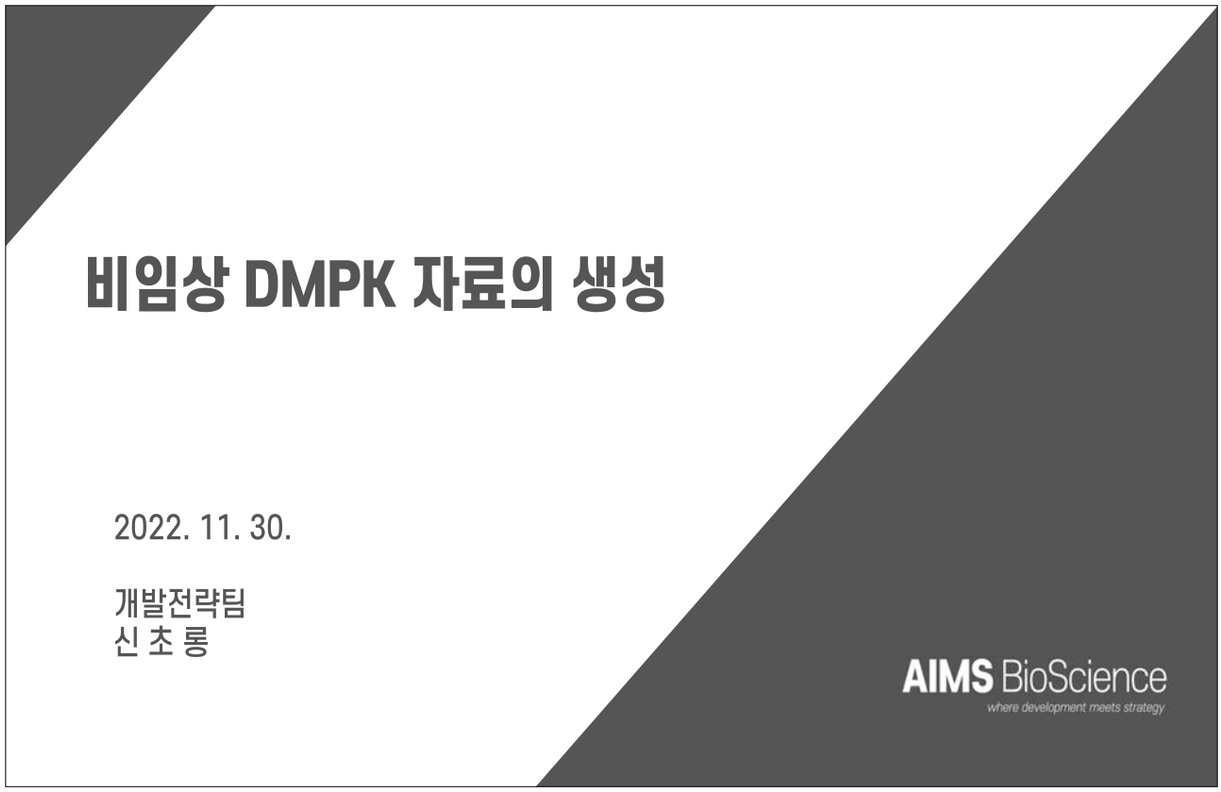
비임상 DMPK 자료의 생성

신 초 롱



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INTRODUCTION

Drug Discovery and Development

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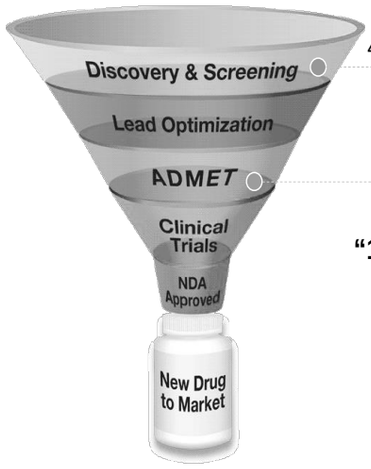

400 compounds

50 compounds

"1 compound"

Discovery stage

- Screening to find druggable compounds

Development stage

- Evaluation of selected compound

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Non-clinical DMPK study list

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Absorption	Distribution	Metabolism	Excretion	Drug-Drug Interactions
Cell Permeability	Plasma protein binding	Plasma stability	Mass balance	CYP Direct inhibition
In vivo PK (C_{max}/T_{max})	Microsomal binding	Metabolic stability	Urinary/Fecal excretion	CYP Time-dependent inhibition
Dose proportionality	Tissue distribution	In vivo PK (CL)	Biliary excretion	CYP induction
Food effect	QWBA	Metabolite ID	In vivo PK (CL)	Transporter substrate
Gender difference	In vivo PK ($V_{d,ss}$)	Reaction phenotyping		Transporter inhibition
		GSH trapping		

