Drug Metabolism by Cytochrome P450 using in vitro/in vivo Metabolic Model

2016/10/14

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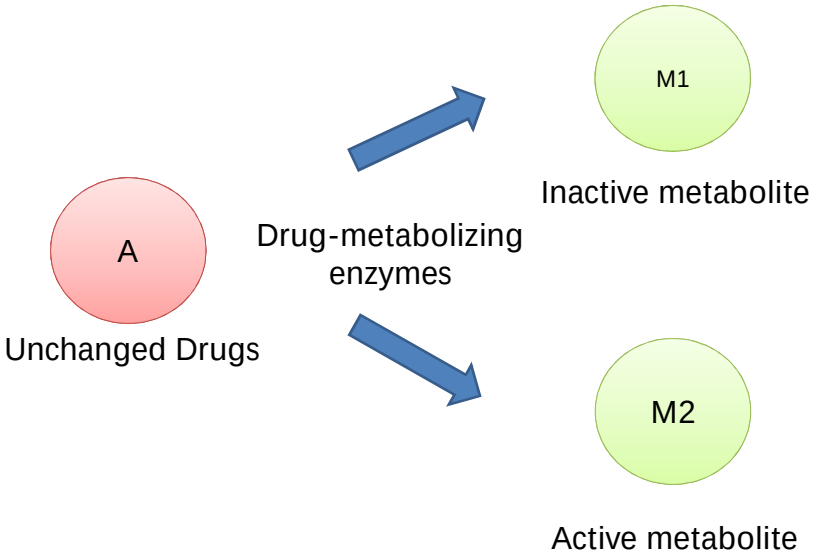
Hiroshima University

Drug Metabolism



Metabolic activation of proestrogens by cytochrome P450

3


毎日新聞

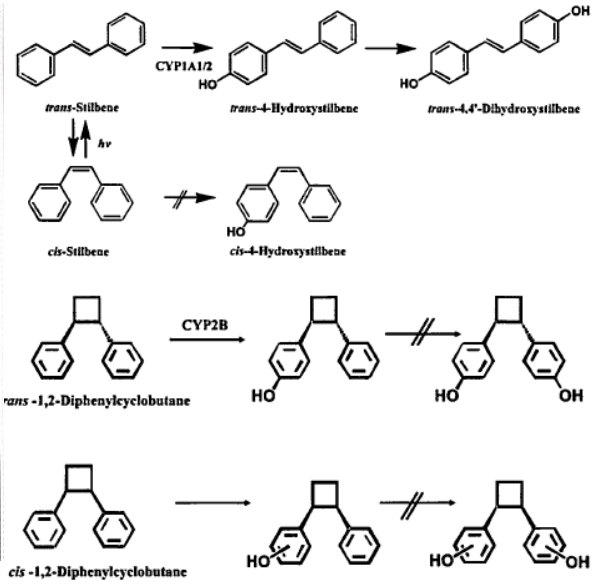
スチレン

環境ホルモン

環境省がリストから外す

女の気持ち

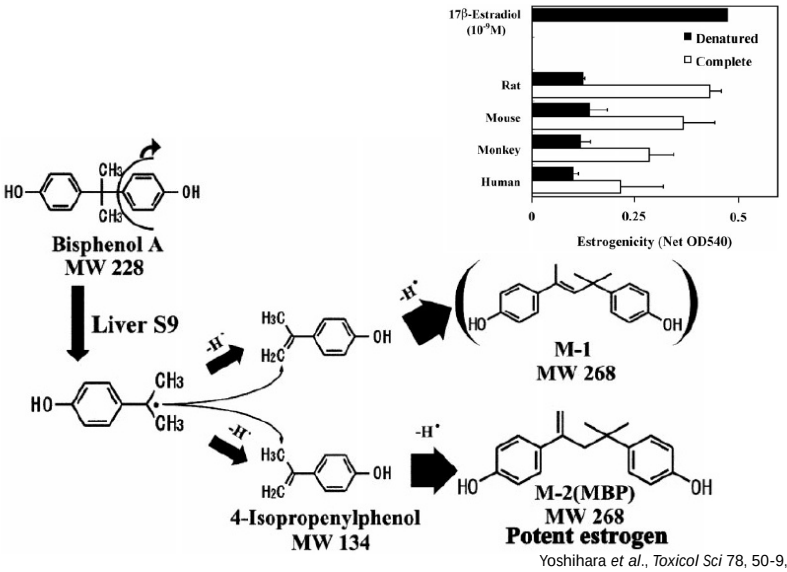
Mainichi Newspapers Feb. 2002



Kitamura et al., *J Health Sci* 54, 343-355, 2008

Potent estrogenic metabolites of bisphenol A formed by liver S9 fraction

4



Yoshihara *et al.*, *Toxicol Sci* 78, 50-9, 2004

In vitro / in vivo metabolic study

5

✓*In vitro metabolic model*

Substrate
O2
NADPH

→ *Cyt P450*

What kind of metabolite?

