Enzymological study on P450 s

Shinshu University Hospital
Shigeru Ohmori, PhD

Martin Klingenberg

(Estabrook RW, Drug Metab Dispos, 31, 1461-1473, 2003)

Pigments of Rat Liver Microsomes[1]

Martin Klingenberg[2]

From the Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pennsylvania

Received August 13, 1957

The Effect of Carbon Monoxide on the Spectra

Upon addition of carbon monoxide to microsomes reduced with either DPNH or dithionite, a rather broad absorption band with a maximum at 450 mμ appears[4] (Fig. 3, curves *A* and *B*). This CO band forms slowly ($t_{1/2co}$ = 10 sec.). In microsomes reduced by DPNH the absorption at 450 mμ (curve *A*) is about 1.5 times as intense as the α-band of reduced cytochrome b_5. The addition of dithionite increases this two to three times. The intensity relationships are not the same for microsomes isolated from other mammalian livers. The trough at 404 mμ indicates the

[3] B. Chance and E. Boeri, unpublished experiments.
[4] Unpublished observations of Dr. G. R. Williams.

- (Arch Biochem Biophys, 75, 376-386, 1958)

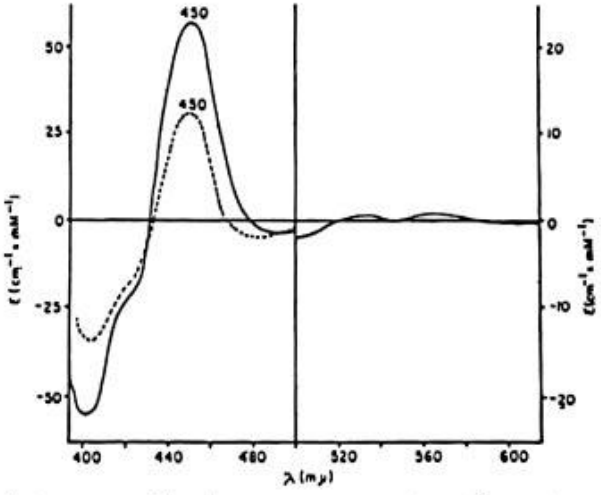


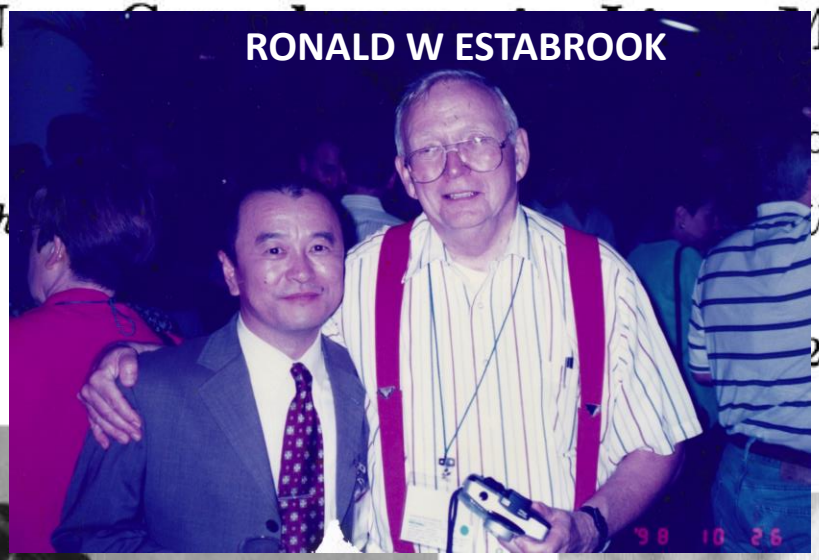
FIG. 3. Carbon monoxide difference spectra of rat liver microsomes. The millimolar extinction coefficients refer to the cytochrome b_5 present in the microsomes. – – – – – – Curve *A*: Carbon monoxide with DPNH reduction. ——— Curve *B*: Carbon monoxide with dithionite reduction.

A N Microsomes

o

From th University, Osaka,

2, 1962)

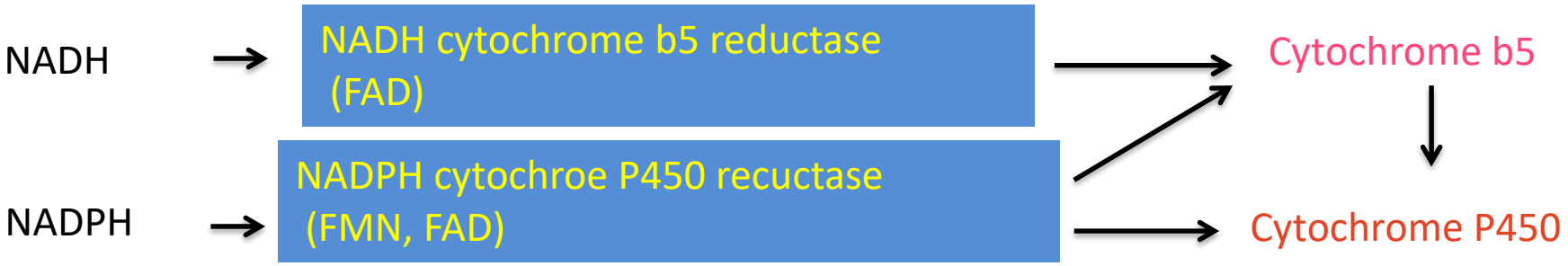


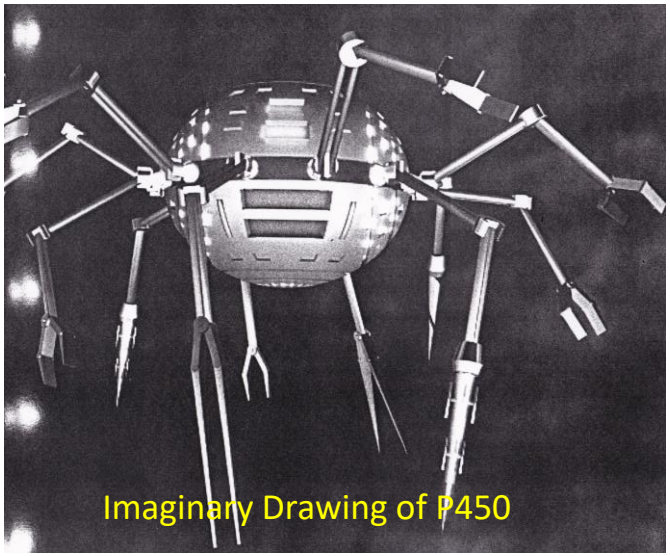
RYO SATO

TSUNEO OMURA

(Estabrook RW, Drug Metab Dispos, 31, 1461-1473, 2003)

Hepatic Microsomal Electron Transport System





Inducibility
Spectral properties
Species Difference
Sex difference
Elecrton pathway
…
…
…

J Gillete
W Levin
A Y H Lu
F P Guengerich
Eric Johnson
F Gonzalez
M I-Sundberg
…
…
…

The theme for my Graduation Thesis :
Purification of Cytochrome P448 from 3-Methylchanthrene Pretreated Rat Liver Microsomes.

Vol. 60, No. 1, 1974 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS

First report for P450 purification for ref.

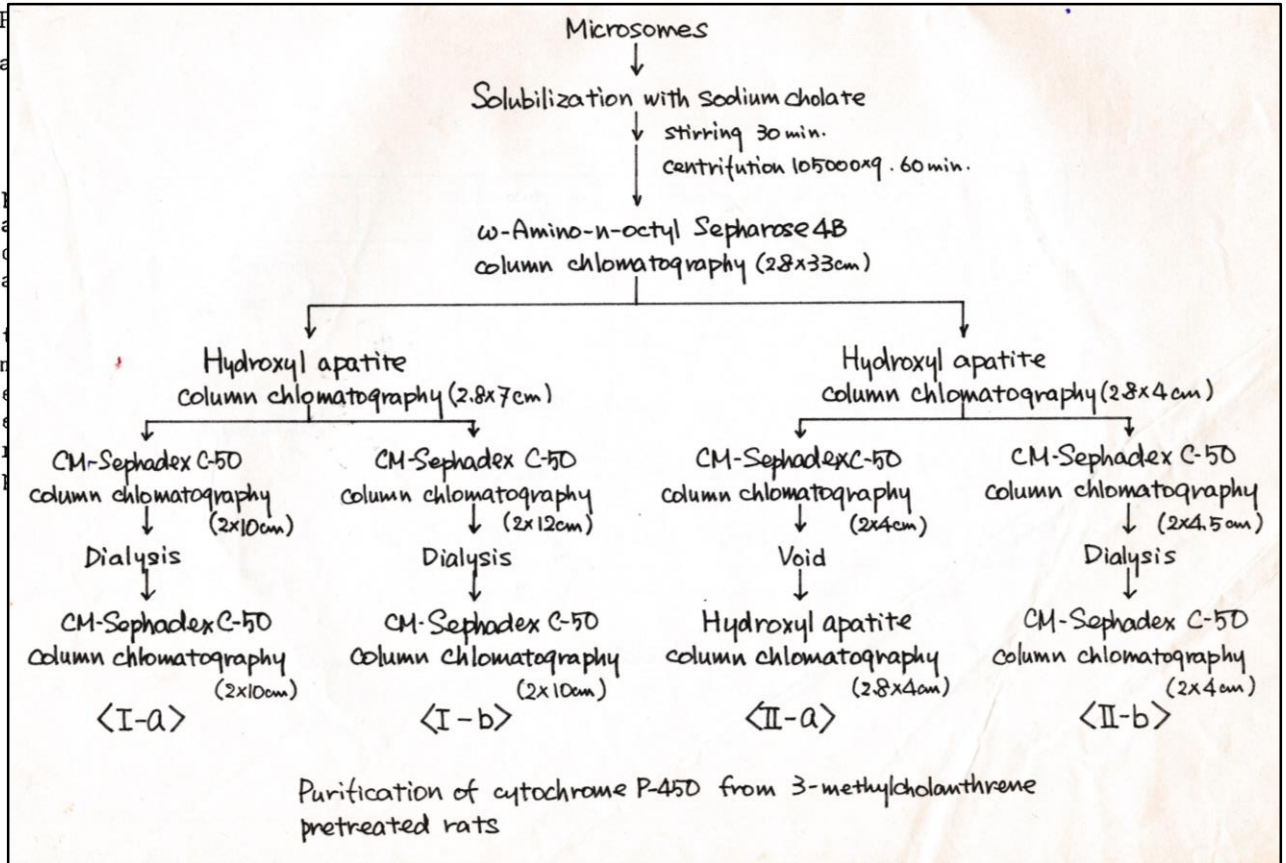
A GEL-ELECTROPHORETICALLY HOMOGENEOUS PREPARATION OF CYTOCHROME P-450 FROM LIVER MICROSOMES OF PHENOBARBITAL-PRETREATED RABBITS

#1/5

Yoshio Imai and Ryo Sato
Institute for Protein R
Suita, Osa

Received July 12,1974

Summary: Cytochrome P-450 was p
barbital-pretreated rabbits to a
per mg of protein with a yield o
yielded only a single protein ba
acrylamide gel electrophoresis,
about 45,000 was estimated for t
of cytochrome b5, NADH-cytochrom
c reductase activities. Aniline
methylase activities could be re
cytochrome with an NADPH-cytochr
by a detergent method) and phosp