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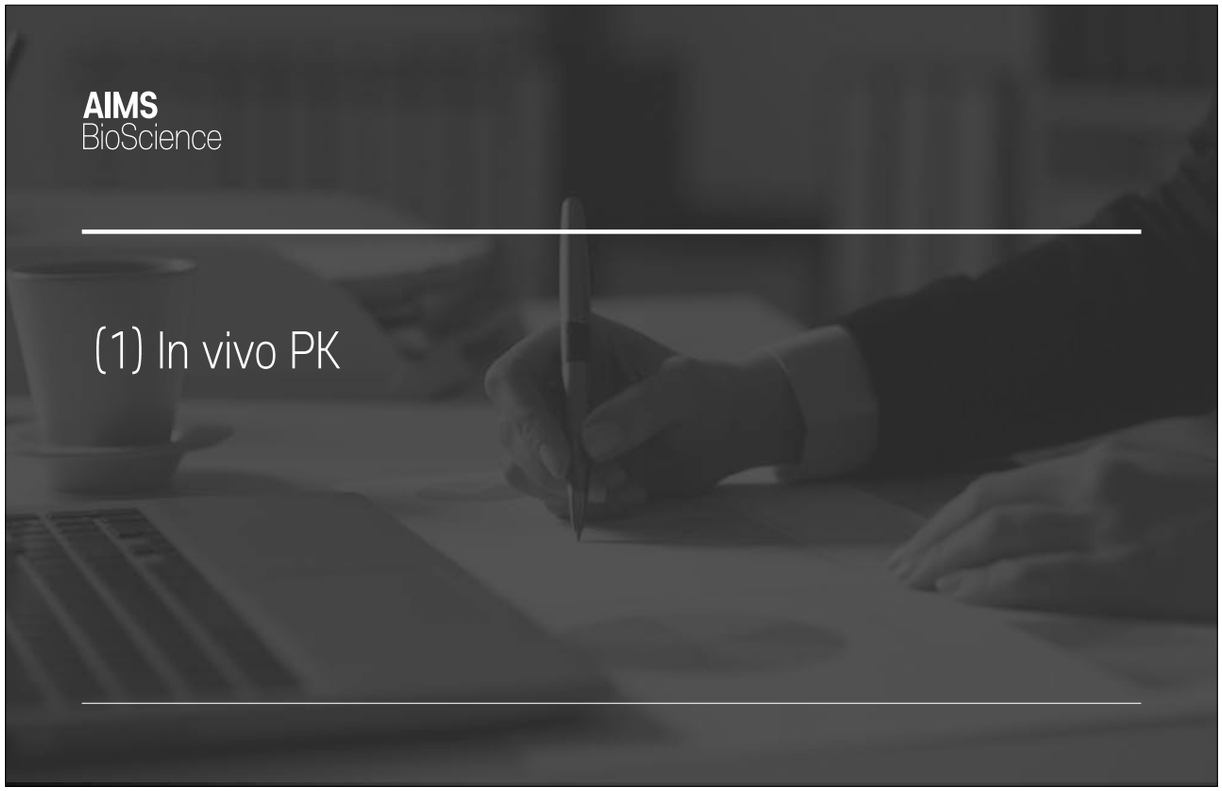
NON-CLINICAL DMPK STUDIES

In vivo PK
Absorption
Distribution
Metabolism
Excretion
Drug-Drug interaction



In vivo PK

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- **시험 목적:** 각 동물 종별 *in vivo* 약물동태 특성 및 dose proportionality 확인
  - Gender difference: male vs. female animals
  - Food effect: fasted vs. fed animals
- **시험 디자인**
  - Species: 3 species 이상에서 평가 권장; Mouse, Rat, Dog (or Monkey)
  - Sampling time points: 7~8 points
  - Group (n=3~6/ group)

Group	Administration Route	Dose (mg/kg)
G1	IV	5 (Low)
G2	PO	5 (Low)
G3		10 (Mid)
G4		20 (High)

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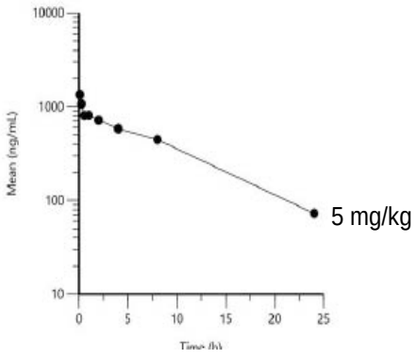
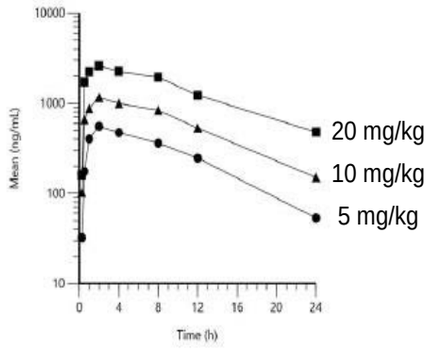
In vivo PK: Example – Dog PK

단회 정맥 투여 (IV)



단회 경구 투여 (PO)



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In vivo PK: Example – Dog PK

투여 경로		IV	PO		
Dose (mg/kg)		5	5	10	20
Parameter	Unit				
C_{max}	ng/mL	-	555	1160	2610
C_0	ng/mL	1520	-	-	-
T_{max}	h	-	2	2	2
C_{last}	ng·h/mL	8330	6030	13600	32500
$t_{1/2}$	h	6.67	5.52	6.53	7.64
CL or CL/F	mL/h/kg	558	817	669	598
$V_{d,ss}$	mL/kg	5230	-	-	-
F	%	-	72	82	98

[Criteria]
- < 0.3 X 간 혈류량: Low
- 0.3 – 0.7 X 간 혈류량: Moderate
- > 0.7 X 간 혈류량: High