Metabolite

✓ *In vivo metabolic model*

Rat
Dog
Monkey
Humanized model

→ *Prediction*

How is human metabolism?

Human

Structure determination of metabolites

6

✓*Standard Approach* *"Defensive Approach"*

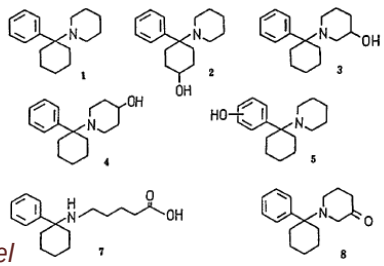
1. Metabolic reaction in vivo/in vitro
2. Separation of metabolites by chromatographic methods (< 1 mg scale)
3. Structure assumption with MS and NMR
4. Structure determination with authentic compound

✓ *Our Approach* *"Offensive Approach"*

1. Oxidation of substrate by CYP450 mimic system (Metal porphyrin, Oxidants etc.)
2. Separation of products by chromatographic methods (～100 mg scale)
3. Structure determination with MS, NMR and elemental analysis
4. Metabolic reaction in vivo/in vitro
5. Structure determination with authentic (synthetic) compound

Application of chemical P450 model system to studies on Phencyclidine metabolism (1)

7



Chemical P450 model

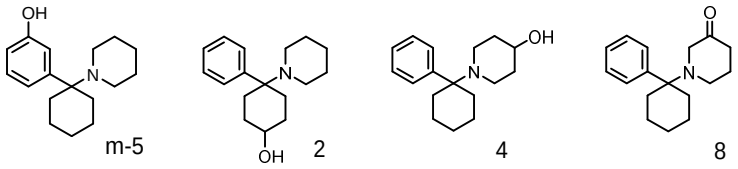
Reaction system	Amount of product (μg/reaction mixture)							
	o-5	*m*-5	*p*-5	2	3	4	7	8
$Fe^{2+}+H_2O_2$	0.345	76.5	24.2	87.70	3.38	N.D.	0.98	—
in $H_2O:CH_3CN=8:3$	(0.2)	(39.6)	(12.5)	(45.4)	(1.8)	(0.0)	(0.5)	—
$Fe^{2+}+H_2O_2$	1.475	107.0	28.2	99.88	12.0	0.697	1.00	—
in $H_2O:CH_3CN=1:9$	(0.6)	(42.7)	(11.3)	(39.9)	(4.8)	(0.3)	(0.4)	—
$Fe^{2+}+O_2$	0.089	0.740	Trace	50.11	21.71	33.53	N.D.	—
+ascorbic acid	(Trace)	(0.7)	(Trace)	(47.2)	(20.4)	(31.6)	(0.0)	—
Fe(III)TPPCl	9.87	0.196	0.152	1.48	2.40	0.679	0.43	250.4
+iodosylxylene	(3.7)	(Trace)	(Trace)	(0.6)	(0.9)	(0.3)	(0.2)	(94.3)
Fe(III)TPPCl	1.31	0.264	0.203	1.90	3.49	1.79	N.D.	93.3
+cumene hydroperoxide	(1.3)	(0.3)	(0.2)	(1.9)	(3.4)	(1.8)	(0.0)	(91.2)
Fe(III)TPPCl	N.D.	0.328	N.D.	40.85	15.77	25.45	N.D.	—
$+Zn+AcOH+O_2$	(0.0)	(0.4)	(0.0)	(49.6)	(19.1)	(30.9)	(0.0)	—

Fe(III)TPPCl = tetraphenylporphinatoiron(III) chloride. —, not measured. N.D., not detected. Trace, <0.1. Relative yields (%) are given in parentheses.

Masumoto et al., *Chem Pharm Bull (Tokyo)*.37(7):1788-94, 1989.

