Studies on the factors affecting pharmacokinetics and mechanisms of drug toxicity in humans

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Contents

Memorable work

* Neurotoxicity of MPTP which causes Parkinsonism in human
* Identification of CYP isoform involved in the metabolism of omeprazole in human
* *SLCO1B1*15* as a genetic marker predisposed to statin-induced rhabdomyolysis

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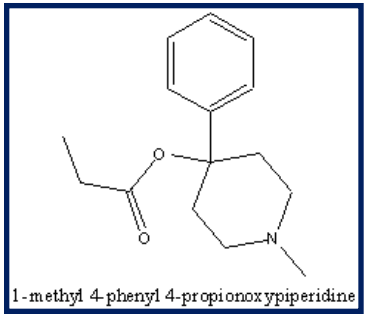
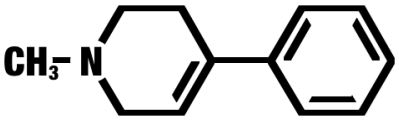
MPTP in California

- Case 1
  - 42 years old male
  - Mute, drooling, flexed posture, profound bradykinesia, cogwheel rigidity, short stepped gait
- Case 2
  - 31 years old female, girl friend of case 1
  - Similar symptoms as case 1
- Case 3,4•••7
  - Young brothers etc.

- All the patients were around 40 or younger
- They were no feature of Parkinson' disease at least three weeks before admission
- It turns out that all of them were drug abusers and took synthetic heroin before the appearance of symptoms
- Dr. Langston, a neurologist, bought the drug from smuggler and analyzed it

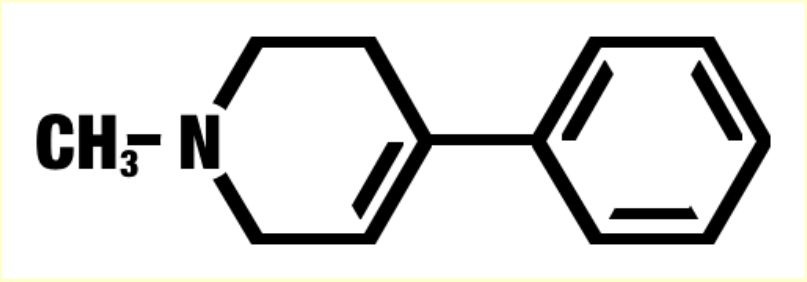
- Group of National Institute of Mental Health administered MPTP to monkey, which also caused Parkinson's disease in monkey





Contaminant
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

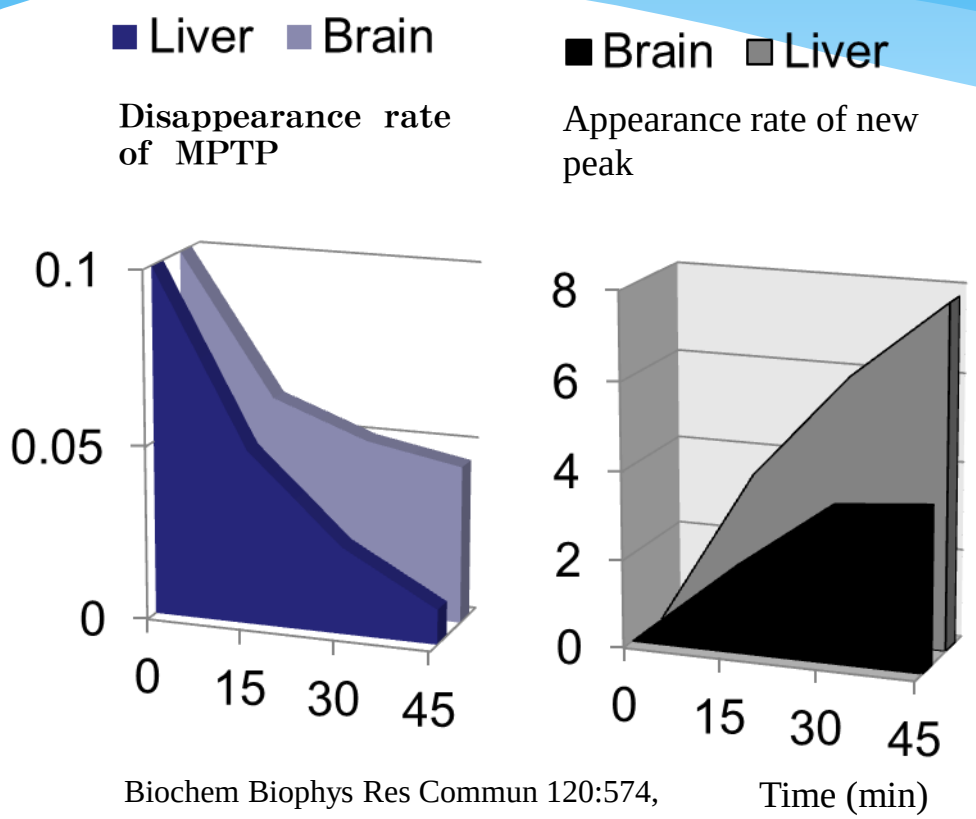
MPTP cause Parkinson's disease in humans. Why and how?



