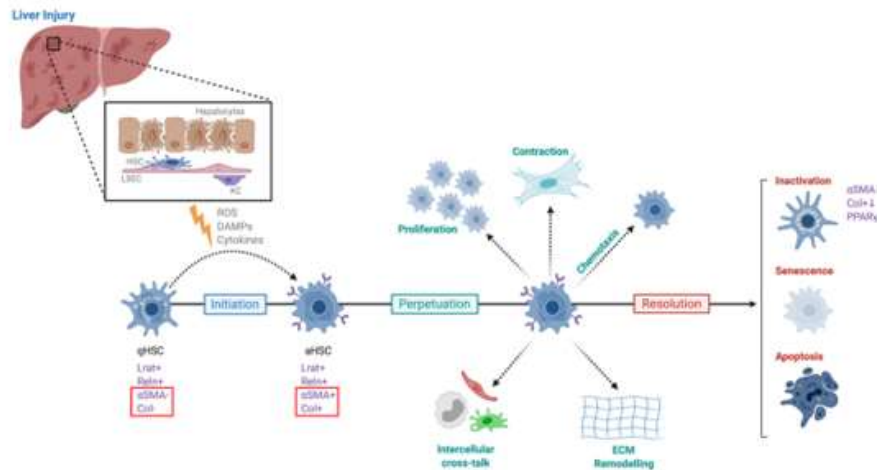
Fernando Bessone et al.
Cellular and Molecular Life Sciences Jan 2019

Role of hepatic stellate cell in liver fibrosis

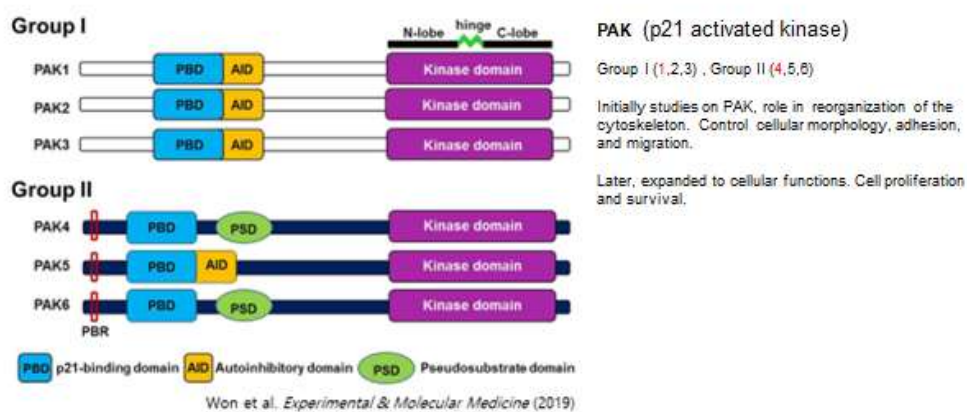


GASTROENTEROLOGY Jun 4, 2016

Activation of hepatic stellate cell



P21-Activate kinase family



Research for PAK4 in liver

Contents lists available at ScienceDirect

Cancer Letters

Journal homepage: www.elsevier.com/locate/canlet

Original Article

Zic2 promotes tumor growth and metastasis via PAK4 in hepatocellular carcinoma

Shi Xun Lu^{a,b,c}, Chen Zhen Zhang^{a,b,c}, Rong Zhen Luo^{a,b,c}, Chun Hua Wang^{a,b,c},
Li Li Liu^{a,b,c}, Ju Fu^{a,b,c}, Lingling Zhang^{a,b,c}, Huamin Wang^a, Dan Xie^{a,b,c}, Jing-Ping Yun^{a,b,c}

^a Key Lab of Laboratory Cancer Control, East Wu Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangdong Cancer Clinical Research Institute, Guangzhou, China

^b Department of Pathology, Southern Medical University, Guangzhou, China

^c Department of Pathology, Southern Medical University, Guangzhou, China

^d Department of Pathology, The University of Hong Kong, Hong Kong, China

^e Department of Pathology, The University of Hong Kong, Hong Kong, China

Biomedical and Biomedical Research Communications, 100 (2020) 3491–3500

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

Journal homepage: www.elsevier.com/locate/bbrc

Original Article

p21-activated kinase 4 as a switch between caspase-8 apoptosis and NF- κ B survival signals in response to TNF- α in hepatocarcinoma cells

Qing Li^a, Xiaomeng Zhang^a, Na Wu^a, Shouren Liu^a, Yamin Liu^{a,b,c}, Hao Wang^{a,b,c,d}

^a Institute of Hepatology, College of Basic Medical Sciences, Chongqing University, 400040, China

^b Department of Hepatology, Chongqing Medical University, Chongqing, 400016, China

^c Department of Hepatology, Chongqing Medical University, Chongqing, 400016, China

^d Key Laboratory of Chongqing, Chongqing Medical University, Chongqing, 400016, China

^e Department of Hepatology, Chongqing Medical University, Chongqing, 400016, China

Metastasis and Cancer Pathobiology

Cancer Research

PAK4 Phosphorylates p53 at Serine 215 to Promote Liver Cancer Metastasis

Hai Tao Xu^a, Wang-Ling Lu^a, Hong-Fu Liu^a, Lei-Lian Wang^a,
Xiao-Lin Hu^a, and Yuxiang Chang^{a,b}

HEPATOLOGY



ORIGINAL ARTICLE | Full Access

p21-activated kinase 4 inhibition protects against liver ischemia/reperfusion injury: Role of nuclear factor erythroid 2-related factor 2 phosphorylation

Yuanzheng Mao, Chang Yeob Han, Lihua Hao, Younho Lee, Jung Beom Son, Hwangjun Choi, Mi Rin Lee,
Jae Do Yang, Suk Kyun Hong, Kyung Suk Suh, Hee Chul Yu, Nam Do Kim, ... See all authors

First published: 02 February 2022 | <https://doi.org/10.1002/hep.32384>

PAK4 research for liver I/R injury

Thank you

2022.11.23.

우수연구자 초청 세미나

아보메드

임원빈 대표님



ARBORMED
Global Leading R&D Innovator
11, 1, 2022

 **ARBORMED**
www.arbormed.com

Corporate Identity

 **ARBORMED**

Novel target : 희귀 난치질환 신약 개발 전문 기업

 Differentiation 차별화된 신약 파이프라인 조기 라이선싱 빠른 시장 출시 가능한 Novel Target 희귀 난치질환 치료제	 Competence 경영진 역량 연구개발과 글로벌 사업 역량	 Profitability 미국 시장 매출 모델 보유 5개 Complex Generic 의약품 미국 허가권 및 판매권 보유
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01. 회사 개요

ABOUT US

 설립일 |
  주요 사업 |
  직원수 |
  대표이사

2017년 12월 | 신약연구개발 | 19명 | 박교진, 임원빈

사업장	Web Site	임직원
본사 서울시 강남	www.arbormed.com	■ 경영, 사업개발
연구소 경기도 판교		■ 연구, 개발
해외 미국 샌프란시스코		

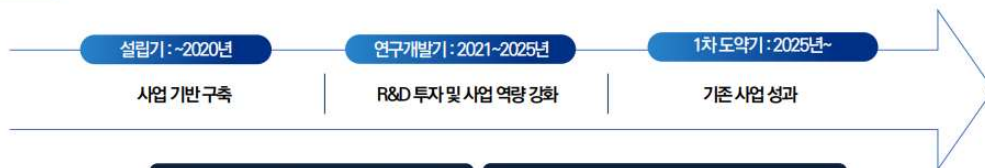
Diversified pharmaceutical company with divisions for
Novel medicines and Complex generics

HISTORY

- ARB-101 율슨병 치료제 효과 가능성 소개 (Wilson Aarhus 2022)
- ARB-201 ARDS 치료제 후보물질 공개 (ATS 2022)
- 판교 R&D 센터 설립
- 공동 연구 개발 기술이전 계약 체결 (연세의료원)
- 추가파이프라인 확보(ARDS, Pendred)
- 보건복지부 희귀질환 국책연구 사업선정
- 외 역원 편입 (일동제약)
- 미국 수출 계약 (한국유나이티드 제약)
- ARBORMED Pharmaceuticals Inc. 자회사 설립
- 미국 수출 계약 (아이큐어)
- 주식회사 아보메드 설립
- 2022
 - Series B 역원 편입(산업은행 외)
- 2021
 - 가죽진화인증기업 선정
 - 여가진화인증기업 선정
 - 내부 파이프라인 확보 신약 연구개발 협약 체결 (일동제약)
 - 병역자랑업체 선정
 - 국가신약개발사업 선정
- 2020~2017
 - 국가신약개발사업 선정
 - 벤처기업 인증
 - Series A 역원 편입 (SBI 등)
 - 신약 파이프라인 확보 (율슨병)

02. 비전

VISION OF ARBORMED



의학적 미충족 수요에 기반한 신약 개발

미국 자회사를 통한 한국 원제의약품 판매 유통

혁신 신약 중심의 글로벌 상업화 바이오텍 회사

01

희귀 난치질환 치료제 라이선싱딜

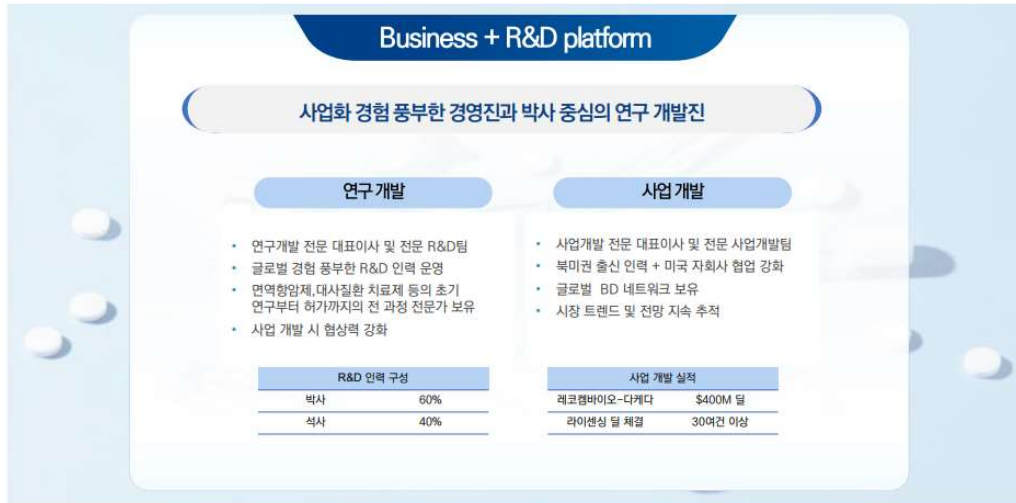
율슨병 upfront \$70M - \$100M 전제 딜사이즈 약 700M

02

미국 시장 매출 발생

매출 1,000억원 EBITDA 200억원

03. 경영진 역량

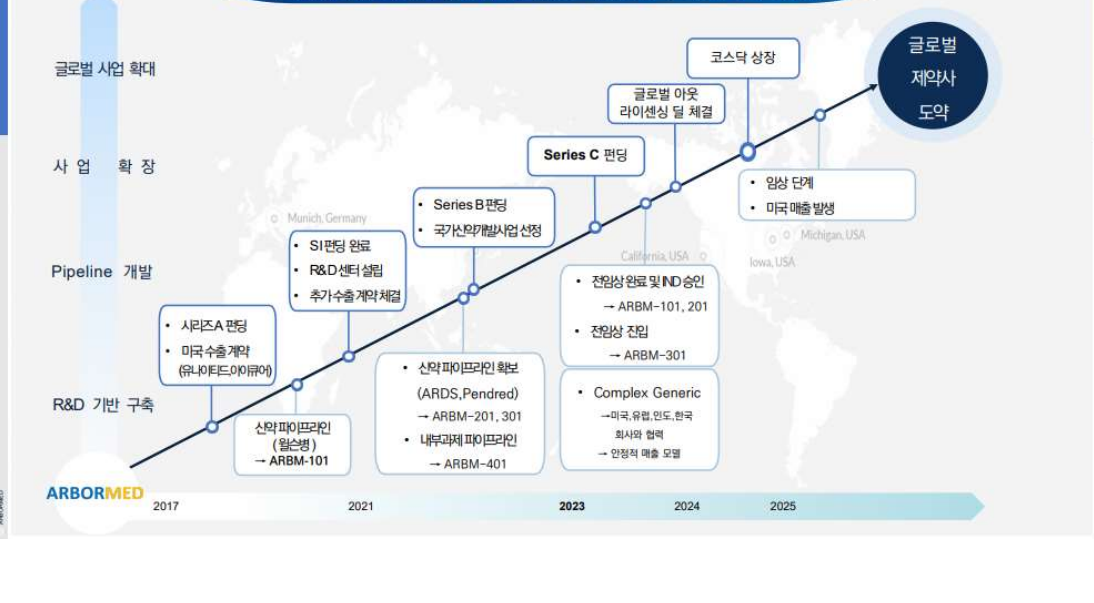


04. 미국 시장 매출 모델 보유



05. Milestone

기존 사업 확장 및 글로벌 사업 추진의 Two-Track 전략으로 지속적인 성장 도모



06. Orphan Drug Market Trend

01 급성장 블루칩 시장

- 희귀의약품은 각 나라마다 파격적인 혜택 제공
- 제약산업의 새로운 블루칩 시장으로 급성장하고 있음
- 연평균 성장률 약 11.2% 예상되며 약 \$217B (250조원)의 매출을 기록할 것으로 예상



02 높은 신약 승인율

- FDA 승인품목 중 '15년 약 37%, '16년 약 47%를 희귀의약품이 차지
- 3상 단계 희귀의약품 성공 가능성은 약 89%로 상당히 높음



03 미국 시장에서의 희귀질환 치료제 개발 편의



- ✓ 고가의 약가
- ✓ 미국 내 39%의 약가
- ➔ 약 10만불

- 신속 심사기간 적용으로 6개월 이내로 심사기간 단축 (보통 1년 ~ 1년 6개월 이상)
- 임상 2상 단계에서 조건부판매 가능
- 심사비용 면제, 임상비용의 50%까지 세금 면제
- License-Out 후보자 다수 존재
- 희귀질환에 대한 지속적인 정부 지원 강화

07. 신약 Pipeline 개발 현황



I. Introduction

월슨병, Wilson's Disease

유일한 치료방법은 간이식, 기존 치료제의 효능 부족 및 장기복용시 부작용 심각

간이 건강해야 몸이 건강하다

월슨병은 유전자 돌연변이로 인해 1:30,000 확률로 생기는 열성 희귀 유전병

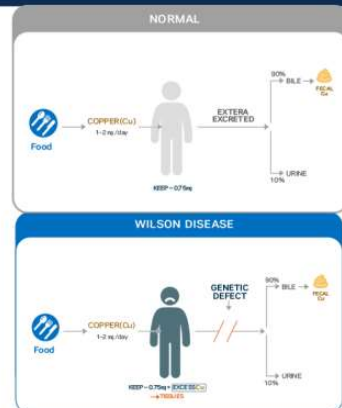
월슨병 환자의 50%가 만성 간경화로 진행
13%는 급성 중증 간경화로 진행 (주요 사망 원인)

아직까지 효과적 치료제 없음

(기존 치료제들은 효능부족, 장기복용시 부작용 심각)



정상 상태와 월슨병 환자의 구리 흡수/대사량과 배설경로



II. MARKET – Competition & Opportunities

기존 약물

- ✓ D-Penicillamine (DPA)
FDA 승인 : 1970
- ✓ Trientine
FDA 승인 : 1970

특징

혈관 속 구리와 결합해 소변으로 배출
(구리와 다른 배설물과 함께 소변으로 배출됨 - 부작용 없음)

간경화 등의 증상 악화 방지 역할

한계

부작용 심함 (30% 이상 환자 복용 중단)

백혈구 감소증, 신부전 등

간에 축적된 구리를 배출하지 못함

경쟁 약물

- ✓ ALXN1840
* WTX101, TTM
(개발 단계 : 임상 3상)

특징

소변과 대변 배출로 효능 개선

간경화 환자군은 임상 3상에서 제외

한계

매일 복용시 장기 독성 우려
(폴리브덴 축적 가능)

소변 배출에 의한 신부전 등 이슈

- ✓ VTX801
* AVV 유전자 치료제
(개발 단계 : 임상 1/2상)

특징

구리 대사 능력 회복

간경화 환자군은 임상 3상에서 제외

한계

급성 중증 간경화 환자에게 부적합
(중증 간경화 환자 - 5주 이상)

간세포 침투 비율에 따라 간 기능 회복 수준에 한계

ARB-101

정맥주사제



Clinical Benefits

간에 과다 축적된 구리를 대변으로 **90% 이상** 배출

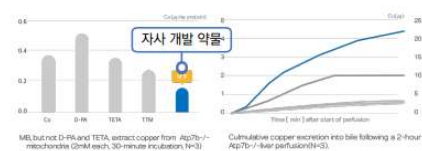
신속하게 과도한 구리 배출로 간 수치 **정상화** 확인
동물 모델의 평균 수명을 정상 수준으로 개선
(비투여 동물 모델 - 랫드)

간경화 증상을 **완화**시키는 작용기전 확인

간헐적 집중 투여 = 장기 투여 독성 우려 **낮음**

II. MARKET - 차별화 포인트

기존 치료제 대비 체내 구리량 비교



- 세포 및 세포내 기관에서 구리량이 정상 수준으로 낮아짐

ARB-101에 의한 간세포 내 구리 배출 효과

과도하게 축적되어 있던 구리의 현저한 감소 확인

세포 및 세포내 기관에서 구리량이 정상 수준으로 낮아짐

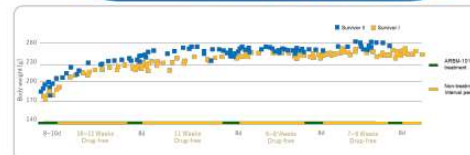
과량의 구리 배출로 인해 회복

구리 축적으로 손상된 세포 회복 - 미토콘드리아



구리로 손상된 세포의 활성 회복 효과 확인

ARB-101 투여로 월수명 동물 수명 연장



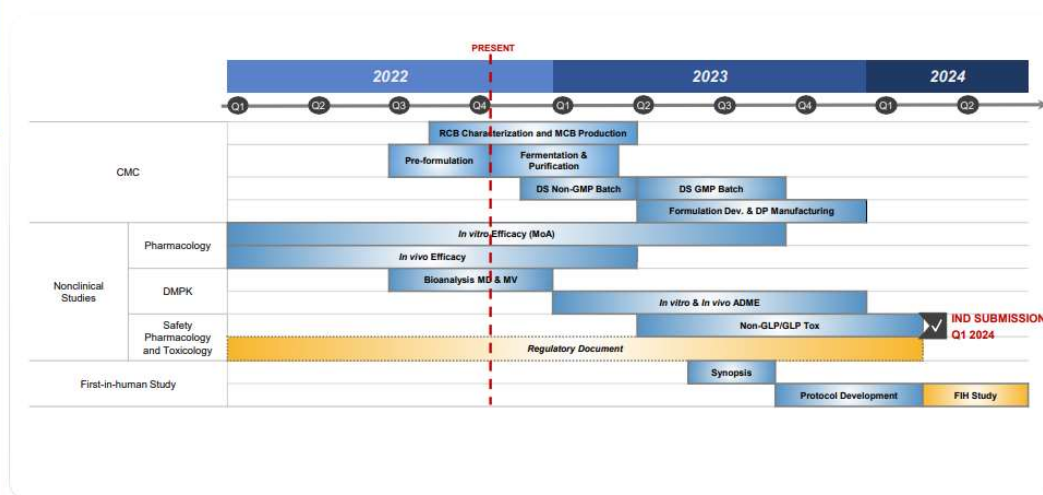
- 비투여 WD 랫드는 평균 수명 110일 (생후 90-95일부터 간수치가 높아짐)

→ ARB-101 투여 WD 랫드는 440일 이상 생존

V. ARBM-101 publication 전략



VII. Timeline



I. Introduction

급성 호흡곤란 증후군, ARDS

다양한 원인에 의한 급성 폐질환, 사망률 40%

생명을 유지하는 첫 번째 보호막: 폐



외상, 감염 (대표적, 폐혈증), 폐질환 등 원인



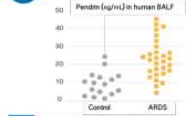
폐포 (alveoli) 에 물이 차서 혈중 산소 수치가 급격하게 감소되어 호흡곤란 야기

아직까지 효과적인 치료제 없음



펜드린 억제제 개발 배경

01 ARDS 환자에서 펜드린 발현 3배 이상 증가



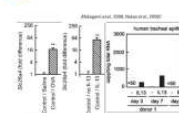
02 Pendred 증후군 환자에서 천식과 고혈압 질환 미발생

Disease status	0	1	2
Asthma	11	2	0
No asthma	135	37	21
Hypertension	15	6	0
No hypertension	187	33	21

03 추가 적응증 확대 가능성 (Rhinovirus)



04 추가 적응증 확대 가능성 (Asthma)



II. MARKET

기존 약물

스테로이드제 · 신경근육차단제



특징

중환자실 자원 기간 shock
인공호흡기 사용 기간 감소

한계

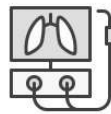
전반적인 면역 체계 파괴

명확하게 입증되지 않은 효능

부작용 가능성 높음

의료 기기 사용

산소호흡기 ECMO



특징

강제적으로 산소공급

한계

단기간 지속 효과

사망을 변화 미미

신기능 부전 초래 가능성

고가의 치료 비용

ARB-201

펜드린 억제제



Clinical Benefits

On-target protein 분포가 폐에 집중

기존 약제 내성과 무관

기존 약제와의 병용 투여 가능성

Risks

On-target 으로 인한 낮은 부작용

II. MARKET and Competition

급성호흡곤란증후군 시장의 성장과 높은 미충족 의료 수요 존재의 지속

글로벌 ARDS 시장 규모 예측



국내 ARDS 시장 규모



COVID-19 같은 호흡기 전염병의 유행

매년 1.5%씩 환자 증가

미국 내 중증 환자는 약 15만명

경쟁 약물 및 개발 현황

물질	개발사(지역)	타겟	단계	투여경로	물질종류
AP-301 (Solnatide)	Apeptico (Germany, Austria) 미국 개발 실패	Epithelial sodium channel activator	발매	흡입/경구	합성 펩타이드
Traumakine	Maruishi(JP) / Faron(Finland)	interferon beta-1a receptor	3상	정맥	재조합 단백질
MultiStem	Athersys(US) / Healos(JP)	-	3상	정맥	세포 치료제
Sabizabulin	Venu(US)	Alpha Tubulin	경구	경구	화합물

Human albumin, 폐 표면활성제 등의
약물 재창출 (drug repurposing)

신경근육 차단제 (neuromuscular blocker)의
약물 재창출

면역반응 조절 및 특정 염증경로 차단하는 기능성 약물

대부분
치료 효과
입증 실패

III. MOA (Mode of Action)

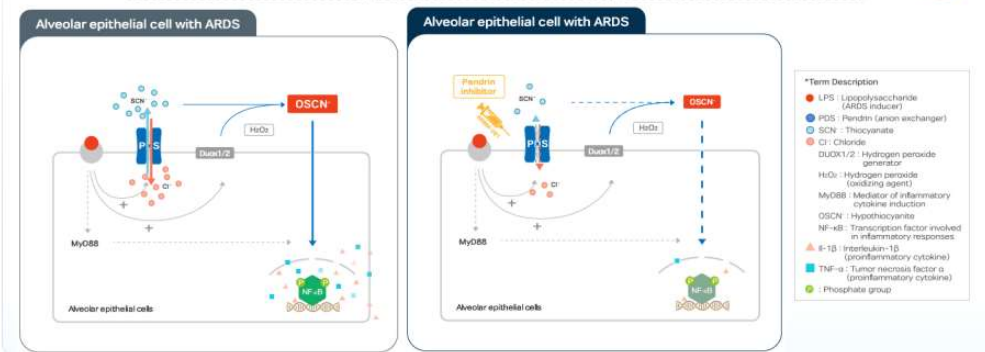
ARBM-201에 의한 항염증 효과

펜드린 발현으로 인한 SCN⁻ 이온 교환 증가
BALF 내에서 염증성 cytokine 농도 증가

펜드린 억제를 통해 다양한 염증성 cytokine 발현을
동시에 억제

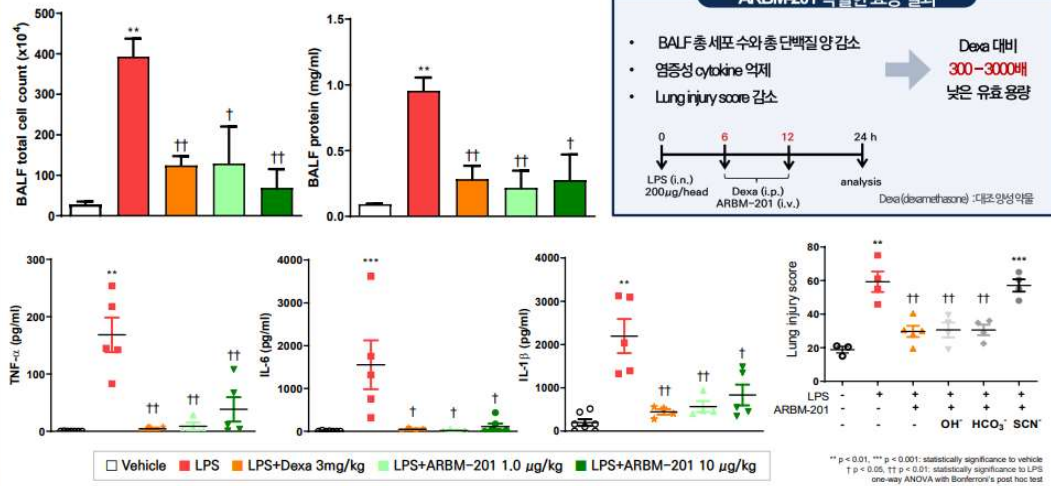
염증 감소에 따른 폐 손상 개선

차별화
포인트



IV. Efficacy

In vivo efficacy (LPS induced mouse model)



V. ARBM-201 publication

아보메드 ATS 2022서 급성호흡곤란증후군 치료 후보물질 발표

△ 메디칼트리플 △ 승인 2022.05.25 10:22 △ 댓글 0

아보메드가 13일부로 18일(현지시간)까지 열린 2022

미국호흡학회 (ATS 2022, 샌프란시스코)에서 급성호흡 곤란증후군(Acute Respiratory Distress syndrome, ARDS) 치료 후보물질인 ARBM-201의 연구 결과를 공개했다.