Application of chemical P450 model system to studies on Phencyclidine metabolism (2)

8



Microsomal system

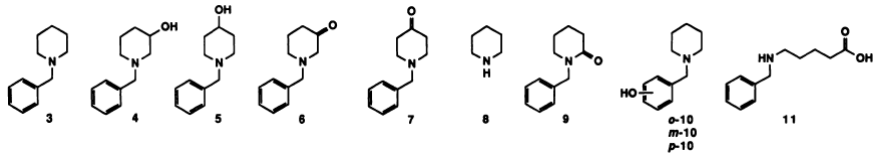
Microsomes	Oxidant	Amount of product (μg/reaction mixture)			
		m-5	2	4	8
Rat non-treated	A	0.40 (4.3)	0.96 (10.4)	4.2 (45.4)	3.7 (40.0)
	B	0.38 (5.4)	3.5 (49.4)	1.7 (24.0)	1.5 (21.2)
Rat PB-treated[a)]	A	0.36 (10.0)	2.4 (66.7)	0.37 (10.3)	0.47 (13.1)
	B	0.34 (3.4)	6.0 (59.2)	1.2 (11.8)	2.6 (25.6)
Rat 3-MC-treated[b)]	A	0.42 (20.7)	0.89 (43.8)	0.28 (13.8)	0.44 (21.7)
	B	0.38 (2.2)	1.6 (9.1)	8.3 (47.2)	7.3 (41.5)
Mouse	A	0.87 (31.4)	1.3 (46.9)	0.24 (8.7)	0.36 (13.0)
	B	0.34 (3.9)	2.6 (30.1)	3.0 (34.7)	2.7 (31.3)

Oxidant: A) cumene hydroperoxide, B) iodosylxylene. *a)* Phenobarbital Na 60 mg/kg d for 3 d i.p. *b)* 3-Methylcholanthrene 30 mg/kg d for 3 d i.p. Relative yields (%) are given in parentheses.

Masumoto et al., *Chem Pharm Bull (Tokyo)*.37(7):1788-94, 1989.

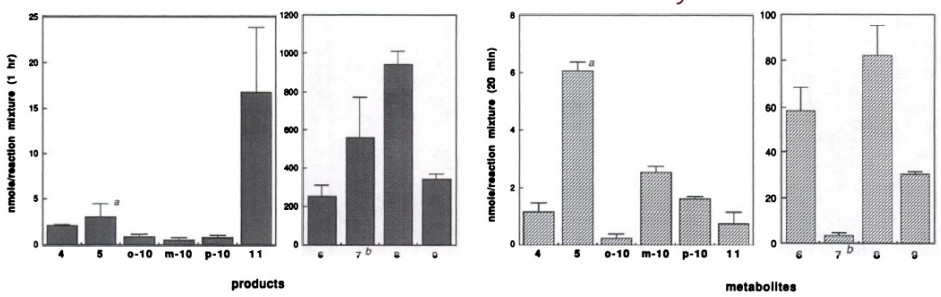
Application of chemical P450 model system to studies on Piperidine metabolism

9



a Chemical P450 model

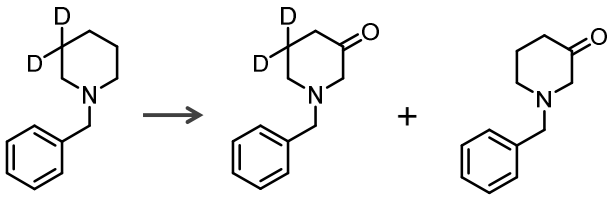
b Microsomal system



Masumoto et al., *Drug Metab Dispos* 19(4):768-80, 1991.