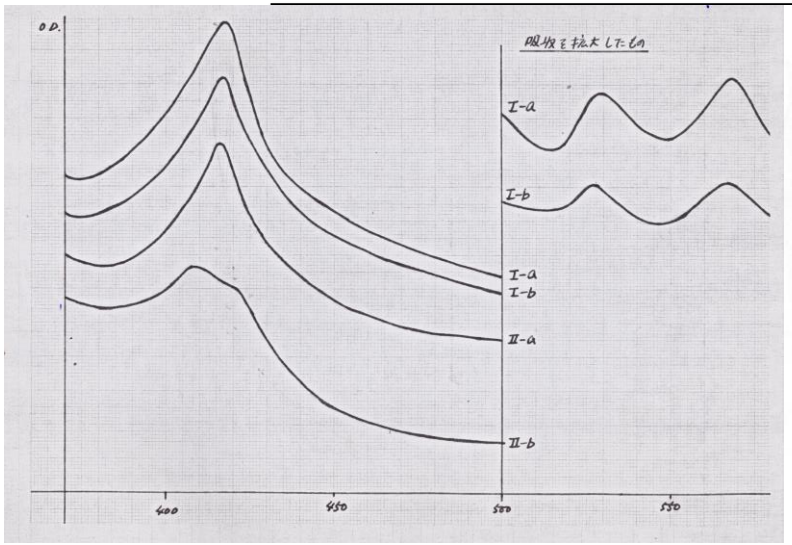


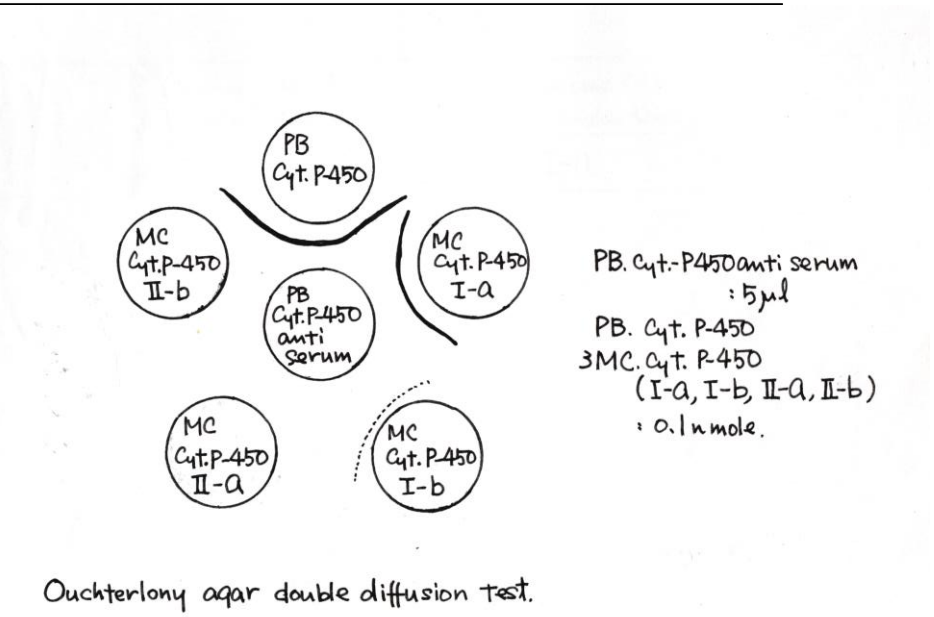
Purification of cytochrome P-450 from 3-methylcholanthrene pretreated rats

The theme for my Graduation Thesis ： Purification of Cytochrome P448 from 3-Methylchanthrene Pretreated Rat Liver Microsomes.

Results were enough to excite me to continue to work for P450

P450I-a P450I-b P450II-a P450II-b





Ouchterlony agar double diffusion test.

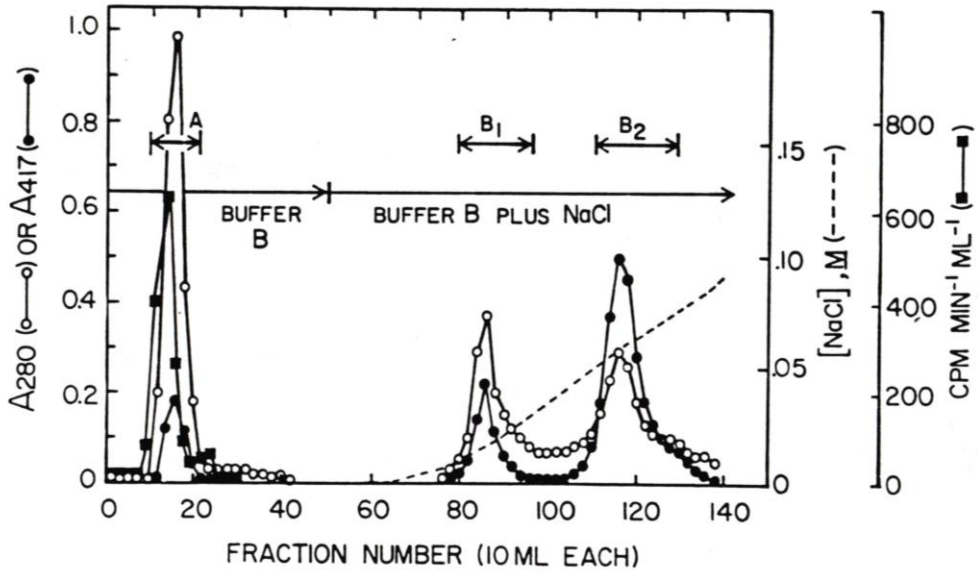
My simple research question:
How many P450s are present in liver microsomes?
To clarify the characteristics of each P450.

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 205, No. 2, December, pp. 365–379, 1980

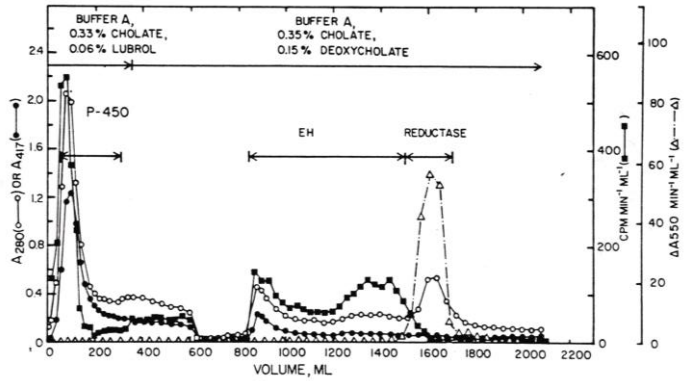
Purification of Cytochrome *P*-450, NADPH-Cytochrome *P*-450 Reductase, and Epoxide Hydratase from a Single Preparation of Rat Liver Microsomes[1]

F. PETER GUENGERICH AND MARTHA V. MARTIN

Chromatography of n-Octylamino-Sepharose 4B P-450 Fraction on DEAE-Cellulose.



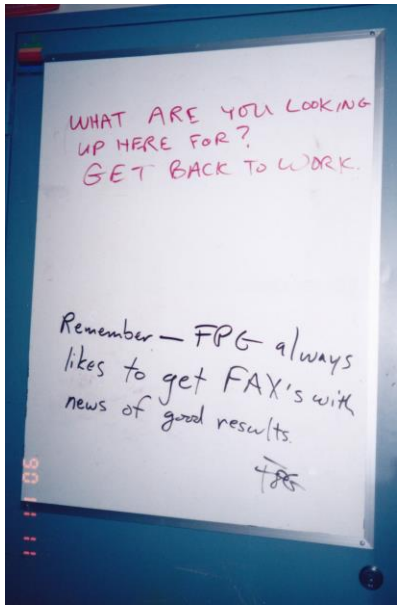
Chromatography of Cholate-Solubilized Rat Liver Microsomes on n-Octylamino-Sepharose 4B.

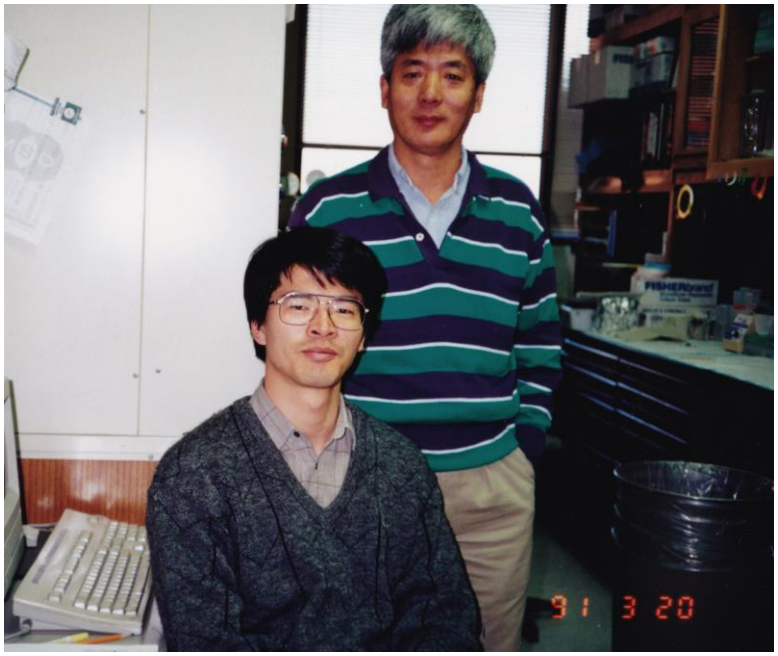


Monkey CYPs Purified and Characterized

	Classified Family	Substrate	Ref
Cynmolgus monkey			
P450CMLa	CYP2B	Banzphetamine	Arch. Biochem. Biophys. (1993)
P450CMLb	CYP2A	Coumarin	Mol. Pharmacol. (1993)
P450CMLc	CYP3A4	Testosterone (6β-OH)	Arch. Biochem. Biophys. (1993)
P450CMLd	CYP2C	S-Mephenytoin	Arch. Biochem. Biophys. (1993)
	CYP2C43		Drug Metab. Pharmacokin. (2002)
Baboon			
P450BLa	CYP3A	Testosterone (6β-OH)	Biol. Pharm. Bull. (1994)

1990: I went to Fred's Lab. to study P450 expression in *E. coli*.





Expression of Modified Human Cytochrome P450 3A4 in *Escherichia coli* and Purification and Reconstitution of the Enzyme

E. G. Gillam, T. Baba, B-R. Kim, S. Ohmori, and F. P. Guengerich

Arch. Biochem. Biophys., 305, 123-131, 1993.

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      1    5        10        15        20        25        30        35
NF1:  M A L I P D L A M E T W L L L A V S L V L L Y L Y G T H S H G L F K K
NF10: M A ———————————————————————————————— Y G T H S H G L F K K
NF12: M A ———————————— L L L A V ———————————————————— F K K
NF13: M A L I P D L A M E T W L L L A V S L V L L Y L Y G T H S H G L F K K
NF14: M A ———————————— L L L A V F L V L L Y L Y G T H S H G L F K K
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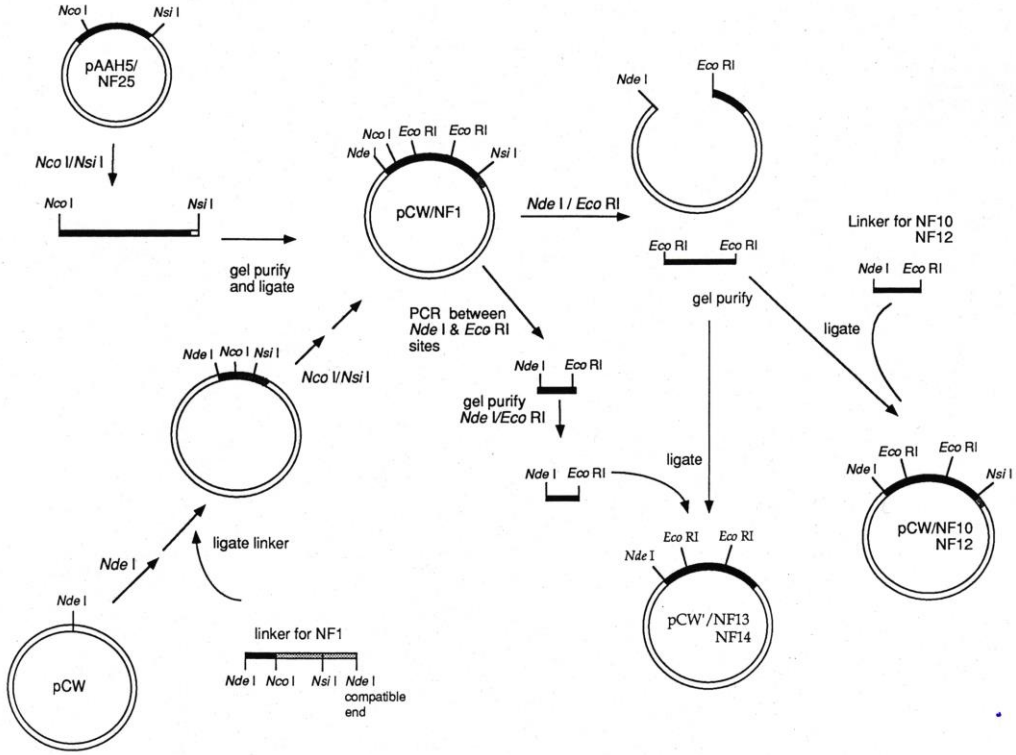
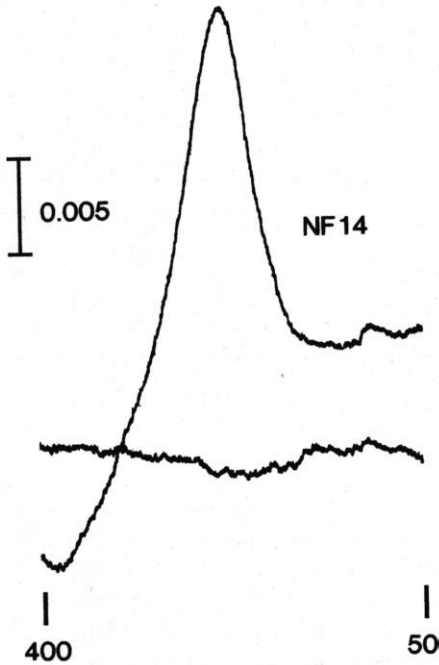
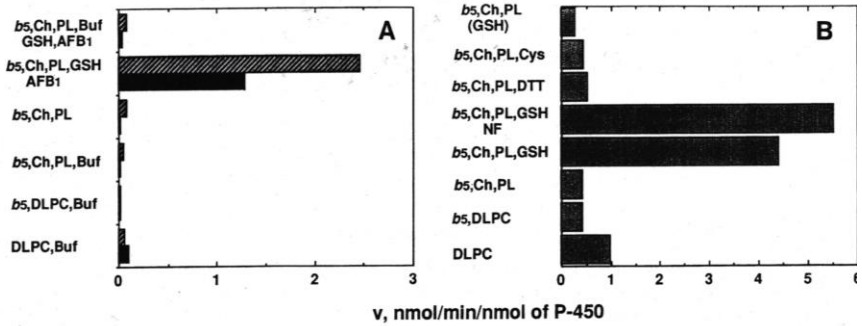