이 후보물질은 폐포 내막에 피모신안과 및 아미노산 시아메이트 이온의 유입을 감소시켜 염증 반응에 관여 하는 단백질 NF-κB의 억제 그리고 염증 사이토카인을 감소시키는 기전을 가진 펜드린 계열이다.

펜드린은 폐포 내막을 손상시키고 있는 단백질인 NF-κB의 억제 및 NF-κB의 억제에 의해 증대된 염증 반응을 억제한다. 급성기 질환 가운데 천식, 만성폐쇄성폐질환(COPD), 알레르기 비염에서 펜드린 발현이 증가한다고 알려져 있다.

사람의 폐포 상피세포에서도 LPS로 인해 과발현된 펜드린이 ARBM-201 투여 후 발현이 억제됐다. LPS 감염 동물모델에서도 ARBM-201 1µg/kg를 1일 2회 정맥 투여하자 펜드린 단백질 발현이 정상 수준으로 억제됐다. 기관지폐포세척액(BALF)의 총 세포수와 단백질 농도도 확연히 줄었다.

염증성 사이토카인 IL-1β, IL-6, TNF-α 모두 크게 감소했으며, 폐조직 병리 검사에서는 손상 정도와 염증성 지표가 크게 개선됐다.

아보메드에 따르면 현재 급성호흡곤란증후군에 효과적으로 사용할 수 있는 치료제가 없는 데다 중환자실에 투여되는 스테로이드제는 효능은 작은 반면 부작용이 매우 크다고 알려져 있다.

또한 약물 대항물(anti-drug antibody)의 특정 면역 반응을 조절하거나 염증 반응을 차단하는 전략에 발의 임상시험 진행 중이지만 치료 효과가 검증되지는 않고 있다.

아보메드 임원인 공동대표는 "펜드린은 염증 반응이 발생하는 경로의 상위에서 다양한 염증 사이토카인을 모두 조절할 수 있어 특정 염증을 억제하는 역할보다 효과적"이라고 예상했다.

ARBM-201은 비임상 단계에서 향후 2년 내 한국 또는 미국에 1상 임상시험계획(IND) 제출할 계획이다.

저작권자 © 메디칼트리플 무단 전재 및 재배포 금지

미국 흉부학회에서 ARDS 후보물질 ARBM-201 연구 결과 공개

호흡기 질환에는 펜드린 발현 증가

ARBM-201 투여 후 발현 억제 확인

급성호흡곤란증후군은 현재 치료제가 없다

전체적인 염증 사이토카인을 모두 조절할 수 있어 더욱 효과적

VI. ARBM-201 publication

01 메디컬 투데이

아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 발표

최민준 기자 | 2022-05-25 10:48:51

▲ 아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 발표. ATS 2022, 11월 10일부터 14일까지 미국 샌프란시스코에서 개최되는 ATS 2022에서 아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 발표.

02 파이낸셜뉴스

아보메드, ATS 2022에서 급성호흡곤란 증후군 치료제 후보물질 발표

김민준 기자 | 2022-05-25 10:48:51

03 증권경제신문

아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 공개

최민준 기자 | 2022-05-25 10:00

04 메디컬트리뷴

아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 발표

최민준 기자 | 2022-05-25 10:00

05 SEN 서울경제 TV

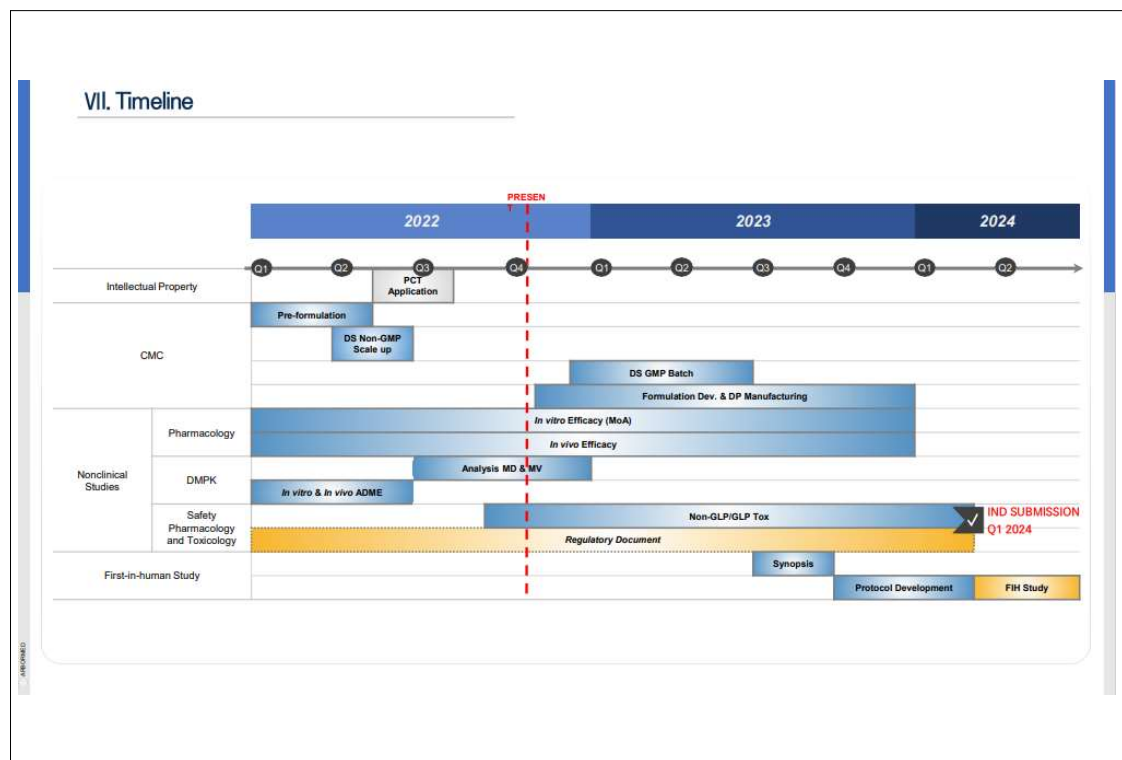
바이오펜터 아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 연구 결과 발표

최민준 기자 | 2022-05-25 10:00

06 증권경제신문

아보메드, ATS 2022서 급성호흡곤란 증후군 치료제 후보물질 발표

최민준 기자 | 2022-05-25 10:00



I. Introduction

펜드리드 증후군, Pendred Syndrome

선천성 난청 환자의 약 15%를 차지하는 유전 질환 치료제의 필요성

제 2의 인체, 인체의 축소판 : 귀

펜드린 유전자 SLC26A4의 변이로 발생하는 유전 질환

내이의 pH 불균형 등을 유발하여
전정수도관 확장증 = 선천성 난청 유발

유소아기때부터 난청이 시작되어 진행성 청력 손실로 이어짐



아직까지 효과적인 치료제 **없음**
(보청기와 인공 와우 수술)

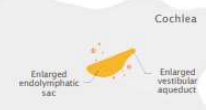


펜드린 Inhibitor 로서의 개발 배경

NORMAL INNER EAR



PENDRED SYNDROME



II. MARKET – Unmet Need & Positioning

신생아 약 1,000명 중 1.7의 비율로 난청 발생



미국
난청신생아
약 6,500명

펜드린
돌연변이로
인한 난청
약 650명

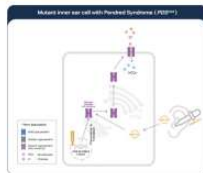
• 미국 시장성은 500명 x \$400,000/year = \$200M

인공와우 이식술은 청력 소실 이후 실시



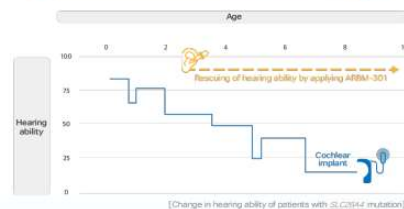
• 기존 청력 유지 및 회복으로 자연음을 들을 수 있게 조기 예방 및 치료 가능한
약물 대체 방법 필요성

국소적 약물 투여를 통한 전신 부작용 회피의 장점

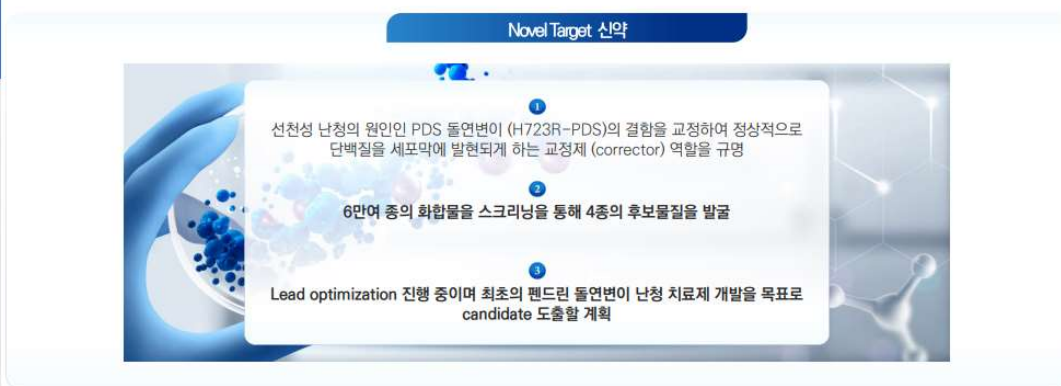


• ARBM-301이 PDS/SLC26A4 돌연변이에 의해 생긴 단백질 구조 결함을
교정하여, 세포표면에서의 발현을 정상화

펜드리드 증후군의 청력 변화 및 corrector의 청력 개선 효과



III. 개발현황 및 계획



감사합니다

주식회사 아보메드 www.arbormed.com

본사: 서울시 강남구 논현로 538 HJ빌딩 6층(역삼동)

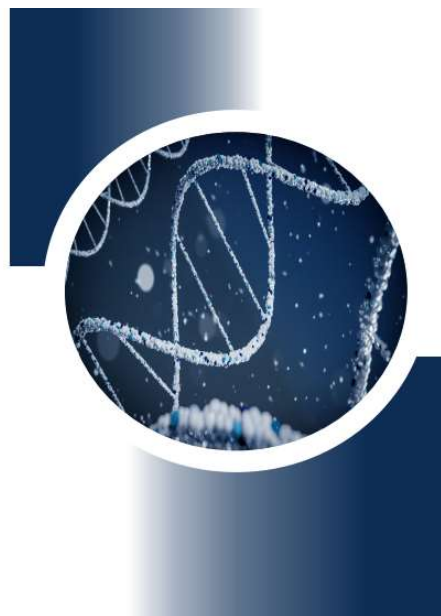
R&D센터: 경기도 성남시 분당구 민교로 190, 일파타워 202호

TEL.: 02-6953-1207 (본사) / 031-8039-6219 (R&D센터)

EMAIL: ykang@arbormed.com



ARBORMED



2022.11.30.

약품물리학/유기화학 전공 대학원생 발표 세미나

유성원 학생

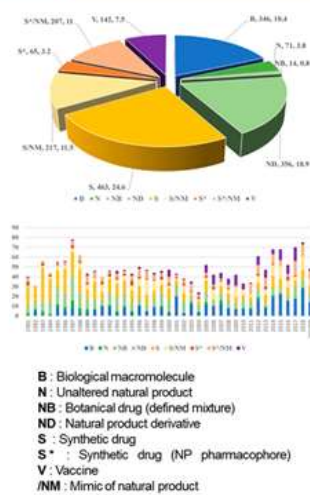
Total Synthesis of (-)-Madurastatin C₁ and Their Stereoisomers to Probe the Stereoselectivity of the Fe(III) Uptake Machinery

Yu Seongwon
Jeonbuk National University School of Pharmacy
Medicinal Chemistry and Organic Synthesis

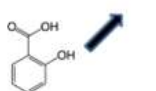
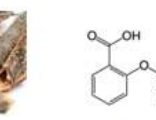
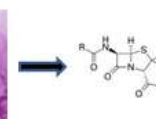
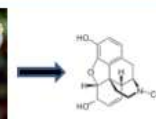
Madurastatin C₁

- Total synthesis of natural product
- Madurastatin C₁
- Retrosynthesis
- Synthesis
- Plan

Total synthesis of Natural Product



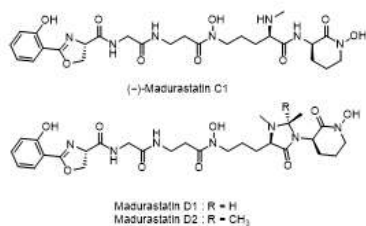
J. Nat. Prod. 2020, 83, 770-803



Total synthesis of Natural Product



Madurastatin C₁

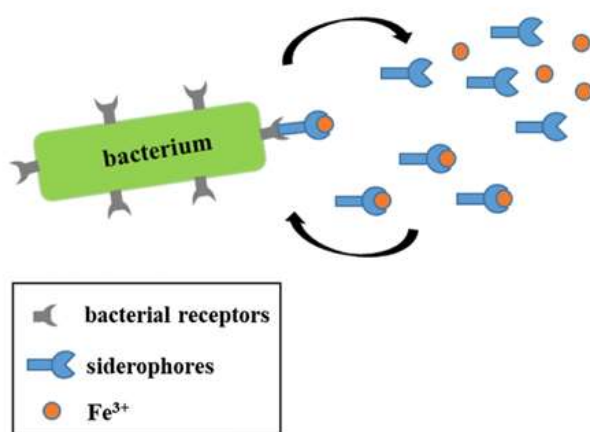


- N-hydroxyamide
- Oxazoline
- Linear peptide
- Siderophore

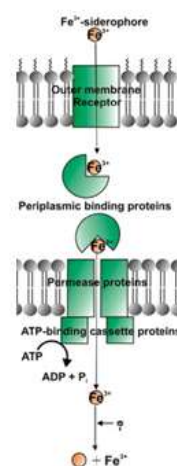
Madurastatins from *Actinomadura* with maduraferrin

Org. Lett. 2019, 21, 6275–6279

Siderophore

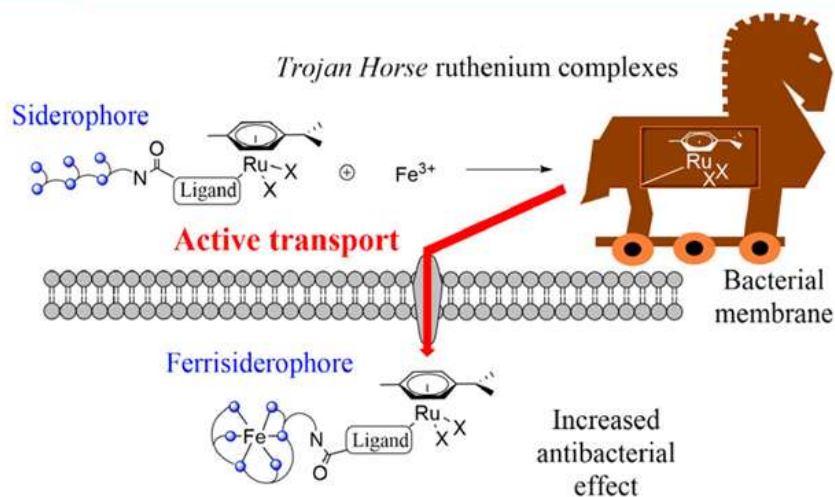


Environ Chem Lett 17, 1485–1494 (2019)



Biochimica et Biophysica Acta 1778 (2008) 1781–1804

Siderophore



Organometallics 2018, 37, 6, 915–923

Siderophore

- ✓ Up to 128-fold more active than cefaclor
- ✓ Intracellular delivery by fimsbactin B
- ✓ *Acinetobacter*-selective

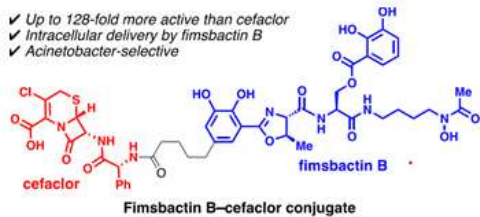


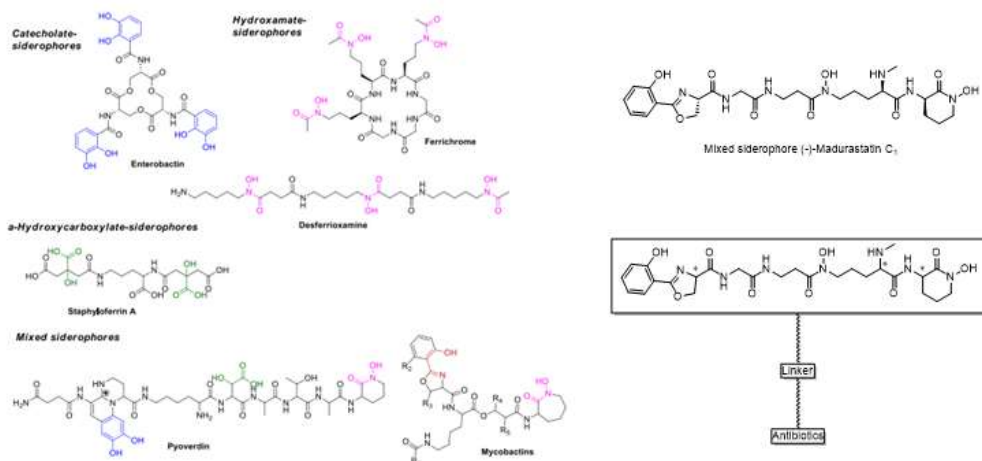
Table 1. Antimicrobial Activity Assay Results^a

entry	strain	DP concentration (μM) ^b	18a	18b	18c	Cec
1	<i>A. baumannii</i> ATCC 17978	300	0.5	1	2	64
2		0	16	16	64	>64
3	<i>A. baumannii</i> ATCC 19606	300	16	16	16	>64
4		0	>64	>64	>64	>64
5	<i>A. nosocomialis</i> ATCC 17903	150	16–32	16	16	>64
6		0	>64	>64	>64	>64
7	<i>E. coli</i> K12	300	>64	>64	>64	64
8		0	>64	>64	>64	64
9	<i>P. putida</i> ATCC 12633 ^c	300	>64	>64	>64	>64
10		0	>64	>64	>64	>64



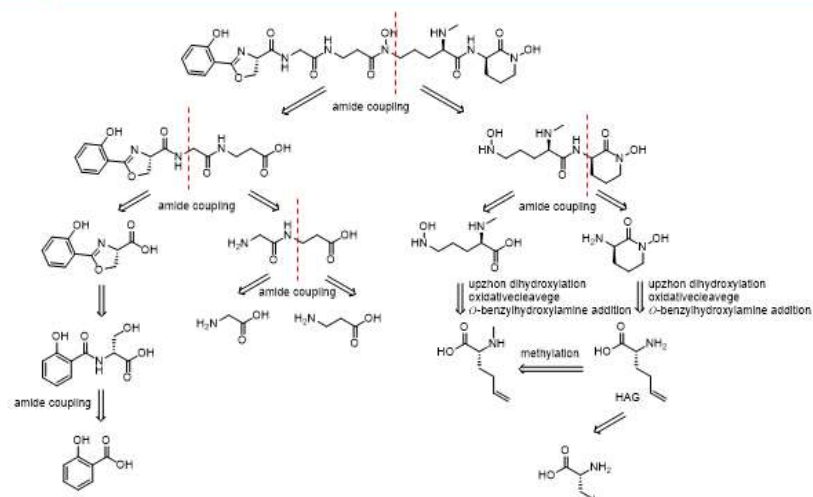
Org. Lett. 2021, 23, 13, 5256–5260

Siderophore

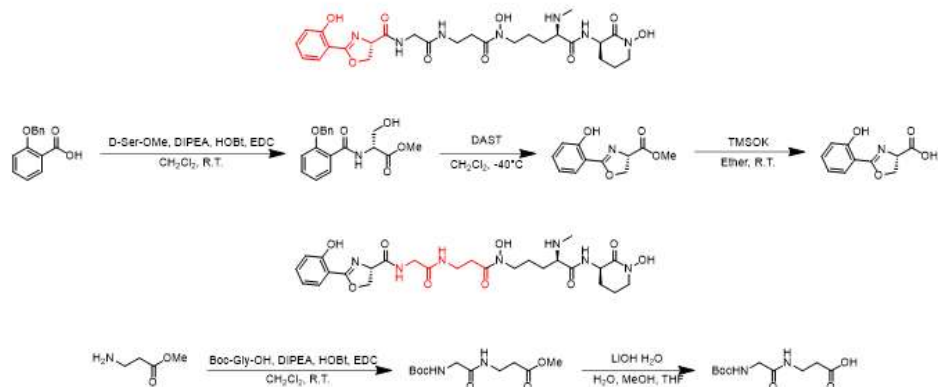


European Journal of Medicinal Chemistry 208 (2020) 112791

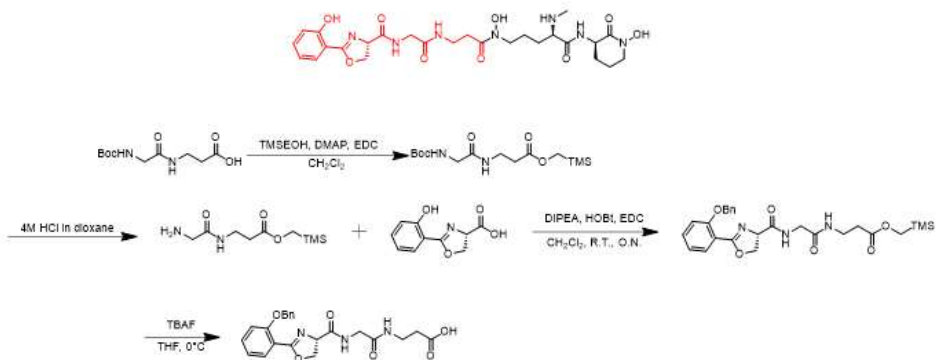
Retrosynthesis



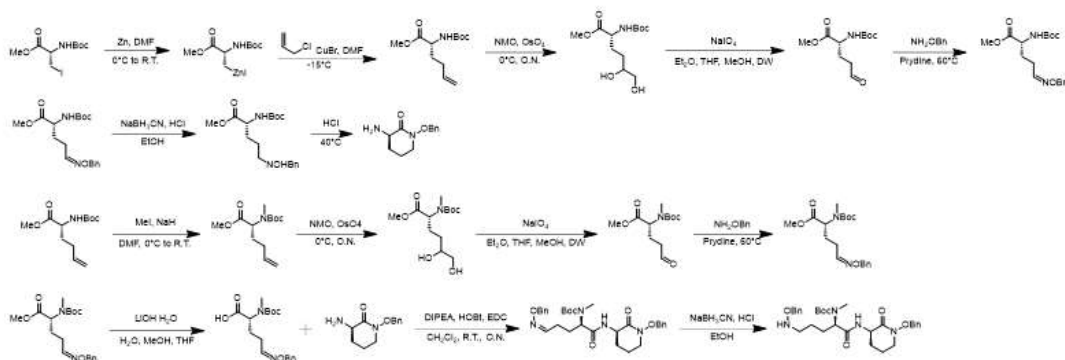
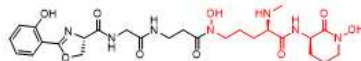
Synthesis



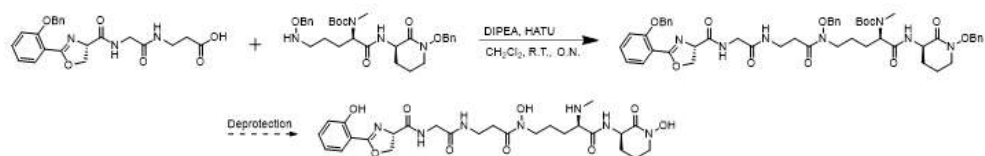
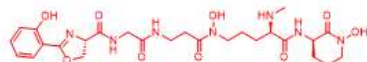
Synthesis



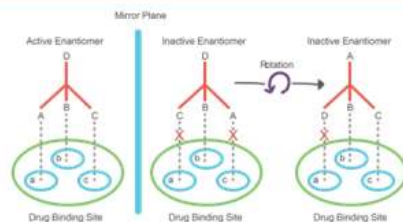
Synthesis



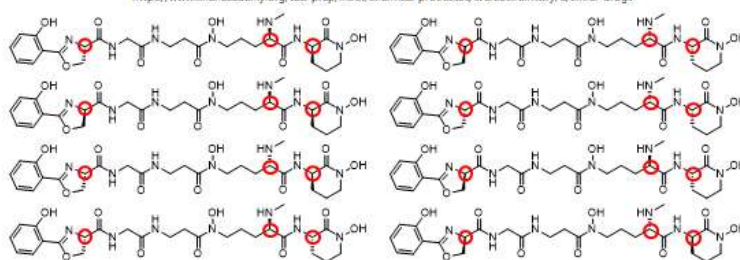
Synthesis



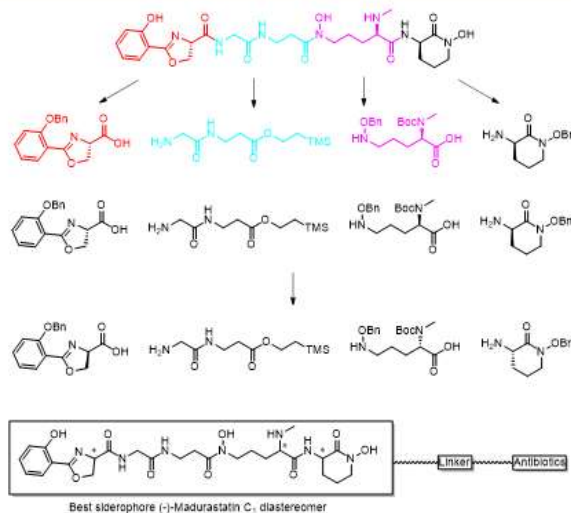
Plan



<https://www.khanacademy.org/test-prep/mc/at/chemical-processes/stereochemistry/a/chiral-drugs>



Plan



감사합니다

2022.11.30.