Intramolecular product isotope effects on the β-oxo-formation

10



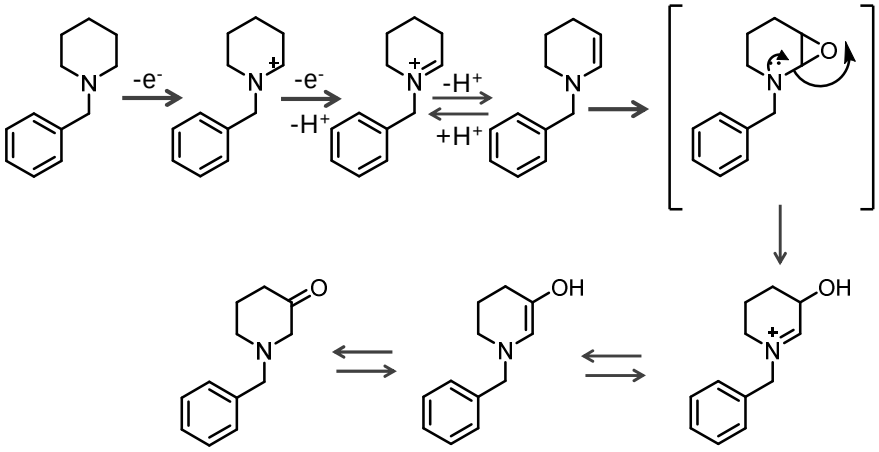
Rat Liver Microsomes-NADPH/O_2 System		Fe(III)TPPCl-Iodosylxylene System	
Reaction Time	k'_H/k'_D[a]	Reaction Time	k'_H/k'_D
min		*min*	
5	1.43 ± 0.02[b]	50	0.88 ± 0.20
11	1.08 ± 0.05	75	1.13 ± 0.09
16	1.19 ± 0.07	100	0.97 ± 0.04
20	1.14 ± 0.03		

[a] Molar ratio of 6-d_2/6 formed.
[b] Each value represents mean ± SD of three determinations.

Masumoto et al., *Drug Metab Dispos* 19(4):768-80, 1991.

11

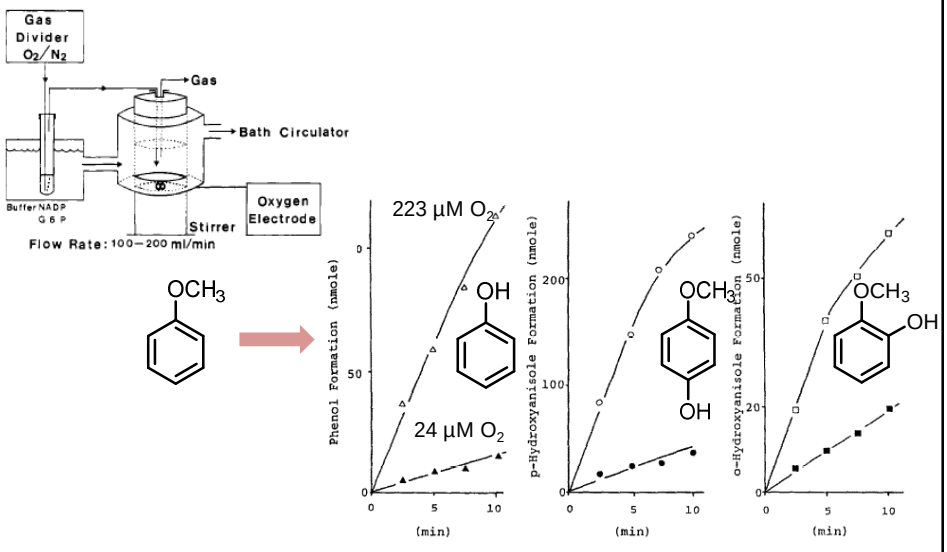
Mechanism of piperidine metabolism



Masumoto et al., *Drug Metab Dispos* 19(4):768-80, 1991.

12

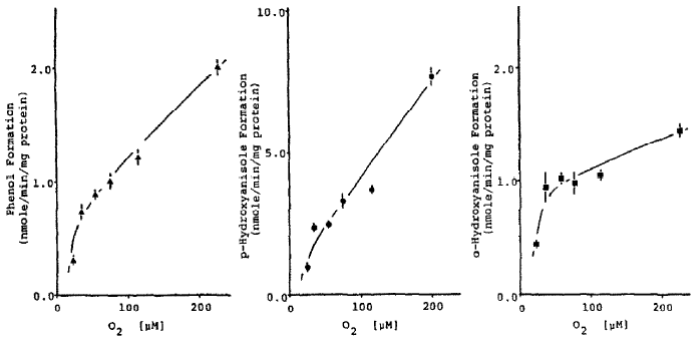
Effects of oxygen concentration on the metabolic pathway of anisole



Takahara et al., *Biochem Pharmacol*. 35(3):541-4.,1986

Rates of anisole metabolite formation by rat liver microsomes as a function of oxygen concentration