Next year, P450 1A1 was also reported:. Expression of Modified Human Cytochrome P450 1A1 in *Escherichia coli* : Effects of 5' Substitution, Stabilization, Purification, Spectral Characterization,

Zuyu Guo, E. G. Gillam, S. Ohmori, R. H. Tukey and F. P. Guengerich. Arch. Biochem. Biophys., 312, 436-446, 1994.

Drug Interaction Case report

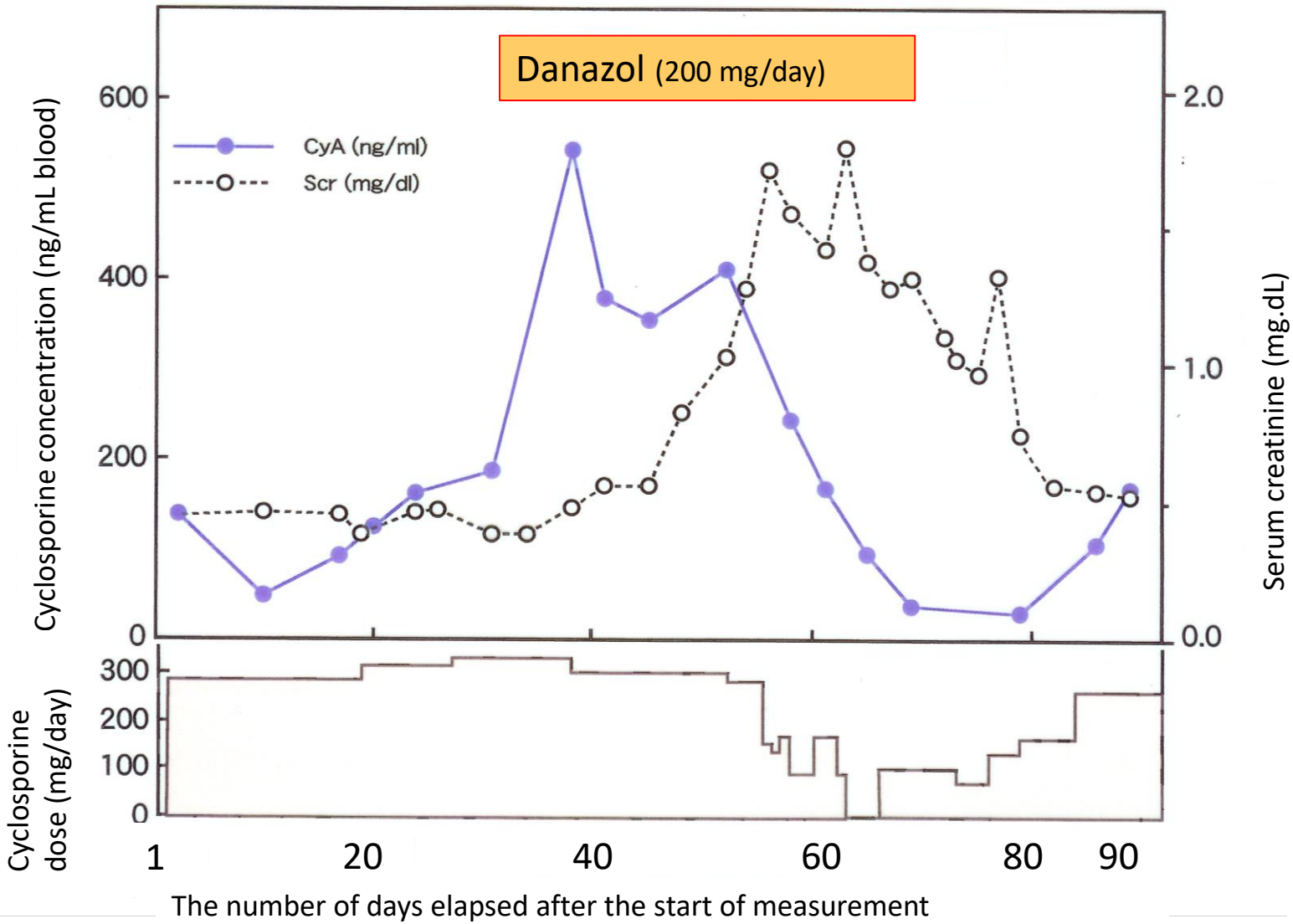
1， Cyclosporine and Danazol

2， Changes in Theophylline Clearance

Prediction of Drug Interaction

Elucidation of the mechanism of Drug Interaction

Case report; Drug Interaction Between Cyclosporine and Danazol



The patient : Aplastic anemia, 12-year old male with a height of 153 cm and a BW of 44.5 kg. aplastic anemia
Concomitant medications : Danazol, Prednisolone, Famotidine, Polymyxin B, Amphotericin B, S-T mix, and Magnesium oxide.

Changes in Therphylline Clearance and the Concominant Drug Usage

Hospital Day	Theophylline Clearance (L/h)	Theophylline Dosage (mg/d)	Clarithromycin Dosage (mg/d)	Levofloxacin Dosage (mg/d)
1		400[a]		
4	1.40	400[a]		
15		400[a]	400	
17		400	400	
18		400	400	300
20	0.84	400	400	300
21	0.80	400	400	300
22		300	400	300
24	0.80	300	400	300
26		300	400	300
39	1.51	300	400	

[a]Continuous intravenous infusion of aminophylline 500 mg/d.

Levofloxacin: CYP1A2 inhibitor

Clarithromycin: CYP3A4 inhibitor

Nakamura H et al. ; Annal Pharmacother, 2001; 35, 691-693.

Drug Interaction Case report

1， Cyclosporine and Danazol

2， Changes in Theophylline Clearance

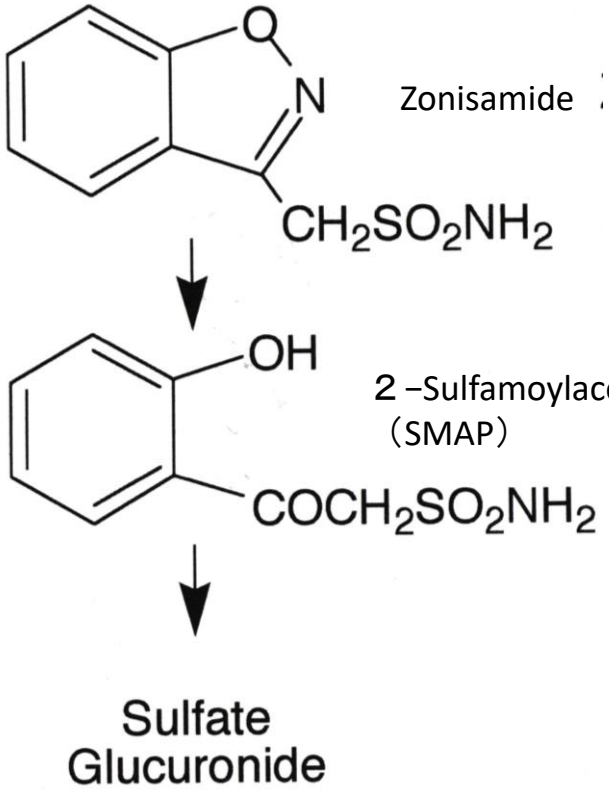
Prediction of Drug Interaction

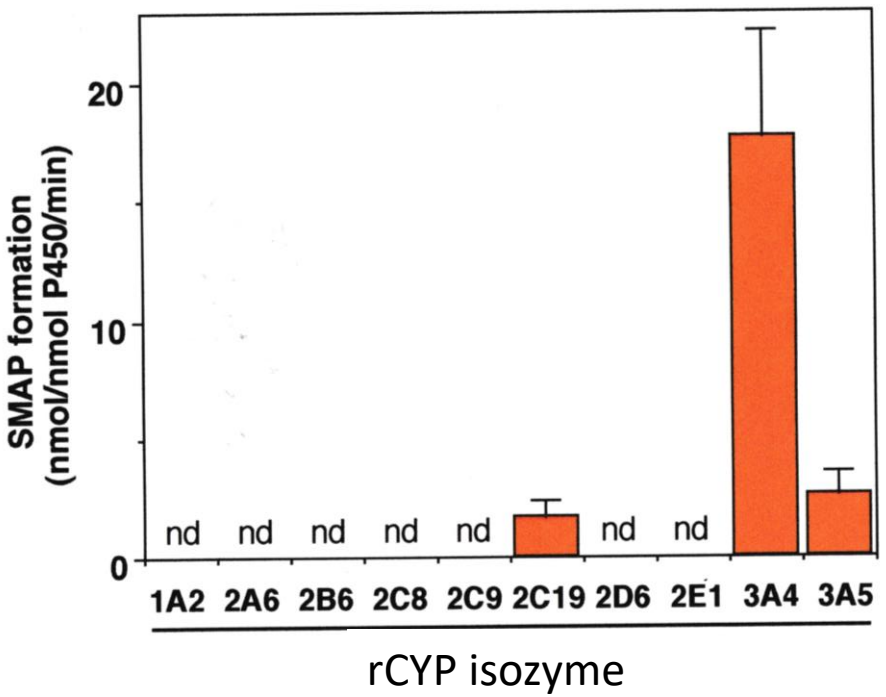
Elucidation of the mechanism of Drug Interaction

Prediction of Drug Interaction : Zonisamide

Metabolic Pathway of Zonisamide

Zonisamide is a medication used to treat the symptoms of epilepsy and Parkinson's Disease.
Zonisamide is reductively metabolized by P450 under anaerobic conditions.
CYP3A4 is predominantly responsible for the reductive metabolism of zonisamide in human liver microsomes (HLM).