약품물리학/유기화학 전공 대학원생 발표 세미나

김지환 학생

Development of cannabidiol-loaded polypeptide-based nanocarrier with long-sustained drug release for effective treatment of breast cancer

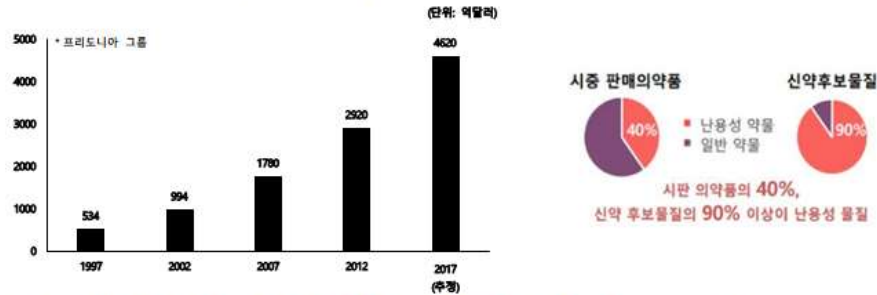
202150333 전북대학교 약학과 김지환



Contents

- Part 1** 연구 배경 I (DDS, 나노 입자, 수동 표적, 능동 표적)
- Part 2** 약물전달체에 사용되는 나노 입자
- Part 3** 연구 배경 II (Cannabidiol, Breast cancer)
- Part 4** 연구 목적, 서론
- Part 5** 결과
- Part 6** 최종 실험 계획

➤ 약물전달 시스템 (Drug Delivery System, DDS)



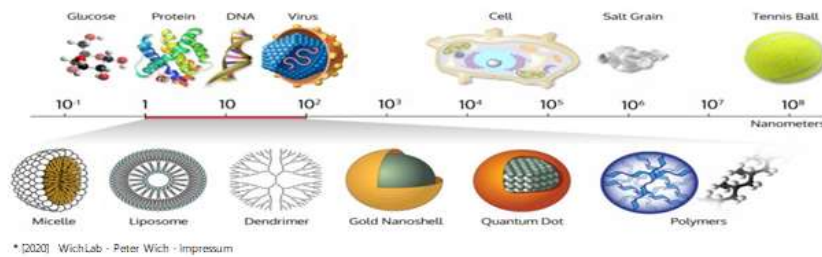
- 기존 의약품의 부작용을 최소화, 효능을 극대화 하기 위해 제형을 설계하여 최적화 하는 기술
- 약효는 우수하지만 낮은 용해도, 낮은 생체 내 이용률의 약물을 전달 시스템으로 개선이 가능
- Off-target effect를 줄여 부작용을 줄이고, 약효를 극대화하도록 제형을 설계
- 약물의 유효성을 극대화하며, 투여 편의성을 증대 시킬 수 있는 제형을 설계

➤ 암 발생자수, 성별 주요 암종 발생분율



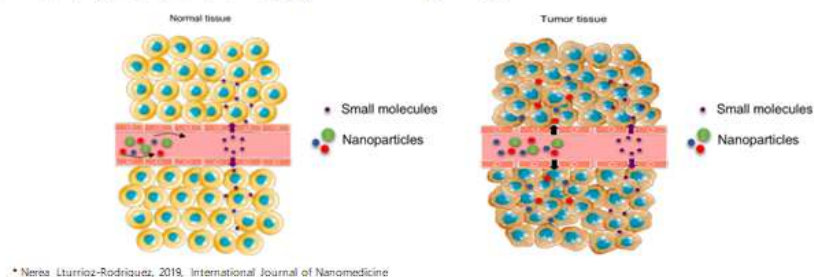
▶ 전립선암, 유방암은 높은 비율을 보이며, 지속적으로 증가하는 추세를 나타냄

➤ 나노 입자 (Nanoparticles)



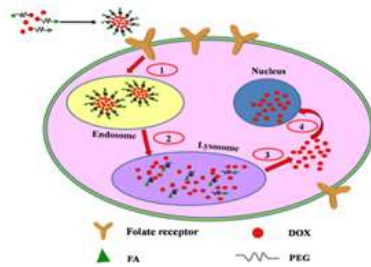
- 나노 입자는 적어도 한 차원이 100 nm 미만인 미립자 물질
- 크기가 작기 때문에 큰 분자 보다 효율적으로 세포에 흡수가 되어 치료제의 한계 극복 가능
- 기존 항암제의 단점인 off-target effect를 줄여 암세포에만 효과적으로 약물 전달
- 나노 입자는 DNA, RNA와 같은 핵산, 난용성 약물을 전달하는데 용이함

➤ 나노 입자 약물 전달 수동 타겟 (Passive targeting)



- 종양 환경 고유의 특징으로 종양 미세환경 내에서 나노입자 축적
- 수동표적기전 (Enhanced Permeability and Retention) EPR 효과
- 나노 입자의 전달은 크기, 모양 및 표면 전하와 같은 나노 입자 자체 고유의 요소에 의해 결정
- Size : 10 ~ 200 nm / 10 nm ↓ 신장 여과에 의한 제거, 200 nm ↑ Opsonization, 대식세포의 포식

➤ 나노 입자 약물 전달 능동 타겟 (Active targeting)

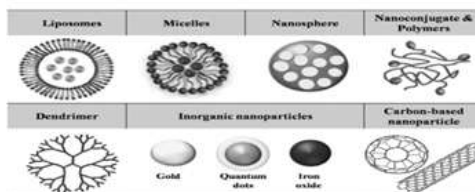


* Wei-liang Ye etc., 2014, PLOS One

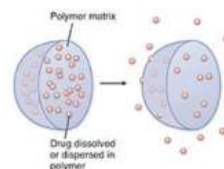
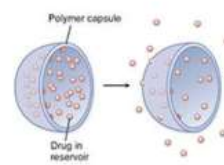
Ligand		Receptor
Herceptin	→	Antigen HER2 receptor
Folate	→	Folate receptor
Transferrin	→	Transferrin receptor
Hormone EGF	→	EGF receptor
Hyaluronic acid	→	CD44 receptor

- 암이 계속 성장하기 위해 영양분이 필요하기 때문에 수용체들이 과잉으로 발현
- 종양세포에서 발현되는 수용체인 Folate, Transferrin, EGF 등을 표적
- 나노 입자 표면에 Ligand, 항체를 결합하여 종양세포만 표적으로 타겟이 가능

➤ 약물전달시스템에 사용되는 나노 입자 종류

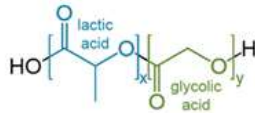


* Ji Won Park, 2014, BT News



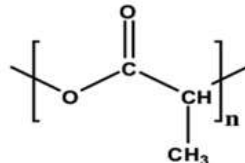
- 약물전달시스템에 활용되는 나노입자는 크기, 표면의 특성 등에 따라 구분
- 인지질의 친수성인 헤드, 소수성인 꼬리 영역을 지닌 양친매성 리포좀
- 자가유화를 이용한 친수 및 친유성 그룹을 갖는 copolymer의 micelle
- 생체 내 자연분해 폴리머를 이용한 고분자 폴리머 전달체
- 덴드리머는 분자량, 크기, 구조 예측이 가능하며 다양한 작용기 도입 가능

➤ 약물 전달체에 사용되는 고분자

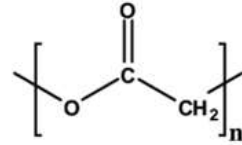


x and y indicate the number of times each unit repeats.

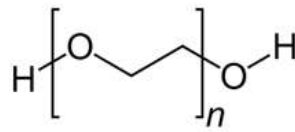
Poly(lactic-co-glycolic acid)
PLGA



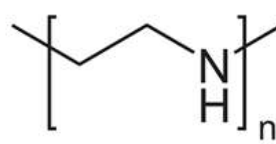
Poly(lactic acid)
PLA



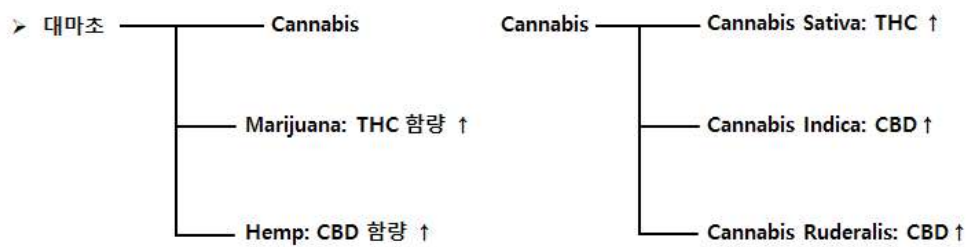
Poly(glycolic acid)
PGA



Poly(ethylene glycol)
PEG



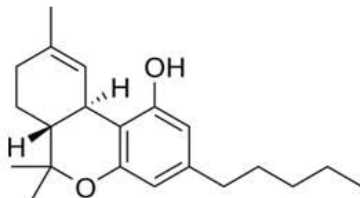
Poly(ethylene imine)
PEI



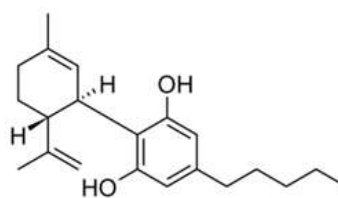
- Cannabis는 삼과에 속하는 꽃이 피는 식물로 흔히 대마로 부름
- 미국→2018년 농업 개선법을 통해 THC가 0.3% 이하는 농작물로 법제화
- 대마초는 오락용, 의료용으로 사용되어 왔음



➤ Cannabidiol (CBD)



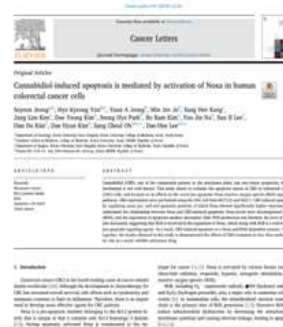
Δ-9 THC(tetrahydrocannabinol)



CBD(cannabidiol)

- Cannabidiol, Tetrahydrocannabinol은 대마초 식물에서 400여가지 이상의 생리 활성 성분 중 100여가지 이상의 카나비노이드 성분 중 하나임
- THC와 CBD는 분자 구조는 같지만, 원자 배열 방법의 구성이 다르며, 신체에 각각 다른 영향을 미침
- CBD는 항불안제, 항산화제, 항우울제, 항염증제, 진통제 등과 같은 의학적인 목적으로 사용
- 최근 연구에 대한 결과로 CBD는 다양한 암에서 항증양제 역할을 한다고 보고됨

➤ Cannabidiol (CBD)



➤ Breast cancer



- 유방암은 전 세계적으로 여성이 앓고 있는 암 중 24%를 차지
- 주로 악물 내성, 림프절, 뼈, 간과 같은 중양의 전이 능력 발달로 사망률이 15%를 차지
- 원인은 규명되지 않았지만 유전, 에스트로겐, 방사선, 서구화 식습관으로 인한 요인으로 추정
- 치료 방법으로는 수술, 방사선, 항호르몬, 화학요법 등이 있음

➤ Breast cancer

호르몬 수용체 (에스트로겐, 프로게스테론)	HER2	유방암 분류
O	X	호르몬 양성 유방암
X	O	HER2 양성 유방암
O	O	호르몬 양성, HER2 양성 유방암
X	X	삼중 음성 유방암

- 에스트로겐, 프로게스테론 중 하나가 양성이거나 둘 다 양성일 경우에 타목시펜,아나스트로졸과 같은 항에스트로겐 제제를 사용 → 수용체와 결합하여 중양의 증식을 억제 및 호르몬 생성을 억제
- 유방암의 세포 증식과 관련된 HER2의 신호 전달체계를 차단하는 허셉틴, 라파티닙이라는 치료제가 존재
- 하지만 항호르몬제의 부작용으로 자궁내막암, 심부전증, 골다공증 등이 있음
- 항호르몬제의 효과가 없는 경우, 화학 요법을 하게 되는데, 정상 세포에서도 영향을 미치기 때문에, 부작용으로 인한 탈모, 혈소판 감소증 등이 존재한다는 문제점이 있음

➤ 연구의 목적

- CBD는 유방암에 항종양 효과가 있다는 선행 연구 결과들이 보고됨
- CBD는 낮은 생체이용률과, 난용성으로 인해 약물 전달에 있어 어려움이 존재함
- 기존 항암제의 문제점인 Off-target effect의 부작용을 줄이고 암을 표적하여 치료할 수 있는 약물 전달체 개발
- 전달체를 도입하여 더 효과적이고 안정성이 우수하며 항암 효능을 높이기 위함
- 전달체가 없는 CBD에 비해 전달체를 도입한 CBD가 생체내에 주입했을 때, PK나 생체분포의 향상으로 항암 치료 효능이 우수할 것이라는 설정을 두고 실험을 진행

➤ Pharmacokinetics and biodistribution of DOX formulations in vivo



Calcium mineralized polypeptide nanoparticle for intracellular drug delivery in osteosarcoma chemotherapy

Yu Li^{1,2}, Li Li^{1,2}, Li Zhao¹, Yanghe Chang^{1,2}, Xi Zhang^{1,2}, Yan Guo^{1,2}, Danyu Zhang¹

¹Department of Pharmacy, Anhui Medical University, 171 Xigao Road, Hefei, Anhui 230025, China; ²Department of Pharmacy, Anhui Medical University, 171 Xigao Road, Hefei, Anhui 230025, China

ARTICLE INFO

Keywords:
Calcium mineralized polypeptide nanoparticle
Drug delivery
Osteosarcoma chemotherapy

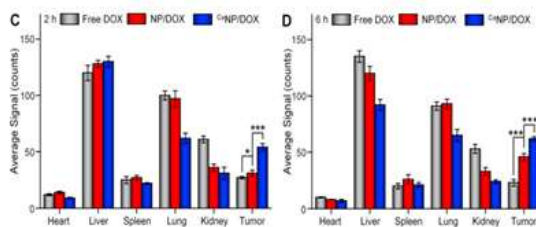
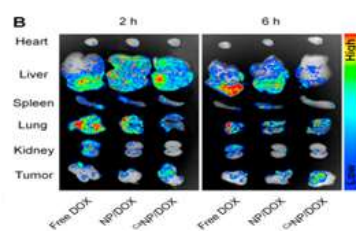
ABSTRACT

The study demonstrates a novel drug delivery system for the development of a calcium mineralized polypeptide nanoparticle (CMNP) for intracellular drug delivery in osteosarcoma chemotherapy. The CMNP was synthesized by the self-assembly of calcium mineralized polypeptide (CMNP) and doxorubicin (DOX). The CMNP showed a high loading capacity and a high stability in the blood circulation. The CMNP showed a high cellular uptake and a high intracellular release. The CMNP showed a high tumor targeting and a high tumor inhibition. The CMNP showed a high safety and a high biocompatibility. The CMNP showed a high efficacy in the treatment of osteosarcoma.

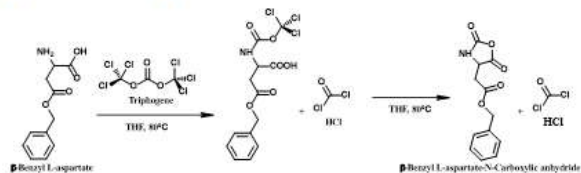
1. Introduction

Osteosarcoma is the most common primary bone tumor in children and young adults. It is a highly aggressive malignancy with a poor prognosis. The mainstay of treatment for osteosarcoma is a combination of surgery and chemotherapy. However, the efficacy of chemotherapy is limited by the poor pharmacokinetics and biodistribution of the drugs.

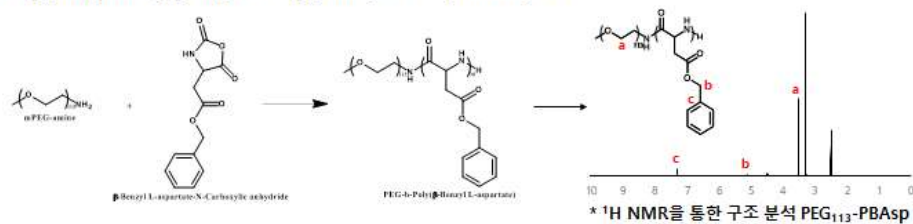
Calcium mineralized polypeptide nanoparticle (CMNP) is a novel drug delivery system for the treatment of osteosarcoma. The CMNP is composed of calcium mineralized polypeptide (CMNP) and doxorubicin (DOX). The CMNP shows a high loading capacity and a high stability in the blood circulation. The CMNP shows a high cellular uptake and a high intracellular release. The CMNP shows a high tumor targeting and a high tumor inhibition. The CMNP shows a high safety and a high biocompatibility. The CMNP shows a high efficacy in the treatment of osteosarcoma.



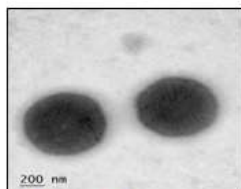
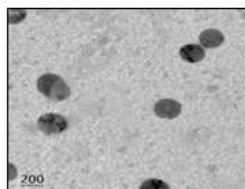
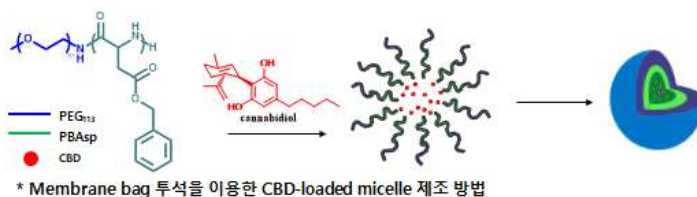
➤ β -Benzyl-L-Aspartate N-carboxyanhydride 합성



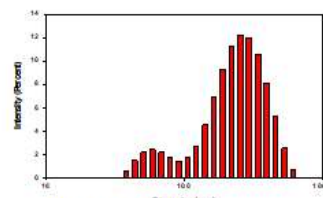
➤ Poly(ethylene glycol)-b-Poly(Benzyl-L-Aspartate) 합성



➤ CBD-loaded micelle



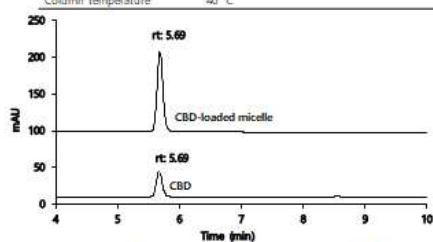
* TEM을 통한 형태학적 이미지 측정 (Sphere shape)



* DLS를 통한 크기 분포 측정

➤ CBD-loaded micelle 특성 분석

Parameter	Condition
Mobile phase	A: 0.1% Formic acid in water B: 0.1% Formic acid in ACN
HPLC	ThermoFisher 3000 HPLC
Column	Hypersil GOLD C18, 250 x 4.6 mm column
Column temperature	40 °C

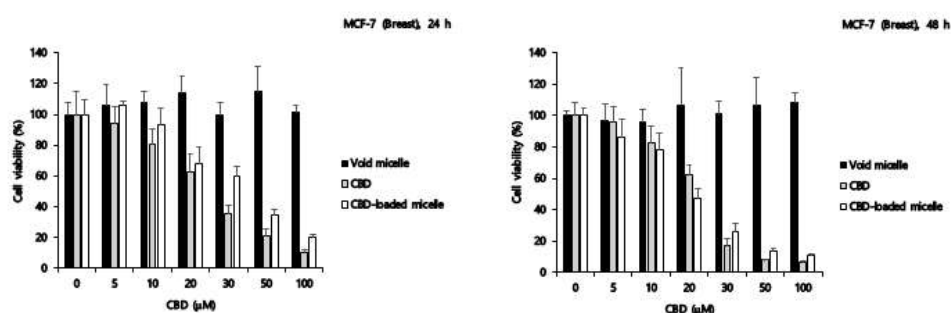


* HPLC를 사용하여 Standard CBD와 CBD-loaded micelle 측정

→ Standard CBD와 같은 retention time과, nm 파장 확인

→ Encapsulation Efficiency: 89%, Loading content: 8.17%

➤ CBD-loaded micelle 세포 독성 확인 (1/2)



* CCK-8 kit으로 측정된 Cytotoxicity 24, 48시간

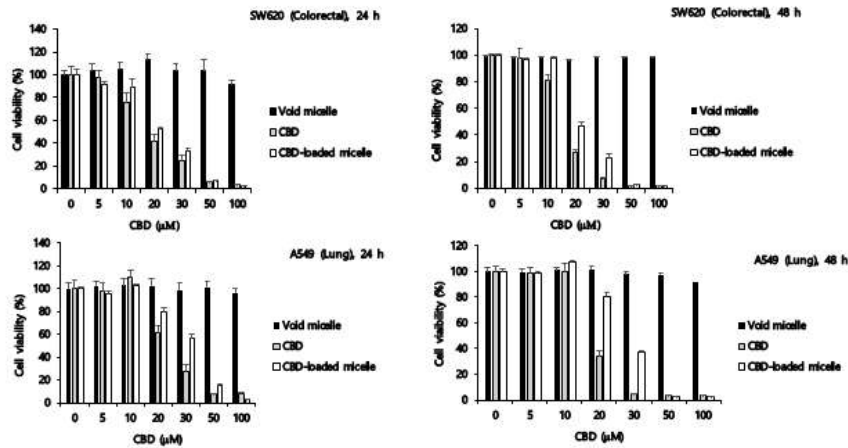
→ Void micelle (10 ~ 200 μg/mL)에서 독성이 보이지 않았음 / 약물이 없는 Micelle 자체의 독성은 없음

→ CBD (0 ~ 100 μM)에서 독성 확인

→ CBD-loaded micelle (0 ~ 385 μg/mL)에서 확인

※ CBD의 독성이 더 높은 이유는 확산에 의해 빠르게 이입

➤ CBD-loaded micelle 세포 독성 확인 (2/2)



* SW620 (대장암 세포), A549 (폐암 세포) 다른 Cancer cell line에서도 Cytotoxicity를 보임 → 다양한 암에서 항암제의 역할을 기대

Therapeutic Dictionary

Cannabidiol Induces Programmed Cell Death in Breast Cancer Cells by Coordinating the Cross-talk between Apoptosis and Autophagy

Author: Shrawan, Preeti M, Rastogi, Jyoti S, Singh, and Anil Prasad

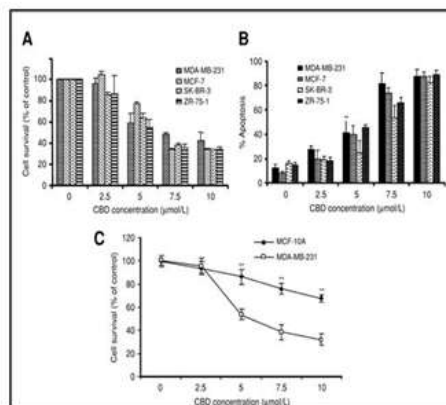
Abstract

Cannabidiol (CBD) is a major non-psychoactive constituent of cannabis. It is considered an antineoplastic agent as the basis of its in vitro and in vivo activity against tumor cells. However, the exact molecular mechanism through which CBD induces the activity is yet to be elucidated. Here, we have shown CBD-induced cell death of breast cancer cells, independent of cannabinoid and vanilloid receptor activation. Evidence supporting cannabidiol-mediated apoptosis is shown by the induction of caspase-3 and caspase-9. Evidence supporting autophagy is shown by the induction of Beclin-1 and LC3-II. CBD-induced cell death is inhibited by the inhibition of caspase-3 and caspase-9. CBD-induced cell death is inhibited by the inhibition of Beclin-1 and LC3-II. CBD-induced cell death is inhibited by the inhibition of caspase-3 and caspase-9. CBD-induced cell death is inhibited by the inhibition of Beclin-1 and LC3-II.

Introduction

Breast cancer is the second leading cause of cancer-related death in women in the United States [1]. Current breast treatment options are often limited by toxicity or acquired resistance, and novel agents are needed. We studied the effects of the Cannabis sativa constituent cannabidiol (CBD), a phytocannabinoid compound with reported activity in breast cancer cell lines, and elucidated its effects on the signaling pathway.

Cannabidiol is a major non-psychoactive constituent of cannabis. It is considered an antineoplastic agent as the basis of its in vitro and in vivo activity against tumor cells. However, the exact molecular mechanism through which CBD induces the activity is yet to be elucidated. Here, we have shown CBD-induced cell death of breast cancer cells, independent of cannabinoid and vanilloid receptor activation. Evidence supporting cannabidiol-mediated apoptosis is shown by the induction of caspase-3 and caspase-9. Evidence supporting autophagy is shown by the induction of Beclin-1 and LC3-II. CBD-induced cell death is inhibited by the inhibition of caspase-3 and caspase-9. CBD-induced cell death is inhibited by the inhibition of Beclin-1 and LC3-II. CBD-induced cell death is inhibited by the inhibition of caspase-3 and caspase-9. CBD-induced cell death is inhibited by the inhibition of Beclin-1 and LC3-II.