[Criteria]
- 총 체수분량과 비교

- CL: 558 mL/h/kg < Dog's hepatic blood flow (1800 mL/hr/kg)의 30% (= 600 mL/h/kg) → low
- $V_{d,ss}$: 5230 mL/kg > Dog's total body water vol. (1 L/kg) → large, 혈액보다 조직에 더 많이 분포

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In vivo PK: Example – Dog PK

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투여 경로		IV	PO		
Dose (mg/kg)		5	5	10	20
Parameter	Unit				
C_{max}	ng/mL	-	555	1160	2610
C_0	ng/mL	1520	-	-	-
T_{max}	h	-	2	2	2
AUC_{last}	ng·h/mL	8330	6030	13600	32500
$t_{1/2}$	h	6.67	5.52	6.53	7.64
CL or CL/F	mL/h/kg	558	817	669	598
$V_{d,ss}$	mL/kg	5230	-	-	-
F	%	-	72	82	98

- Systemic exposure: as C_{max} & AUC_{last}
- Dose ratio = 1 : 2 : 4 → C_{max} ratio = 1 : 2.1 : 4.7 / AUC_{last} ratio = 1 : 2.3 : 5.4
  - Systemic exposure was increased dose proportionally at the dose range of 5~20 mg/kg following oral administration.

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In vivo PK: Dose proportionality

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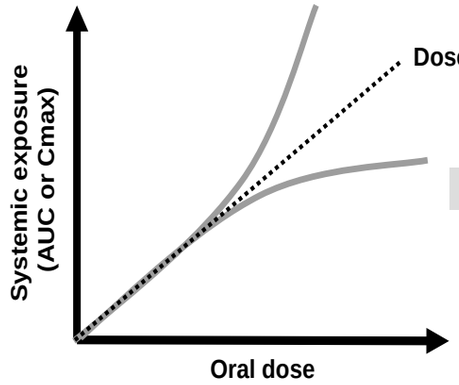
More than dose proportional

- Substrate of efflux transporter
- Saturation of metabolism



Less than dose proportional

- Lower solubility (dissolution process)
- Saturation of uptake transport

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(2) Absorption

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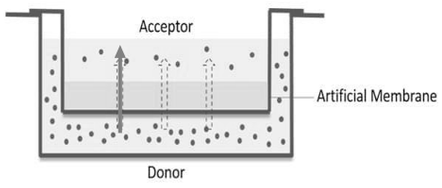
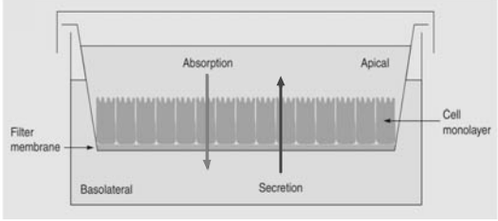
Absorption: Cell permeability

- **시험 목적:** 약물의 세포 투과도 평가 *(in vitro)*
  - PAMPA (artificial membrane)
  - Caco-2 cells, MDCK cells…
- **시험 디자인**
  - Test conc. : 1 conc.
  - Caco-2 or MDCK cell assay: efflux ratio 산출 (efflux transporter에 대한 기질성 확인 가능)





1) The Role of Intestinal Permeability in Gastrointestinal Disorders and Current Methods of Evaluation, Tim et al., Sec. Nutritional Immunology.
2) https://www.orsolyaszathmari.com/post/inreased-intestinal-permeability-what-it-is-and-why-you-should-care-about-it

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Absorption: Cell permeability

- **Caco-2 permeability 시험 결과 (Example)**

[Criteria] (x10^{-6} cm/sec)
- High: >10
- Moderate: 1 - 10
- Low: <1

[Criteria]
- Efflux Ratio > 2

Compound	Test conc. (uM)	Mean P_{app} (x10^{-6} cm/sec)		Efflux ratio
		A to B	B to A	
AIMS-001	1	7.44	59.9	8.06

- $P_{app, A\ to\ B}$: 7.44 (x10^{-6} cm/sec) → moderate
- Efflux ratio (= $P_{app, B\ to\ A}$ / $P_{app, A\ to\ B}$) : 8.06 → possible to be a substrate of efflux transporters

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(3) Distribution

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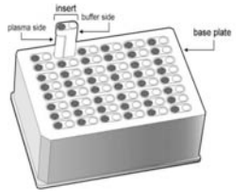
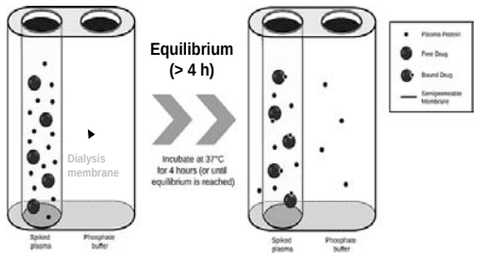
Distribution: Plasma protein binding

- **시험 목적:** 동물 종의 혈장 내 단백 결합률 확인 *(in vitro)*
  - Equilibrium dialysis, Ultrafiltration, Ultracentrifugation
- **시험 디자인**
  - Test species: Human + Animals (약효 및 독성 동물 종 포함)
  - Test conc. : 1 conc.