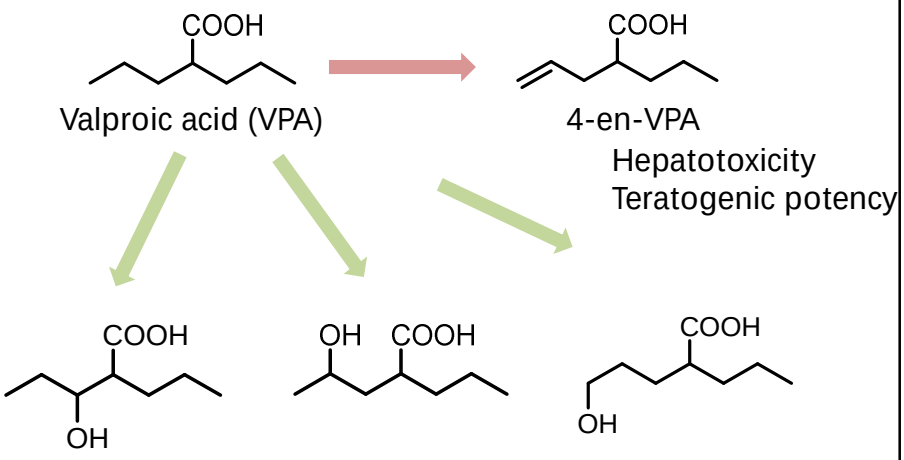
13



Takahara et al., *Biochem Pharmacol*. 35(3):541-4.,1986

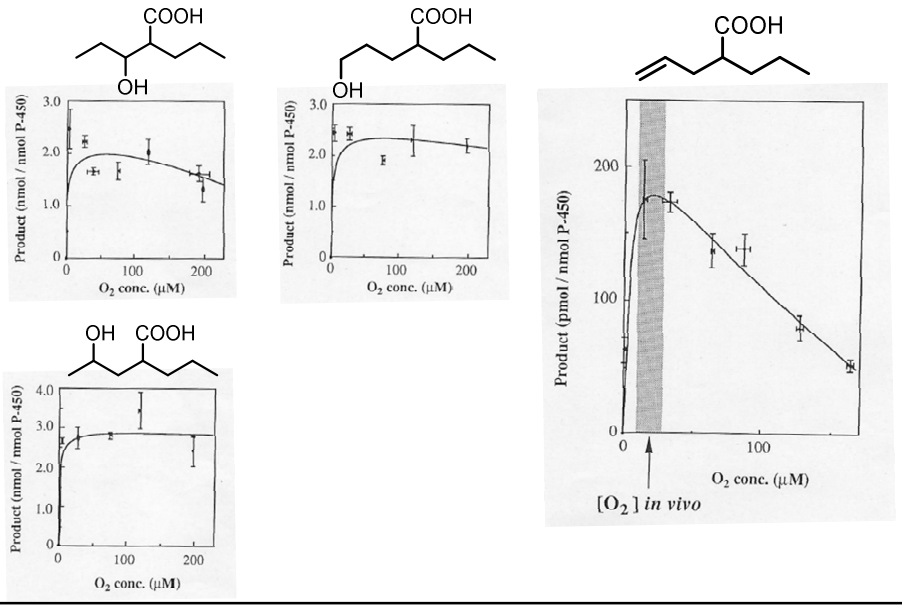
Metabolic Pathways of Valproic acid

14



15

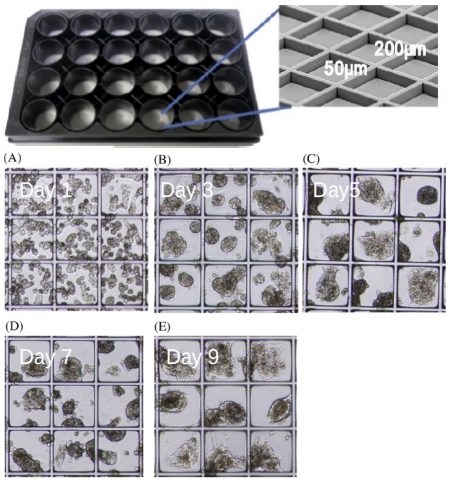
Effects of O_2 concentration on VPA metabolism