* 선행 논문에서 Cannabidiol이 호르몬 수용체와는 관계 없이 breast cancer cell에서 모두 독성을 보이는 결과가 있었음
- MDA-MB 231 (삼중음성유방암), MCF-7 (ER 양성), SK-BR3 (HER2 양성), ZR-75-1 (ER or PR or HER2 양성 유방암)

➤ 최종 실험 계획

- Time-dependent RT-PCR을 통해 CBD가 MCF-7 세포의 ER stress, Noxa signals을 확인하여 암세포 사멸 경로의 mRNA expression level 확인
- Time-and Dose-dependent Western blot을 통해 ER stress, Noxa signals을 확인하여 암세포 사멸 경로의 protein expression level 확인
- CBD-loaded micelle에 의해 유도된 세포사멸을 FACS를 통해 apoptosis, necrosis 확인
- Confocal laser scanning microscopy를 통해 CBD-loaded micelle의 세포 내 분포 확인
- *In vivo* P.O (Oral administration), IV (Intravenous injection)를 통한 PK, 생체 내 분포 확인

감사합니다.

2022.12.07.

우수연구자 초청 세미나

전북대 약대

정재훈 교수님

디지털치료제와 전자약에 의한 의약 산업의 패러다임 전환

전북대학교 약학대학 정재훈

약에 대한 패러다임의 변화

약사법 제2조(정의)

4. “의약품”이란 다음 각 목의 어느 하나에 해당하는 물품을 말한다.

가. 대한민국약전(大韓民國藥典)에 실린 물품 중 의약외품이 아닌 것

나. 사람이나 동물의 질병을 진단·치료·경감·처치 또는 예방할 목적으로 사용하는 물품
중 기구·기계 또는 장치가 아닌 것

다. 사람이나 동물의 구조와 기능에 약리학적(藥理學的) 영향을 줄 목적으로 사용하는 물품
중 기구·기계 또는 장치가 아닌 것

➤ A chemical substance that is used to prevent, diagnose, treat, or relieve symptoms of a disease or abnormal condition, or to promote well-being.

➤ A substance that affects the functioning of living things and the organisms.

➤ A substance which produces a biological effect in living organism.

4차 산업혁명과 의·약의 미래



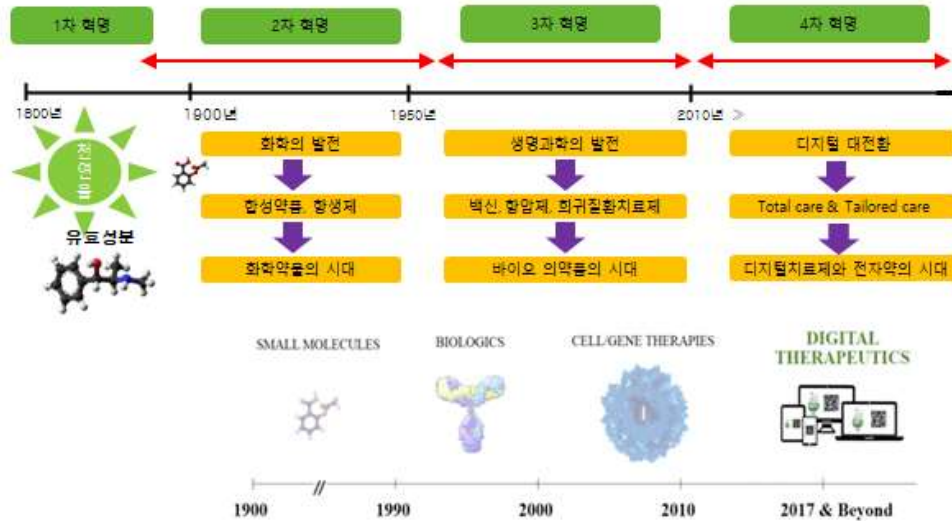
- 초연결(사물인터넷)과 초지능(인공지능), 초융합에 기반한 다양한 산업 분야의 혁명: 인공지능(AI), 사물인터넷(IoT), 마이크로 로봇, 드론, 자율주행, 가상현실(VR), 메타버스, 신소재 등
- 디지털전환(Digital transformation): 데이터·네트워크·인공지능(D.N.A)에 기반한 새로운 디지털 기술을 산업분야에 빠르게 접목시켜 업무를 혁신하고 새로운 시장과 고객들을 창출하고 있음
- Digital health care & Digital therapeutics, Electroceuticals

약에 대한 패러다임의 변화

Natural products	- BC 2000 수메르인들의 의약품제조법(정토판) - BC 1550 800여종의 처방(이집트 파피루스) - BC 250 365종 생약 기록(신농본초경) - AD 1610 동의보감 1805 Morphine(Freidrich Wilhelm Adam Serturner) ✓ quinine(1820), santonine(1830), cocaine(1860) 1885 Ephedrine(Nagai) 1897 황명수(민병초)
합성 의약품	1884 Antipyrin 1897 Aspirin(직접 합성 의약품) 1890 디프테리아 혈청 1900 Hormone 1910 Vitamin 1907 Salvarsan(P. Erlich) - Farbwerke Hoechst A. G. 1928 penicillin 1940s Antibiotic era 1950s DNA - new technology 1960s Etiology - Targeting 1970s Drug-design, Biotechnology(유전자재조합, 단일클론항체 제작 기술)
바이오 의약품	1980s Commercialization of drug discovery, Combinational chemistry ✓ 유전자재조합기술 - 인슐린(1982 릴리사), Interferon(1986) 1990s Antibody drugs(monoclonal antibody), Robotics 2000s 생물학적제제, 단백질의약품, 항체의약품, 세포치료제, 유전자치료제 ✓ 2012년 매출상위 10개 의약품 중 바이오의약품이 7종 ✓ 2019년 글로벌 바이오의약품 시장 규모 3180억 달러, 2023년 4420억 달러
디지털 치료제 & 전자약	2015 세계 첫 약물중독 치료용 소프트웨어 'reSET' - Pear Therapeutics 2017 아빌리파이 + 초소형 칩셋 = 치료 효율 상승 2018 아편중독치료용 어플리케이션- reSET-O FDA 승인 2019 electrical stimulation - 노인의 단기 기억력 20대 수준 회복(N. Neuro 러인하르프) ✓ 시장 확대: 2019년 3조 → 2025년 10조 - FDA 승인 제품: ▲피어 테라퓨틱스의 리셋(약물중독 후유증 치료) ▲카이아의 카이아 COPD 테라피 앱(만성폐쇄성폐질환 증상 감소) ▲네추럴 사이클의 모바일 앱(피임, 임신예방 효과) ▲레시프로일 펄스의 모바일 앱과 센서 '프로펠러헬스(COPD 증상 감소)' ▲테바의 프로에어 디지털일러(COPD 증상 감소) 등

약에 대한 패러다임의 변화

➤ 의약품 개발



약에 대한 패러다임의 변화

- 제3의 물결: 첨단 기술과 의약품 접목한 치료제 - 알약 형태의 전통적인 의약품에서 벗어나 스마트폰-웨어러블에 탑재된 치료용 소프트웨어나 전기 자극 치료
- 디지털치료제(Digital therapeutics, DT): 근거 기반의 고도화된 소프트웨어(모바일 애플리케이션, 게임, 가상현실, chat bot, 인공지능 등)를 활용하여 질병이나 장애를 예방, 관리, 치료하는 기술
- 전자약(Electroceuticals, EC): 전자(Electronic)와 약품(Pharmaceutical)의 합성어, 전류나 자기장 등의 에너지로 뇌 또는 기관, 신경, 특정세포를 자극해서 생리 기능을 변화시키고 치료 효과를 내는 기술
- 기술 특성: 생체표적 기술 + IT·AI 기술 + 음파노이드 + 마이크로 로봇 기술 등의 융합
- ▲ 독립형(Standalone) ▲ 증강형(Augment) ▲ 보완형(Complementary)
- DT 시장: 미국 시장조사기관인 Allied market Research의 2020년 보고서에 따르면 글로벌 디지털치료제 시장은 2018년 21.2억 달러에서 연평균 19.9%씩 성장하여 2026년에는 96.4억 달러에 이를 것으로 전망
- “바이오 의약품에서도 경험하지 않았는가? 국내 제약사들이 손을 놓고 있을 동안, 셀트리온이 바이오시밀러로 세계적인 기업이 되었다. 제약기업군이 디지털 치료제와 전자약 시장에서도 머뭇거리면 10년 이후 제약 시장의 파이를 모두 내어줄 수도 있다. 그런 위기감을 느끼지 않는 것이 이상하다.”



세계 디지털치료제 시장 전망
[출처: Euromonitor International(19), 의학저 제가공(2021 KFDA) 임상학회 발표자료]

디지털치료제(DT)의 개념

■ 디지털치료제 개념의 정립

- 2012년 미국특허와 상표사무소(USPTO)가 “디지털치료제(Digital Therapeutics)”라는 용어를 공식적으로 사용
- 2013년 Omada Health의 CEO였던 Sean Duffy가 그들의 온라인 코칭 소프트웨어를 디지털치료제로 묘사하면서 디지털치료제를 “의료적 또는 정신적 이상을 치료하기 위하여 디지털과 온라인 건강 기술을 활용하는 보건 영역과 치료 옵션(a health discipline and treatment option that utilizes digital and online health technologies to treat a medical or psychological condition)”으로 정의
- 2015년 Sepah 등이 디지털건강(Dital Health)을 세분화하며 디지털치료제의 개념과 범위를 다시 정함
 - ✓ “evidence-based behavioral treatments delivered online that can increase accessibility and effectiveness of healthcare(의료의 접근성과 효율성을 높일 수 있는 온라인으로 제공되는 근거 기반 행동 치료)”
- 2017년 디지털치료제연합(Digital Therapeutics Alliance, DTA)이 창설되어 새로운 분야에 대한 글로벌 표준을 제시
 - ✓ DTA는 디지털치료제를 “delivering evidence-based therapeutic interventions to patients that are driven by software to prevent, manage, or treat a medical disorder or disease(질병이나 의료적 장애를 예방, 관리 또는 치료하기 위하여 소프트웨어로 환자에게 행해지는 근거기반 치료 중재)”로 정의
 - ✓ **핵 개념**: 근거 기반의 고도화된 소프트웨어(모바일 애플리케이션, 게임, 가상현실, chat bot, 인공지능 등)를 활용하여 질병이나 장애를 예방 또는 관리, 치료하는 기술

■ 디지털치료제와 건강추적 앱의 차이 [출처: Perspect Clin Res. 2020; 11(2): 54-58]

- ✓ 디지털치료제가 사람에게 적용되기 위해선 그 제품의 안전성과 유효성이 입증되고 적응증 등을 심사·검증 받아야 함

가이드 고리	디지털치료제	건강추적 앱
적용 범위	특정 증상 또는 질환에 초점	다양한 건강상태에 초점
전제 조건	규제 요건: 의료 인력 중 한 명에서 자기감 주파워 배포 시험	앱 스토어에서 다운로드하기 위한 최소 기술 요건
치료적 접근	실제(일상) 증거 수집 및 분석을 통한 안전성과 유효성 입증	임상적 이질에 관한 입증되지 않은 주장
적용 기작	기업 주도 기업과 개인간 거래(B2C)	기업과 개인간 거래(B2C)
복합성과 지조	응수도 또는 응수도(Compliance) 재해가 발생 또는 주어진 시간 내에 반복 중재	사용자 수/ 응수 직접 해설적
확급 접근	응수도에 따라 지불자에 의해 확급될 수 있음	소비자만 확급 접근

디지털치료제(DT)의 개념

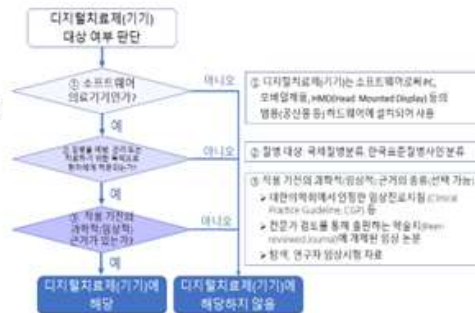
■ 디지털치료제의 특성

구분	기존 치료제(화합물)	디지털치료제	비고
공통점	• 병태생리적 변화의 유도 • 임상적으로 검증된 근거에 기반하여 질병의 예방과 치료, 그 증상의 완화 목적으로 사용할 수 있도록 허가됨		
차이점	핵심 기술	생체기술 + ICT 기술	• BIO-ICT 융합 • 생체표지-온가노이드 기술 • 비약물 요법의 디지털화 • ICT-AI, 가상증강현실 등
	작용기전	물리적 작용기전 또는 복합적 작용기전	연상 자극과 광-전-자기적 자극들과 생리 변화에 의한 병태생리 변화
	유효성	단일 또는 다중 효과 조정 가능	• 개인 맞춤형 심리·Life style • 의약품 등 여타 치료와 병용이 용이함 • 의약품+ 디지털치료제 병용을 통한 최적 치료 가능
	특성과 부작용	물리-약리적 특성과 부작용	디지털치료제의 특성과 부작용을 상대적으로 약하거나 아주 적게 고려할 수 있음
	투약 (복약)	• 다양한 투여 경로 • 다중 질환과 증상에 다 약제 동시 복용 • 별도의 복용관리 필요 • 상호작용 위험	• 투여 또는 처치 장치 • 하나의 장치로 다중 처치 가능 • 복용 실시간·지속 관리 가능 • 복용 관리 가능
	환자 모니터링과 자료화	• 한번 설치된 장치나 프로그램으로 환자 상태 등의 모니터링이 가능 • 자동적 자료화와 분석 • 추적 자료 근거 맞춤형 중재 가능	• 환자 상태 모니터링을 통해 진행 중인 처치의 조정과 추가 처치가 가능하고 자동 자료화와 분석 가능 • 환자 맞춤형 치료 가능
	개발비용	저비용과 고효율, 개발 절차와 기간, 비용이 상대적으로 적음	유효성과 안전성, 품질, 안정성을 확보하기 위한 비용이 상대적으로 매우 저렴
	사용비용	회합을 약품의 지속적 공급(복용) 비용이 요구됨	한번 설치된 장치나 프로그램의 재사용으로 추가 비용이 발생되지 않음

디지털치료제(DT)의 개념

■ 우리나라에서 디지털치료제의 개념

- ▶ 식품의약품안전처가 2020년 8월에 발표한 「디지털치료기기허가심사가이드라인」에선 “Digital Therapeutics”를 **디지털치료기기**로 정의
- ▶ 가이드라인에서 Digital Therapeutics 정의: 의학적 장애나 질병을 예방, 관리, 치료하기 위해 환자에게 근거기반의 치료적 개입을 제공하는 소프트웨어 의료기기 (SaMD, ※ 디지털치료기기의 사용은 치료적 개입이 필요한 “환자”를 대상으로 함)
- ▶ 소프트웨어 의료기기(Software as a Medical Devices, SaMD): 하드웨어에 종속되지 않고 의료기기의 사용목적에 부합하는 기능을 가지며 독립적인 형태의 소프트웨어만으로 이루어진 의료기기



- 의약품: 사람 또는 동물의 질병의 진단·치료·경감(輕減)·처치 또는 예방의 목적으로 사용되는 것으로 기구·기계가 아닌 것

디지털치료제(DT)의 개념

■ 디지털치료제의 범위



[출처: J. Family Med. Primary Care, 9(5): 2207-2213]

- ▶ 치료 범위: 만성질환에서 ⇄ 뇌질환과 항암까지

▶ 사업화 방식

- ✓ 국외: 다국적 제약사가 기술-벤처를 인수 합병 또는 합작회사 설립, MOU 예) GSK + Verily(구글 자회사) ⇒ Bioelectronic medicines 개발을 목표로 Galvani Bioelectronics를 설립
- ✓ 국내: 병원+벤처회사 협력 연구 개발, MOU 수준 예) 휴온스와 뉴아인의 MOU
- ▶ 국내 전문가 양성: 의과대학과 병원, 공과대학이 주도

■ 디지털치료제 관련 이해관계 군



디지털치료제(DT)의 개념

다국적 제약사의 디지털 치료제 관련 스타트업과 협력 내용

회사	시기	협력 참여 글로벌 제약사	협력 내용
Amblyotech	2020년	Novartis	인수
Voluntis	2017년	Sanofi	당뇨병 치료제 관련 비독점적 협력
	2018년 (종료)	Roche	고형암 관련 공동개발
	2018년	AstraZeneca	난소암 관련 공동개발
	2019년	Novartis	유방암 관련 공동개발
	2020년	BMS	알 관련 공동개발
Mysugr	2017년	Roche	인수
Noom	2019년	Novo Nordisk	비만 치료제 개발 관련 협력
Omada health	2019년	Abbott	당뇨병 치료제 개발 관련 협력
Pear Therapeutics	2018년	Sandoz (Novartis 자회사)	reSET과 reSET-O의 시장 출시 협력
Happify Health	2019년	Sanofi	공동연구 및 라이선싱 계약 체결
Click Therapeutics	2019년	오츠카 제약	\$302 M 규모 계약, 우울증 치료제 공동 개발
	2020년	Boehringer Ingelheim	\$500 M 이상 규모 계약, 조현병 치료제 공동 개발
Harman (삼성전자 자회사)	2020년	Roche	자폐증 환자 디지털 치료제 공동 개발
Akili interactive	2019년	Shionogi	125M\$ 규모 계약, ADHD, 자폐증 치료제 공동 개발

출처 : 최윤섭의 헬스케어 이노베이션(www.yoonsupchoi.com) 및 HIT뉴스(2020), 동아비즈니스리뷰(2020), 바이오스펙테이터(2020) 등에서 일부 수정 및 편집

KISTEP 기술개발로드맵 2020-15

디지털치료제 개발 사례

〈표 2〉 해외 디지털 치료제 미국 FDA 승인 사례

개발사(국가)	제품 이름	기능	장치	파트너 제약회사	승인 날짜
Pear Therapeutics (미국)	reSET	약물중독 치료	모바일 앱	Novartis Sandoz	2017. 9. 14
	Somryst	불면증 치료	-	-	2020. 3. 26
Palo Alto Health Science(미국)	Freespira	외상 후 스트레스 및 공황장애 치료	앱, 호흡 측정 장치	-	2018. 8. 23
Akili Interactive (미국)	EndeavorRx	소아 ADHD 치료	게임	Shionogi (일본, 대만)	2020. 6. 15
Voluntis(프랑스)	Insulia	2형 당뇨병 치료	모바일 앱	Safino	2017. 11. 7

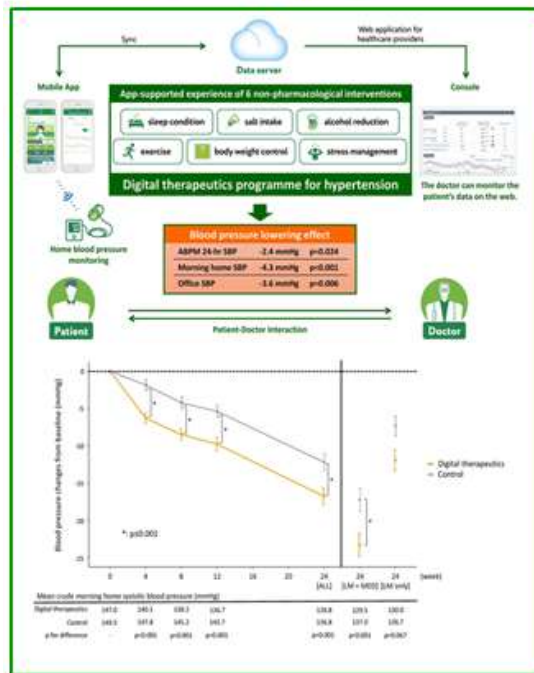
자료: DTA(2020), "FDA Clearances received by DTA Member Companies"; 각 회사 웹사이트



- 399명의 환자 중 대조군(CTP 단독)이 17.6%가 약물사용을 중지한 반면 reSET 처치를 받은 사람의 40.3%가 약물사용을 금지하였다.
- Kaplan-Meier 방법으로 약물사용 유지의 개연성을 평가한 결과 코호트 II에서 대조군의 개연성이 36.8%인 반면 reSET 처치군은 23.8%였다.



디지털치료제 개발 사례: 혈압강하제



ESC European Society of Cardiology
FASTTRACK CLINICAL RESEARCH Evidence-based and prevention

Efficacy of a digital therapeutics system in the management of essential hypertension: the HERB-DH1 pivotal trial

Kazumasa Kario^{1,2}, Akihito Yamura^{3,4,5}, Norihito Harada⁶, Ayako Ohtsuka⁷, Kiyoshi Nakagawa⁸, Tetsuya Taniyama^{9,10}, and Etsuko Hara¹¹

■ 총 390명 대상 임상시험

- ✓ 수면 조건과 염분 섭취, 알코올, 체중 조절, 스트레스 관리와 같은 6종 비약물적 중재를 시행
- ✓ 그 중에는 8단계 즉, 생활습관, 심리적, 사회적 데이터에 기초한 교육 중재와 앱-지원 생활 개선, 자가 계획과 평가 도구
- ✓ 스마트폰을 통해 HERB 소프트웨어 방식으로 12주와 24주간 효과를 관찰

■ 결과

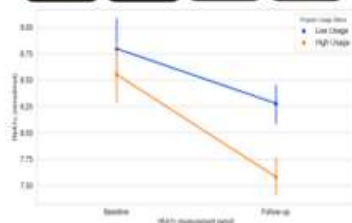
- 24시간 수축기 혈압이 디지털치료 시행(HERB)으로 4.9 mmHg 감소, 낮 동안 수축기 혈압이 7.3 mmHg 감소, 밤 동안 1.0 mmHg 감소, 아침 시간 수축기 혈압이 10.6 mmHg, 저녁 시간 8.5 mmHg, 업무 시간 13 mmHg 감소.
- 디지털치료의 시행으로 12주간 아침 시간 수축기 혈압이 10.6 mmHg 감소한 것은 기존 치료법의 혈압강하제 복용(62.6일간)으로 11.5 mmHg 감소한 것과 비교할 만 한 수준
- 24주간 디지털치료를 시행한 경우 아침 시간 수축기 혈압이 17.1 mmHg 감소.
- 시가 루서로 속도로 특정 분야에서 인간의 뇌 역할을 수행하였듯이 빅데이터와 시가 강력한 디지털 치료 기술은 빠른 시간 내에 혈압강하제(화학적 약물)의 효능성(유효성과 안전성)을 수행할 것이 자명

디지털치료제 개발 사례: 혈당강하제



■ Vida Health 프로그램: 258명, 90일간 HbA1c의 값의 추적

- 평균 HbA1c가 -0.81 감소하는 것으로 나타났고, 고위험군으로 간주되는 참가자(기초 HbA1c ≥8)에서 HbA1c의 평균 감소(평균 -1.44점, SD 1.86) 값이 더 높음
- 근거 기반의 디지털 중재는 이미 당뇨병 관리와 HbA1c 개선에 효과적인 솔루션 도입 수 있듯이 입증



■ 스마트인슐린

스마트인슐린을 환자의 당뇨관리 앱이 탑재된 스마트폰과 연동시킴으로써 단순히 혈당을 모니터링할 뿐만 아니라 환자의 전반적 정보에 기초하여 인슐린의 최적 투약 서비스가 진행될 수 있도록 진화

디지털치료제에 따른 혈당변동성(GV) 개선 효과 [출처: J. Diabetes Sci. Technol. 2021, 15(5):1188-1189] 참조: GV 평균



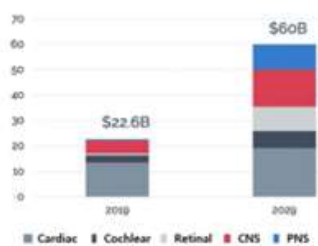
스마트인슐린의 로드맵 [출처: J. Diabetes Sci. Technol. 2021, 15(5):1188-1189]

전자약 (Electroceutical)

- 물리적 신호로 신경을 포함한 다양한 세포-조직-장기 등을 자극하여 병태 생리환경을 개선 또는 정상화 함
- 0000메디헤어는 헬멧처럼 쓰는 탈모 치료 전용 의료 기기인데, 식품의약품안전처에서 '의료용 레이저 조사기 3등급'에 해당하는 의료 기기로 허가받음
- 성인 남녀 46명을 대상으로 진행한 임상 결과에 따르면 0000 메디헤어를 27분 모드로 주 3회씩 총 16주간 사용한 참가자들의 모발은 대조군과 비교해 1cm당 밀도가 21.64%, 모발 굵기는 19.46% 증가



Bioelectronic Medicine Market Value, US\$ Billions, 2019-2029



출처: Alliance for Advancing Bioelectronic Medicine, "Building a bio-electronic medicine movement 2019: insights from leaders in industry, academia, and research, Bioelectron Med, vol. 6, 2020, 1.

그림 8 전자약 시장 성장 규모 예측

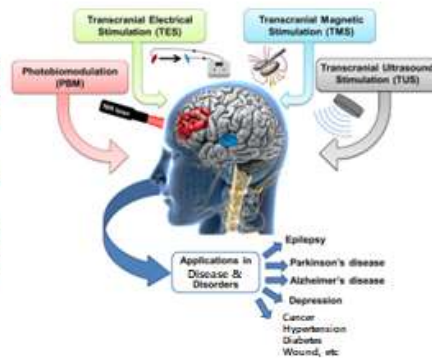
LG화학 헬멧형 탈모기 상용화



전자약 (Electroceutical) 개념

- 전자약 - **인체에 미식 (부작, 비접촉?)하는 치료용 전자 장치**, 전자(electronic)와 약품(pharmaceutical)의 합성어로 약물 대신 전기자극을 줘서 질병을 치료하는 약을 일컫음
- IT산업과 제약산업이 결합해 태어난 융합의 결과물로 뇌와 신경세포에서 발생하는 전기신호로 질병을 치료하는 전자장치를 말함. 넓은 의미에서는 심장박동기나 인공고각 등 전통적인 의료용 미식장치도 전자 약으로 분류됨
- 1958년 순환기내과에서 시작: 스톡홀름에 있는 Karolinska 연구소의 Ake Senning 박사가 심부전증으로 고생하던 43세의 남성 환자에게 삽입형 심장박동기(implantable pacemaker)를 처음으로 설치
- 2013년 영국 GSK의 Kristoffer Famm이 'Nature'지에 "A jump-start for electroceuticals"를 게재하며 전자약을 미래 치료 기술로 소개
- The Brave New Future - Mishra(2017)

- 원리: 인위적으로 또는 신체 신호에 반응하여 생성되도록 설계된 장치를 통해 발생하는 전기 신호로 신경을 포함한 다양한 세포-조직-장기 등을 자극하여 병태 생리 환경을 개선 또는 정상화함
- 기존 의약품이 화학적 변화를 통하여 생체 환경을 조절 하였다면 전자약은 전기적 변화로 생체 환경을 조절
- 화합물질이 제한된 생체 환경에 적정 강도로 개입하는 것은 어렵지 않았지만, 인위적 전기신호를 개입시켜 최적의 생체 환경을 형성하는 것은 매우 복잡하고 어렵다.