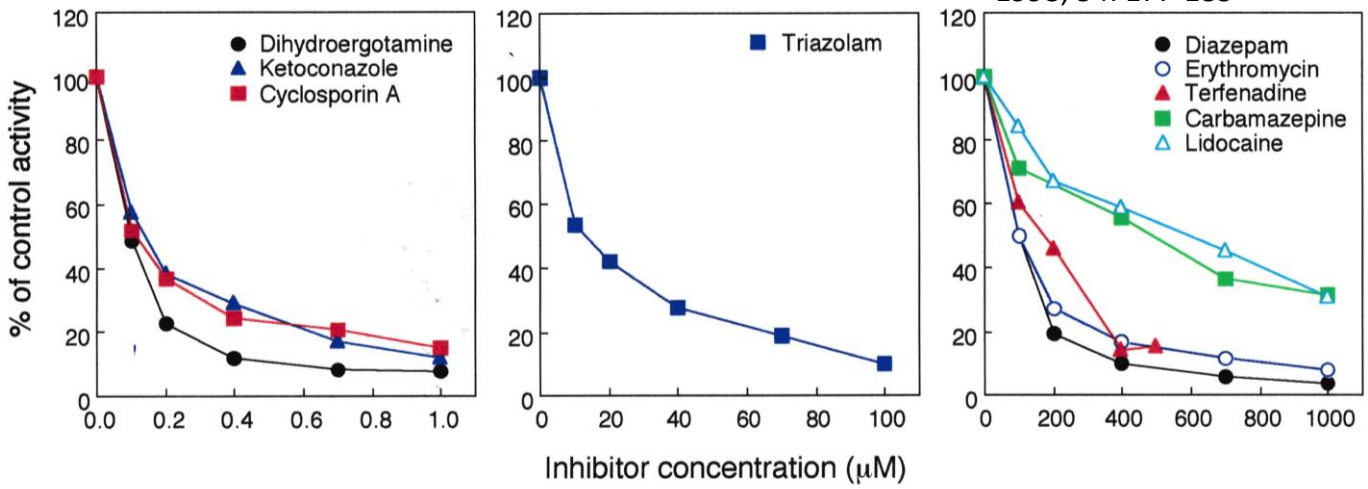




Nakasa H et al. ; Mol Pharmacol, 1993; 44: 216-221.

The Inhibitory Effect of Various Drugs on Zonisamide Metabolism in Human Liver Microsmes (HLM)

Nakasa H et al.; Eur. J. Clin. Pharmacol 1998; 54: 177-183



Each value represents the mean of duplicate determinations. The range of control values were 0.097 to 0.128 nmol/mg/min.

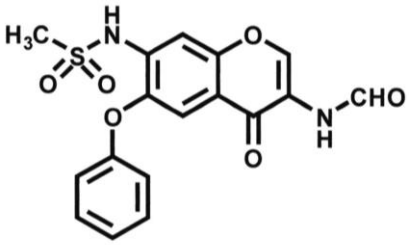
Prediction from *in vitro* Data of Change of Zonisamide Clearance by Other Drugs

	Ki (μM)	u	$I_{u\,max}$ (μM)	$1 + I_{u\,max}/Ki$	Decrease percentage of predicted clearance a)
Ketoconazole	0.18	0.012	0.08	1.4444	30.8
Cyclosporin A	0.19	0.1	0.057	1.3000	23.1
Miconazole	0.84	0.020	0.1682	1.2003	16.7
Fluconazole	61.4	0.879	4.0348	1.0657	6.2
Carbamazepine	180	0.2	10.1583	1.0564	5.3
Itraconazole	0.22	0.002	0.0014	1.0064	0.6
Dihydroergotamine	0.19	0.07	0.0012	1.0063	0.6
Triazolam	8.19	0.099	0.0014	1.0002	0.0

a) Decrease percentage of predicted clearance = $100 - 100 / (1 + I_{u\,max} / Ki)$

Drug Interaction Between Igratimod and Warfarin

Yamaori. Y et al., Biol. Pharm. Bull., 27, 653-657, 2012



Iguratimod

Igratimod:
A novel disease-modifying antirheumatic drug

Instructions: Care should be taken on combined use of igratimod and warfarin.
Iguratimod prolonged prothrombin time when taken together with warfarin in rats.

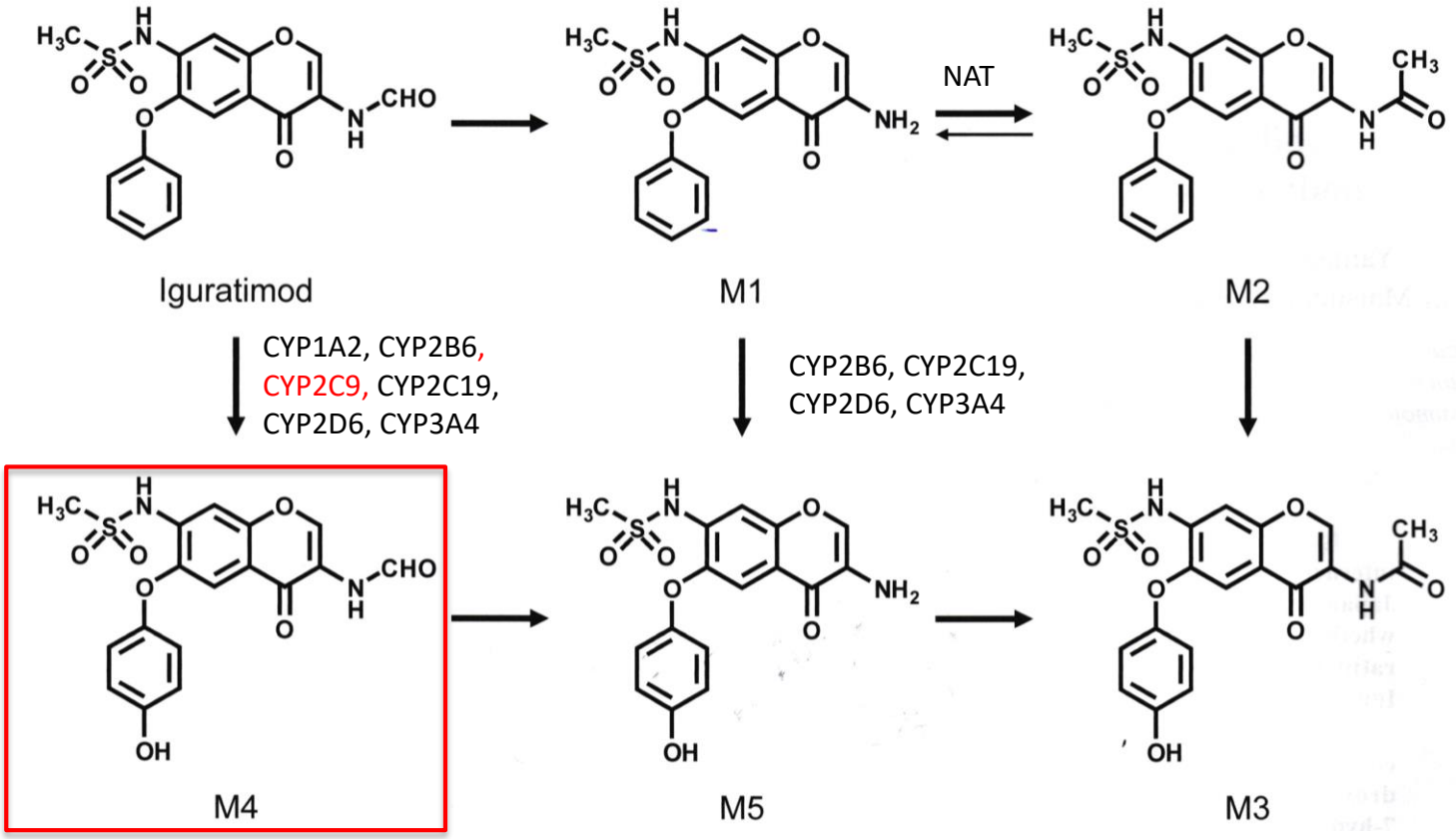
Despite this instraction, a few patients with severe bleeding were reported.

→Igratimd in combination with warfarin has been changed from a precaution to contraindication.

→Drug interaction between igratimod and warfarin.

→The drug interaction may be caused by P450 inhibition. But, precise mechanism is unclear.

Metabolic Pathway of Igratimod in Humans



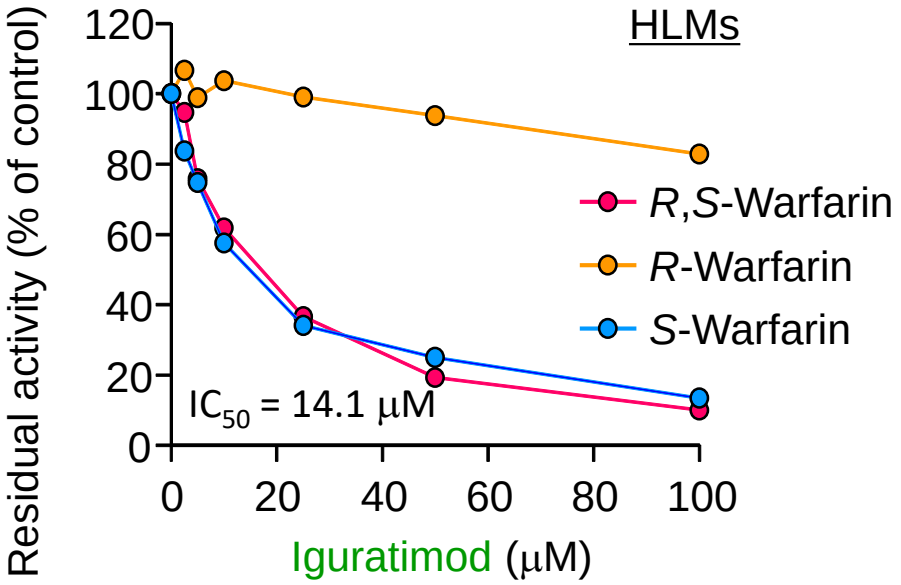
◎ Igratimod (100 μM) inhibited the Tolbutamide 4-hydroxylase activity by approximately 50 %. Based on the inhibitory effect and maximum unbound hepatic input concentration, it was estimated that the possibility of interactions between iguratimod and drugs metabolized by CYP2C9 is low.

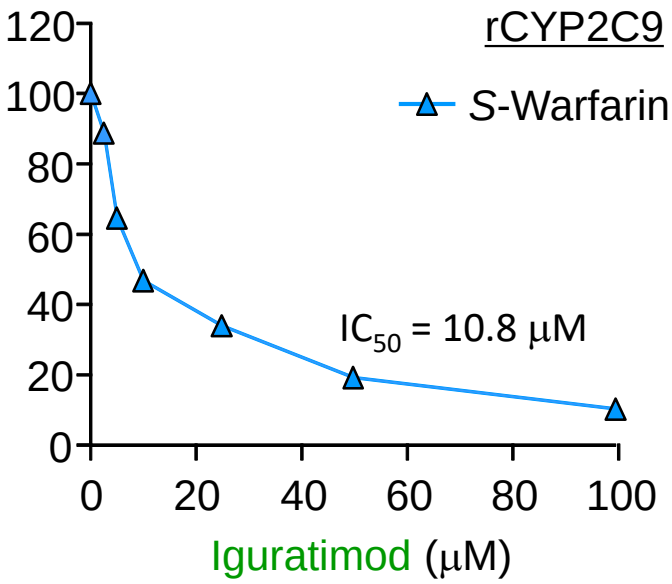
The interview form (4th edition) of Careram® Tablets 25 mg

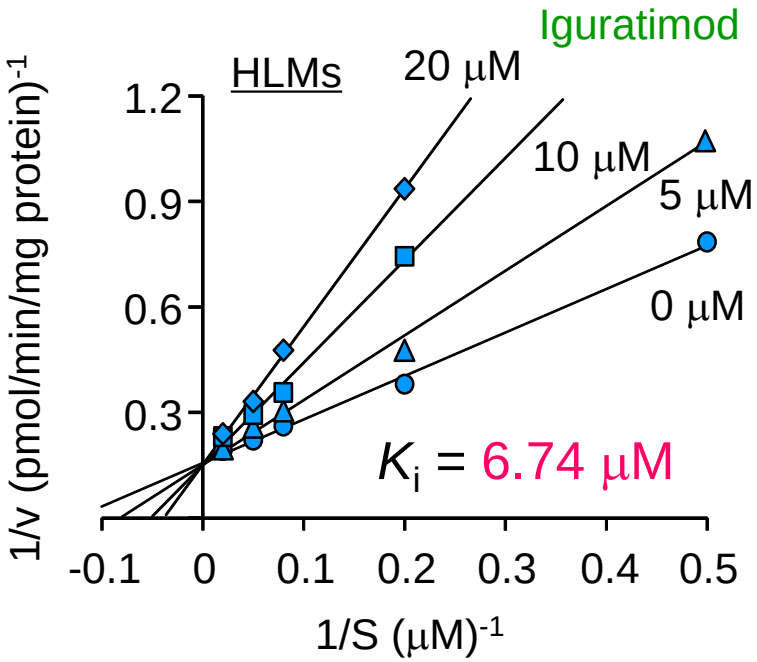
→ The inhibitory potencies of CYP2C9 inhibitors have been shown to vary depending on the substrate used.