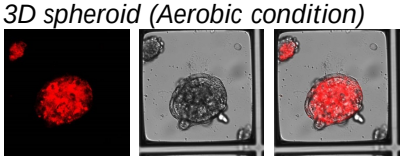
16

Intracellular O_2 levels in hepatocyte spheroids

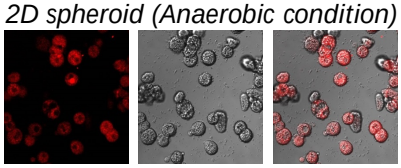
Micro-Space Cell Culture Plate (Kuraray Co., Ltd.) (Elplasia®, Kuraray Co. Ltd.)
24 well plate (200 μm-length×200 μm-width×50 μm-depth)



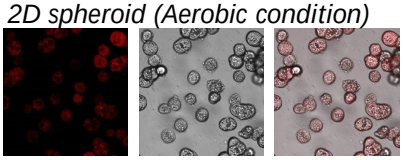
3D spheroid (Aerobic condition)



2D spheroid (Anaerobic condition)



2D spheroid (Aerobic condition)



Sanoh et al., *Toxicol in Vitro* 28: 1176-1182 (2014)

Humanized model mice

17

<Genetically humanized mice>

Advantages
- Permanent model without recreation
- Low cost of production
- High consistency between individual mice
- Human gene expressed in various organs
- Human gene expressed in all liver cells
- Availability of knockout controls
- Usually healthy

Disadvantages
- Expression of selected human gene only
- Different genes of interest require different mouse lines
- High effort to generate donor variability
- No infection with human specific pathogens
- Non-transplantable with human cells
- More challenging human extrapolation through IVIVC
- Potential compensatory gene expression changes

<Liver Chimeric mice>

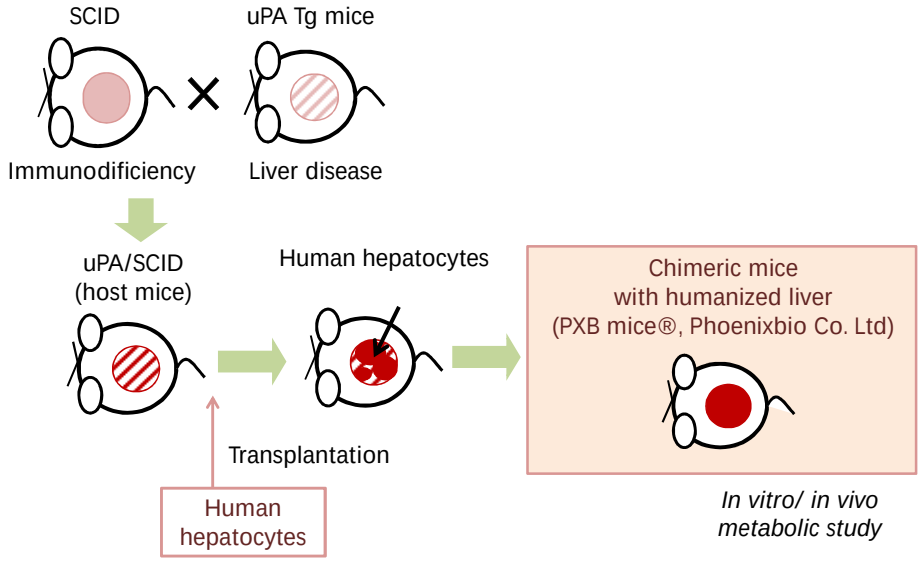
Advantages
- Human hepatocytes express all human genes
- One mouse line fits different purposes
- Ease of generating donor variability
- Susceptible to human-specific pathogens
- Transplantable with various human cells
- Combined use with human hepatocytes supports extrapolation to humans
- No compensatory gene expression changes reported

Disadvantages
- Continuous recreation required
- High cost of production
- Varying degree of humanization
- Humanization restricted to the liver
- Residual mouse hepatocytes express murine gene
- Knockout controls usually not available
- Immune compromised

Scheer and Wilson., *Drug Discov Today*. 2015. pii: S1359-6446(15)00342-6.