Life Sciences 307: 120869, 2022

전자약 (Electroceutical) 개념

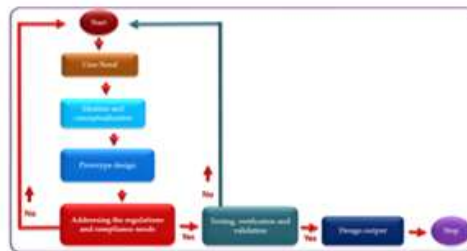
- 전자약이 되기 위해선?
 - 전기 신호로 생체 신호를 조절할 수 있어야 함: 생체 반응 유도
 - 신호를 개입시킬 위치를 특정하는 것
 - 특정 부위에 제한 적으로 신호를 부과하는 것
 - 적정 전기신호의 강도를 설정하는 것
 - 전기신호의 시점과 지속기간, 빈도를 설정하는 것
 - 안전성과 편리성, 경제성을 확보하는 것 등

전자약으로 정복 가능한 질병들



Getting better with electroceuticals: Interventions & Care 2016

- 전자약을 위한 기술?
 - Bio-Tech: organoid, bio-sensor
 - Digital Tech
 - Micro-Robot
 - Electrical generator: Nanogenerator
 - AI
 - Material engineering



Micromachines 2022, 13(2), 161; <https://doi.org/10.3390/mi13020161>

전자약 기술: 부착형, 삽입형



Lo et al., 2021, Advanced Science
Wearable and Implantable Electroceuticals for Therapeutic Neurostimulation

소형화(miniaturization)와 지능화(intellectualization)로 무장한 전자약이 유연성(flexibility)과 신뢰성(reliability), 편리성(convenience)을 갖춘다면 미래의 주요 치료기술을 넘어 건강 증진 또는 예방 기술로 확장될 것이 확실해 보인다.

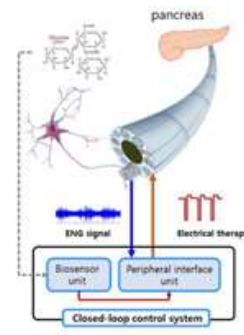
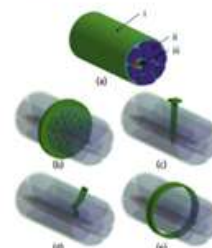


그림 5 삽입형 전자약 시스템 개념도



출처: Reproduced from C. Roud et al., "Peripheral nerve tissue interface: a review of electrodes," 2016, vol. 5, pp. 11-16, CC BY 4.0.

그림 5 삽입형 전자약 시스템 (a) 신경근육계(neuromuscular), (b) 자율신경계(autonomic), (c) 심혈관계(cardiovascular), (d) 호흡기계(respiratory)

전자약 (Electroceutical) 개발

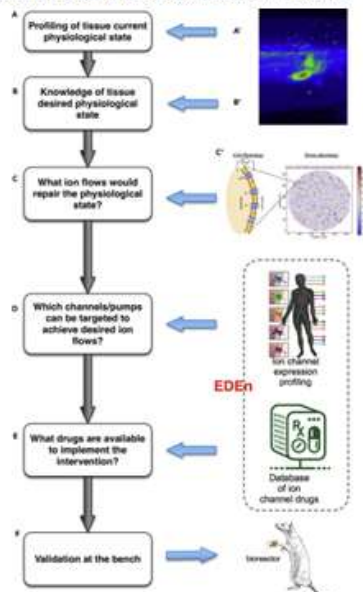
Table 1. World's electroceutical device developing companies.

Retinal Prosthesis Devices	Implantable VNS Devices and External VNS Devices	Cardiac Pacemaker Devices	Deep Brain Stimulation Devices
- Philips Healthcare - Retina Implant - Second Sight Medical Products - Bionic Eye Technologies - Bionic Vision Australia - Vision Care Ophthalmic Tech. - Abbott Laboratories	- Enteromedics, Inc. - ElectroCore, Inc. - Boston Scientific Corp. - NeuroMetrix, Inc. - Inspire Medical Systems, Inc. - LiveNova, PLC. - Electrocore, LLC. - Parasym Ltd. - Innovative Health Solutions - IVNS Technologies GmbH	- Medtronic, PLC. - Boston Scientific Corp. - Biotronik - LiveNova, PLC. - Abbott Laboratories - Medco S.P.A. - Shree Paceronix Ltd. - Ospika Medical, Inc. - Lepu Medical Group	- Medtronic plc. - Boston Scientific Corp. - Abbott Laboratories - Aleva Neurotherapeutics SA - Deep Brain Innovations, LLC - Beijing Pins Medical Co., Ltd.

Micromachines 2022, 13(2), 161; <https://doi.org/10.3390/mi13020161>

전자약 (Electroceutical) 사례

EDEn – Electroceutical Design Environment: An Ion Channel Database with Small Molecule Modulators



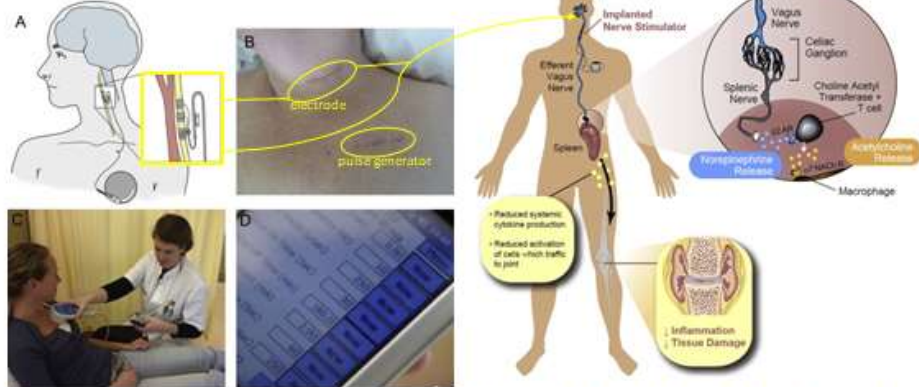
iScience, 2019, 11: 42-56



- 1988년 이식형 미주신경자극 장치가 사람에게 적용
- 1997년 미국 FDA는 불응성 뇌전증 치료를 위한 이식형 미주신경자극 장치(VNS Therapy®)의 사용을 승인
- 그 이후 그 용도를 우울증과 편두통, 군발 두통, 비만까지 확대
- 2005년 미국 FDA는 우울증 치료를 목적으로 Cyberonics가 제조한 미주신경 자극 장치를 승인
- 미주신경 자극이 노르아드레날린과 세로토닌 신경 전달을 강화

전자약 (Electroceutical) 사례

류마치스 치료: Vagus nerve stimulation



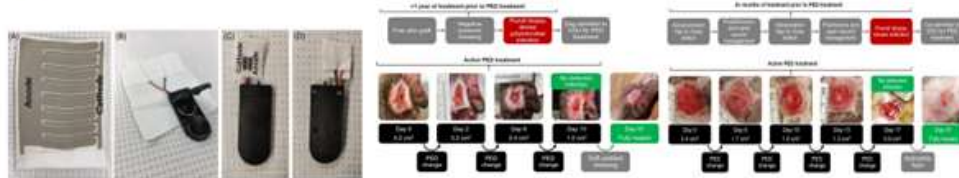
Vagus nerve stimulation (VNS) from surgery to stimulation settings. A. Implantation of the three helices around the left vagus nerve containing a positive electrode, negative electrode and an anchor tether. The electrodes are connected to the lead, which is attached to the pulse generator. B. Marks of the implantation 6 months after the operation. There are two incisions made to implant the VNS device. The first horizontal incision is made in the neck to implant the helices around the vagus nerve, the lead is tunneled under the skin and connected to the pulse generator located below the left clavicle on the pectoral muscle, which requires the second horizontal incision. C. With a wand above the pulse generator and a read-out handheld computer, the settings of the pulse generator can be changed or stimulations can be evaluated. D. Example of stimulation settings on the handheld computer.

Electrical stimulation of the parasympathetic vagus nerve leads to activation of the sympathetic splenic nerve which produces norepinephrine in close proximity to choline acetyltransferase positive (CHAT+) T-cells. The CHAT+ T-cells are able to produce the anti-inflammatory mediator acetylcholine, which can bind to the nicotinic acetylcholine receptor type 7 ($\alpha 7nAChR$) and reduces cytokine production. This results in reduced inflammation in the joints.

Best Practice & Research Clinical Rheumatology, 2014, 28(4): 625-638

전자약 (Electroceutical) 사례

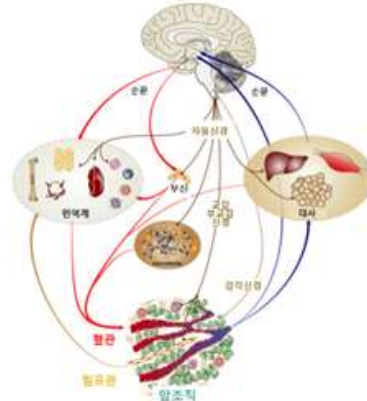
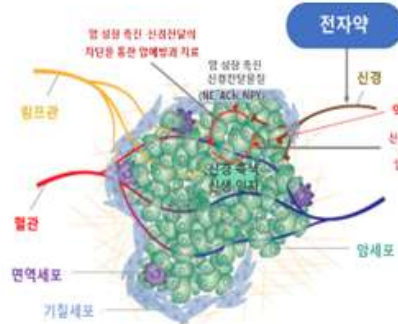
Printed Electroceutical Dressing(PED) – wound healing



개(왼 편)와 고양이(오른 편) 상처에 PED를 적용한 결과

- **원리:** 감염이 일어나면 세균의 생물막(biofilm) 형성 등으로 인해 그 관리와 치료에 어려운 상황들이 지속된다. 박테리아는 상처부위에 정착하기 위해 정전기적 상호작용이 필요한데 이 과정에 전기적 방해를 가하면 상처부위에서 박테리아의 서식을 막을 수 있고, 박테리아의 서식이 없으면 상처조직의 치유 본능에 따라 손상 조직이 복구된다.
- **장치:** 10k Ω 안정 저항기를 통해 6V 배터리에서 최대 전류 600 μ A(또는 0.6mA)까지 적용 - (A) 맞춤형 디자인의 전극이 있는 7.5 × 7.5cm PED, (B) 및 (C) 개에 사용된 7.5 × 7.5cm PED는 의료 테이프로 가려진 회로가 6V 배터리 팩에 연결, (D) 고양이에게 사용된 1 × 2-cm PED는 동일한 크기의 양극과 음극을 사용하여 전극을 단순화하였고 6V 배터리에 연결되었음, 두 PED에는 전류를 0.6mA로 제한하는 안정기(10k Ω)가 설치되어 있다.
- **결과:** 개의 상처는 PED 적용 2일째부터 변화가 나타나기 시작하여 10일째부터 그 크기가 유의적으로 감소하였고 생검에서 감염이 감지되지 않았으며, 67일째 완전히 치료되었다. 고양이의 상처는 6일째부터 변화를 확인할 수 있었고 17일째 크기가 유의적으로 감소하고 생검 시험에서 감염이 감지되지 않았으며, 47일째 완전히 치료되었다.

전자약 (Electroceutical) 사례

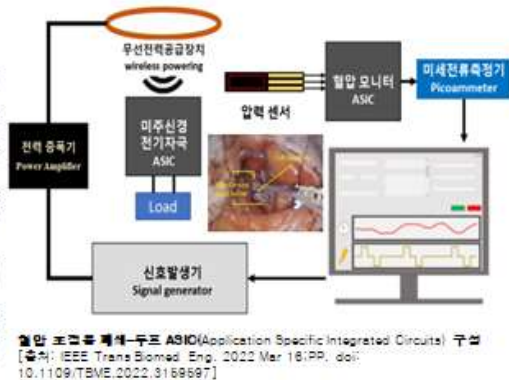


- 암 조직에 신경이 개입하여 종양 성장을 자극하는 신경 전달 물질 즉, 아세틸콜린(Ach), 노르에피네프린(NE), 신경 펩티드 Y(NPY) 등을 유리하고 그 물질들이 암세포의 증식과 전이를 촉진한다.
- 종식으로 증가한 암세포들은 신경 성장 인자(NGF)를 유리하고, NGF에 의해 새로운 신경 가지 즉, 축삭이 생성되며 그 결과로 신경전달이 강화되며, 그 강화가 암세포 증식을 더 촉진하는 악순환의 고리가 형성된다.
- 전자약이 그 악순환의 고리를 차단할 수 있고 아래의 기작을 통하여 항암작용을 나타낼 수 있다.
 - ① 종양세포에서 유래하는 신경 자극을 통하여 암의 발생과 진행에 영향을 준다.
 - ② 신경계는 DNA 돌연변이 및 종양유전자 관련 신호전달에 영향을 미친다.
 - ③ 신경계는 종양 관련 면역 반응을 조절한다.
 - ④ 종양 조직에 신경이 개입(innervation)한다.
 - ⑤ 신경에서 유리되는 신경전달물질은 종양의 성장과 전이에 영향을 미친다.
 - ⑥ 신경 종양의 분해 또는 조절이 암의 발생과 진행에 영향을 미친다.
 - ⑦ 종양 조직에서 유리되는 물질이 신경계에 영향을 미친다.

전자약 (Electroceutical) 사례

심부전과 고혈압 치료

- Dworkin 등이 대동맥과 갈동맥에 0.086-0.086 Hz로 전기 자극하면 수초 내 혈압이 조절될 수 있음을 확인
- 2018년 미국 FDA는 OVRx가 개발한 심부전증과 고혈압 치료에 위한 신경자극장치인 "Barostim Neo"를 시판전 승인(pre-market approval, PMA) - 이식하여 갈동맥들의 압수축을 자극하는 장치로 개발-부조 방식
- 메세-루프 방식의 Application Specific Integrated Circuits (ASICs: 응용 특이 집적회로)과 Microscale Pressure Sensor (MPS: 미세압력 센서)를 위에 적용하여 혈압 조절 효과를 달성
- ✓ 실험 쥐(Long Evans rats)에 두 개의 ASIC 칩: 마이크로-스케일 압력 감지 압력 센서로 혈압을 모니터링하고, 다른 회로로 전기 자극
- ✓ 압력센서가 대동맥에서 혈압을 감지하여 컴퓨터 정량하여 보내 컴퓨터가 이를 해석하여 (초 평균압력들 기준)으로 설정된 압력 역치에 따라 오른쪽 측부에서 미주신경에 심어진 전극(전기 자극기)이 켜지거나 꺼짐
- ✓ 전기자극으로 혈압이 떨어지는 것을 확인하고 결과를 통하여 얻어진 자료에 기초하여 대동맥압이 실험 역치에 도달하면 자극기가 켜지고 하혈역치에 도달하면 자극기가 꺼지도록 조절
- ✓ 전기자극의 강도(current amplitude of 626 μ A, pulse width of 1 ms, and frequency of 16Hz)를 고정



혈압 조절용 메세-루프 ASIC(Application Specific Integrated Circuits) 구성
[출처: IEEE Trans Biomed Eng. 2022 Mar 16;PP. doi: 10.1109/TBME.2022.3168997]

- 실험동물에서 37회의 전기 자극(미주신경 자극)으로 평균 혈압이 16.677 ± 8.125 mmHg 감소, 이는 임상적으로 유효할 수 있는 결과

DT와EC가 열어갈 미래

▪ Digital Therapeutics(DT)와 Electroceuticals(EC)의 작동원리

- ▶ 생체의 작동과 표현은 생체 내 물리적·화학적 변화에 기초한다. 운동신수의 멋진 활동과 수드사의 심오한 영상 모두 전기·화학·생물학적 기작으로 설명할 수 있다. 덕분에 전기·화학·생물학적 자극이 생각과 신체 활동을 변화시킬 수 있고, 질병이나 장애를 정상화할 수 있다. **DT와 EC의 작용 포적 역시 생체 분자이고 DT와 EC의 효과도 병태생리의 변화에 기초한다.**
- ▶ 생각훈련(심리 또는 알성치료 등)이나 행동 훈련(미술, 음악, 운동, 여행 치료 등)도 전기·화학·생물학적 변화를 일으킬 수 있다. 약물은 자극의 강도와 기간을 일정하게 통제할 수 있어서 생물학적 시계계를 기반으로 치료의 표준화가 쉬운 반면, 생각훈련이나 행동 훈련은 자극 강도를 계량화하기가 매우 어렵다 뿐만 아니라 반응자(반응체계)의 개체차가 심해서 표준화가 매우 어렵다. **디지털 기술이 비물리적 자극강도의 미세조절과 반응체계의 표준화·개별화를 가능하게 하고 있다.**
- ▶ 사람들이 오래 건강하게 살고 싶다는 욕구가 소멸되지 않는 한, **DT와 EC 기술은 의과학적 근거에 기반하여 치료 효능을 높일 수 있는 새로운 모드들을 창출하고 지속적으로 up-grade될 것이다.** 환자수준의 개선이나 맞춤형 치료모드 개발, 부작용 최소화, 과학적 치료 기전 규명 등 과제도 산재해 있지만 다수의 원천 아이디어들이 속속 임상시험에서 성공하고 있기 때문에 나머지 문제들은 돈과 시간이 해결해 줄 것이다.

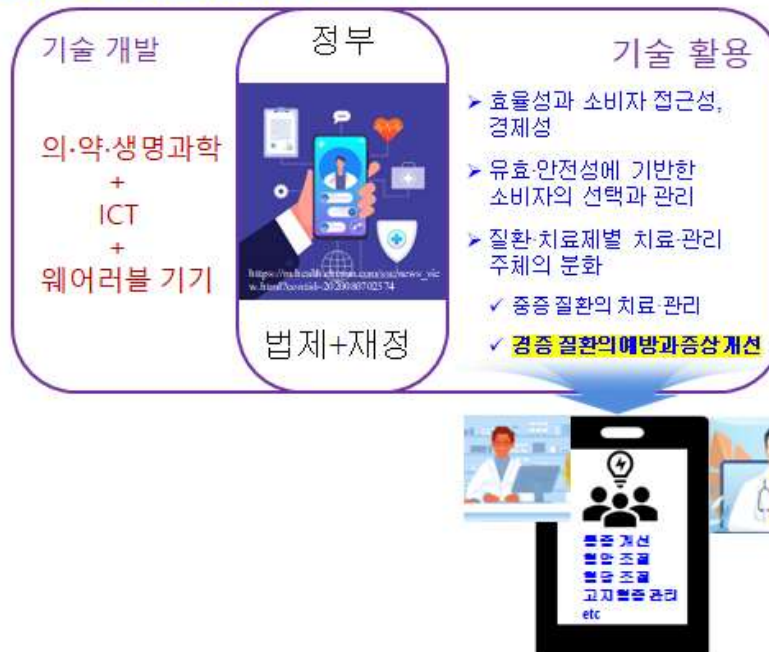
▪ DT

- ▶ 디지털치료제가 안목요법으로 질병을 진단·치료·경감(輕減) 또는 예방할 수 있음이 의과학적으로 입증되었고, 사람이나 동물에서 생화·심리·물리적 자극으로 생체의 전기·화학·생물학적 변화를 유도하여 신체의 구조·기능에 악리학적 효과를 유발한다.
- ▶ 대부분의 국가들은 디지털치료제를 새로운 의약품 수반으로 인정하고 적절한 제도를 마련하고 있고, 대다수 디지털치료기술 개발의 Leader는 생체 정보를 잘 이해하고 있는 의과학자들임을 유의
- ▶ **Bio-와 IT의 융합이지만 아이디어와 본리는 의과학 역량에 기반하고 있고, 이제, 만성질환 또는 관리 질환을 포적으로 하는 디지털 치료 기술의 개발은 속도전으로 진행되고 있음**

▪ EC

- ▶ 신경의 회화와 글리즘의 위축, 전신쇠약 등과 같은 증상들을 치료하기 위하여 전기 치료가 사용되고 있는데, EC는 각각에 대하여 맞춤형 치료 방안을 제시할 수 있고 환자가 병원에 가지 않고서도 지속적으로 치료할 수 있는 편리한 방안이 될 수 있음
- ▶ 웨어러블 EC는 근육사용 장애에 따른 근 위축과 마비를 포함한 근육 기능 손실을 예방할 수 있고, 정확한 매핑에 근거하여 특정 근육을 직접 자극할 수 있는 EC-모드가 개발되었고, 미세 신경 영역의 구축화와 나노스케일 전극의 개발로 그 효능성은 더욱 높아지고 있음
- ▶ **소형화(miniaturization)와 지능화(intellectualization)로 무장한 전자약이 유연성(flexibility)과 신뢰성(reliability), 편리성(convenience)을 갖춘다면 미래의 주요 치료기술을 넘어 건강 증진 또는 예방 기술로 확장될 것이 확실해보임**

DT와EC의 개발과 활용에서 약사의 역할



Thank you very much!

P팜뉴스



다제암치료제와 전이암에 의한 악성 신장암의 경우다
임 천환

주요 키워드: 다제암치료제, 전이암, 악성 신장암, 임 천환



임 천환은 다제암치료제와 전이암에 의한 악성 신장암의 경우다. 다제암치료제와 전이암에 의한 악성 신장암의 경우다. 다제암치료제와 전이암에 의한 악성 신장암의 경우다.



<https://www.pharmnews.com/news/>

2022.12.14.

우수연구자 초청 세미나

경희대 약대

이경태 교수님

New Drug Seeds and Development of Health Functional Food



Hydrangea serrata Serrige
(Nickname : Korean hydrangea)



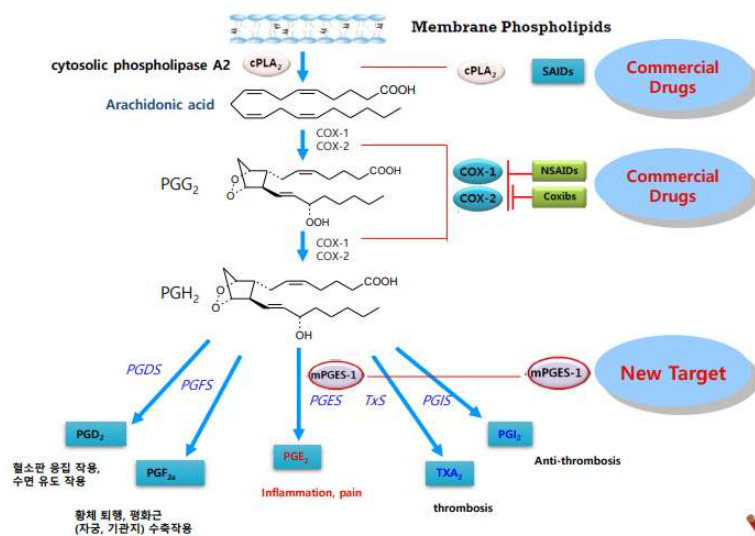
PART 1

mPGES-1 inhibitors as anti-arthritis efficacy



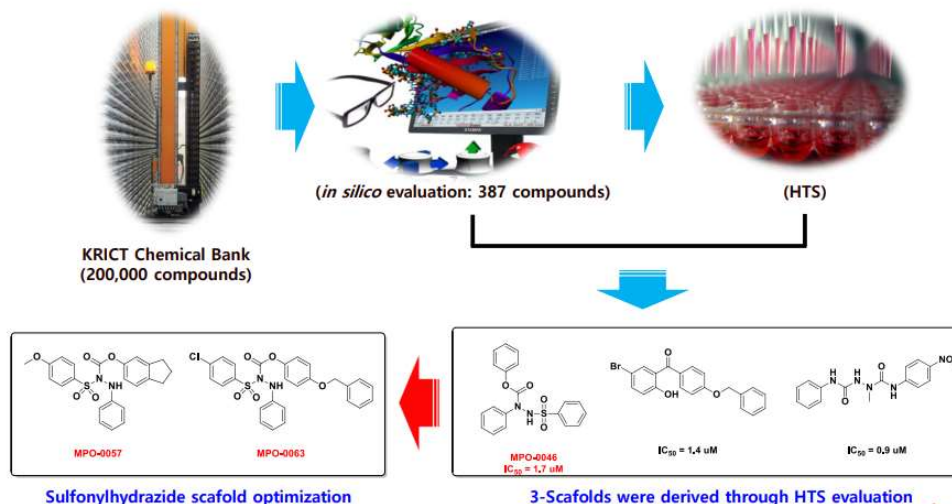
Unmet Medical Needs : Why mPGES-1 inhibitor?