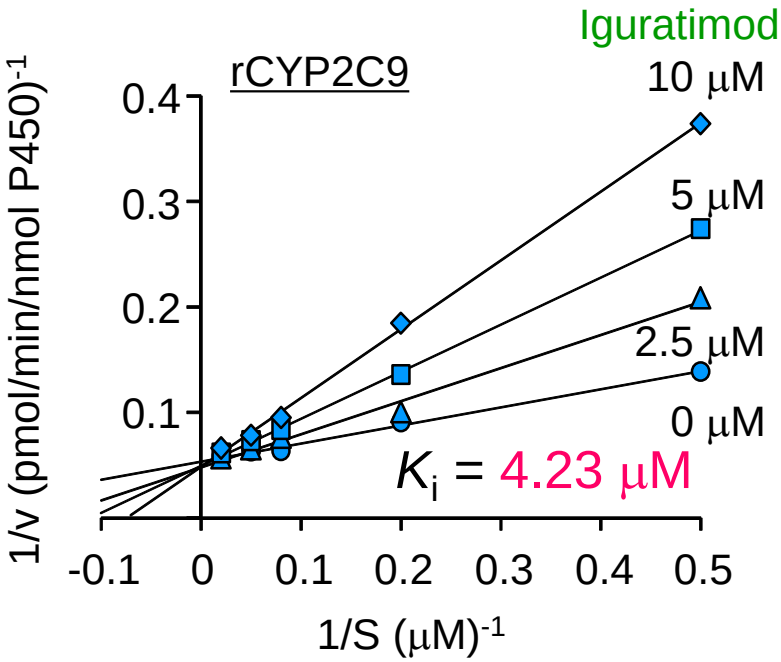
→ We reviewed the mechanism underlying this interaction.

Effect of Iguratimod on 7-Hydroxylations of *R*,*S*-Warfarin, *R*-Warfarin and *S*-Warfarin by HLMs and Recombinant CYP2C9









Kinetic Parameters for CYP2C9-mediated *S*-Warfarin 7-Hydroxylation in the Presence or Absence of Iguratimod

Enzymes	Iguratimod	K_m	V_{max}		V_{max}/K_m (Intrinsic clearance)
	(μM)	(μM)	(pmol/min/mg protein)		(μL/min/mg protein)
HLMs	0	6.68	5.97		0.894
	5	10.5	6.08	-35 %	0.579
	10	13.7	5.64		0.412
	20	23.4	6.24		0.267
			(pmol/min/nmol P450)		(μL/min/nmol P450)
rCYP2C9	0	3.15	18.6		5.90
	2.5	5.38	19.4	-58 %	3.61
	5	7.68	18.9		2.46
	10	10.9	19.0		1.74

Yamaori. Y et al., Biol. Pharm. Bull., 27, 653-657, 2012

Prediction for *In Vivo* Drug Interactions of *S*-Warfarin and Iguratimod

Ratio of AUC with inhibitor to control AUC*

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{\left(\frac{f_{m(CYP2C9)}}{1 + \frac{[I]_{in\ vivo}}{K_i \cdot f_p}} \right) + (1 - f_{m(CYP2C9)})}$$

$$= 2.3$$

$f_{m(CYP2C9)}$; 0.91 (fraction of metabolism of *S*-warfarin by CYP2C9)

$[I]_{in\ vivo}$; 0.806 μM (maximum unbound hepatic input concentration)

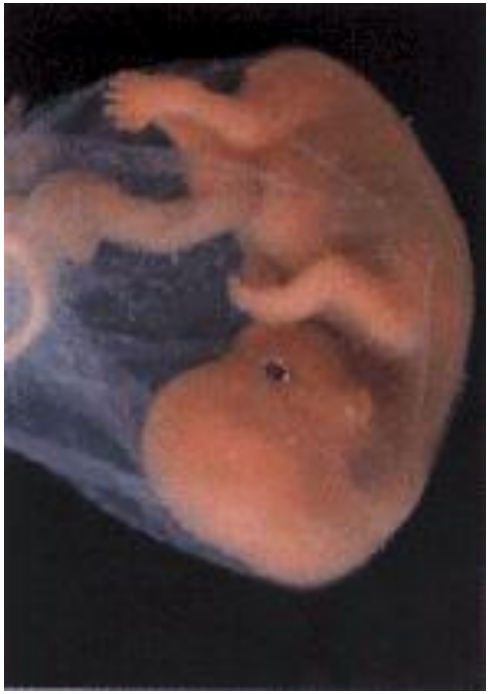
K_i; 6.74 μM (inhibitory potency of iguratimod obtained in this study with HLMs)

f_p; 0.07 (assumed that the fraction of iguratimod unbound to liver microsomal proteins, fu_{HLMs}, is equal to fu_p)**

* Obach RS *et al.*, *J. Pharmacol. Exp. Ther.*, **316**, 336-348 (2006)

**The interview form (4th edition) of Careram® Tablets 25 mg

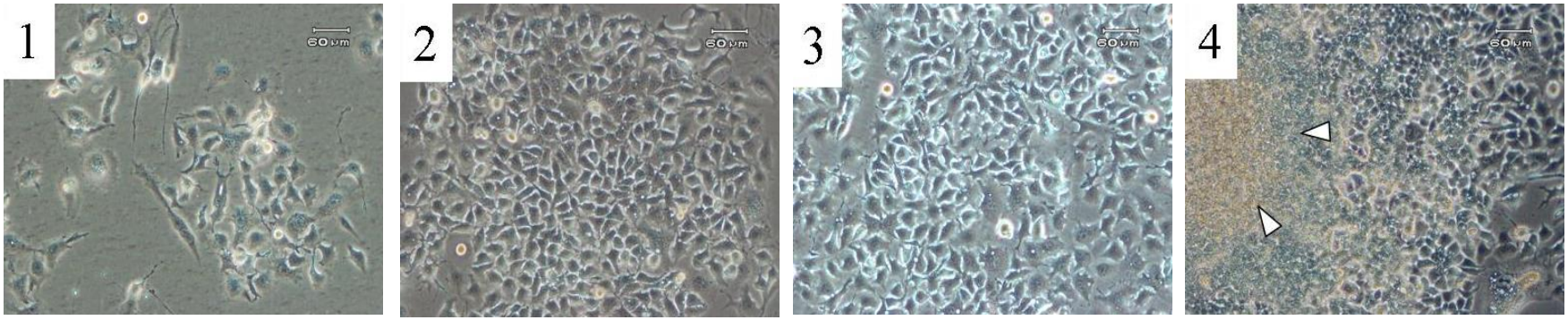
P450 mRNA expression in Human Fetal Liver



Matsunaga T et al; Drug Metab Pharmacokin, 27, 653-657, 2012.

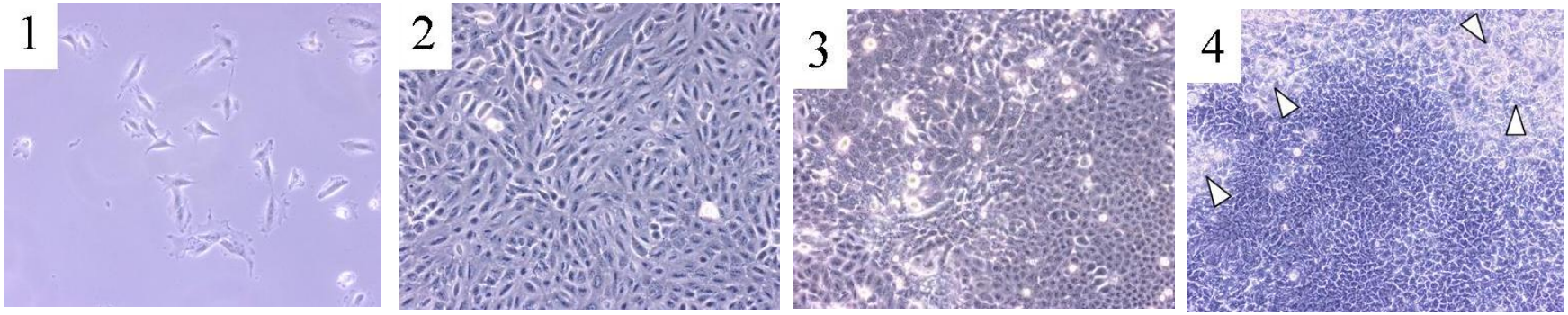
Phase-contrast micrographs of HepG2 and Human Fetal Liver (HFL） cell cultures

HepG2



2 culture days　6 culture days　9 culture days　19 culture days

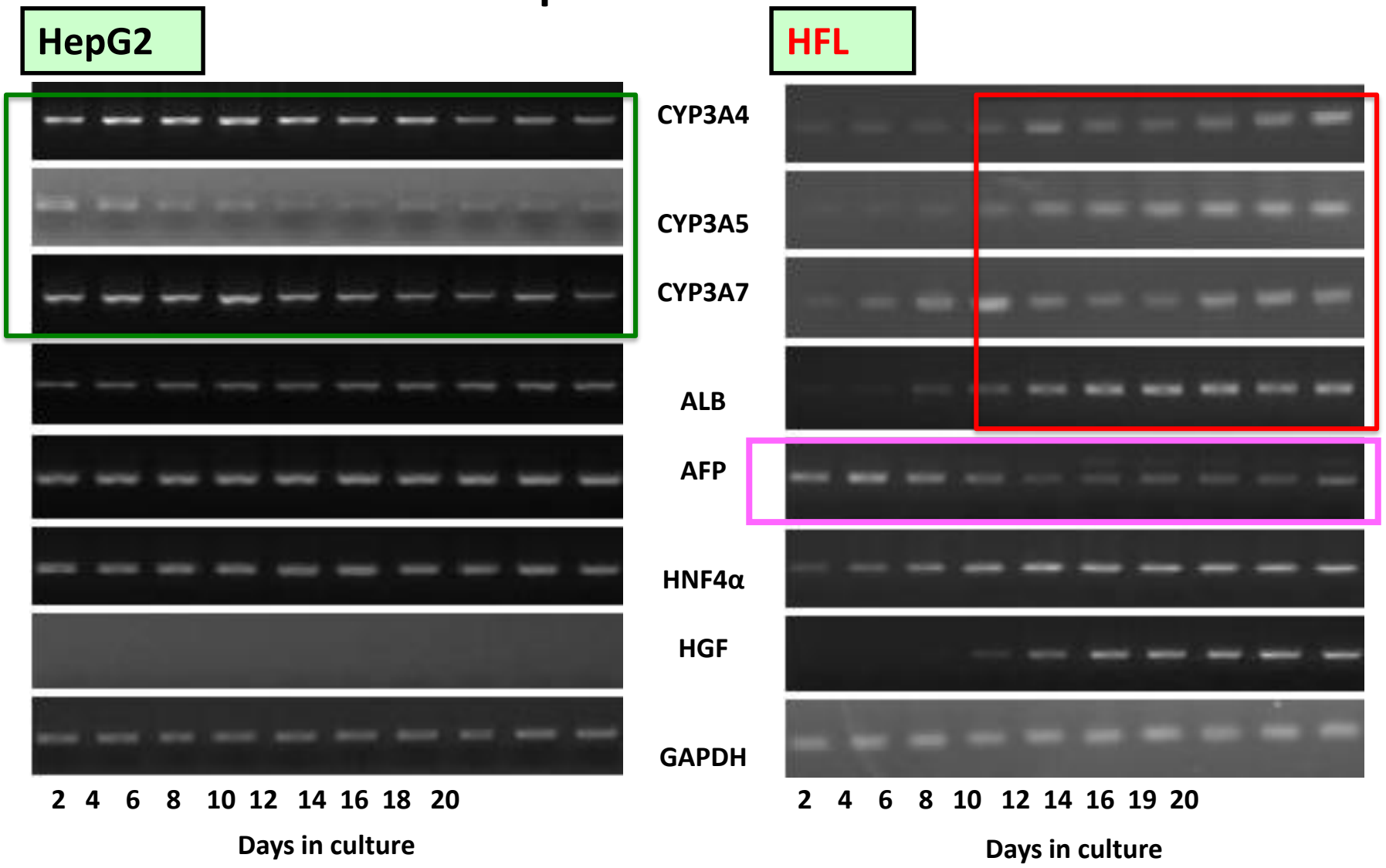
HFL



2 culture days　6 culture days　9 culture days　19 culture days

HepG2 and HFL cells were seeded 1 x 10^4 cells/well onto 6 well-plate coated with type I collagen. Arrow heads indicate the cluster.