Chimeric mice with humanized liver

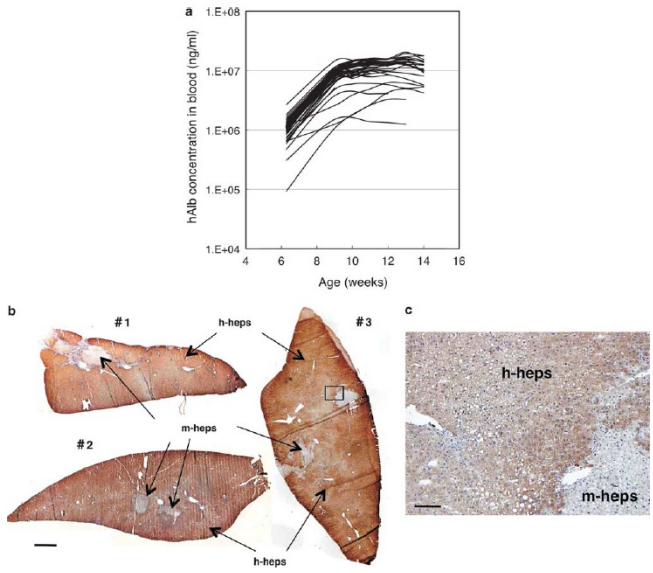
18



Tateno *et al., Am J Pathol.* 2004 ;165:901-12.

Replacement of human hepatocytes in mouse liver

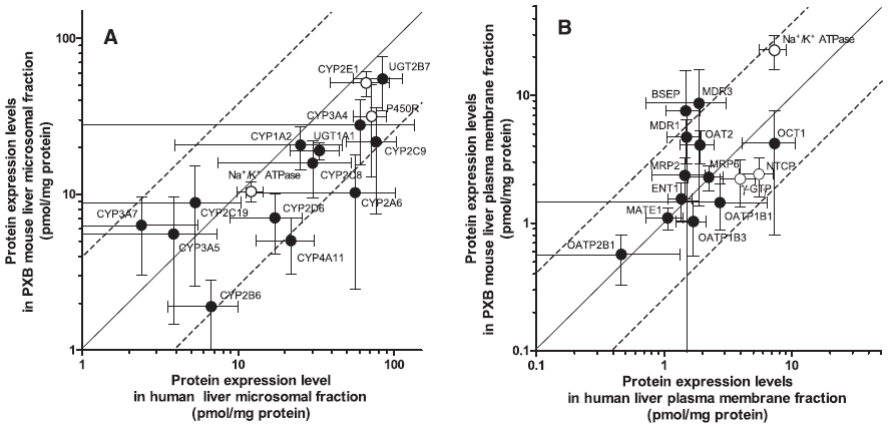
19



Tateno *et al., Lab Invest.* 2013 ;93:54-71

Expression of human specific drug-metabolizing enzymes and transporters in chimeric mice

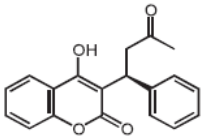
20



Ohtsuki *et al.*, *Drug Metab Dispos* 2014;42: 1039-1043

Predictability of drug metabolism

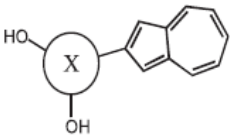
21



S-Warfarin
7-Hydroxywarfarin

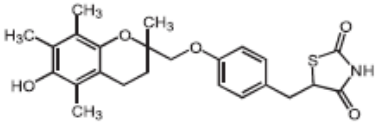
Inoue *et al*., *Drug Metab Dispos* 2008; 36: 2429-2433

Inoue *et al*., *Drug Metab Pharmacokinet* 2009; 24: 153-160



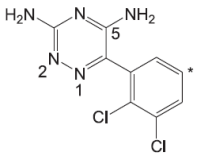
Compound A
O-Glucuronidation, hydroxylation, sulfation

Kamimura *et al*., *Drug Metab Pharmacokinet* 2010;25: 233-235



Troglitazone
Glucuronidation, sulfation, quinone form

Schulz-Utemoehl *et al*., *Xenobiotica* 2012;42: 503-517



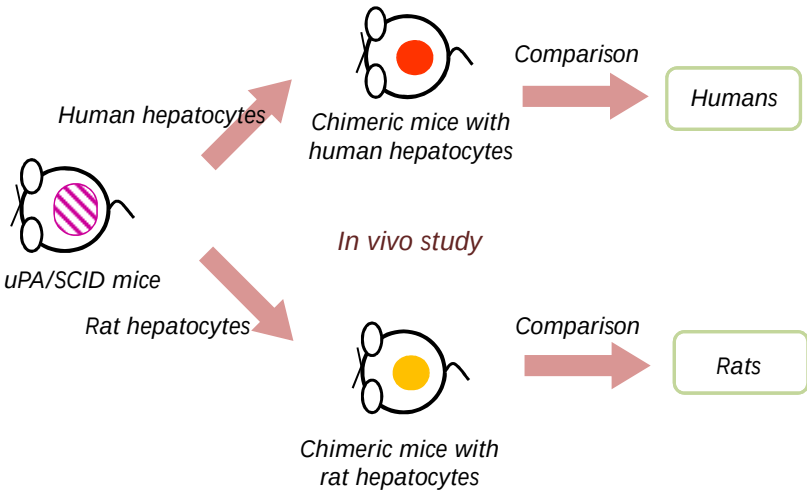
Lamotrigine
N-Glucuronidation, *N*-oxidation