Biosynthesis of Prostaglandins (PG)



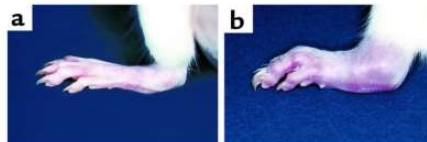
Synthesis of new skeletal compounds

3-Scaffolds were derived through HTS evaluation

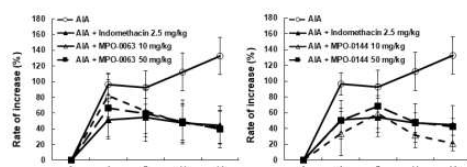


In vivo study

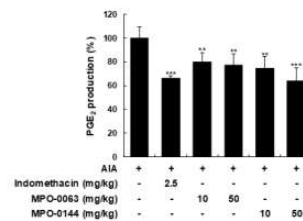
Inhibitory effects of MPO series on adjuvant-induced arthritis (AIA) model



Paw edema



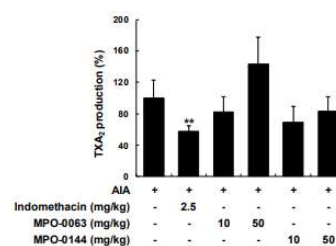
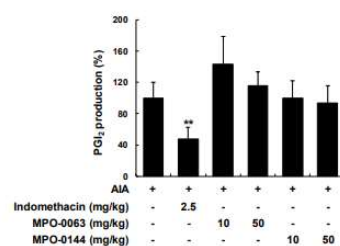
PGE₂ production



Cardiovascular- and gastritis-related side effects

PGI₂ & TXA₂

• Plasma

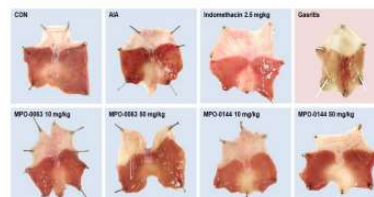


Liver/Kidney

plasma		AIA				
		Indomethacin 2.5 mg/kg	MPO-0063 10 mg/kg	MPO-0063 50 mg/kg	MPO-0144 10 mg/kg	MPO-0144 50 mg/kg
GOT (KamenimL)	34.55 ± 1.71	45.00 ± 4.50***	36.96 ± 0.07	38.73 ± 5.91	37.17 ± 9.66	33.73 ± 6.17
GPT (KamenimL)	25.59 ± 3.83	35.55 ± 2.89**	22.57 ± 4.93	27.42 ± 7.00	23.53 ± 9.06	17.72 ± 4.53
BUN (mg/dL)	8.88 ± 1.22	14.87 ± 3.76***	9.58 ± 1.86	10.33 ± 1.05	9.88 ± 2.68	10.94 ± 2.16
Troponin I (%)	100.00 ± 11.26	174.47 ± 30.39***	108.51 ± 42.48	86.93 ± 18.85	125.96 ± 34.39	109.57 ± 11.72



Gastric lesion

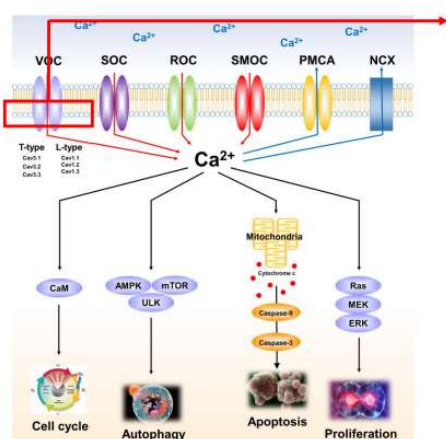


PART 2

Pharmacological Properties of KCP10043F, T-type Calcium Channel Blocker, as a Potent Chemotherapeutic Agent



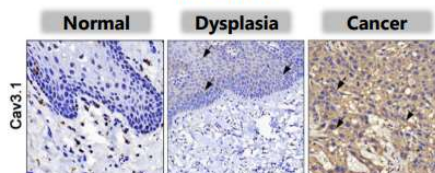
Calcium signaling & T-type calcium channel



*Sallán MC et al, T-type Ca^{2+} Channels: T for Targetable. *Cancer Res.* 2018, 78(3), 603-609.*

*Barceló C et al, T-Type Calcium Channels: A Potential Novel Target in Melanoma., *Cancers (Basel)*, 2020, 12(2), 391.*

T-type calcium channel ↑ in cancer

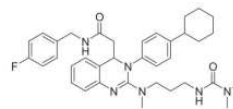


T-type Calcium Channel Blocker

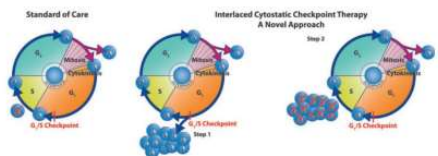


Mibefradil

FDA approval (orphan drug)
Ovarian cancer (2007)
Pancreatic cancer (2008)
Glioblastoma (2009)



KCP10043F



Compound	T-type Ca^{2+} Channel (α_{1G}) ¹ (IC ₅₀ : μM)
Control	—
KCP10043F	0.89 $\pm$ 0.06
KYS050905	0.51 $\pm$ 0.02
Mibefradil	1.34 $\pm$ 0.49

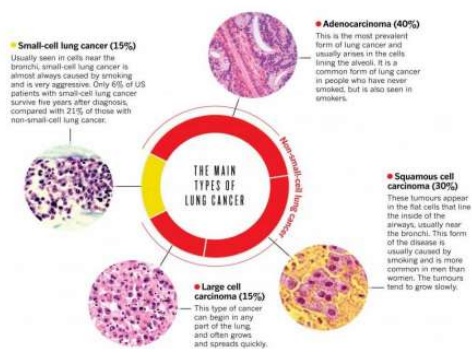
- Cell cycle arrest $\uparrow$
- Synergistic effect with etoposide

Matthias H *et al*, Timed sequential therapy of the selective T-type calcium channel blocker mibefradil and temozolomide in patients with recurrent high-grade gliomas, *Neuro-Oncology*, 2017, 19(6), 845–852,

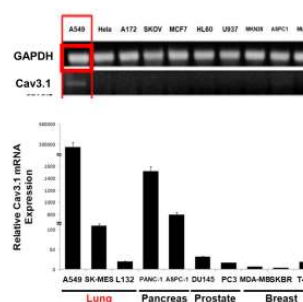
Byun JS *et al*, In vitro synergistic anticancer activity of the combination of T-type calcium channel blocker and chemotherapeutic agent in A549 cells, *Bioorg. Med. Chem. Lett.*, 2016, 26(3), 1073–1079.



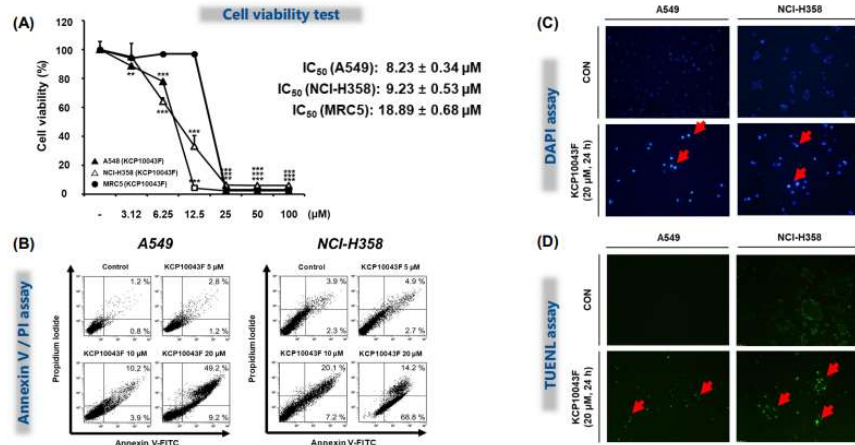
Non-small cell lung cancer (NSCLC)



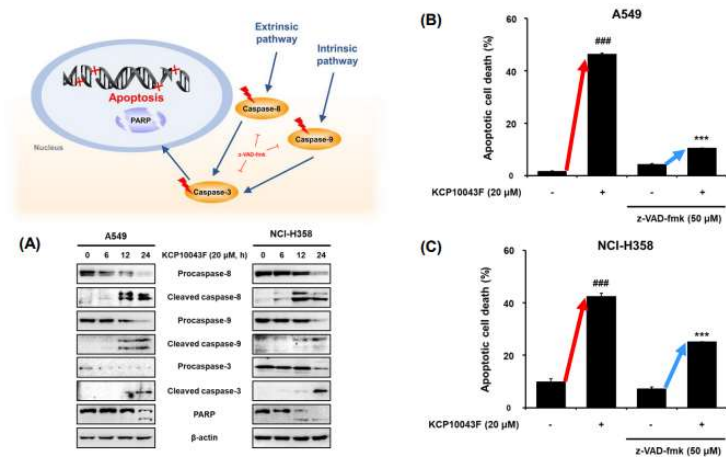
Cav3.1 expression



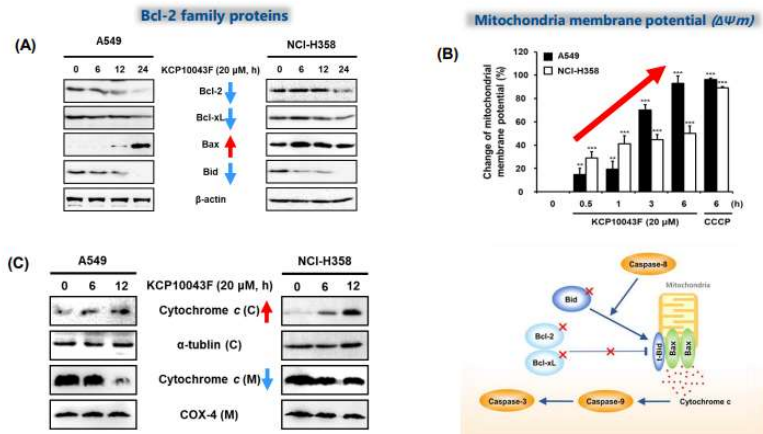
KCP10043F inhibits the proliferation of human NSCLC cells by inducing apoptosis



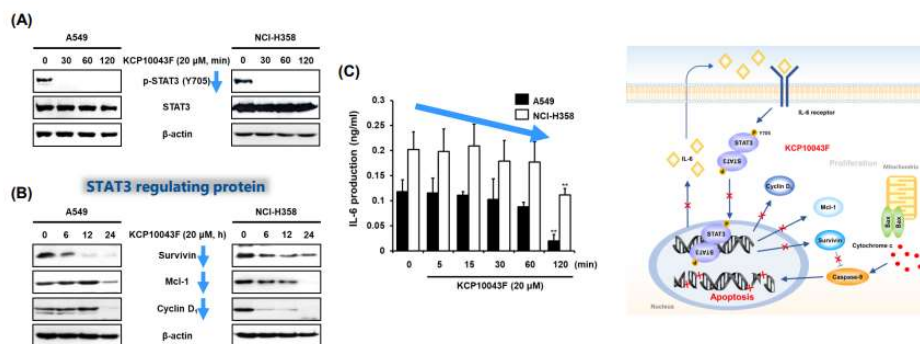
KCP10043F induces caspase-dependent apoptosis



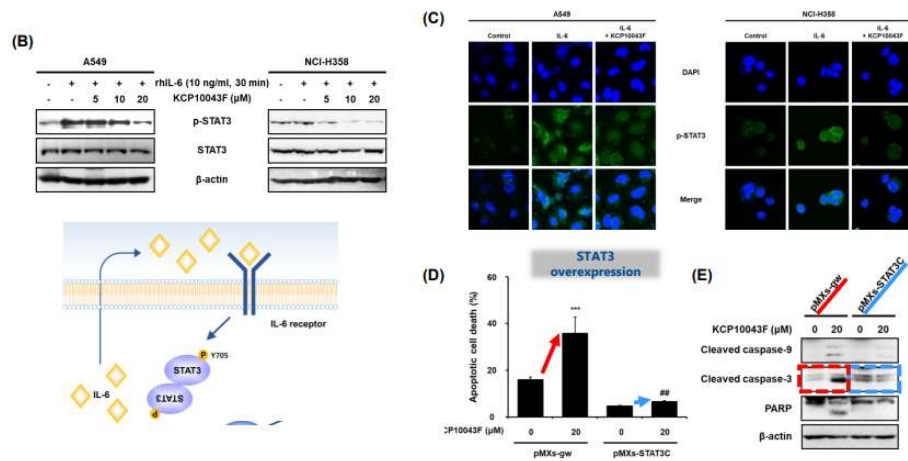
KCP10043F induces the release of cytochrome *c* into the cytosol and loss of $\Delta\Psi_m$



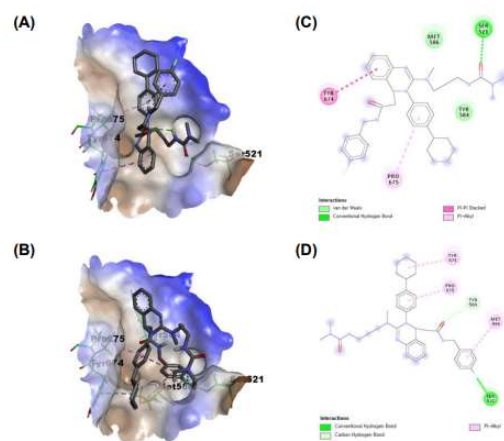
KCP10043F affects the STAT3 signaling pathway in NSCLC cells



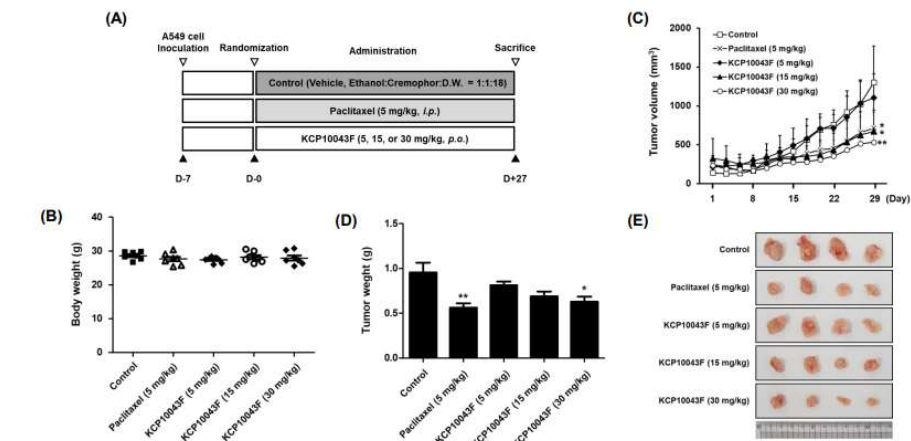
KCP10043F affects the STAT3 signaling pathway in NSCLC cells



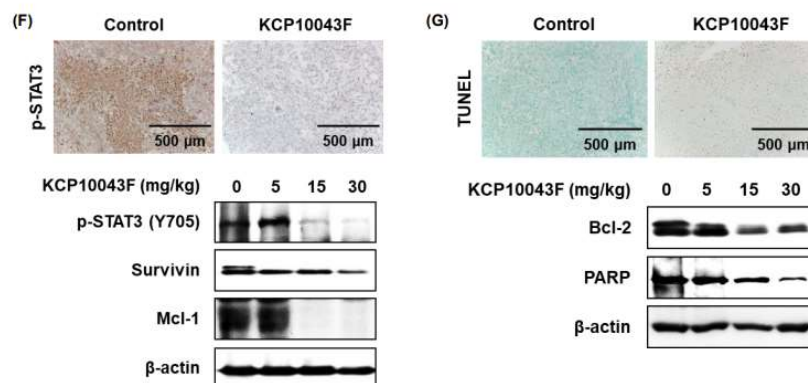
KCP10043F docks into the SH2 domain of STAT3



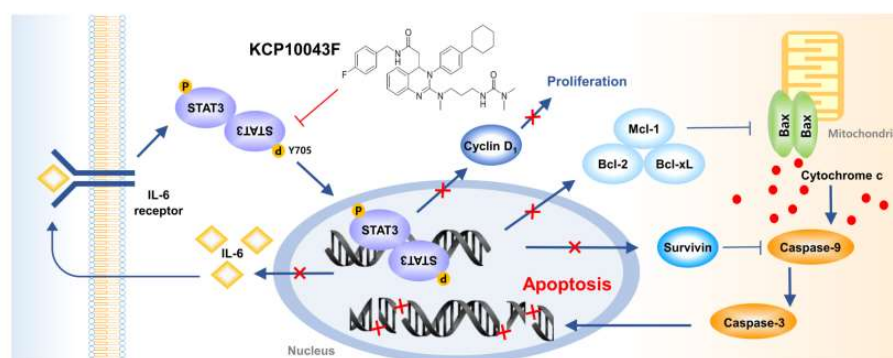
KCP10043F suppresses tumor growth through apoptotic cell death in A549 xenograft model



KCP10043F suppresses tumor growth through apoptotic cell death in A549 xenograft model



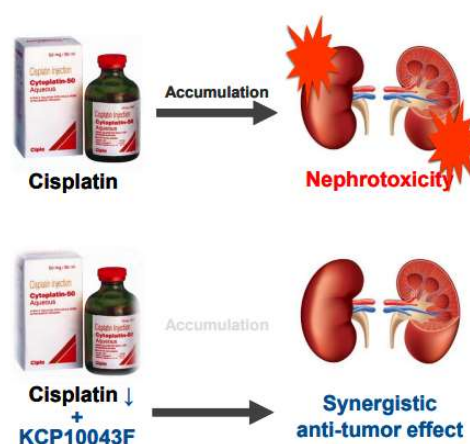
Conclusion



Combined therapy with cisplatin

Drug combination	CF	Interpretation ^a
Control	1.9	
KCP10043F (2.5 μM)	1.6/1.3 ^b	
KCP10043F (5 μM)	1.0/1.3	
KCP10043F (10 μM)	7.6/4.0	
Etoposide (50 μM)	33.0	
Etoposide (50 μM) + KCP10043F (2.5 μM)	36.9	Additivity
Etoposide (50 μM) + KCP10043F (5 μM)	43.4	Synergism
Etoposide (50 μM) + KCP10043F (10 μM)	83.1	Synergism
Cisplatin (30 μM)	17.0	
Cisplatin (30 μM) + KCP10043F (2.5 μM)	18.6	Additivity
Cisplatin (30 μM) + KCP10043F (5 μM)	24.8	Synergism
Cisplatin (30 μM) + KCP10043F (10 μM)	52.3	Synergism

Byun J.S. et al., Bioorg. Med. Chem. Lett., 26(3):1073-9, 2016.



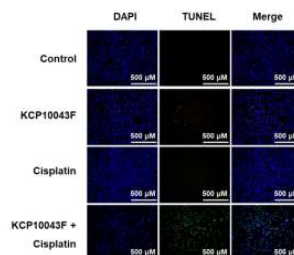
Combined treatment with KCP10043F and cisplatin induces apoptotic cell death in A549 lung cancer cells

(B)

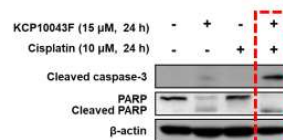
Combination index (CI)

KCP10043F (μM)	Cisplatin (μM)	Fa	CI
10	0.6	0.061	1.001
	1.2	0.078	0.944
	2.5	0.065	0.983
	5	0.07	0.968
	10	0.104	0.882
15	0.6	0.328	0.971
	1.2	0.371	0.932
	2.5	0.407	0.902
	5	0.446	0.895
	10	0.419	0.9

(C)



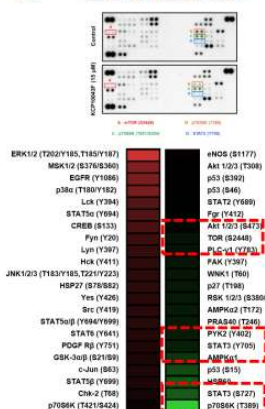
(D)



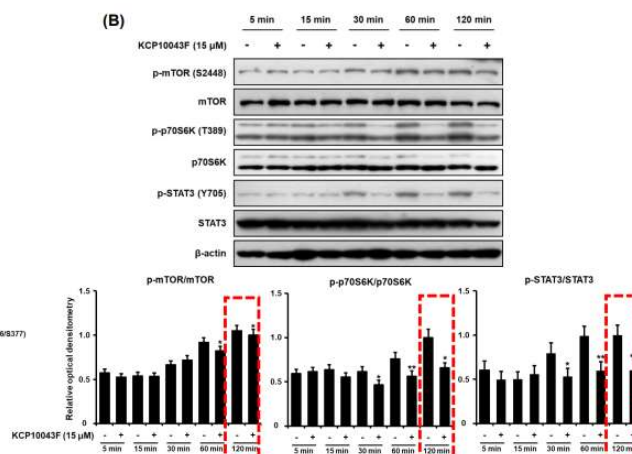
Combined treatment with KCP10043F and cisplatin synergistically downregulates the mTOR/p70S6K and STAT3 pathways in A549 human lung cancer cells

(A)

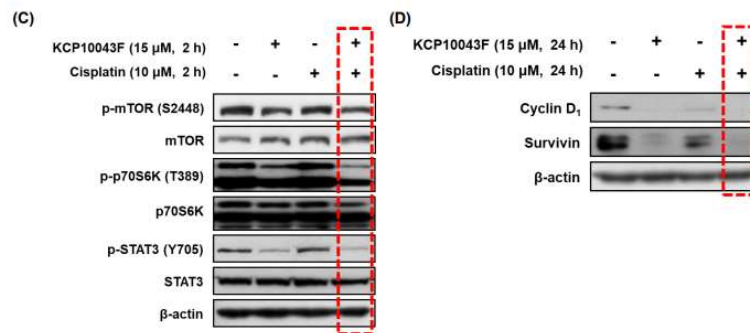
Phospho-kinase array



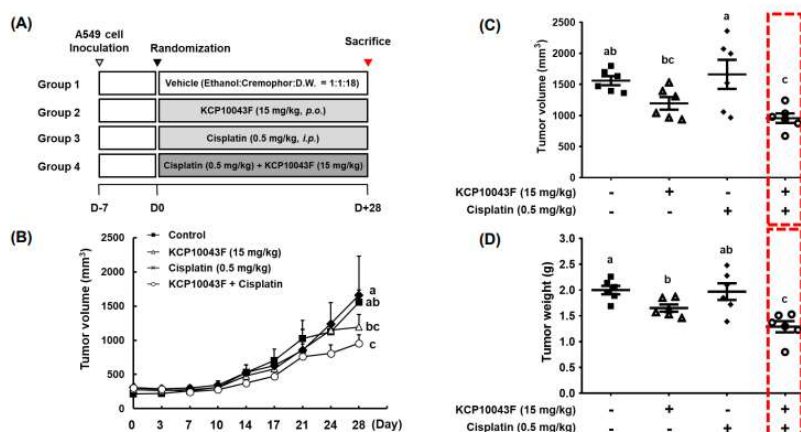
(B)



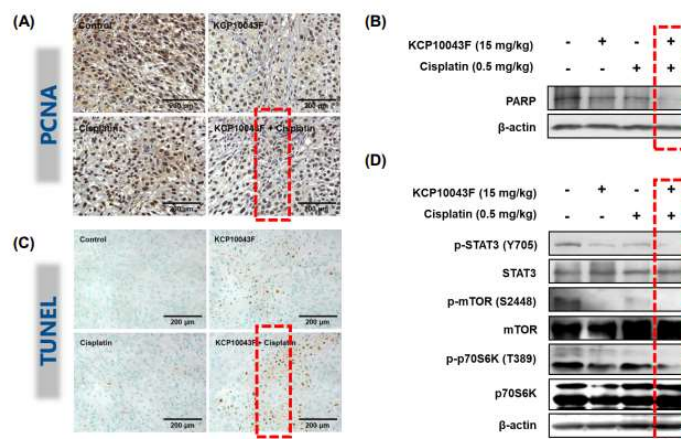
Combined treatment with KCP10043F and cisplatin synergistically downregulates the mTOR/p70S6K and STAT3 pathways in A549 human lung cancer cells



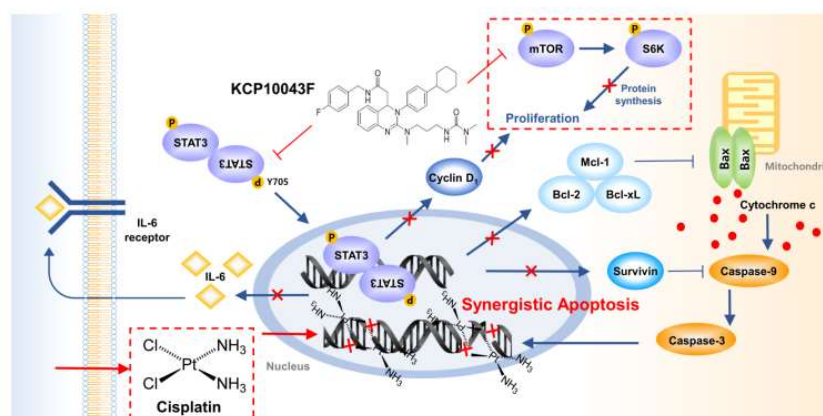
Combined treatment with KCP10043F and cisplatin exerts synergistic antitumor effects in an A549 xenograft animal model



Combined treatment with KCP10043F and cisplatin exerts synergistic antitumor effects in an A549 xenograft animal model



Conclusion



PART 3. Executive Summary : Standardized *Hydrangea serrata* (Thunb.) Ser. Leaves Extract

Ingredient Description



- History of use : Traditional tea in Korea
- Dosage Form : Powder
- Origin : **Plant based**
- Edible countries : **Korea, China, Japan, USA, Europe**
- Daily Doses : **300 ~ 600 mg**
(100% *Hydrangea serrata* leaf extract)

Functionality

Inner Beauty

Skin Health



- Skin hydration
- Protection on skin damages by UV rays

Body Care



- Weight management



R&D Background : Distinguish the Raw Materials

Morphological distinction

	<i>Hydrangea serrata</i>	<i>Hydrangea macrophylla</i>
Picture		
Scientific Name	<i>Hydrangea macrophylla</i> (Thunb.) ser. subsp. <i>serrata</i> (Thunb.) Makino	<i>Hydrangea macrophylla</i> (Thunb.) Ser.
Distribution	Jeonnam, Chungbuk, Gangwon	Gyeongbuk, Jeonnam, Gyeongnam
Description	Deciduous broad-leaved shrub	Deciduous broad-leaved shrub
Leaves	Oval or egg-shaped	Oval or broad oval
Flower	The bisexual flowers bloom from July to August	The neutral flowers bloom from June to July
Seeds	Ovoid or oval-shaped	-
Habitat	Mountain valley, a shady valley that grows up in the alley	It grows well in neutral soils, semi-chromatic plant

Genetic Distinction

- *Hydrangea serrata* seringe showed mutations in the base sequence by insertion/deletion, substitution, at 45, 83, 116, 170, 434, 435 and 574 in 1-nrDNA ITS results.
- The results of cpDNA trnL-trnF showed variations in the base sequence due to insertion/deletion, substitution at 558 and 587 to confirm the difference between the applied material and the similar species.