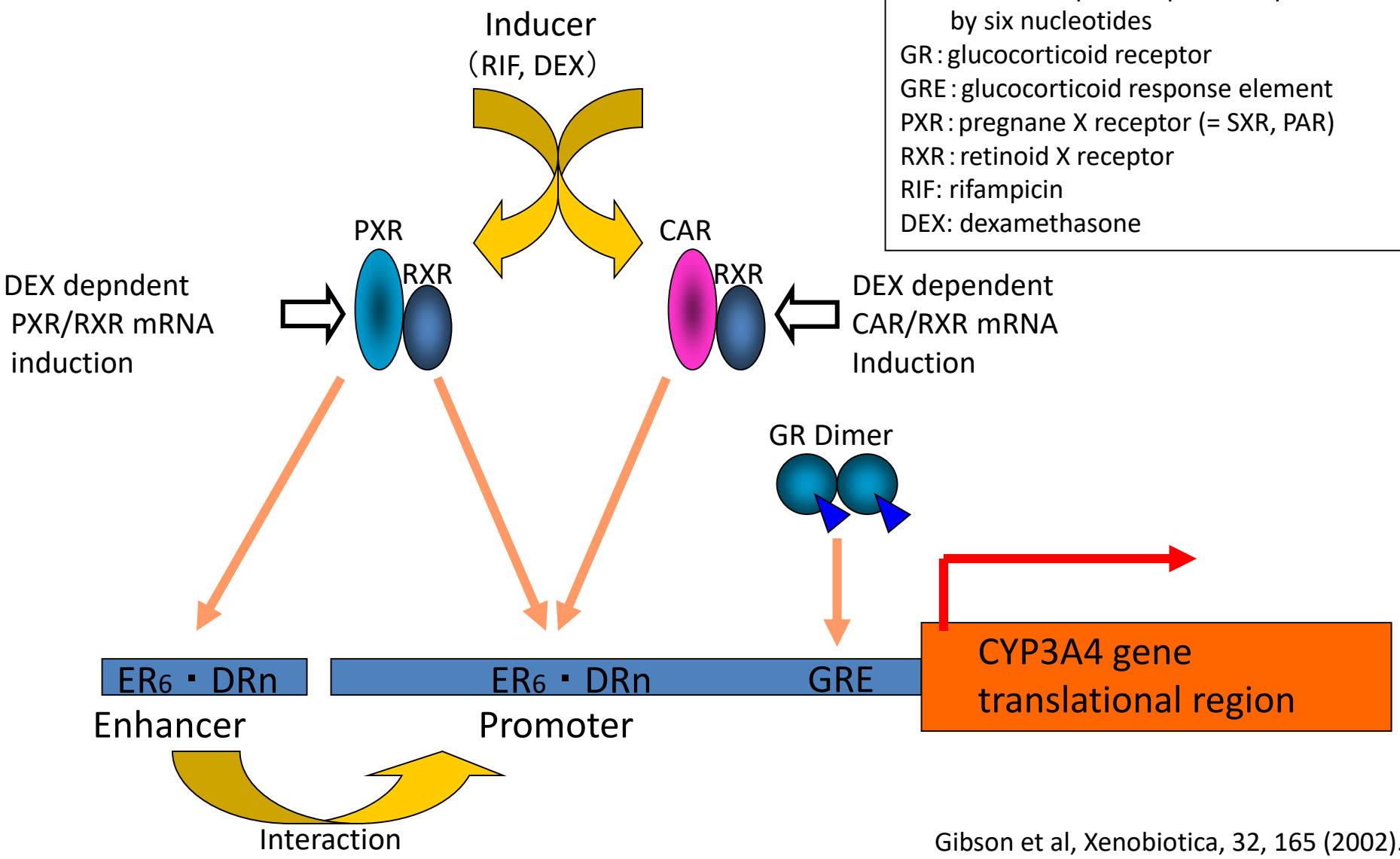
Time-dependent changes of gene expression profiles in HepG2 and HFL cells



albumin (ALB), a-fetoprotein (AFP), hepatocyte nuclear factor 4a (HNF4a), hepatocyte growth factor (HGF), glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

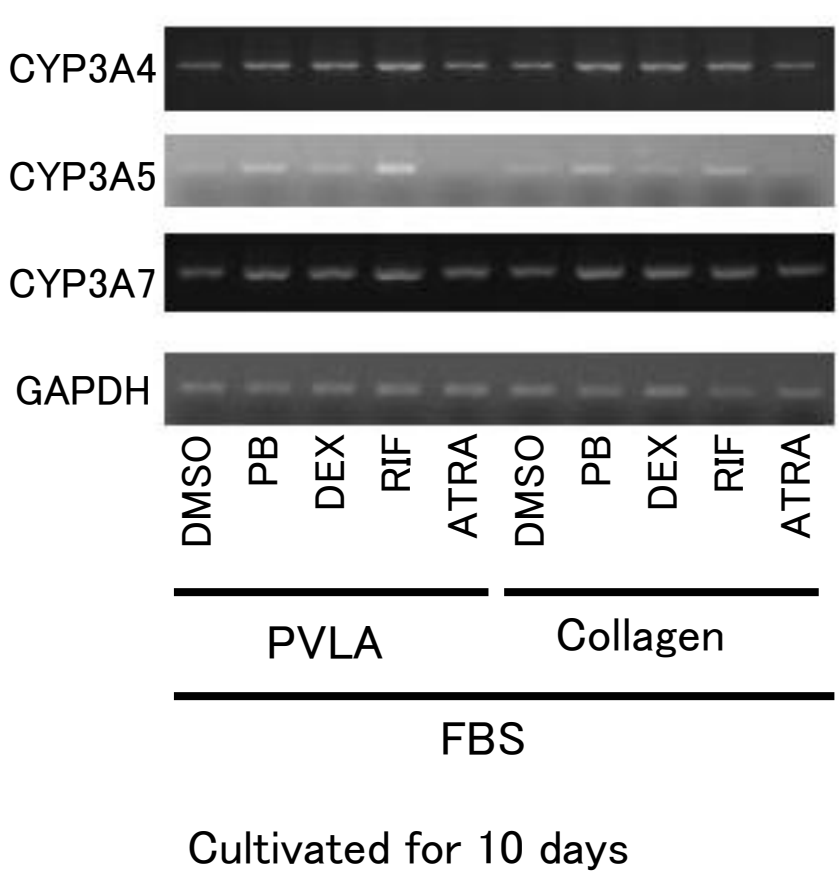
Proposed CYP3A4 gene regulation mechanism

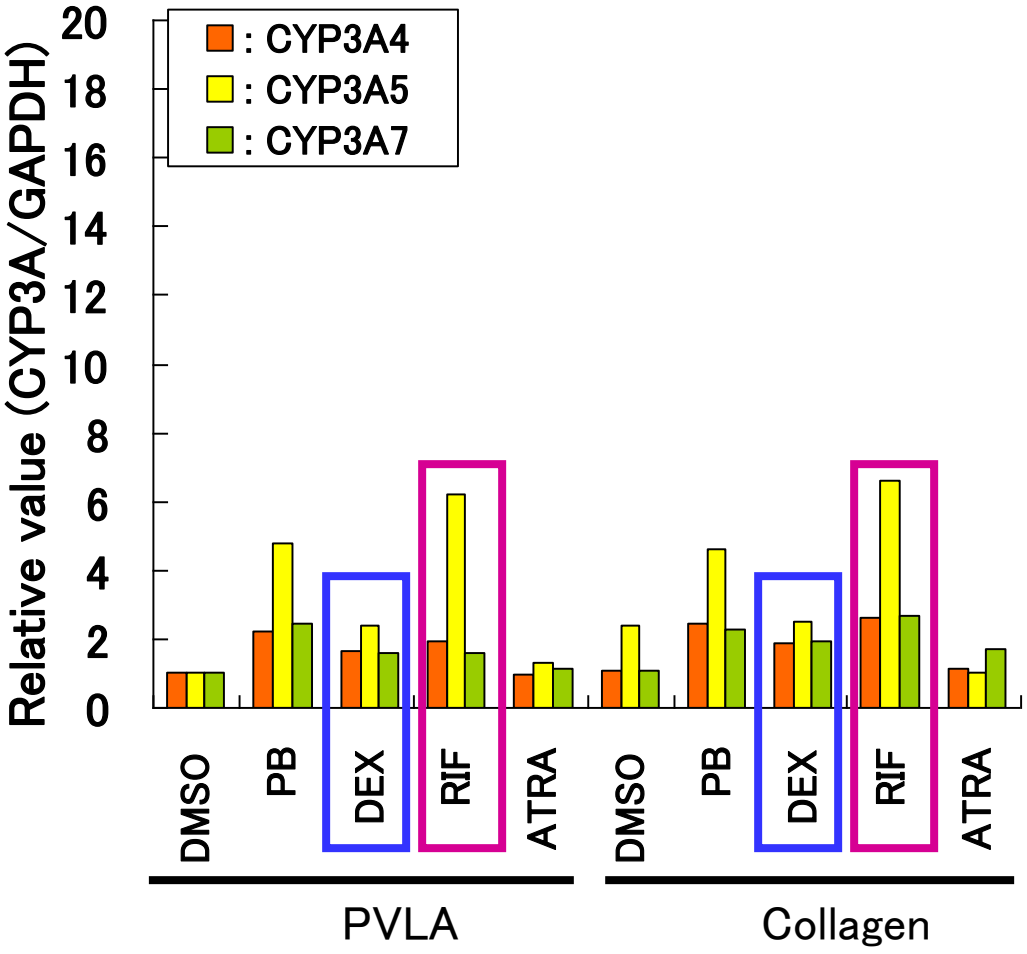
CAR : constitutive androstane receptor
CYP : cytochrome P450
DR : hormone/xenobiotic response element separated by nucleotides
ER6 : everted repeat sequence separated by six nucleotides
GR : glucocorticoid receptor
GRE : glucocorticoid response element
PXR : pregnane X receptor (= SXR, PAR)
RXR : retinoid X receptor
RIF: rifampicin
DEX: dexamethasone



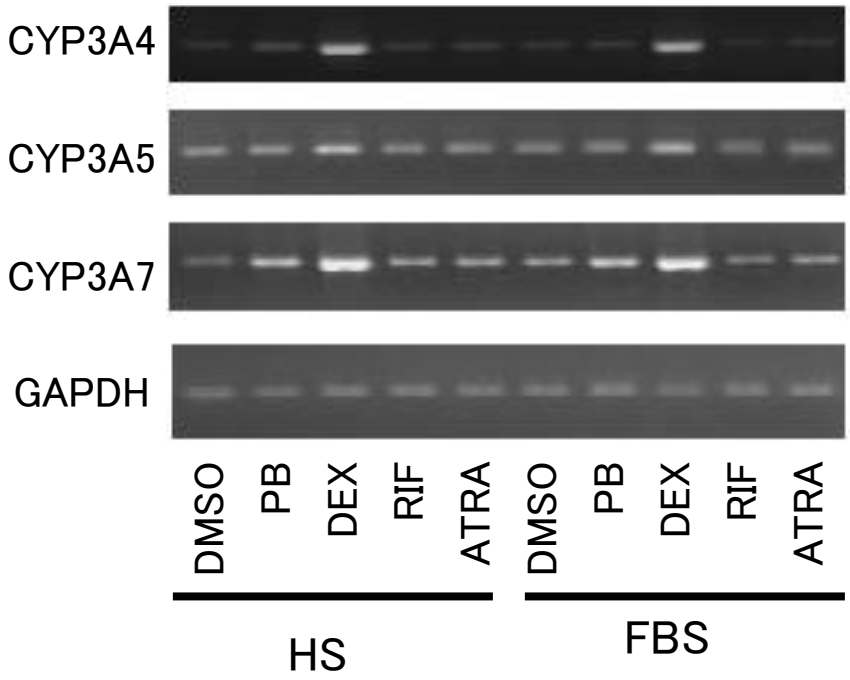
Gibson et al, Xenobiotica, 32, 165 (2002).