MPTP
(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

- **Hypothesis**
  - MPTP would be bioactivated to neurotoxic substance by brain MAO-B
- **Reasons**
  - MPTP is chemically stable but it may be transformed to a reactive compound in the brain
  - MAO is abundant in the brain and it metabolizes tertiary amine like MPTP
  - MAO-B but not MAO-A was believed to exist in dopaminergic nerve cells
- I studied metabolism of MPTP using brain mitochondrial fraction which contains MAO

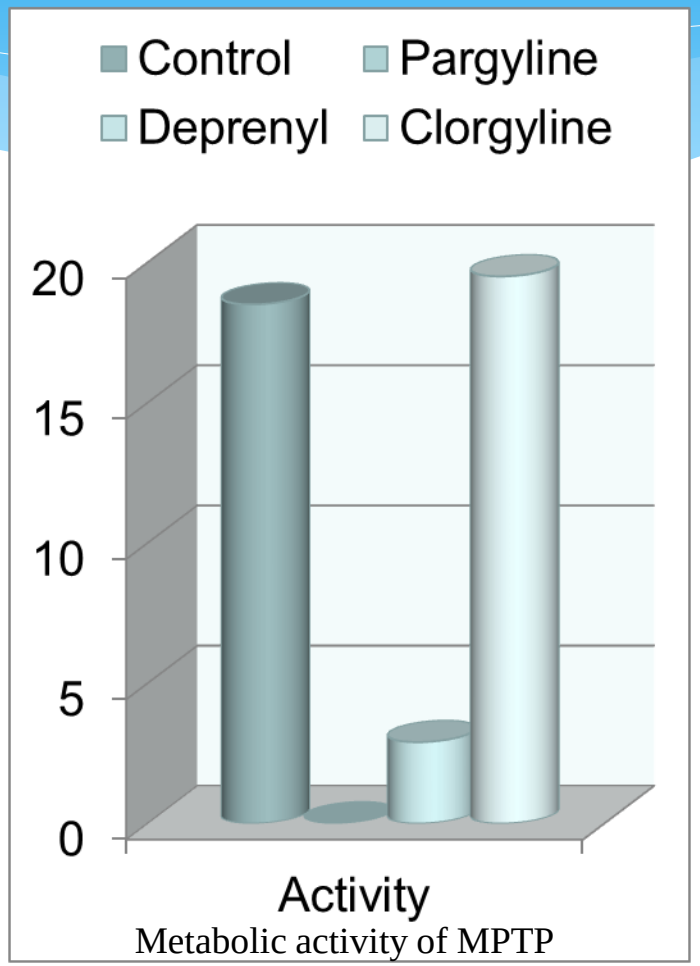
Involvement of MAOB in the metabolism of MPTP in the brain



Disappearance rate of MPTP

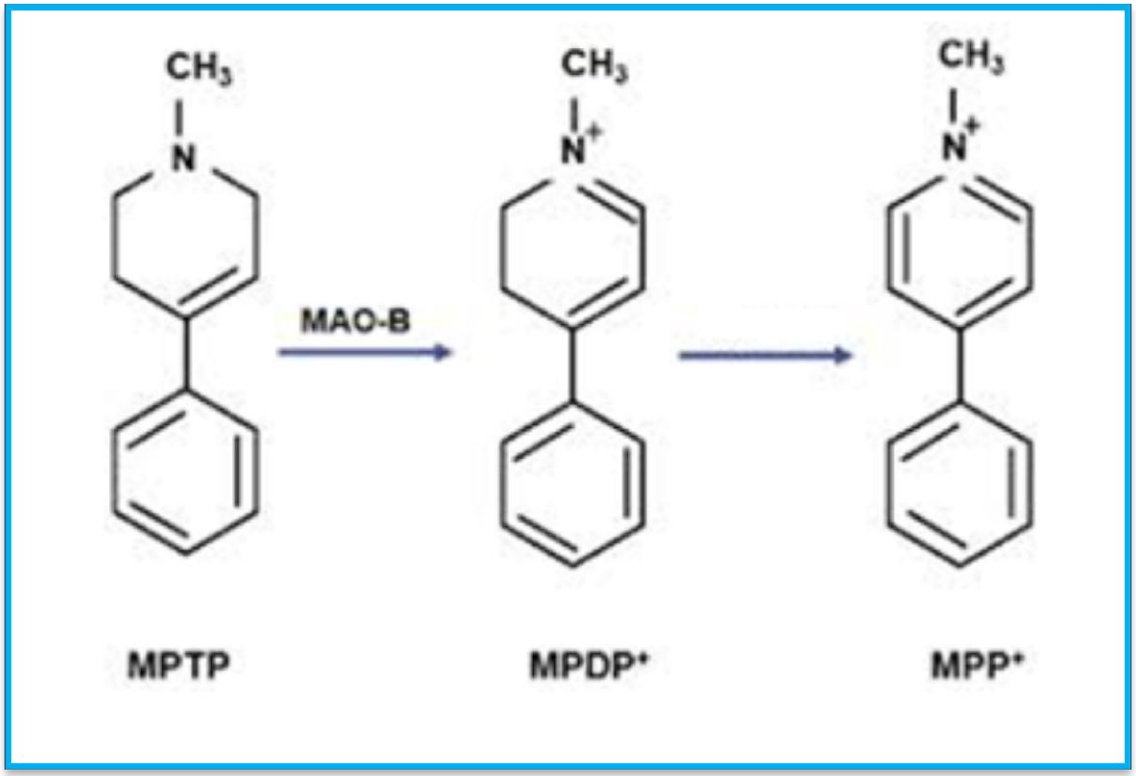
Appearance rate of new peak

Biochem Biophys Res Commun 120:574, 1984



Metabolic activity of MPTP

Bioactivation process of MPTP in the brain



- A group of Stanford University found that MAO-B inhibitor protects animal from MPTP neurotoxicity

MPTP appears to be the first case showing the possibility that xenobiotics exhibit neurotoxic effect on human brain by the metabolic activation

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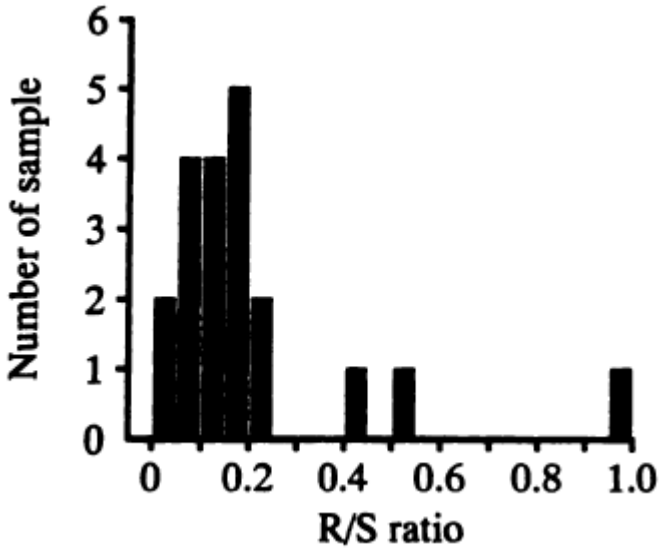
Identification of CYP2C19 as an isoform of CYP involved in 5-hydroxylation of omeprazole in human