DNA	1-nrDNA	cpDNA trnL-trnF
Description	Nuclear DNA	Chloroplast DNA
1-nrDNA		
cpDNA		



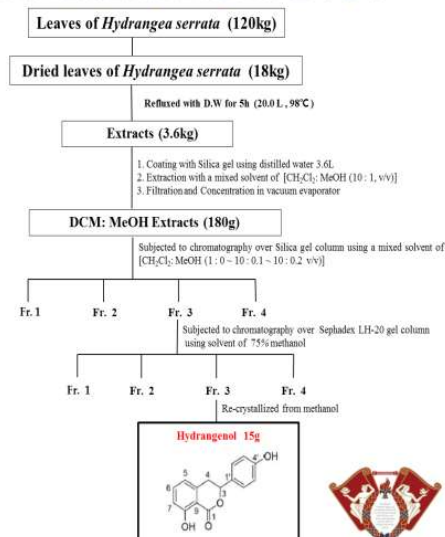
Active Ingredients and Standardization of *Hydrangea serrata*

수국잎추출물 활성물질 (지표물질 확인)

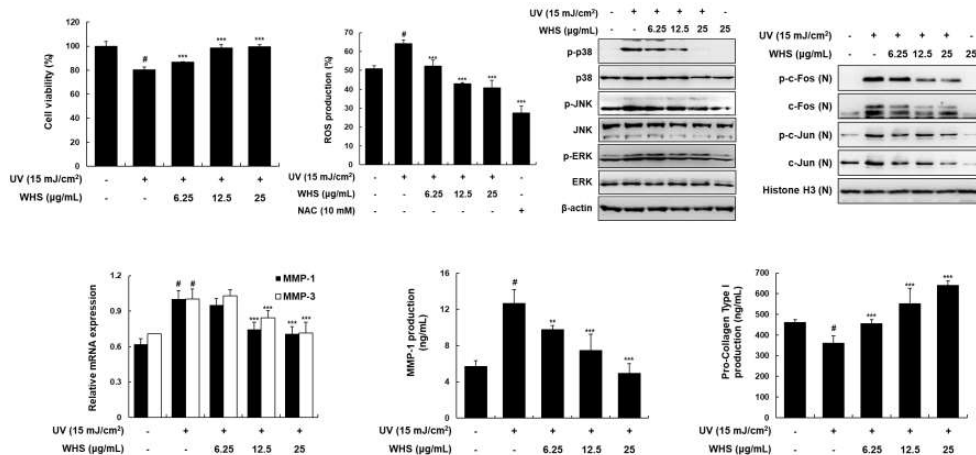
Compound (1 μ M)	HsGT			Hs8		
	Cytosynthetic effect	HA	MMP-1	Cytosynthetic effect	Pro-collagen Type I	MMP-1
Hydrangenol	0	0	X	0	0	0
Diam-sep5-Rt8.2	X	0	X	0	X	0
Thunberginol F	0	X	Δ	0	X	0
Thunberginol C	X	X	X	0	0	0
Thunberginol A	X	0	Δ	0	0	0
Hydrangenoside C	X	X	X	X	0	0
Hs 37-1	X	0	X	X	X	Δ
Hs 37-2	X	X	X	0	Δ	X
5-glucopyranosyl-hydrangeic acid	0	X	0	X	X	0
Hydrangenoside A	0	X	X	X	0	0

Compound (10 μ M)	HsGT			Hs8		
	Cytosynthetic effect	HA	MMP-1	Cytosynthetic effect	Pro-collagen Type I	MMP-1
Hydrangenol	X	0	0	0	0	X
Diam-sep5-Rt8.2	X	X	Δ	0	0	X
Thunberginol F	X	X	Δ	0	0	X
Thunberginol C	X	X	X	X	0	X
Thunberginol A	X	X	X	0	X	0
Hydrangenoside C	X	X	Δ	X	Δ	X
Hs 37-1	X	X	0	Δ	Δ	X
Hs 37-2	X	X	X	0	Δ	X
5-glucopyranosyl-hydrangeic acid	X	X	0	0	Δ	X
Hydrangenoside A	X	X	0	X	0	X

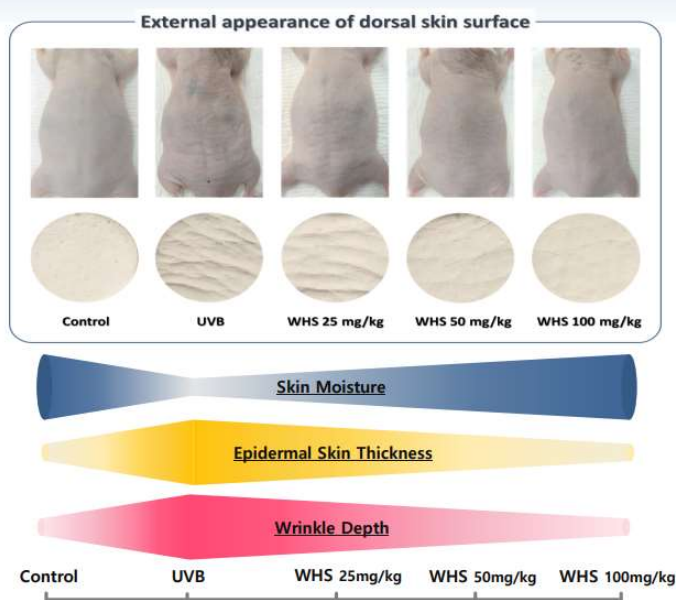
하이드란제놀 (Hydrangenol) 분리 정제



Skin health effects of WHS in UVB-induced Hs68 fibroblasts

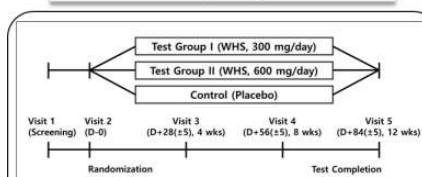


In Vivo Study of WHS for Skin Health



Clinical Trial of WHS for Skin Health

Study Design



○ Number of subjects included in the analysis of the results :

	300 mg	600 mg	Placebo	Total
Safety Set	50	50	51	151
PP Set	48	49	49	146

○ Outcome Measures

• Primary outcome measures

(1) Skin wrinkle (Visual assessment, Mark-Vu, Skin Visiometer® SV700)

(2) Skin moisture contents (Corneometer® CM825)

• Secondary outcome measures

(1) TransEpidermal Water Loss (Tewameter® TM300)

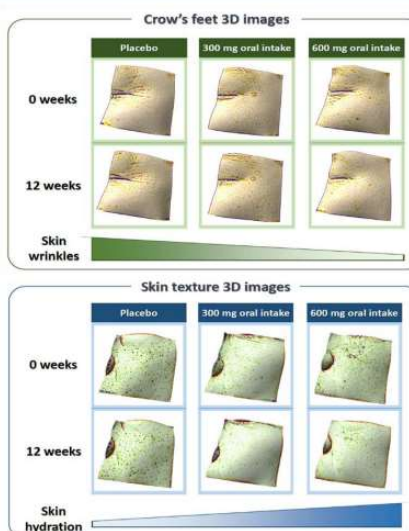
(2) Elasticity (Cutometer® Dual MPA580)

(3) Texture (Antera 3D® CS)

(4) Wrinkle roughness (Antera 3D® CS)

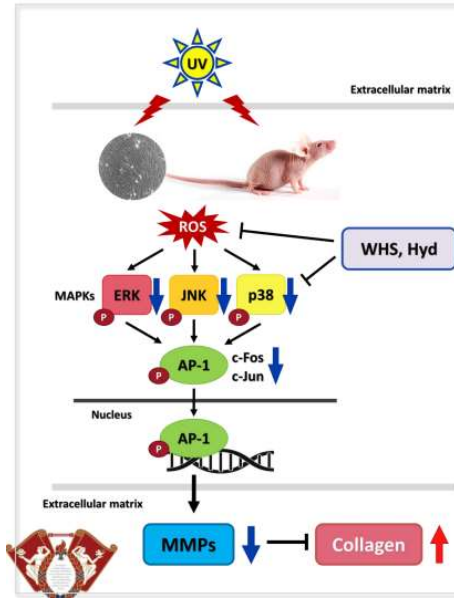
(5) Subject-assessed Global Aesthetic Improvement Scale (GAIS)

(6) Investigator-assessed GAIS



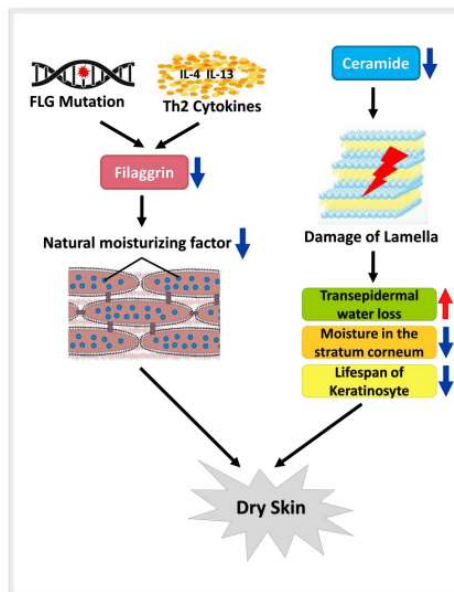
* Research Site : Global Medical Research Center

Help to maintain skin health from skin damage by UV radiation



기전	Biomarker	In vitro	In vivo	Human
피부 보호	ROS 함량	유의감소	-	-
	염증성 사이토카인 (TNF- α , IL-1 β , IL-6 및 IL-8)	유의감소	유의감소 (IL-1 β 및 IL-6)	-
	AP-1 (c-Fos, c-Jun), STAT1 및 MAPK (p38, JNK, ERK)	감소	감소	-
End point	MMP-1 및 MMP-3 발현	유의감소 (production, mRNA)	유의감소 (mRNA, protein)	-
	피부 주름 (육안평가, Mark-Vu 및 Skin Visiometer®) (평균깊이, 면적, 평균깊이 및 최대깊이)	-	유의감소	유의감소
	시험자의 개선도 평가	-	-	유의감소

Help to improve skin hydration



기전	Biomarker	In vitro	In vivo	Human
피부 보습	세포 생존력	유의증가	-	-
	MMP-1 및 MMP-3 발현	유의감소 (production, mRNA)	유의감소 (mRNA, protein)	-
	Pro-collagen type 1	유의증가	유의증가	-
	Pro-COL1A1 발현	-	유의증가	-
	Elastase	유의감소	-	-
	콜라겐 섬유	-	감소	-
	Hyaluronic acid	-	유의증가	-
	Filaggrin, Involucrin 및 Aquaporin-3 mRNA 발현	-	유의증가	-
End point	HYAL 1 및 HYAL 2 mRNA 발현	-	유의감소	-
	피부 수분량	-	유의증가	유의증가
	피부 거칠기	-	-	유의감소
	피부 탄력	-	-	유의증가
	경피수분손실 (TEWL)	-	유의감소	감소
시험자의 개선도 평가	시험자의 개선도 평가	-	-	유의감소

KFDA approval – Dual Functions

Help to improve skin hydration
(KFDA approval, No. 2020-7)

[별지 제3호서식] (일부)

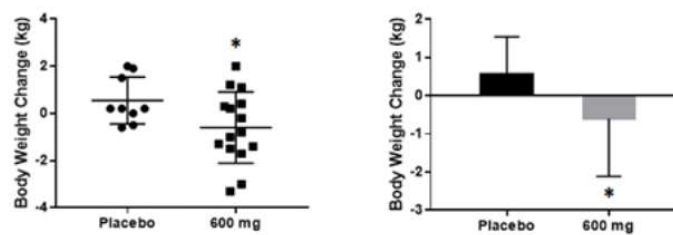
건강기능식품 기능성 원료 인정서	국명	주요
제2020-7호	대한민국	수출
대 표 자 : 김명수 업 계 명 또는 기 관 명 : (주)에스케이(주) 소재 지 : 충청남도 제천시 하이호텔리3로 30 원 료 명 : 수국잎열수추출물	수출국 제조회사명 : 수출국 제조회사 소재지 :	
「건강기능식품 기능성 원료 및 기준·규격 인정에 관한 규정」 제10조제1항에 따라 건강기능식품 기능성 원료로 인정합니다.		
2020년 9월 21일		
식품의약품안전처장		
* 붙임서류 1. 원료명 2. 제조기준(원재료, 제조방법, 기능성분(또는 지표성분), 제조 시 유의사항) 3. 규격 4. 제품차 요건(기능성 내용, 유효성, 유효성, 유효 시 주의사항, 기타사항) 5. 시험방법		
230mm×297mm(일반용지 60g/㎡(제출용지))		

Help to maintain skin health from skin
damage by UV radiation
(KFDA approval, No. 2020-8)

[별지 제3호서식] (일부)

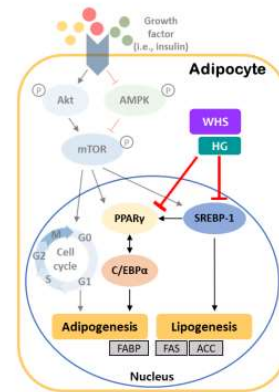
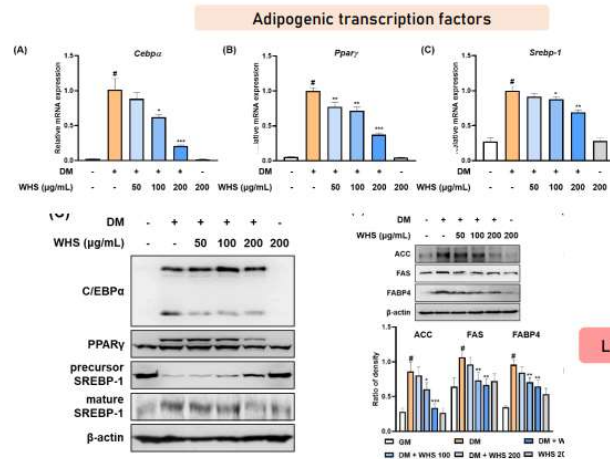
건강기능식품 기능성 원료 인정서	국명	주요
제2020-8호	대한민국	수출
대 표 자 : 김명수 업 계 명 또는 기 관 명 : (주)에스케이(주) 소재 지 : 충청남도 제천시 하이호텔리3로 30 원 료 명 : 수국잎열수추출물	수출국 제조회사명 : 수출국 제조회사 소재지 :	
「건강기능식품 기능성 원료 및 기준·규격 인정에 관한 규정」 제10조제1항에 따라 건강기능식품 기능성 원료로 인정합니다.		
2020년 9월 21일		
식품의약품안전처장		
* 붙임서류 1. 원료명 2. 제조기준(원재료, 제조방법, 기능성분(또는 지표성분), 제조 시 유의사항) 3. 규격 4. 제품차 요건(기능성 내용, 유효성, 유효성, 유효 시 주의사항, 기타사항) 5. 시험방법		
230mm×297mm(일반용지 60g/㎡(제출용지))		

Decreased body weight of WHS

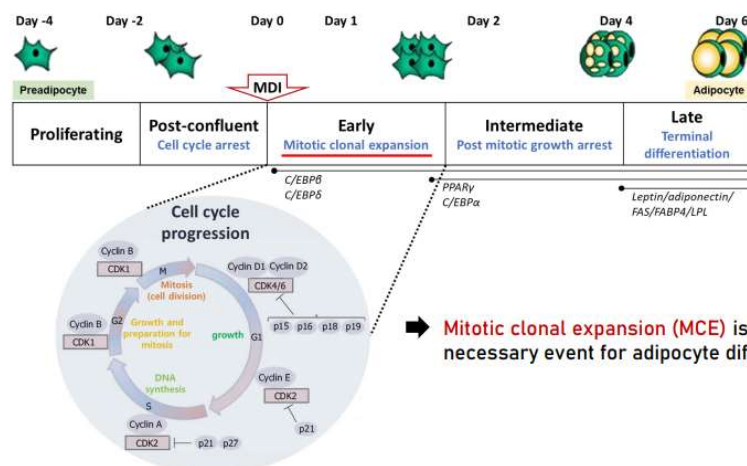


- BMI 25~34.9 kg/m² 피험자 24명, 12주 섭취 ;
- 대조군 9명, 수국잎열수추출물(WHS) 600 mg 시험군 15명

		WHS (μg/mL)		
GM	DM	DM + 50	DM + 100	DM + 200



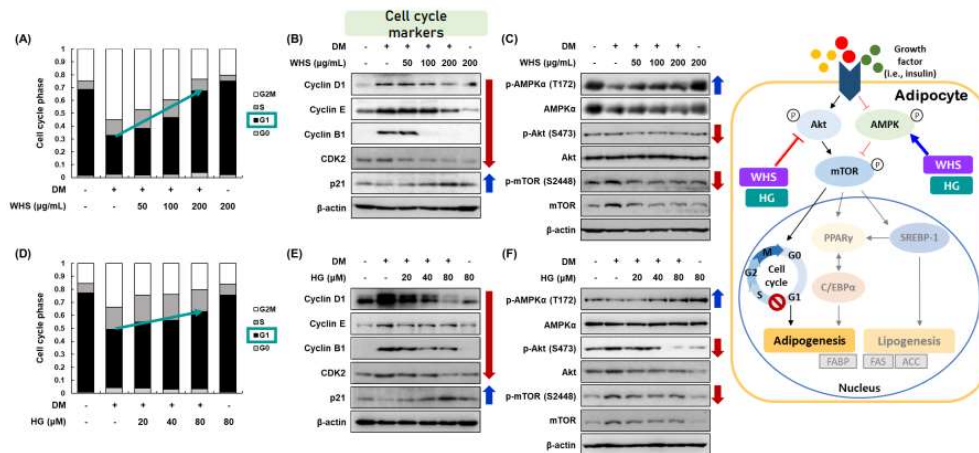
Lipogenic proteins



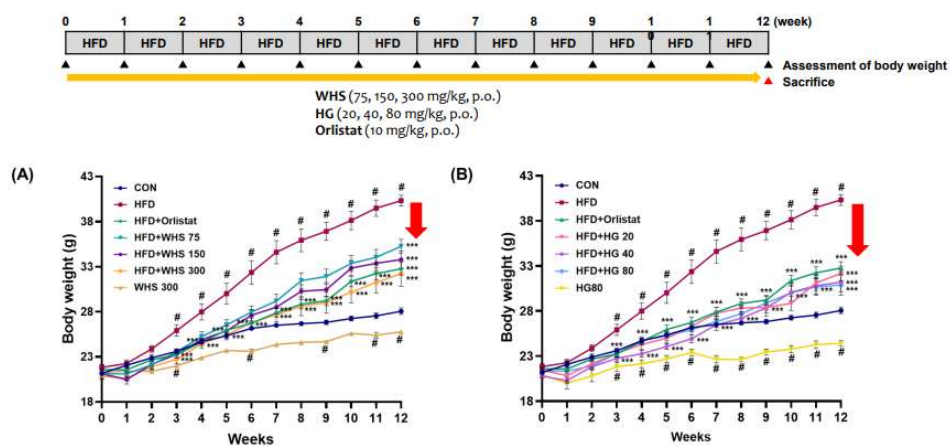
➡ **Mitotic clonal expansion (MCE)** is a necessary event for adipocyte differentiation



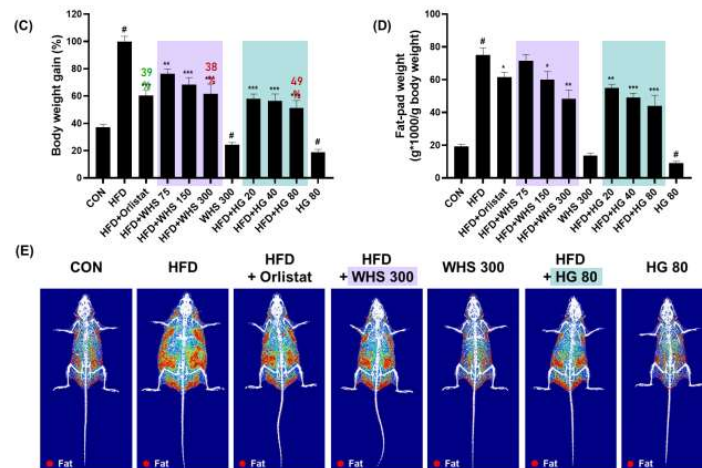
Effects of WHS and HG on cell cycle progression by regulating AMPK and Akt/mTOR pathways in 3T3-L1 cells



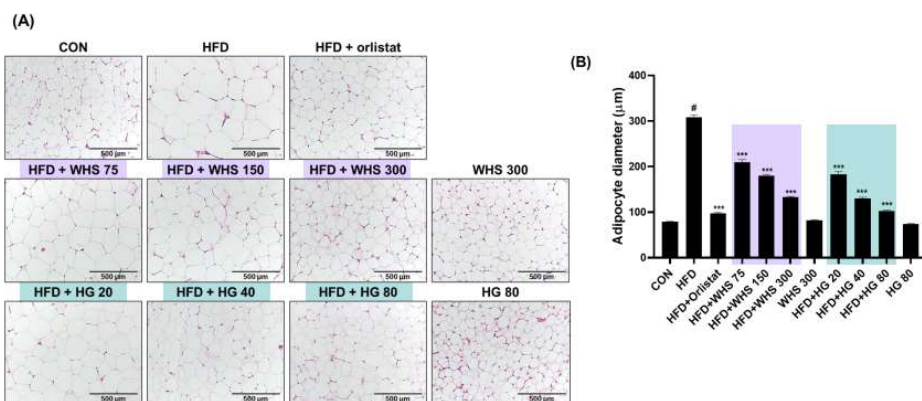
Effects of WHS and HG on body weight in HFD-induced obese mice



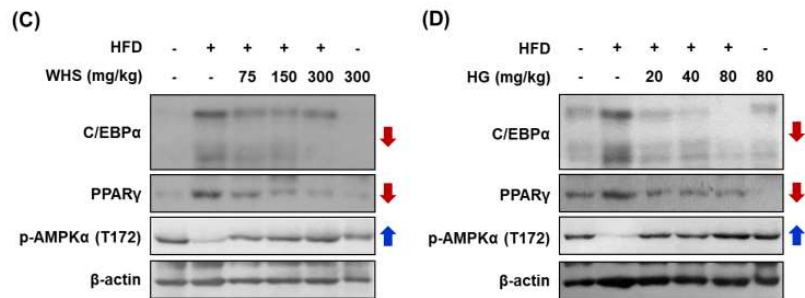
Effects of WHS and HG on body fat weight in HFD-induced obese mice



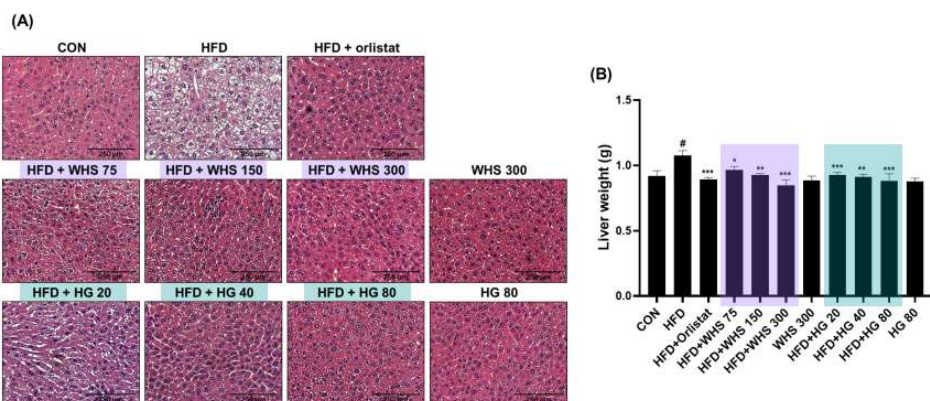
Effects of WHS and HG on adipocyte hypertrophy in white adipose tissues of HFD-induced obese mice



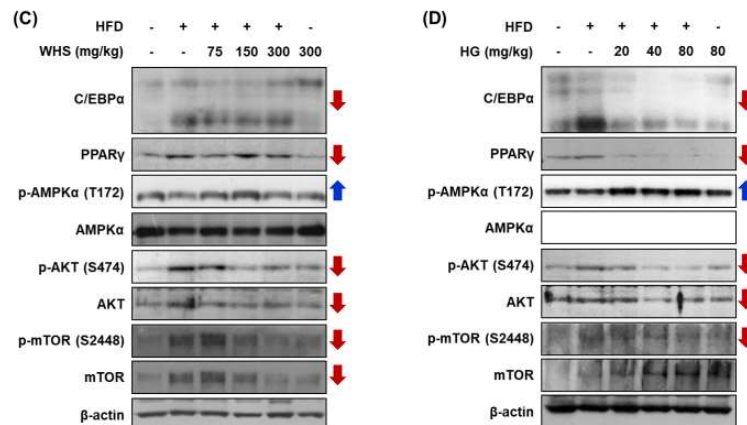
Effects of WHS and HG on adipogenesis in white adipose tissues of HFD-induced obese mice