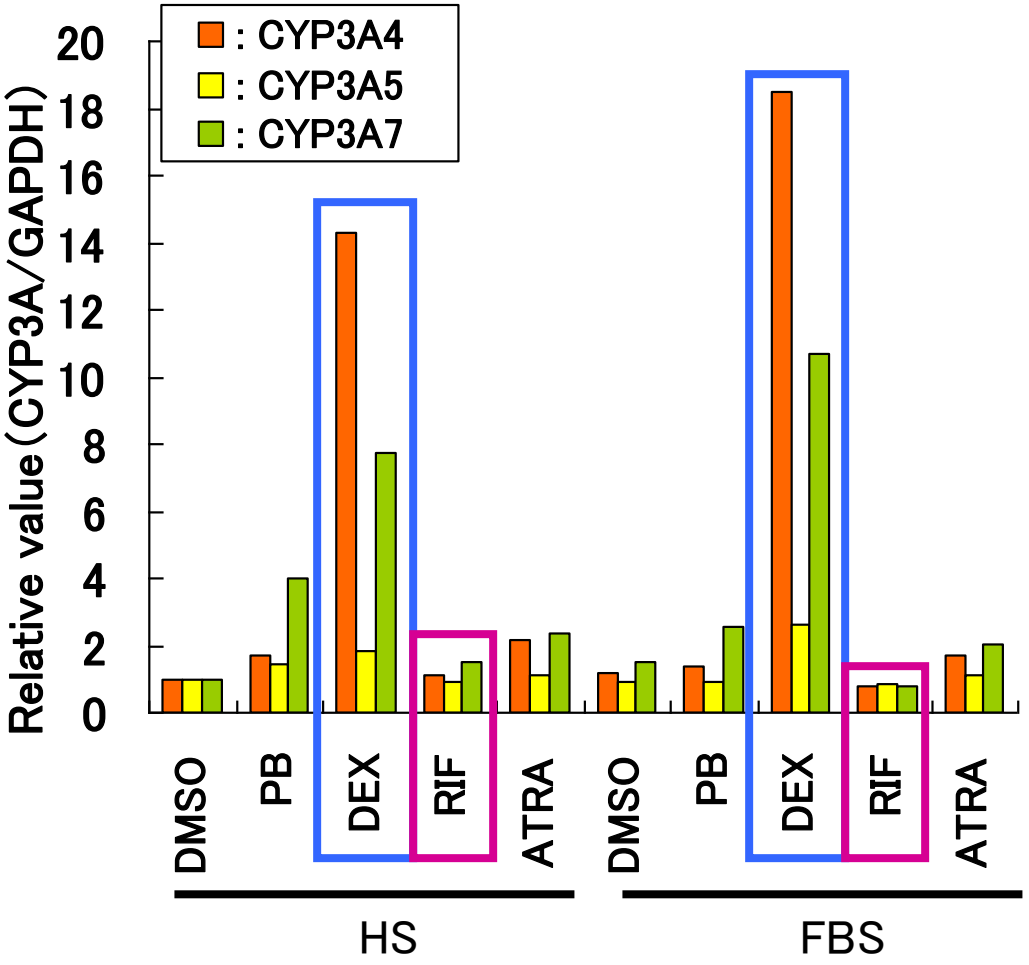
Effects of Various Inducers on CYP3As mRNA Expression in HepG2 Cells





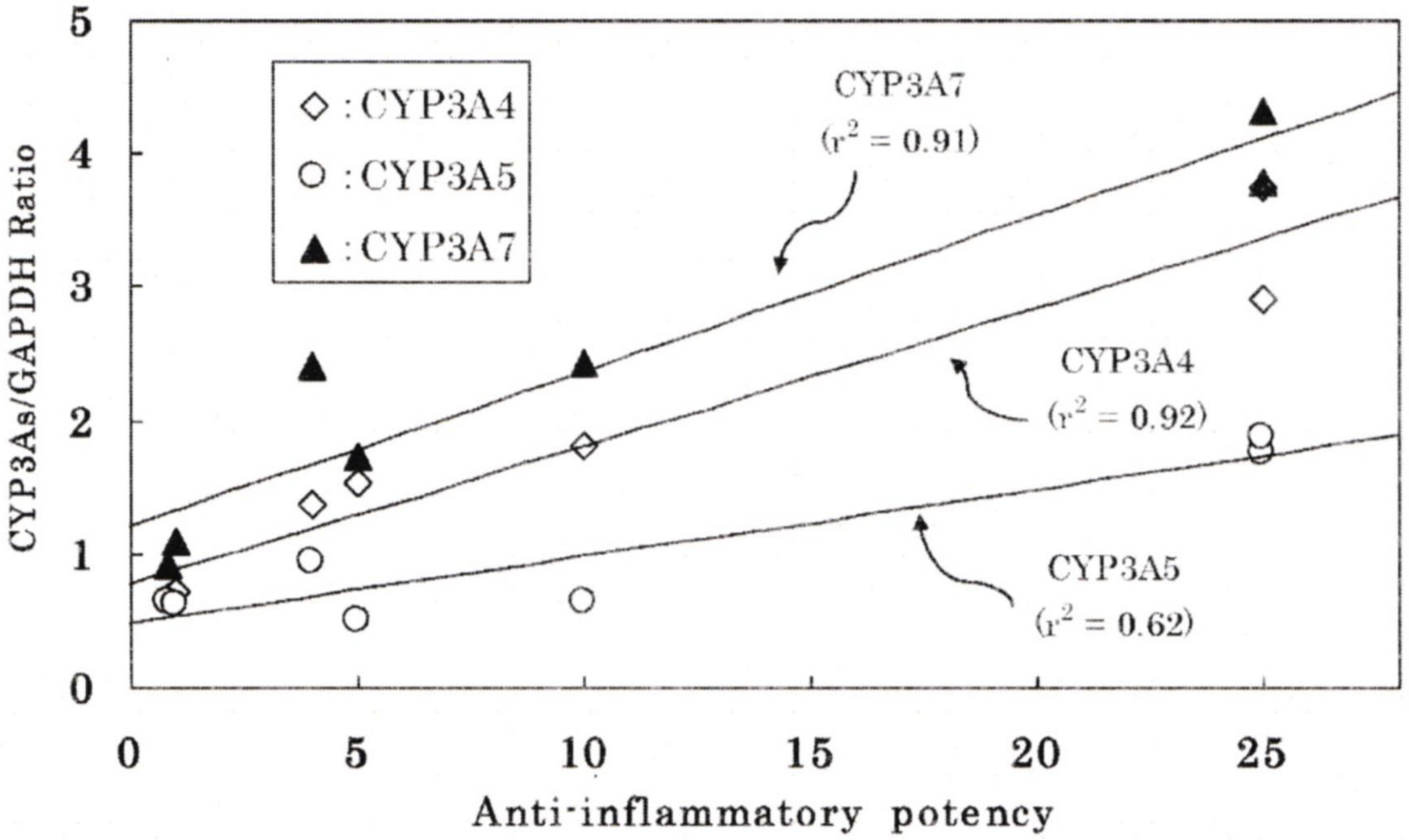
Effects of Various Inducers on CYP3As mRNA Expression in Human Fetal Liver(HFL) Cells





Extracellular Matrix: Collagen
Cultivated for 20 days

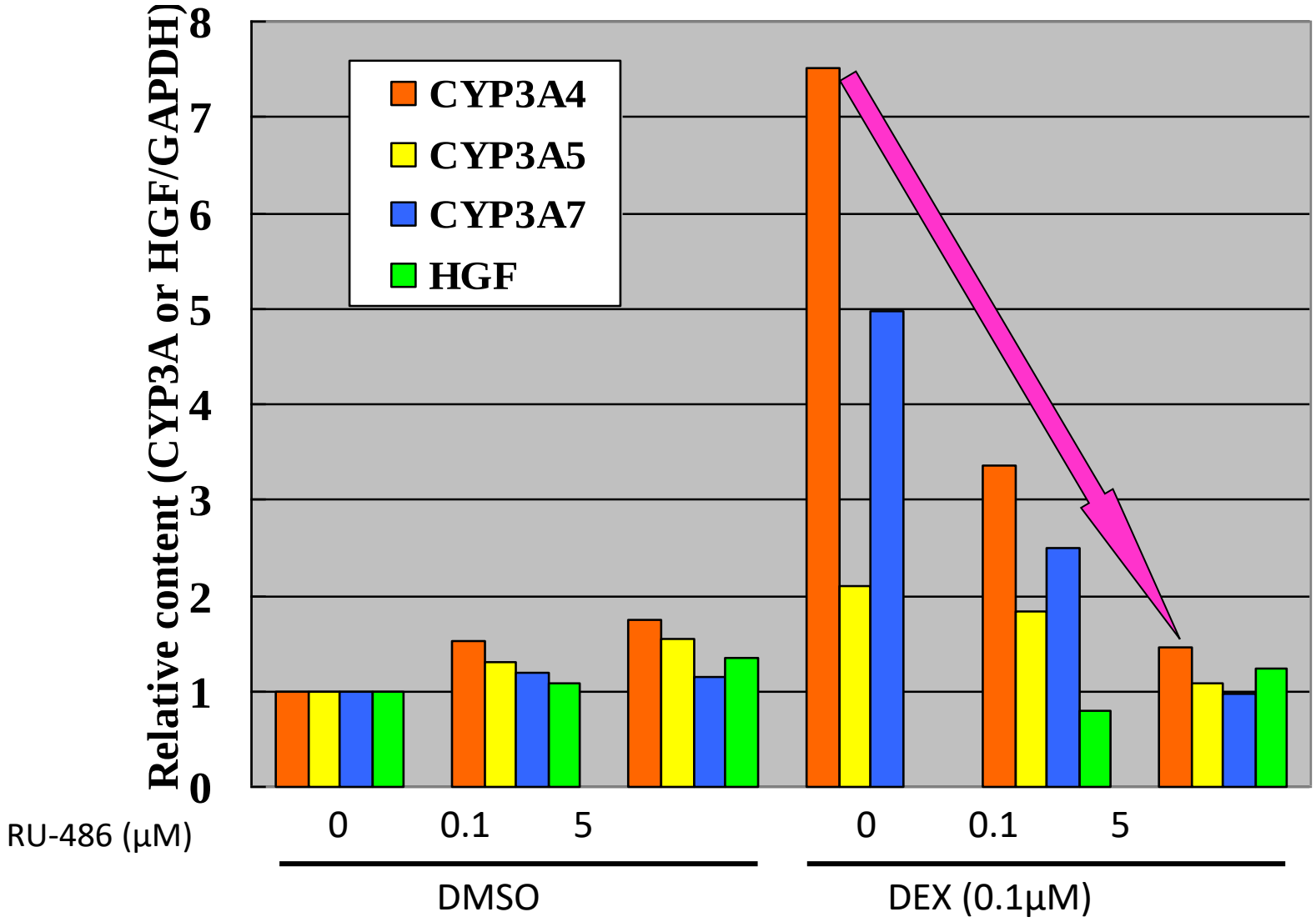
Correlation between the anti-inflammatory properties of glucocorticoids and the CYP 3A-induction ability