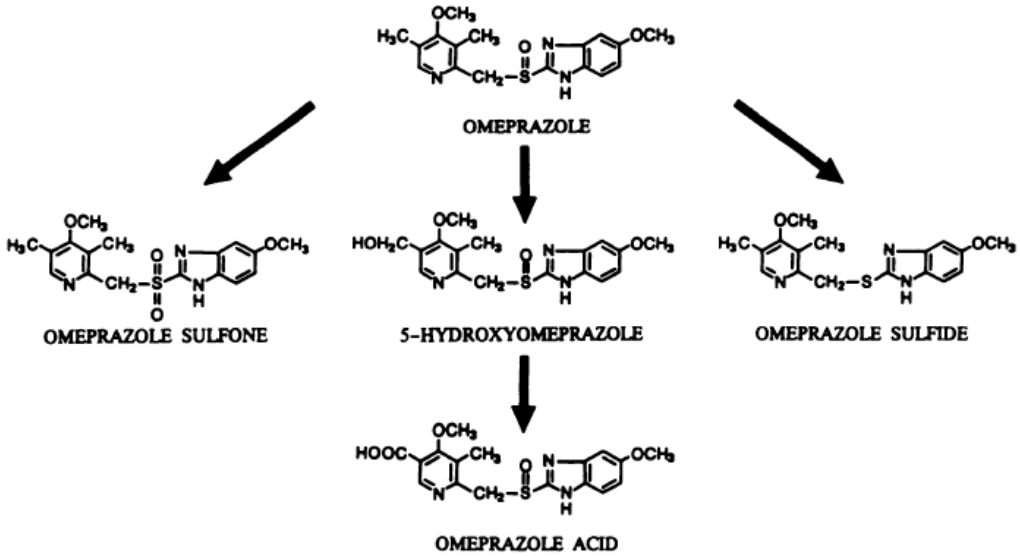
* A report indicating that there are slow- and rapid metabolyzers of omeprazole and diazepam interacts with omeprazole only in rapid metabolizer of omeprazole
* Disposition of diazepam was suggested to be co-segregated with S-mephenytoin

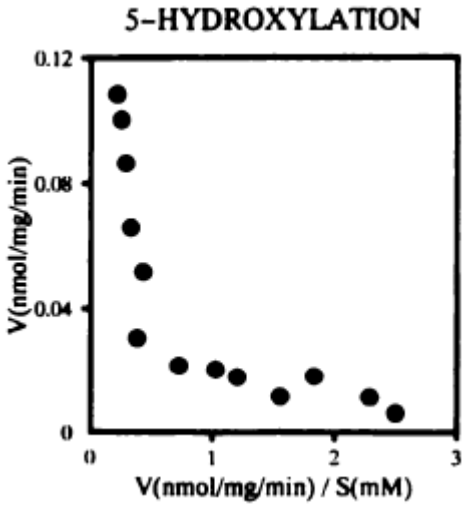


R/S ratios of mephenytoin in 20 Japanese patients underwent hepatectomy

Drug Metab Disps 21:747, 1993

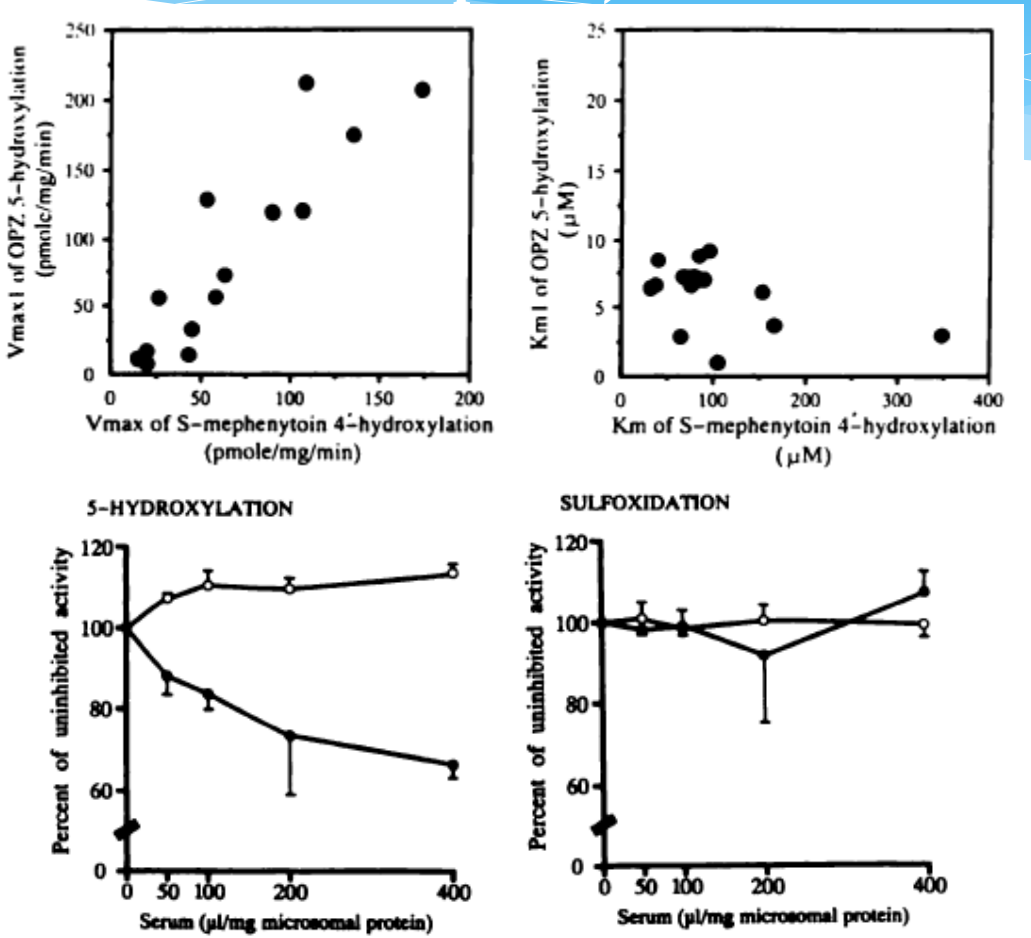
Process and kinetics of omeprazole metabolism in human