RED (Rapid equilibrium dialysis) device



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Distribution: Plasma protein binding

- **시험 결과 (Example)**

	Mouse	Rat	Dog	Monkey	Human
fraction bound (% Bound)	0.993 (99.3%)	0.996 (99.6%)	0.993 (99.3%)	0.995 (99.5%)	0.996 (99.6%)
fraction unbound (% Unbound)	0.007 (0.7%)	0.004 (0.4%)	0.007 (0.7%)	0.005 (0.5%)	0.004 (0.4%)

- Fraction unbound ($f_{u,p}$) = 1 - Fraction bound
- Highly bound in the plasma protein of all species

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Distribution: QWBA

- **시험 목적:** 약물의 조직 분포 확인 *(in vivo)* – target organ, eye uveal or pigmented skin
- **시험 디자인**
  - Test article: Radio-labeled compound
  - Test species: Rat
  - Dose: 1 dose
  - Animals will be sacrificed at each sampling time points

Group	Species	Sampling time points
G1	SD rats (Albino)	> 5 (up to 96 h)
G2	Long-Evans rats (Pigmented)	> 5 (up to 168 h)

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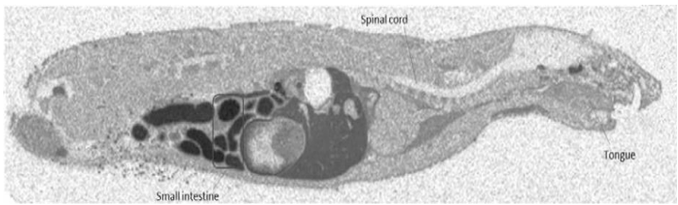
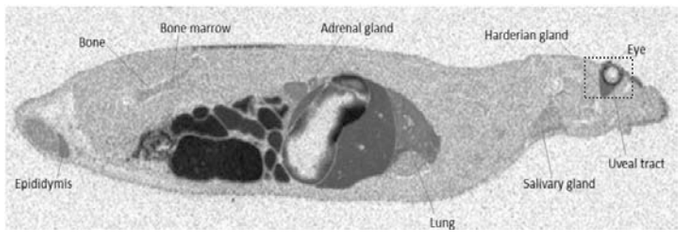
Distribution: QWBA

- **시험 결과 (Example)**

G1: SD rats (Albino)



G2: LE rats (Pigmented)



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Distribution: QWBA

- **시험 결과 (Example)**

In the pigmented rats, highly bind to eye uveal and skin and remain longer
→ **potential for melanin binding**

G2: LE rats (Pigmented)

Tissue/Organ	C_{max} (ng-eq/g)	t_{max} (h)	$t_{½}$ (h)	(ng-eq*h/g)	Pl
Plasma(LSC)	2330	4.0	167	**72,557**	
Aorta	929	4.0	NC	**5,302**	
Blood(heart)	1740	4.0	NC	**8,093**	
Whole brain	NA	NA	NA	**NA**	NA
Spinal cord	NA	NA	NA	**NA**	**NA**
Eye	3048	8.0	229	241,907	3.33
Crystalline lens	NA	NA	NA	**NA**	**NA**
Uvea	8307	8.0	162	1,102,109	15.19
Non-pigmented skin	835	4.0	NC	**3,829**	**0.05**
Pigmented skin	1454	4.0	NC	**23,340**	**0.32**
Adrenal gland	1908	4.0	NC	**11,492**	**0.16**
Adrenal cortex	2019	4.0	NC	**11,673**	**0.16**
Adrenal medulla	1823	4.0	NC	**10,477**	**0.14**
Hypophysis cerebri	1815	4.0	NC	**9,631**	**0.13**
Thyroid	1300	4.0	NC	**6,744**	**0.09**
Brown fat	1029	4.0	NC	**11,190**	**0.15**
White fat (abdominal cavity)	NA	NA	NA	**NA**	**NA**
Esophagus	3662	1.0	NC	**13,866**	**0.19**
Stomach wall (glandular region)	31505	1.0	NC	**63,775**	**0.88**
Stomach wall(Non-glandular region)	33830	1.0	NC	**58,641**	**0.81**
Small Intestine Wall	11336	1.0	NC	**31,040**	**0.43**
Large intestine wall	17894	4.0	35.3	**295,349**	4.07

Tissue/Organ	C_{max} (ng-eq/g)	t_{max} (h)	$t_{½}$ (h)	AUC0-t (ng-eq*h/g)	Tissue: Plasma*
Lymph gland(neck)	898	4.0	NC	**5,917**	**0.08**
Spleen	1433	4.0	NC	**9,224**	**0.13**
Thymus	806	4.0	NC	**4,746**	**0.07**
Kidney	3033	4.0	NC	**14,985**	**0.21**
Renal cortex	2657	4.0	NC	**27,606**	**0.38**
Renal Medulla	3480	4.0	NC	**17,781**	**0.25**
Liver	3903	4.0	NC	**20,337**	**0.28**
Bladder wall	2974	8.0	NC	**10,623**	**0.15**
Heart	1211	4.0	NC	**6,584**	**0.09**
Muscle (thighbone)	837	4.0	NC	**5,743**	**0.08**
Prostate	716	4.0	NC	**4,130**	**0.06**
Testes	NA	NA	NA	**NA**	**NA**
Lung	1444	4.0	NC	**7,970**	**0.11**
Turbinal	656	4.0	NC	**3,795**	**0.05**
Trachea	882	4.0	NC	**4,618**	**0.06**
Lacrimal gland	1270	4.0	NC	**8,583**	**0.12**
Harderian gland	1748	4.0	NC	**9,188**	**0.13**
Pancreas	1385	4.0	NC	**8,781**	**0.12**
Salivary gland	1703	4.0	NC	**9,637**	**0.13**
Bone marrow (thighbone)	1420	4.0	NC	**8,228**	**0.11**
Bone (thighbone)	NA	NA	NA	**NA**	**NA**