Bateman *et al*., *Drug Metab Dispos* 2014;42: 1055-1065

Sanoh and Ohta, Biopharm Drug Dispos. 2014; 35: 71-86

Approach of drug metabolism using chimeric mice

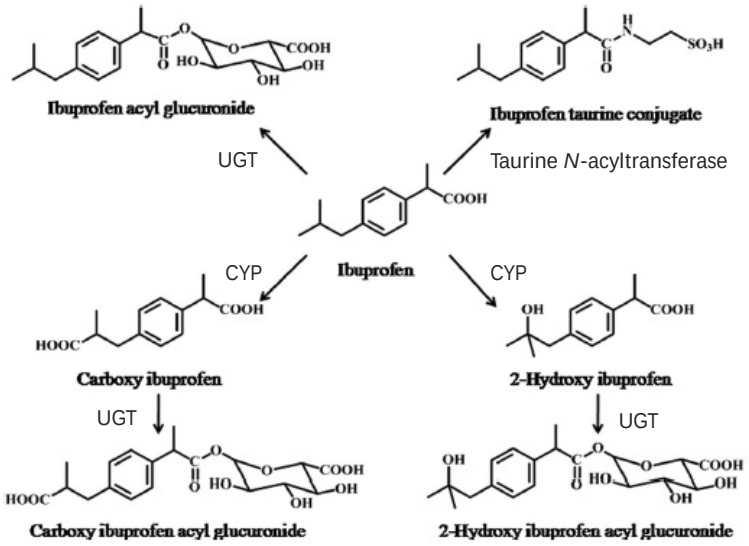
22



Tateno *et al*., *Am J Pathol*. 2004 ;165:901-12.
Sanoh and Ohta, *Biopharm Drug Dispos*. 2014; 35: 71-86

Metabolic pathways of ibuprofen in humans

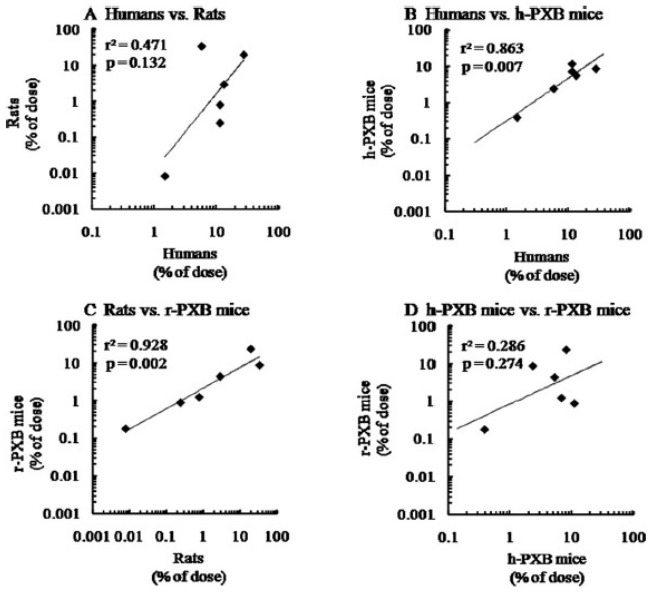
23



Shirley et al., *J Pharmacol Exp Ther* 269:1166–1175 (1994).
Kepp et al., *J Chromatogr B Biomed Sci Appl* 696:235–241 (1997)

Comparison of urinary excretion of ibuprofen metabolites

24



Sanoh *et al.*, *Drug Metab Dispos.* 2012;40(12):2267-2272

Acknowledgement

University of Tokyo
・Prof. M. Hirobe 廣部 雅昭 先生

Hiroshima University
・Prof. S. Yoshihara 吉原 新一 先生
・Prof. S. Kitamura 北村 繁幸 先生
・Prof. K. Sugihara 杉原 数美 先生
・Dr. Y. Kotake 古武 弥一郎 准教授
・Dr. S. Sanoh 佐能 正剛 助教