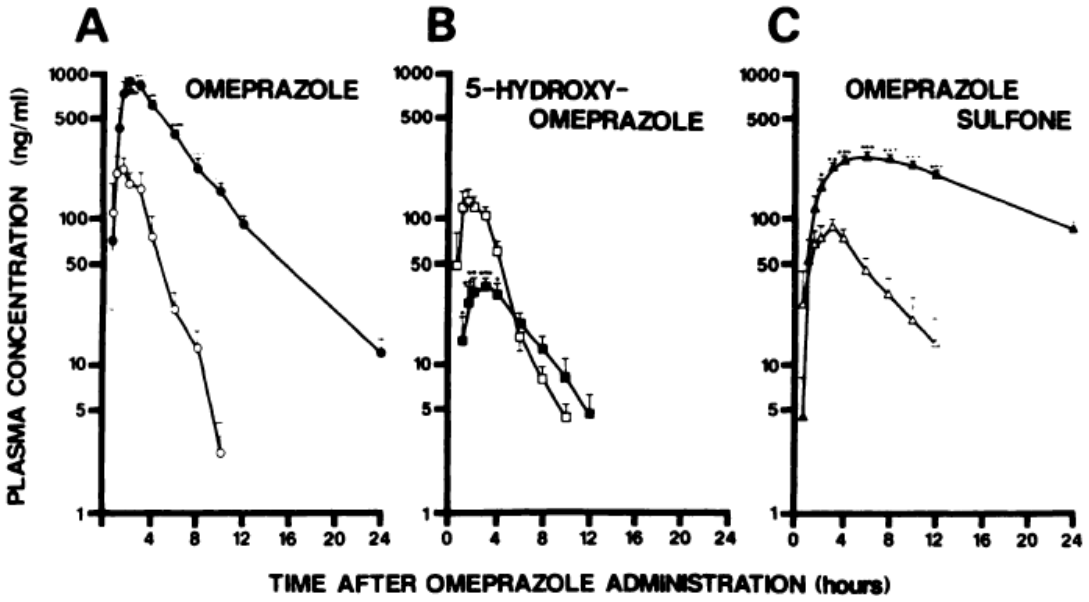
$$V = V_{max1} \cdot S/(K_{m1} + S) + V_{max2} \cdot S/(K_{m2} + S)$$

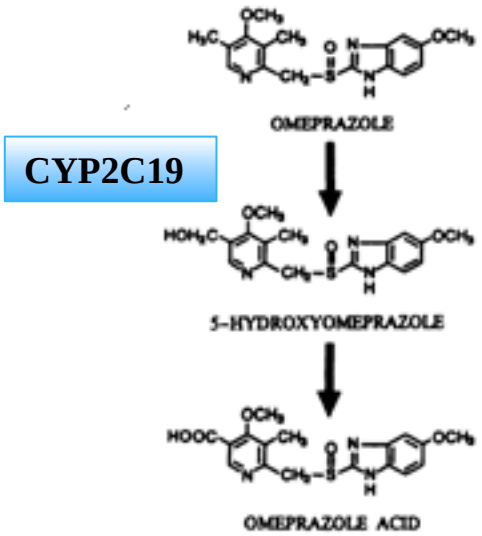
Correlation of omeprazole and mephenytoin metabolism (upper panel) and effect of CYP2C antibody on the metabolism of omeprazole in human liver microsomes (lower panel)



J Pharmacol Exp Ther 266:52, 1993

Effect of S-mephenytoin hydroxylation deficiency on the disposition of omeprazole in human volunteers





J Pharmacol Exp Ther 262:1195、1992

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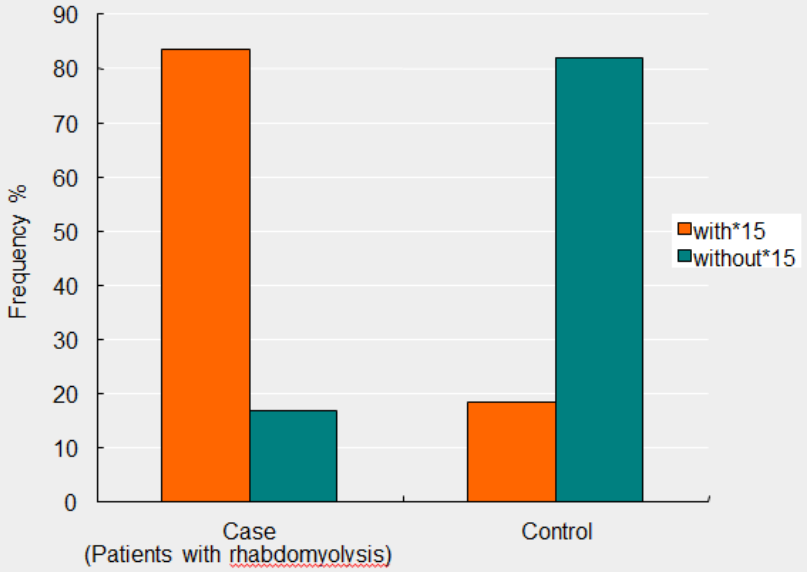
Stain-induced rhabdomyolysis