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(4) Metabolism

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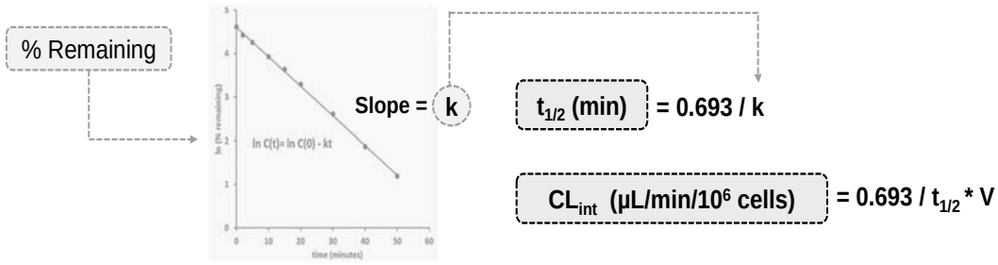
Metabolism: Metabolic stability

- **시험 목적:** 간 대사에 대한 약물의 안정성 평가 *(in vitro)*
  - Liver microsome or S9
  - Hepatocyte

- **시험 디자인**
  - Test species: Human + Animals (Mouse, Rat, Dog, Monkey…)
  - Test conc. : 1 conc.
  - Incubation time: > 60 min



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Metabolism: Metabolic stability

- **시험 결과 (Example)**

Species	Test conc. (uM)	$t_{1/2}$ (min)	CL_{int} (μL/min/10^6 cells)	Category
Mouse		59.1	23.5	High (>17.8)
Rat		10.8	129	High (>27.5)
Dog	1	34.2	40.5	High (>10.5)
Monkey		3.59	386	High (>28.3)
Human		39.7	34.9	High (>19.0)

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Metabolism: Metabolite Identification

- **시험 목적:** 종 간 대사체 프로파일 비교 및 대사체 구조 규명 *(in vitro)*
  - Liver microsome or S9
  - Hepatocyte
- **시험 디자인**
  - Test species: Human + Animals (Mouse, Rat, Dog, Monkey…)
  - Test conc. : 1 conc.
  - Incubation time: > 60 min

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Metabolism: Metabolite Identification

- **시험 결과 (Example)**

Proposed assignment		Relative Abundance (UV Peak Area %)				
		Human	**Mouse**	**Rat**	**Dog**	**Monkey**
Parent		80.1	89.1	42.9	89.7	79.3
M1	Oxidation+Desaturation	-	-	1.73	-	-
M2	Oxidation	**1.14**	7.71	5.29	2.81	3.34
M3	Oxidation	**5.79**	-	37.8	-	-
M4	Oxidation	**2.92**	-	2.38	1.37	-
M5	2x Oxidation	-	-	2.71	-	-
M6	Oxidation + Sulfation	-	-	1.65	2.17	-
M7	Oxidation+sulfation	-	-	1.09	-	-
M8	Glucuronidation	**2.55**	2.16	3.52	3.94	7.05
M9	Oxidation + Glucuronidation	**1.17**	-	2.87	-	-

- No human unique metabolites
- 사람과 가장 유사한 대사체 프로파일을 보인 동물 종: Rat, Dog

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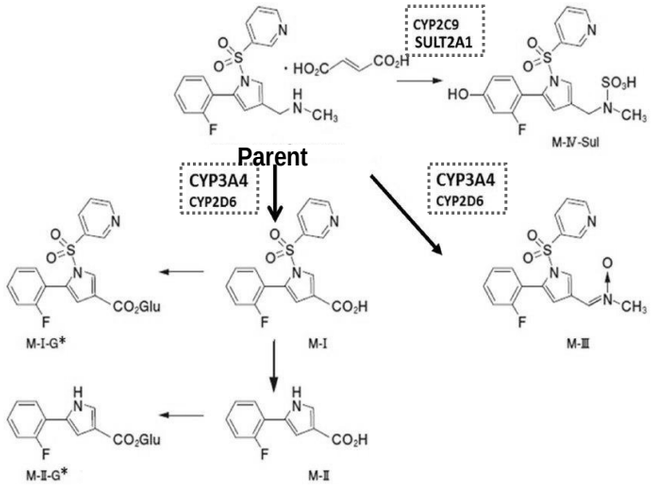
Metabolism: Metabolite Identification

Proposed metabolic pathway



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Metabolism: Reaction phenotyping

- **시험 목적:** 약물의 human CYP (and/or UGT) enzymes에 대한 기질성 확인 *(in vitro)*
  - Human recombinant CYPs/UGTs
  - Human liver microsomes (using chemical inhibitors)