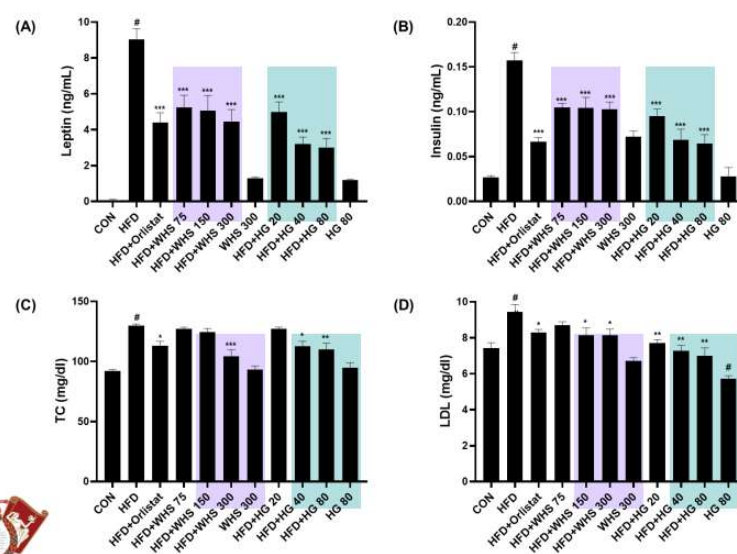
Effects of WHS and HG on liver weight and fat accumulation in liver tissues of HFD-induced obese mice



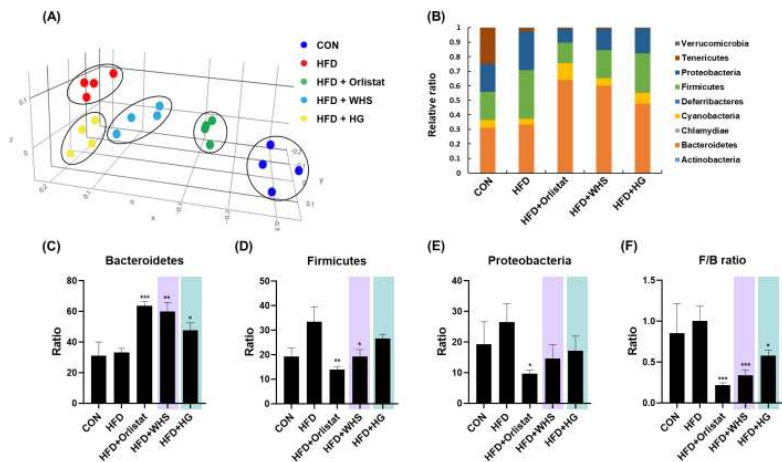
Effects of WHS and HG on adipogenesis in liver tissues of HFD-induced obese mice



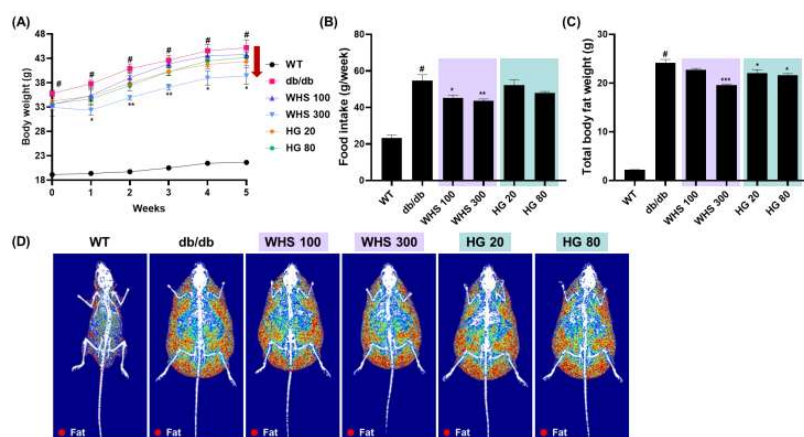
Effects of WHS/HG on plasma leptin, insulin, total cholesterol, and LDL levels in HFD-induced obese mice



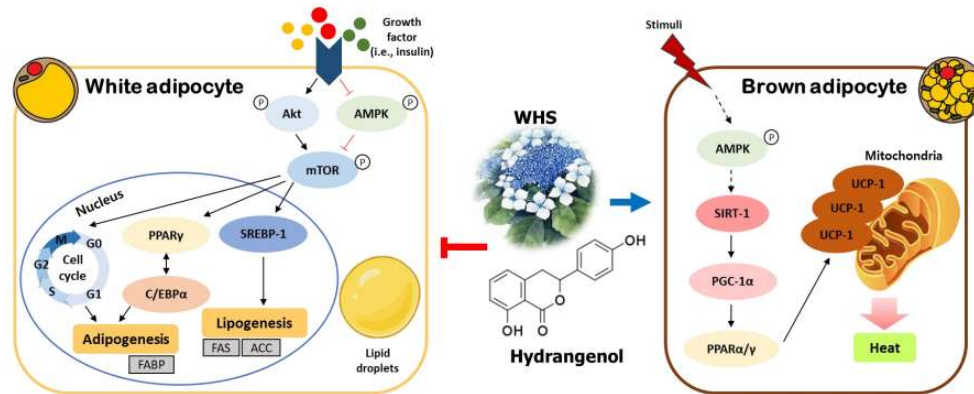
Effects of WHS and HG on the gut microbiota composition in HFD-induced obese mice



Effects of WHS and HG on body weight, food intake, and body fat weight in db/db obese mice

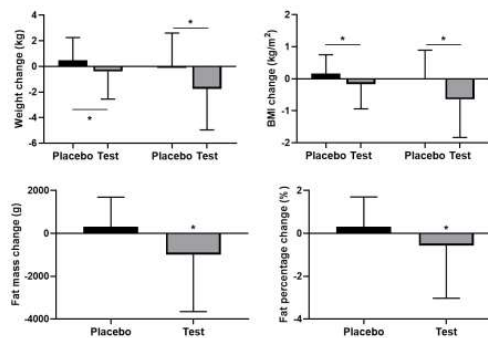
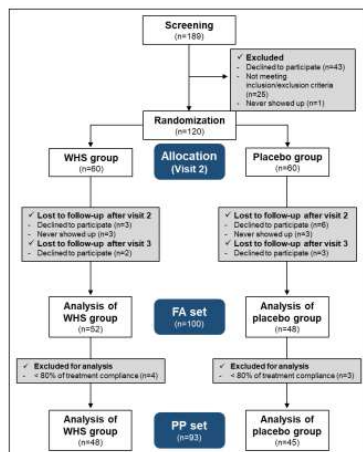


Conclusions



Clinical study

Randomized, double-blinded, placebo-controlled clinical trial - WHS



1. Primary Outcome Measures

1) Body fat mass through DEXA

2. Secondary Outcome Measures

- 1) Weight
- 2) Waist, Hip, Waist to hip ratio
- 3) Body Mass Index (BMI)
- 4) Body fat mass and percentage through DEXA
- 5) Visceral fat area, subcutaneous fat area, total abdominal fat area, visceral/subcutaneous fat area ratio through CT
- 6) Blood lipids: Total Cholesterol, HDL-Cholesterol, LDL-Cholesterol, TG
- 7) hs-CRP
- 8) Adiponectin, Leptin



Additional KFDA approval-Functions

Help to reduce body fat

[별지 제3호서식]

(앞쪽)

건강기능식품 기능성 원료 인정서	국내	수입
○		
제2020-7호		
대표자: 박정숙 업체명: 푸른기행, 코스믹케이오(주) 소재지: 충청북도 제천시 바이오벤처3로 30 원료명: 수국잎열수추출물		
수출국: 수국국 제조회사명: 수출국 제조회사 소재지:		
「건강기능식품 기능성 원료 및 기준·규격 인정에 관한 규정」 제10조제1항에 따라 건강기능식품 기능성 원료로 인정합니다.		
2020년 5월 21일 식품의약품안전처장		
※ 문입사항 1. 원료명 2. 제조기준(원재료, 제조방법, 기능성분(또는 지표성분), 제조 시 유의사항) 3. 규격 4. 제품의 효능(기능성 내용, 원료 출처명, 실제 시 주의사항, 기타사항) 5. 시험방법		

210mm×297mm(단면두께 0.6mm/서(제출용종))

[복위]

(인정번호: 제2020-7호)

구분	인정내용
원료명	(국문) 수국잎열수추출물 (영문) The hot water extract of hydrangea leaf
제조기준	원재료 (학명: <i>Hydrangea serrata</i> Seringe, 사용부위: 잎) 제조기준: 원재료 → 추출 → 여과 → 농축 → 분무건조 → 기능성 원료
제조방법	원재료의 건조
원재료 명칭(제조기준)	수국잎 열수추출물
기능성분	(또는 지표성분)
원재료 명칭(제조기준)	수국잎 열수추출물
기능성분	(1) 상온: 아미노산 함량이 0.1% 이하인 것 (2) Hydrangein(mg/kg): 7.00(표지량의 80~120%) (3) M(mg/kg): 0.8 이하 (4) K(mg/kg): 1.0 이하 (5) K(mg/kg): 0.1 이하 (6) 수분(mg/kg): 0.1 이하 (7) 저장기간: 6개월 (8) 총 아미노산(mg/kg): 15.0 이하(표지량의 100% 이하)
제조기준	(국문) ① 피부보습에 도움을 줄 수 있음 ② 피부건강에 위한 피부세포의 재생을 촉진하여 피부건강 유지에 도움을 줄 수 있음 ③ 체지방 감소에 도움을 줄 수 있음 (영문) ① May help to maintain skin moisturizing ② May help to maintain skin health from skin damage by UV radiation ③ May help to reduce body fat
유효성분	① 피부보습에 도움을 줄 수 있음 ② 피부건강에 위한 피부세포의 재생을 촉진하여 피부건강 유지에 도움을 줄 수 있음 ③ 체지방 감소에 도움을 줄 수 있음 · 수국잎열수추출물에서 300~600 mg/일 · 상온: 아미노산 함량이 0.1% 이하인 것 · 저장기간: 6개월 또는 그 이상 · 유효성분 함량: 15.0% 이상인 것
시험방법	기타 사항
시험방법	※ 실제 시험방법은 별첨함

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수국잎열수추출물 제품 출시

▶ 홈쇼핑 수국 소재 BOOM UP

I. 홈쇼핑 채널 리딩 브랜드 2社 런칭

skinny lab



헬스밸런스
매출액 639억(2020년)
모델: 배우 이다희
대표제품:
가벼워지는 시서스 다이어트

days on



핀인타네셔널
매출액 447억(2020년)
모델: 장도연/오한진
대표제품:
오한진 프로바이오틱스

II. 메인 홈쇼핑 5社 런칭(3~4월)

롯데홈쇼핑

홈&쇼핑

CJ Oshopping

GS SHOP

한대홈쇼핑

▶ 온라인 OBM SALES UP

III. NU:TRIP 제품 런칭(5월)



NU:TRIP

[배우 이세희]

- ✓ 홈쇼핑을 통한 수국 인지도 확보 후 뉴트리프 제품 출시
- ✓ 기능성 및 소재와 적합한 트렌디 모델 선정으로 헬스&뷰티 이미지 조성

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경청해주셔서 감사합니다.

2022.12.14.

면역학/미생물학 전공 대학원생 발표 세미나

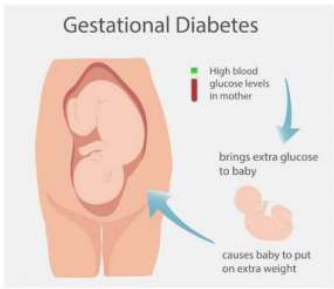
최한희 학생




The study on the effects of early Microbiome composition in infants born to GDM mothers on the development of childhood Obesity

Microbiology and Immunology
Hanhyi Choi

Introduction
Gestational diabetes mellitus(GDM)




Development and initial testing of a GDM information website for multi-ethnic women with GDM, BMC Pregnancy and Childbirth volume 15, Article number: 145 (2015)

<https://www.google.com/>

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Introduction

Gestational diabetes mellitus(GDM)

Gestational Diabetes

high blood glucose levels in mother
↓
extra glucose to baby
↓
baby with extra weight



Baby Complications of GDM Mothers:

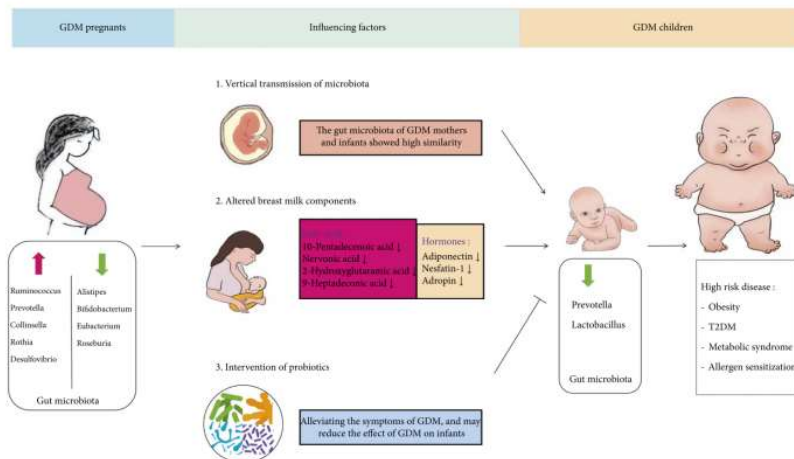
- It affects your baby's growth. This means that your baby may have growth restrictions or grow too tall.
- Elevated sugar levels can cause birth defects. Because of their large size, they can injure the baby during a normal, natural birth.
- Babies are at higher risk of being overweight compared to children of non-GDM mothers

<https://gdm.com.pk/about-gdm/>

3

Introduction

Gestational diabetes mellitus(GDM)

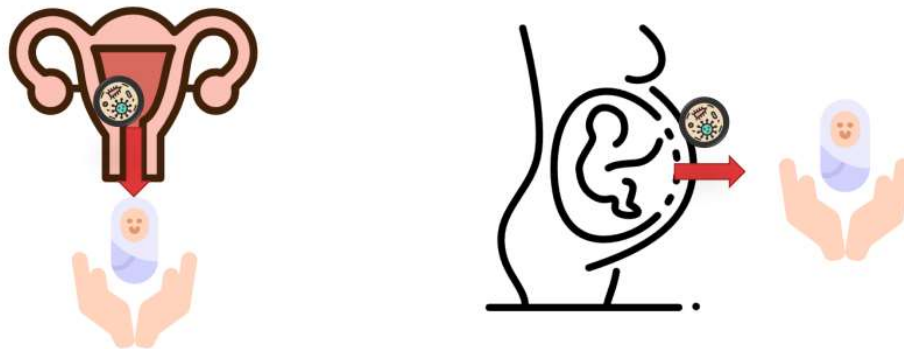


The Intestinal Dysbiosis of Mothers with Gestational Diabetes Mellitus (GDM) and its Impact on the Gut Microbiota of Their Newborns, Can J Infect Dis Med Microbiol. 2021

4

Introduction

Vaginal birth and Cesarean section



<https://www.miricanvas.com/>

5

Introduction

Vaginal birth and Cesarean section

Vaginal Delivery



Introduced to Vaginal Microbes: Lactobacillus

Normal Introduction of Gut Microbes

Normal Development of the Immune System
•Production of specific cytokines for proper immune system development

Richardson; 2013

vs.

Cesarean Delivery



Introduced to Skin Flora: Staphylococcus

Abnormal Microbial Introduction

Disrupted Intestinal Microbial Colonization
•Increase risk for Atopic Diseases, Asthma, Allergic Rhinitis, and Celiac Disease

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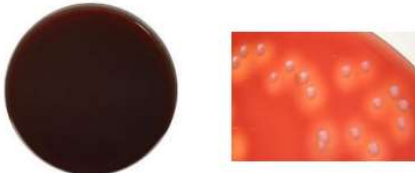
Result	Experimental group	
NC 1	- C-section delivery - Normal mother - Infant's 1day faecal	GDM 1 - C-section delivery - GDM mother - Infant's 1day faecal
NC 3	- C-section delivery - Normal mother - Infant's 3day faecal	GDM 3 - C-section delivery - GDM mother - Infant's 3day faecal

Result	Method
▶ The 16S V4 region was amplified through amplicon sequencing and analyzed using iseq 100(illumina,San Diago,CA). ▶ Feces of infants were diluted with GAM broth(Mbcell,Korea) and spread on BL(Nissui Pharmaceutical,Japan)and DHL (2% Agar), respectively. ▶ BL medium and DHL medium(Eiken,Japan)were used for the smear, and the DHL medium was smeared with $10^{-1}, 10^{-2}$, and the BL medium was smeared with $10^{-5}, 10^{-6}$.	

Result

Culture Media

BL media



- A medium obtained by adding blood from animals such as cows and sheep to the medium from which fiber has been removed.

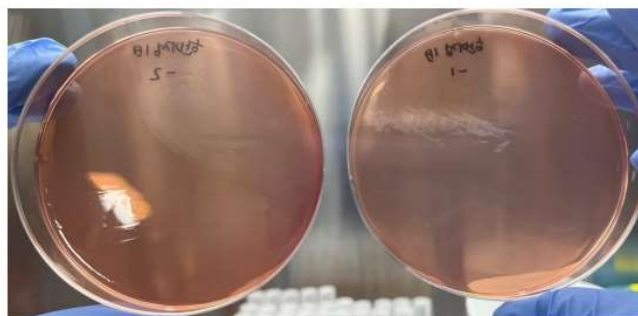
DHL media



- *Salmonella* selective medium

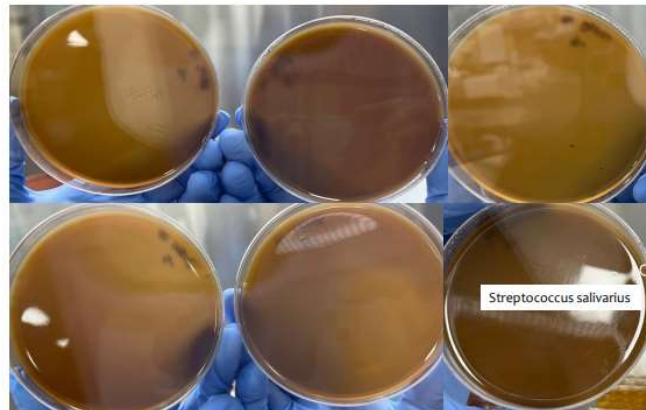
Result

Colony – Normal day₁ DHL



Result

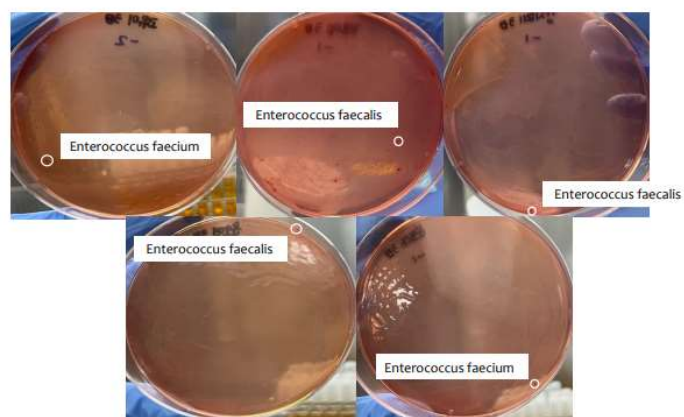
Colony – Normal day1 BL



11

Result

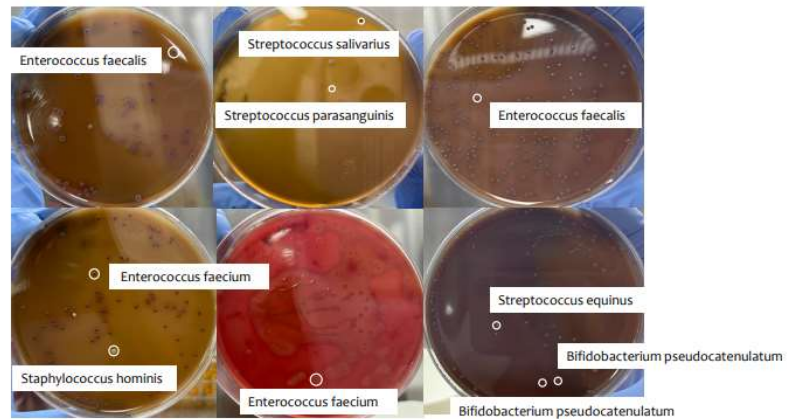
Colony – Normal day3 DHL



12

Result

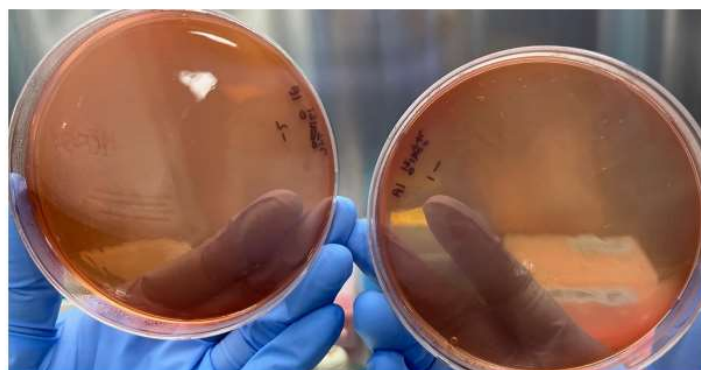
Colony – Normal day3 BL



13

Result

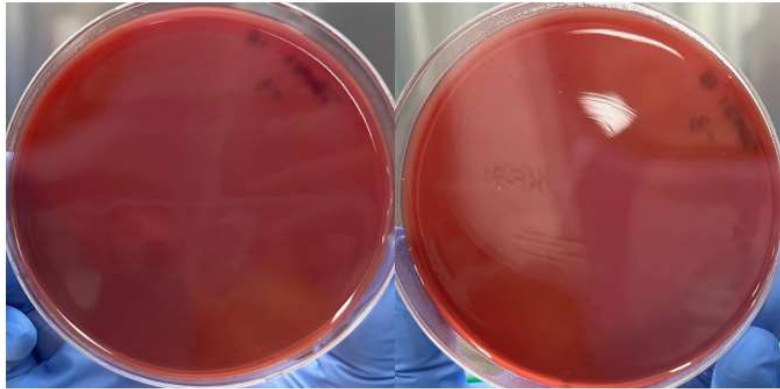
Colony – GDM day1 DHL



14

Result

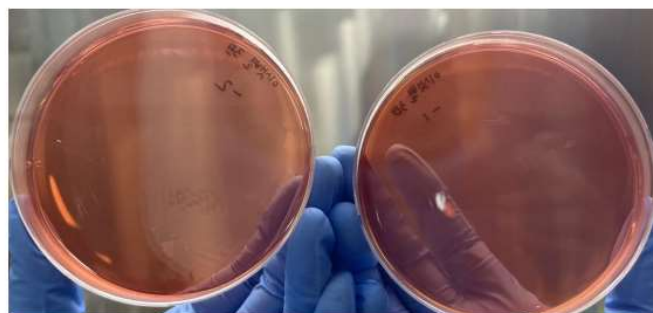
Colony – GDM day1 BL



15

Result

Colony – GDM day3 DHL



16

Result

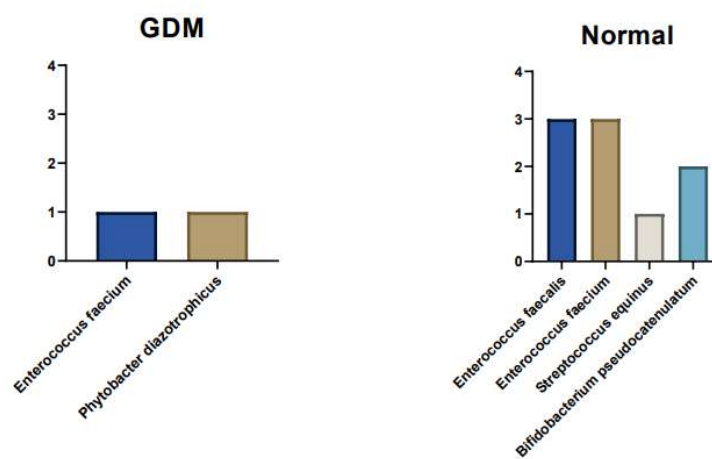
Colony – GDM day3 BL



17

Result

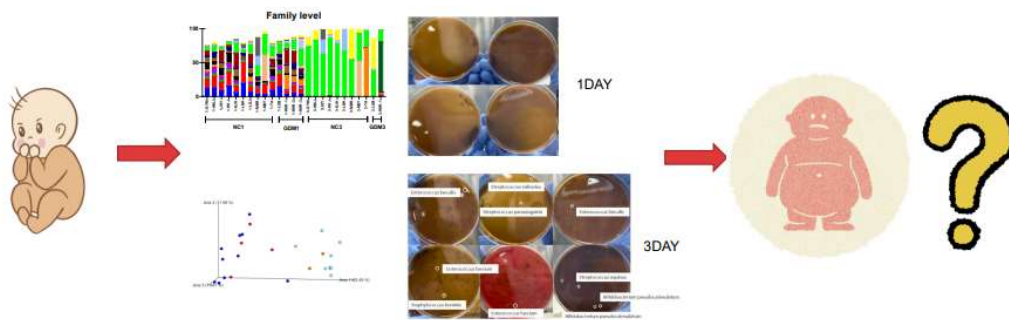
Colony sequencing



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Result

Conclusion



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Result

Future research plan

- ▶ We plan to follow-up the fetuses periodically.
- ▶ Future follow-up studies to find out what proportions of bacteria increase or decrease in microbial communities of fetuses born in GDM mothers.
- ▶ It plans to reveal whether early microbial communities affect the development of childhood obesity.
- ▶ If it affects, it will investigate how it can lead to childhood obesity.

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