- Rhabdomyolysis is a life-threatening adverse reaction of statins
- Although the mechanism of statin-induced rhabdomyolysis was unknown, it had been reported that reduced clearance of statin increases a risk of rhabdomyolysis.
- In addition, inherited rhabdomyolysis is caused by mutation of genes related energy production

- We studied genetic factors responsible for statin-induced rhabdomyolysis with candidate gene approach
- We studied genes which cause inherited rhabdomyolysis (*CPT II*, *VLCDA*, *PYGM*, *LDHA) and* genes related to the disposition of statins (*ABCC2*, *CYP3A4*, *ABCB1*, *SLCO1B1)* in patients with and without myopathy after taking statin

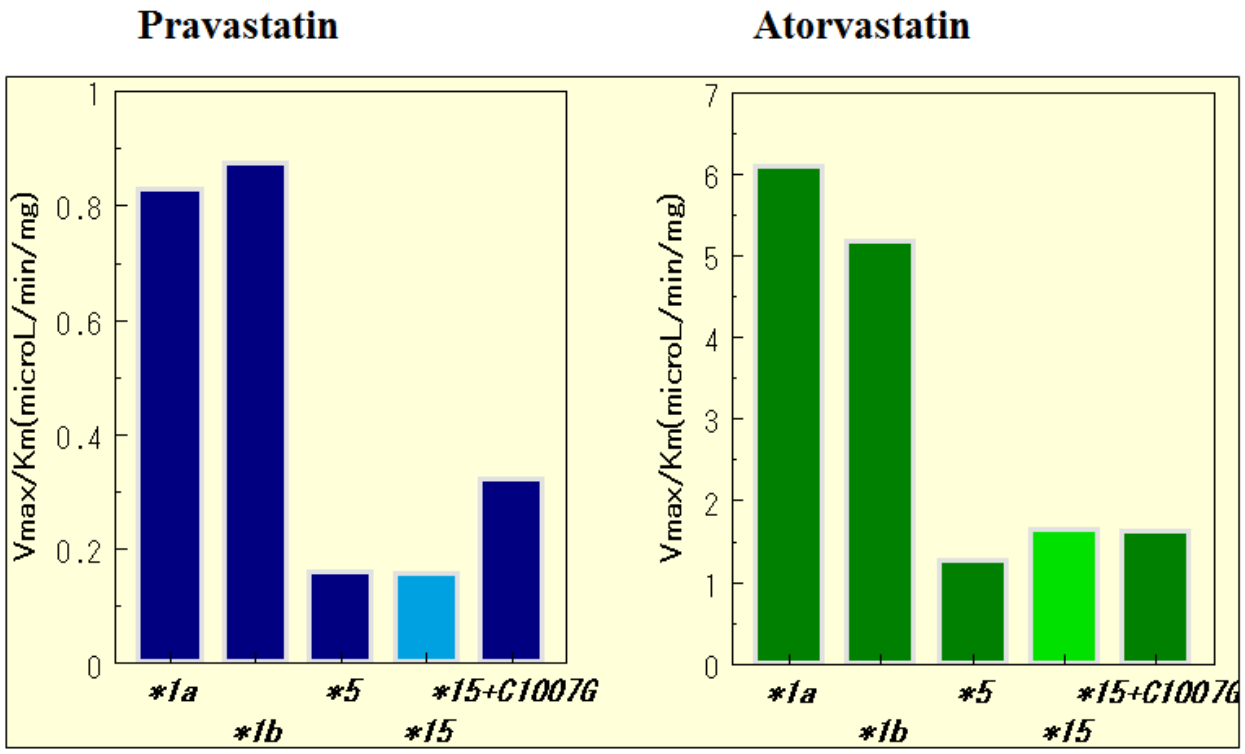
*SLCO1B1*15*-associated myopathy in case (patients with myopathy) and control patients treated with pravastatin or atorvastatin for hypercholesterolemia



	Number		%		Probability		Odds ratio	95% CI
	with *15	without*15	with *15	without*15	χ^2	P value		
Case	5	1	83.3	16.7	**9.175**	**0.002**	**22.5**	(2.03 - 249)
Control	4	18	18.2	81.8			1	

Clin Pharmacol Ther 77:21, 2005

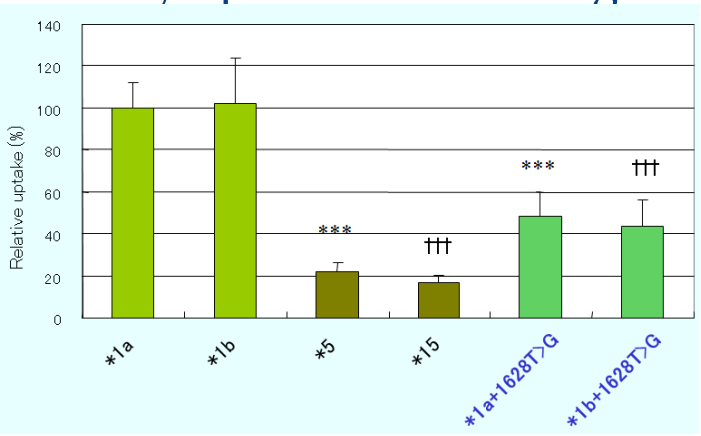
Transporting activities of SLCO1B1 variants expressed in HEK293 cells for pravastatin and atrvastatin



Pharmacogenet Genomics 15:513、2005

A novel variant allele of *SLCO1B1* found in one of two patients who did not carry *SLCO1B1*15* but experienced pravastatin-induced myopathy

* Patient
  - 54-years old (female)
  - Medication：Pravastatin 5 mg/day
  - Symptoms：Severe thigh



Drug Metab Pharmacokinet 19:453, 2004
Pharmacogenomic J 9:185, 209

* The findings suggest that mutation of SLCO1B1 which decreases the function of OATP1B1 should be a predisposing factor responsible for the pravastatin and/or atrovastatin-induced rhabdomyolysis
* Later, genome wide analysis of patients taking simvastatin undertaken by the group of SEARCH showed that this mutation of SLCO1B1 is only a factor responsible for simvastatin-induced rhabdomyolysis (N Engl J Med, 2008).
* These findings suggest that decreased function of OATP1B1 is an predisposing factor of statin- induced rhabdomyolysis