- **시험 디자인**
  - Test Conc: 1 conc.
  - Sampling time points: 4~5 time points

Study item	CYP phenotyping		UGT phenotyping
Isoforms	CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4		UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B15
Test system	Individual rCYPs	Liver microsomes (using chemical inhibitors)	Individual rUGTs
Deliverables	% remaining, $t_{1/2}$	Activity remaining (as % of no inhibitor)	% remaining, $t_{1/2}$

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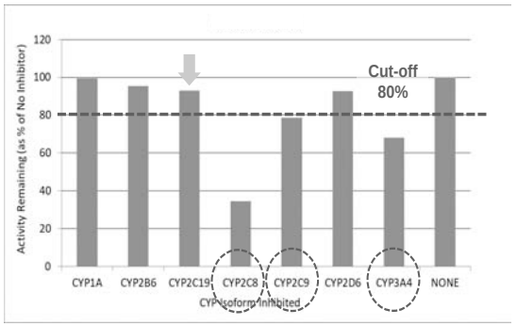
Metabolism: Reaction phenotyping

- **CYP phenotyping 시험 결과 (Example)**

1) Recombinant CYPs

Isoforms	% remaining					t1/2 (min)
	0 min	5 min	15 min	30 min	45 min	
CYP1A2	100	80.1	88.8	84.0	79.0	213
CYP2B6	100	93.9	84.5	92.1	87.1	324
CYP2C8	100	0.15	0.15	0.14	0.13	0.54
CYP2C9	100	60.0	25.3	6.72	3.30	7.77
CYP2C19	100	17.7	0.06	0.06	0.06	2.00
CYP2D6	100	91.4	105	99.5	112	N/A
CYP3A4	100	71.0	48.7	29.9	24.3	17.9

2) Using chemical inhibitors



- Can be metabolized by CYP2C8, CYP2C9 and CYP3A4 (as a substrate)

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(5) Excretion

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Excretion: Mass balance

- **시험 목적:** 약물의 주된 배설 경로 규명 *(in vivo)*
  - Excreta (Urine, Feces, Bile) 내 metabolites profiling study 함께 수행할 수 있음.

- **시험 디자인**
  - Test article: Radio-labeled compound
  - Test species: Rat
  - Dose: 1 dose
  - Sampling time points: 7~8 time points

Group		Sampling time points	Collection matrices
G1	Intact rats	up to 96~120 h	Urine, feces (+ Cage rinse, Carcass)
G2	Bile-duct cannulated rats	up to 72 h	Urine, feces, bile (+ Cage rinse, Carcass)

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Excretion: Mass balance

- **시험 결과 (Example)**

1) Intact Rat

Matrix	**Cumulative % of administered dose**
Urine	17.3
Feces	82.2
Cage rinse	2.2
Carcass	0.6
Total	102.3

2) BDC Rat

Matrix	**Cumulative % of administered dose**
Bile	70.0
Urine	19.4
Feces	9.9
Cage rinse	0.2
Carcass	0.6
Total	100.2

- Main route of excretion: Fecal excretion via biliary excretion

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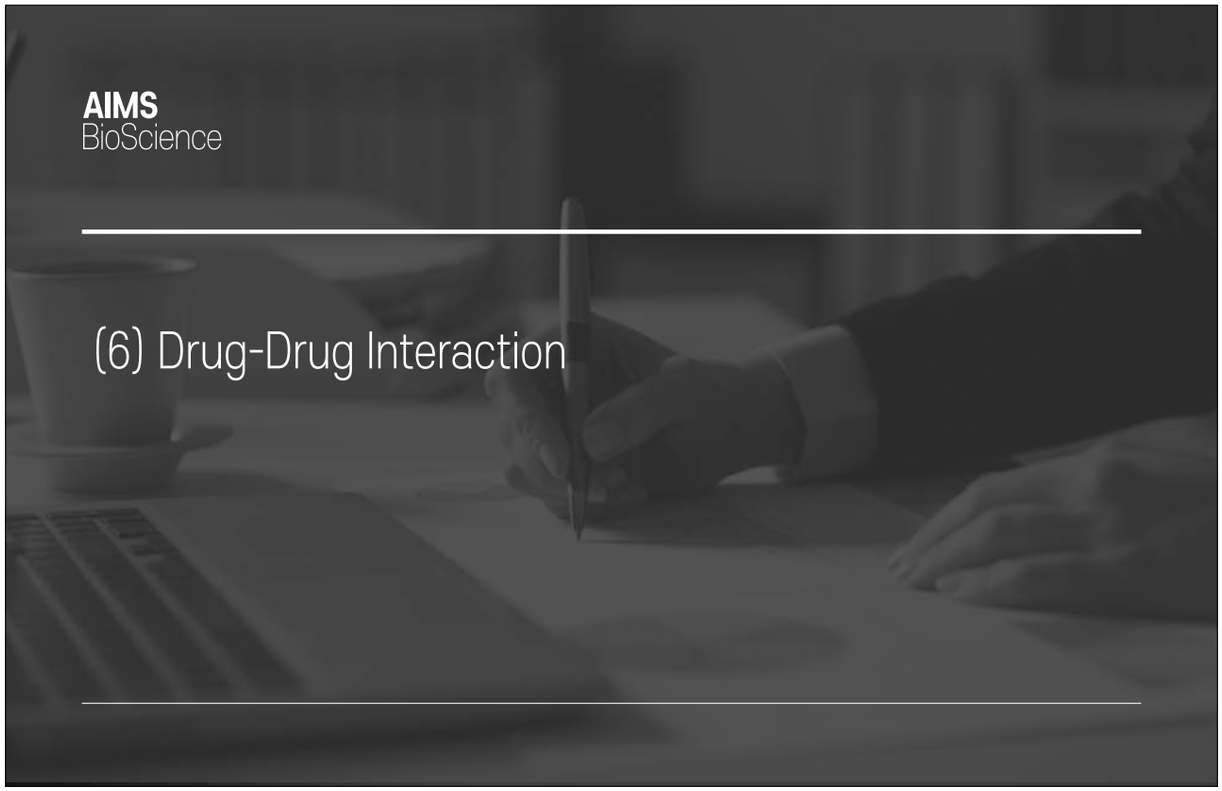