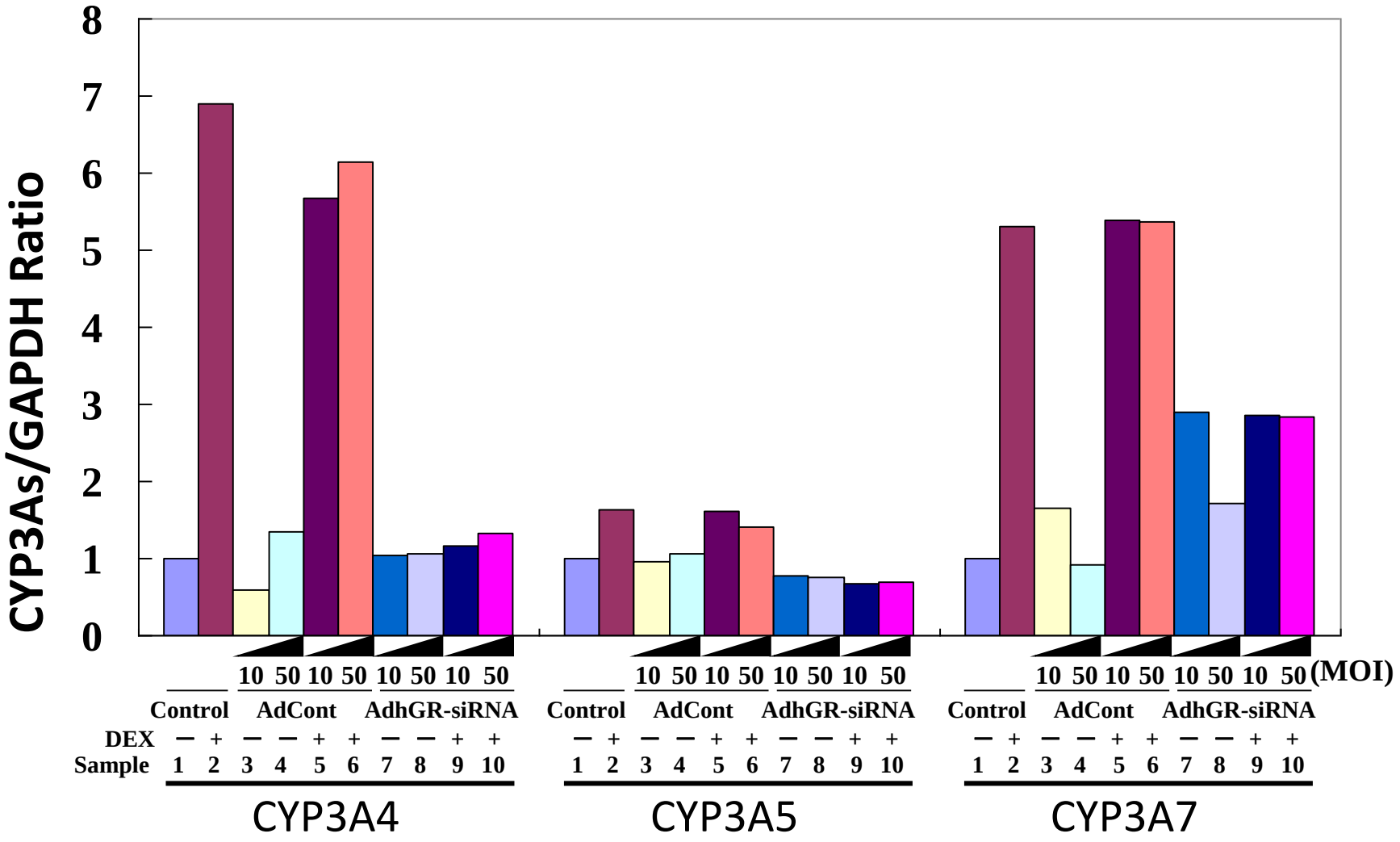
Used cotricosteroids (10 nM): cortisone, hydrocortisone, prednizolone, methylprednisolone, fludrocortisone, betamethasone, and dexamethasone.

Matsunaga *et al*., Drug Metab Pharmacokinet, 27, 653-657 (2012)

Effect of Glucocorticoid Receptor Antagonist (RU-486) on the CYP3As mRNA Inducibilities of Dexamethasone in HFL cells

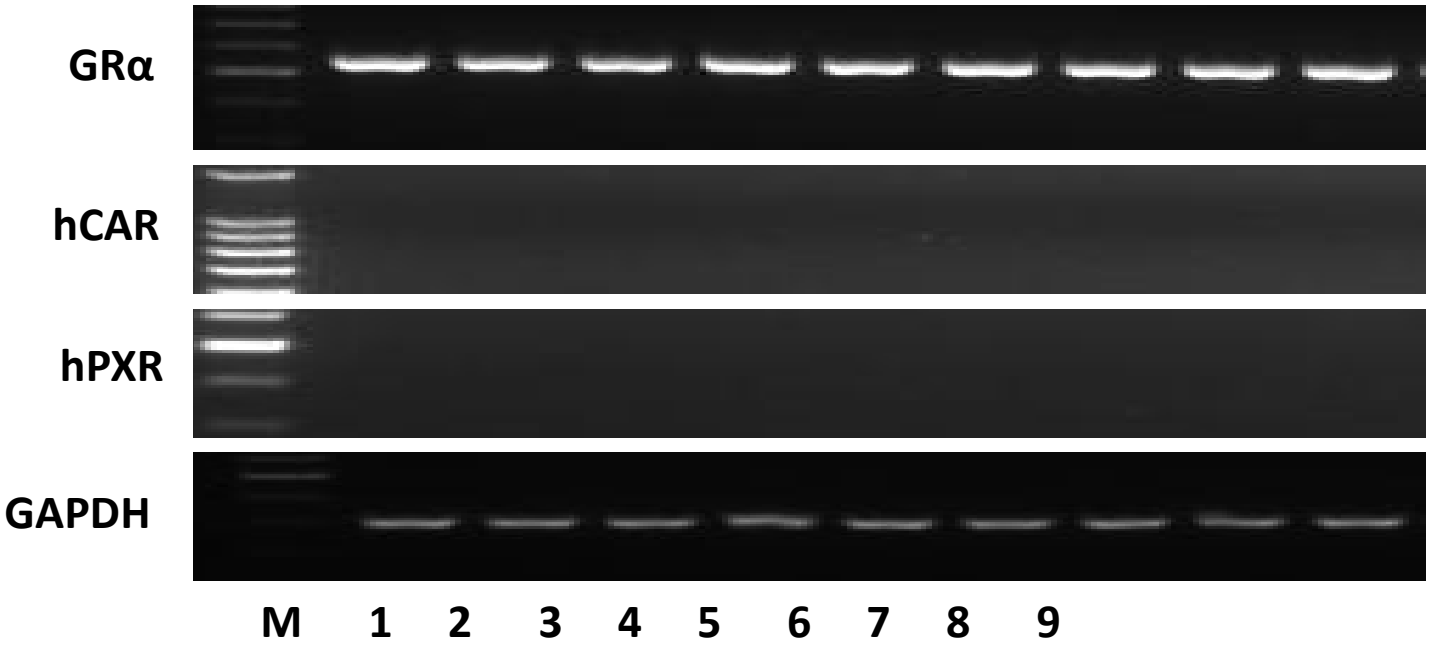


Effects of adenoviral hGR-siRNA expression on CYP3A4, CYP3A5 and CYP3A7 mRNA levels in HFL cells



DEX: 100 μM

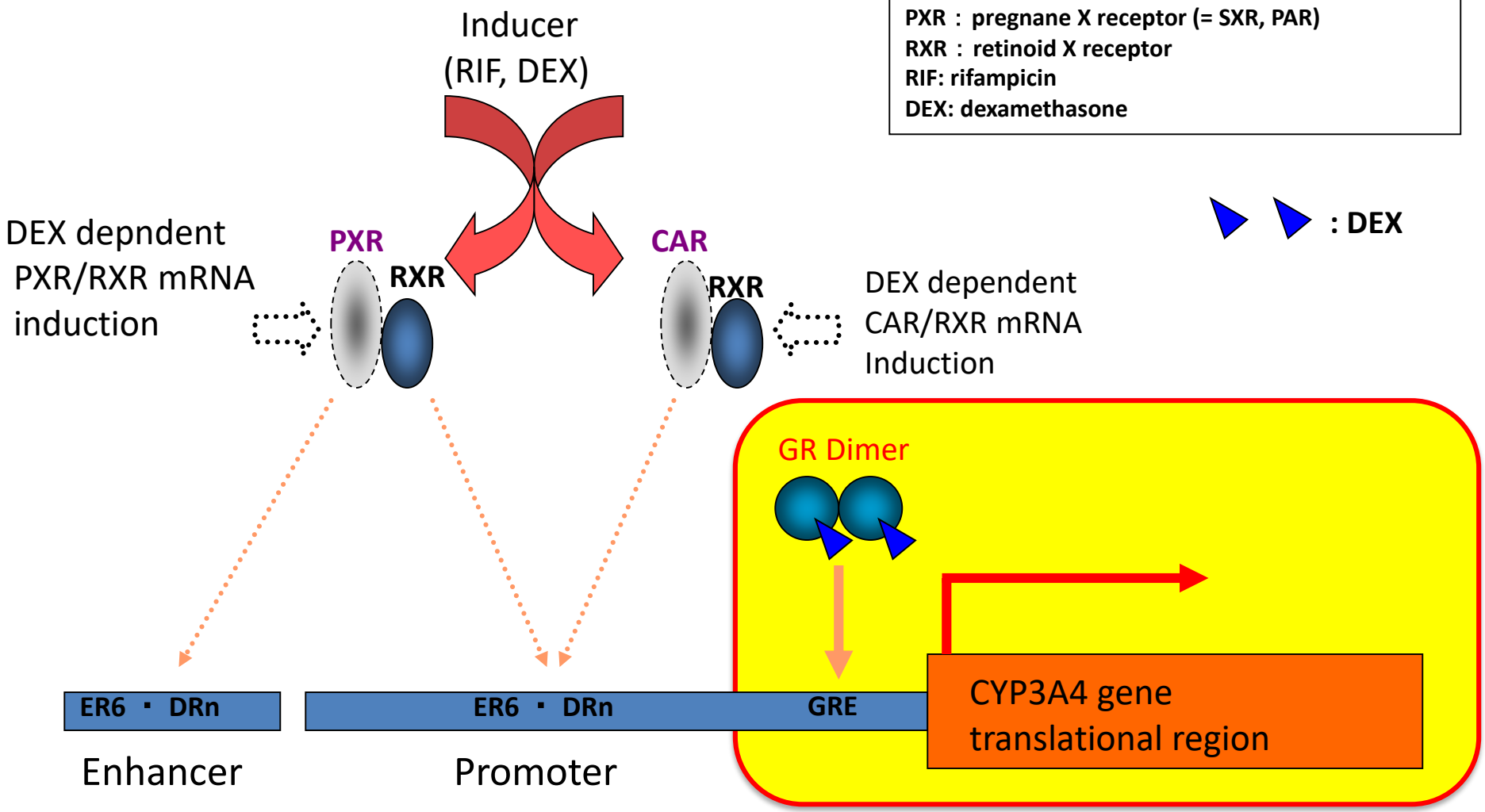
Nuclear Receptor（GRα, hPXR, hCAR) mRNA Expression Analysis in HFL cells



1. DMSO, 2. DEX(100μM), 3. DEX(100 μM),
4. PCN(10μM), 5. PCN+DEX(100μM), 6. PCN+DEX(100 μM),
7. RU-486(5μM), 8. RU-486+DEX(100μM), 9. RU-486+DEX(100 μM)

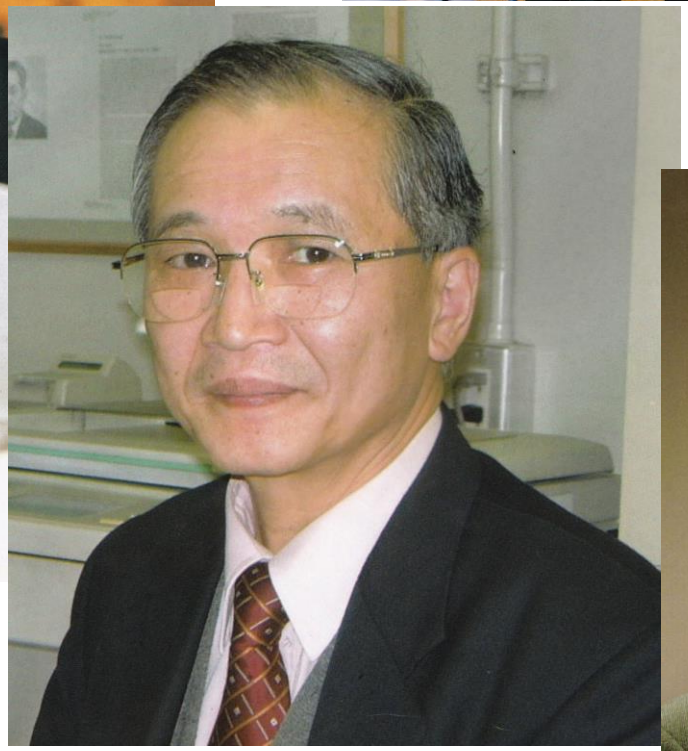
CYP3A4 gene regulation In HFL cells

CAR : constitutive androstane receptor
CYP : cytochrome P450
DR : hormone/xenobiotic response element separated by nucleotides
ER6 : everted repeat sequence separated by six nucleotides
GR : glucocorticoid receptor
GRE : glucocorticoid response element
PXR : pregnane X receptor (= SXR, PAR)
RXR : retinoid X receptor
RIF: rifampicin
DEX: dexamethasone



Gibson et al, Xenobiotica, 32, 165 (2002). With modification

Haruo Kitagawa



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National Treasure
Matsumoto Castle
(Matsumoto, Nagano)