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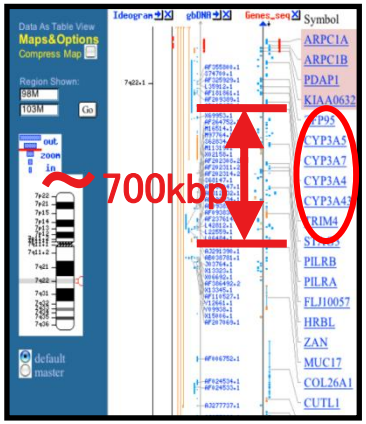
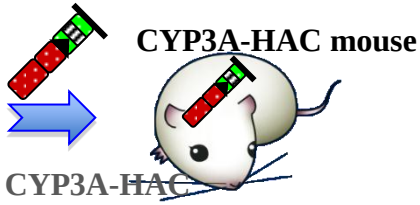
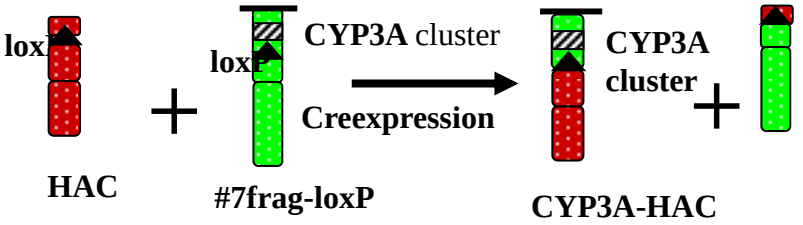
Humanized animal for the prediction of human pharmacokinetics and toxicity

- Prediction of human pharmacokinetics and toxicity using experimental animals is difficult because of species difference
- Humanized animals like transgenic mice are useful tool for such prediction
- However, there is a limitation in size of gene to transfer in the conventional cloning technique
- In contrast, human artificial chromosome vector (HAC), which was developed by Professor Oshimura , Tottori University, has a capacity to carry large genomic loci with their regulatory element, thereby allowing physiological regulation of introduced gene in a manner similar to that of native chromosome
- We have been taking cooperative work with Drs Kazuki and Oshimura, Tottori University, to clarify the usefulness of humanized mouse holding of human CYP3A gene cluster which was constructed using HAC