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DDI: *in vitro* Drug Interaction Studies (FDA)

Classification	Metabolism-mediated	Transporters-mediated

Substrate	• CYPs: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A
	• Use both methods;
	  • Chemical inhibitors in pooled HLMs
	  • Human recombinant enzymes
		• ABC transporters: P-gp, BCRP
		• SLC transporters: OATP1B1/OATP1B3, OAT1/OAT3, MATE1/MATE2-K, OCT2

Inhibition	• CYPs: 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A (midazolam, testosterone)
	• Evaluate in both manners;
	  • Reversible manner
	  • Time-dependent manner
		• ABC transporters: P-gp, BCRP
		• SLC transporters: OATP1B1/OATP1B3, OAT1/OAT3, MATE1/MATE2-K, OCT2

Induction	• CYPs: 1A2, 2B6, 3A4 first • If induce 3A4, should evaluate 2C	*Recommendations are not provided*
Note	• Preferably before first-in-human studies	• Before clinical studies in patients

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DDI: CYP inhibition

- **시험 목적:** 약물의 human CYP enzymes에 대한 저해능 확인 *(in vitro)*
  - CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A (Midazolam/Testosterone)
  - Reversible inhibition & Time-dependent inhibition

- **시험 디자인**
  - Test system: human liver microsome
  - Test conc: > 6 conc. (to estimate IC_{50}); high enough

Reversible inhibition	Time-dependent inhibition
Microsomes (HLM) + CYP substrates + Drugs (Inhibitor) ↓ Incubation for 15 min	Microsomes (HLM) + Drugs (Inhibitor) / Metabolites ↓ Pre-incubation for 30 min + CYP substrates ↓ Incubation for 15 min

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DDI: CYP inhibition

- **Reversible inhibition 시험 결과 (Example)**

IC_{50} (μM)						
CYP1A2	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**
>50	>50	0.482	28.9	7.87	>50	>50

- Strong inhibition potential on CYP2C8 (IC_{50} < 1 μM)

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DDI: CYP inhibition

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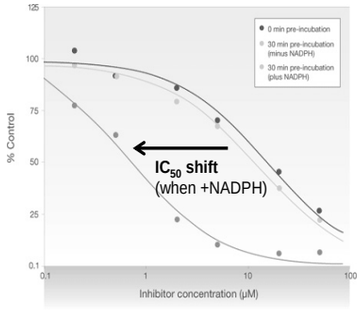
- **Time-dependent inhibition 시험 결과 (Example)**



[Criteria]
- Fold shift ≥ 1.5

Isoforms	IC_{50} (μM)			Fold shift
	0 min pre-incub.	30 min pre-incub. (-NADPH)	30 min pre-incub. (+NADPH)	
CYP1A2	>50.0	>50.0	>50.0	ND
CYP2B6	>50.0	28.3	37.5	0.76
CYP2C8	<0.200	0.25	0.25	0.99
CYP2C9	17.1	18.8	24.0	0.78
CYP2C19	5.71	7.06	6.74	1.05
CYP2D6	>50.0	>50.0	>50.0	ND
CYP3A4 (MDZ)	>50.0	>50.0	23.0	>2.17
CYP3A4 (TST)	>50.0	>50.0	32.3	>1.55

- Time-dependent inhibitor of CYP3A4

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DDI: CYP induction

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- **시험 목적:** 약물의 human CYP enzymes에 대한 유도능 확인 *(in vitro)*
  - CYP1A2, 2B6, 3A4 first
  - If 3A4 inducer → should evaluate induction potential on 2C family

- **시험 디자인**
  - Test system: human hepatocytes (from at least 3 donors)
  - Test conc: > 6 conc.
  - Incubation time: 48~72 h
  - Study endpoints: CYPs mRNA levels (and/or enzyme activity)

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DDI: CYP induction

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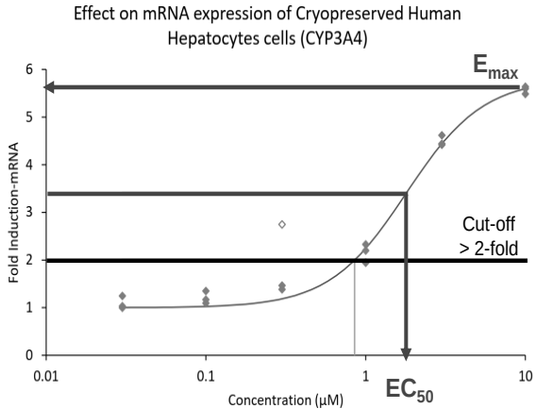
- **시험 결과 (Example)**