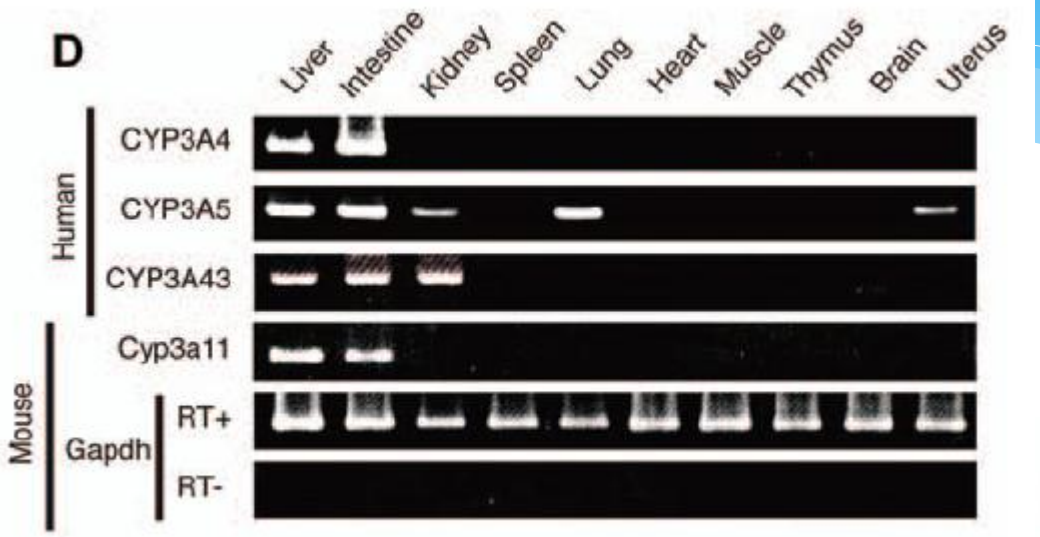
Human CYP3A cluster in chromosome 7

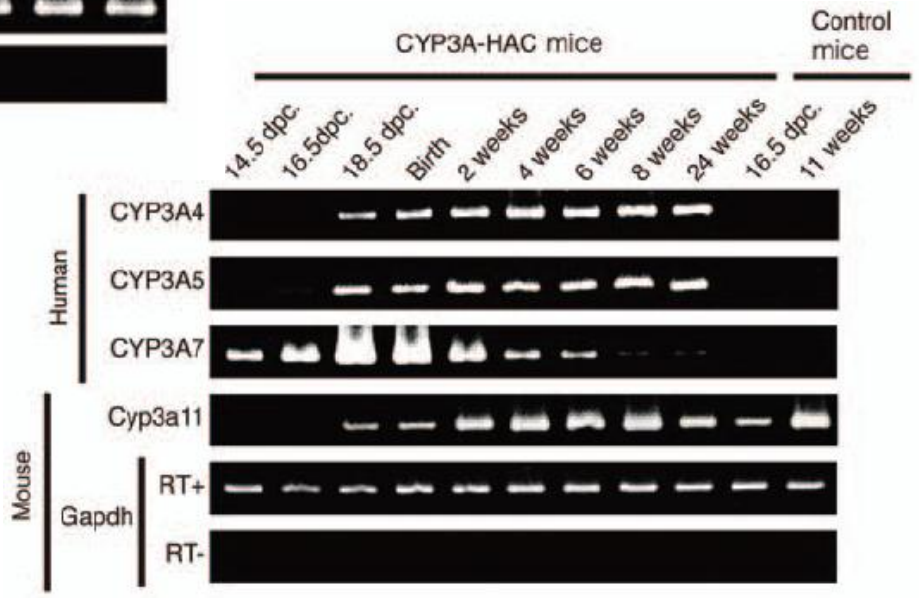






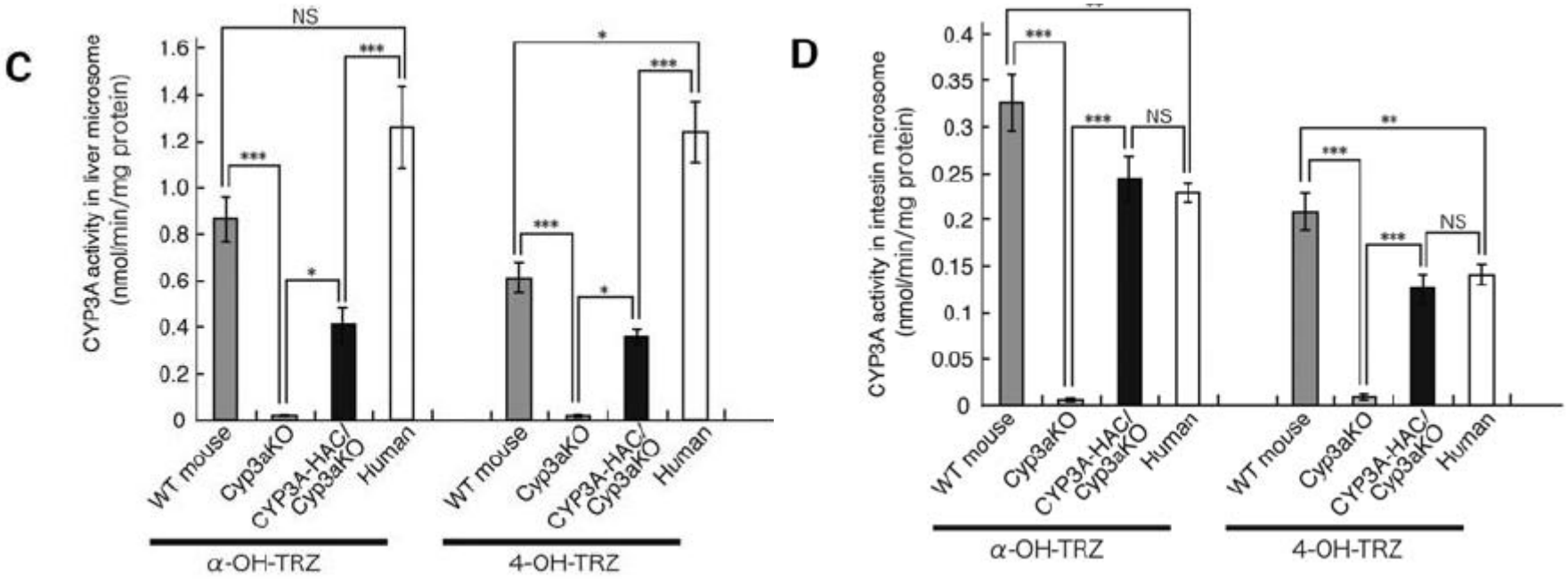
Expression of CYP3A4, 5 and 7 in CYP3A-HAC mouse





Human Mol Genet in press

Hepatic and intestinal activities of CYP3A in CYP3A-HAC mouse



Human Mol Genet in press

Poster 1-P-42, 43, 44, and 45

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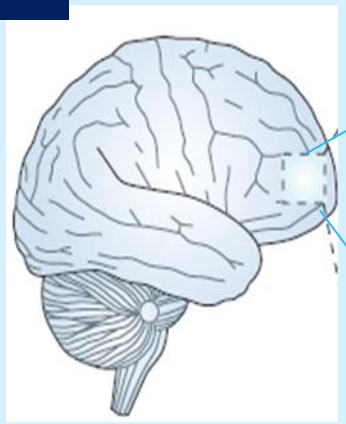
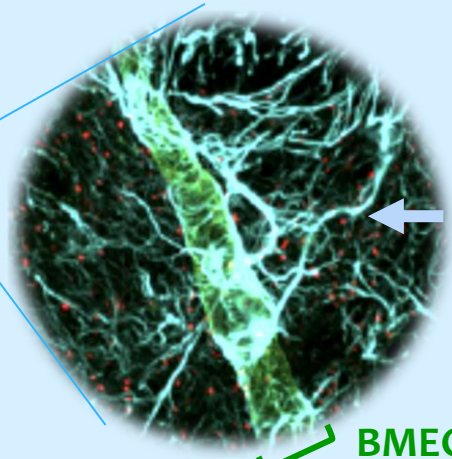
Human in vivo BBB



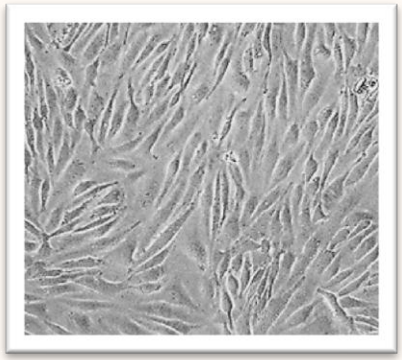


Cecchelli R et al. Nat. Rev. Drug Discov. 2007:6;650-661.

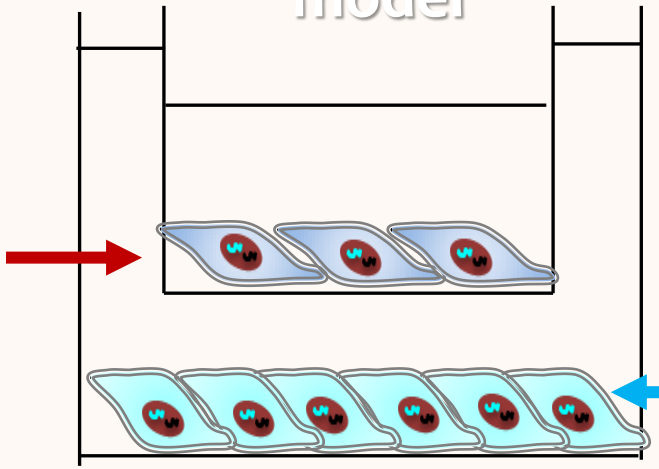
Petzold a& Murthy. *Neuron* 2011;71:783-797.