[Criteria]
- ≥ 2-fold increase

CYP1A2	mRNA expression		
	donor 1	donor 2	donor 3
Maximum Fold induction	NI	NI	1.53
EC_{50} (μM)	NI	NI	NC
E_{max} (μM)	NI	NI	NC
CYP2B6	**mRNA expression**		
	donor 1	**donor 2**	**donor 3**
Maximum Fold induction	NI	5.57	1.93
EC_{50} (μM)	NI	1.80	NC
E_{max} (μM)	NI	5.81	NC
CYP3A4	**mRNA expression**		
	donor 1	**donor 2**	**donor 3**
Maximum Fold induction	NI	1.43	NI
EC_{50} (μM)	NI	NC	NI
E_{max} (μM)	NI	NC	NI

- Induction potential on CYP2B6

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DDI: CYP inhibition/induction

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- **Data interpretation:** Basic kinetic model

Category	Reversible inhibition	Time-dependent inhibition	Induction
R-value equation	$R_1 = 1 + \frac{I_{max,u}}{K_{i,u}}$	$R_2 = \frac{K_{obs} + K_{deg}}{K_{deg}}$ $K_{obs} = \frac{K_{inact} \text{ x } 50 \text{ x } I_{max,u}}{K_{I,u} + 50 \text{ x } I_{max,u}}$	$R_3 = \frac{1}{1 + d \cdot (\frac{E_{max} \text{ x } 10 \text{ x } I_{max,u}}{EC_{50} + 10 \text{ x } I_{max,u}})}$
Cut-off	$R_1 \geq 1.02$	$R_2 \geq 1.25$	$R_3 \leq 0.8$
Note	$I_{max,u}$ = the maximal unbound plasma concentration of the interacting drug at steady-state ($I_{max,u} = C_{max,ss} * f_{u,p}$) $K_{i,u}$ = the unbound inhibition constant determined in vitro ($K_{i,u} = K_i * f_{u,mic}$) $K_{I,u}$ = the unbound inhibitor concentration causing half-maximal inactivation ($K_{I,u} = K_I * f_{u,mic}$) K_{inact} = the maximal inactivation rate constant E_{max} = the maximum induction effect EC_{50} = in vitro concentration causing half-maximum induction d = a scaling factor (assumed to be 1 for the basic model)		

- **If $R_1 \geq 1.02$, $R_2 \geq 1.25$ or $R_3 \leq 0.8$ → Mechanistic models or Clinical DDI studies needed.**

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APPLICATION OF NON-CLINICAL DMPK DATA

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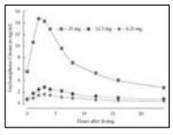
Application of Non-clinical DMPK data

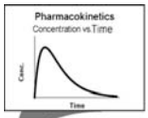
I. Human PK prediction

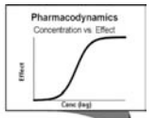
Human PK prediction

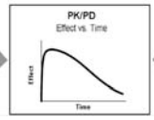
In vivo PK In vitro ADME



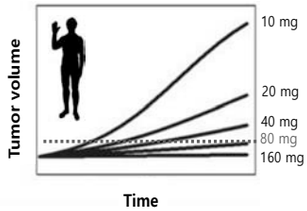
PK-PD modeling in animal model







Simulation for human efficacious dose projection



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Application of Non-clinical DMPK data

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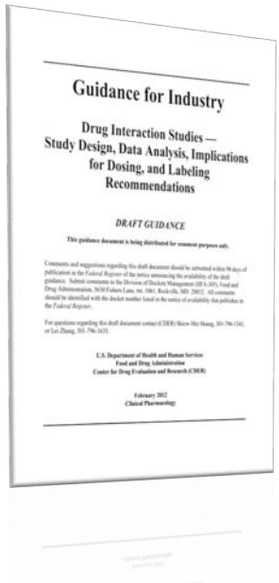
II. Risk assessment for DDI



Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

DRAFT GUIDANCE

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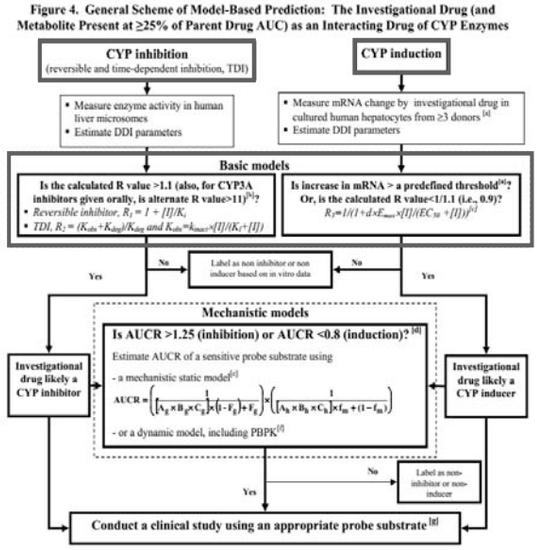
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

February 2012
Clinical Pharmacology



"Clinical DDI studies needed?"

경청해 주셔서 감사합니다.

(Question: cherlyn@aimsbiosci.com)