The new immortalized cell-based in vitro human BBB model

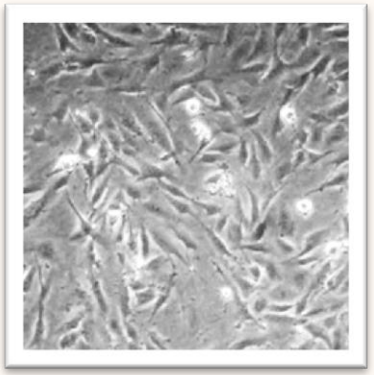
HBMEC/ciβ



Kamiichi A, Brain Res 2012;1488:113-122.

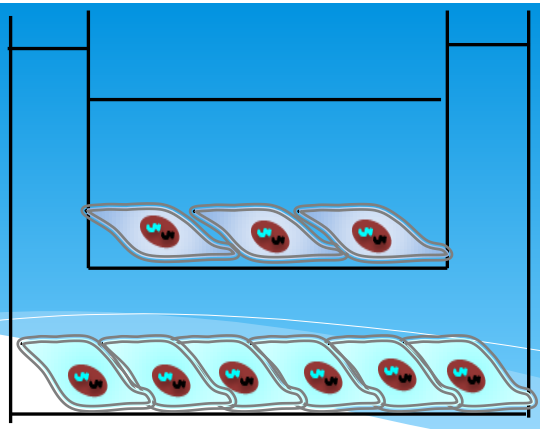


HASTR/ci



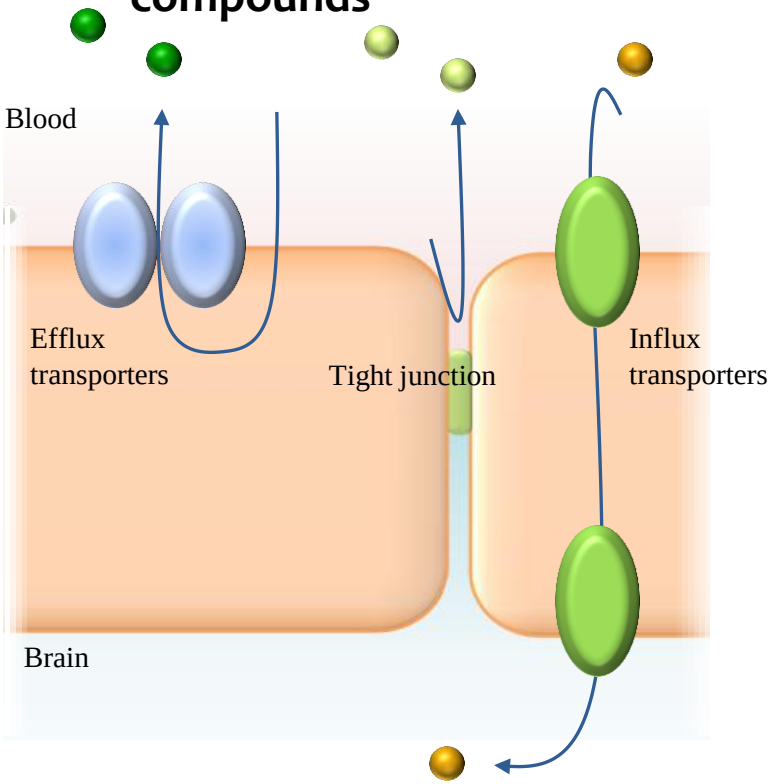
This will be presented in 3P-06

The new in vitro human BBB model

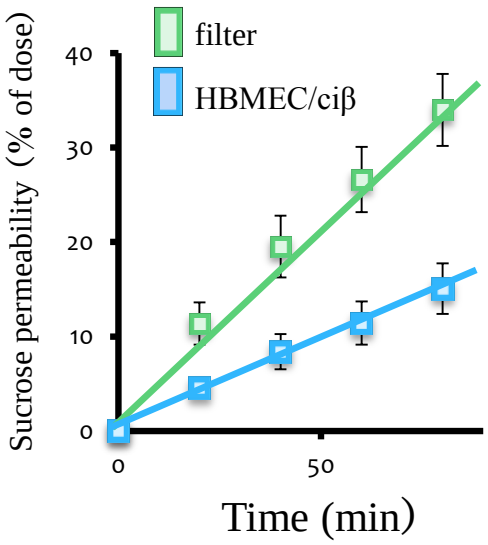


Posters, 1-P-12, 3P-06

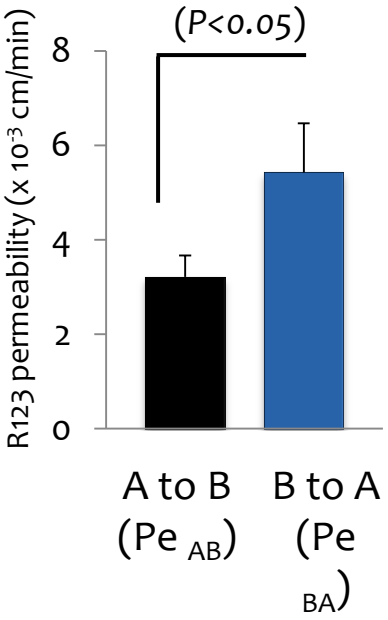
The BBB permeability screening tests for low molecular weight compounds



Sucrose Permeability
Pe = 2.6 × 10^{-3} cm/min



R123 efflux ratio
1.8±0.4



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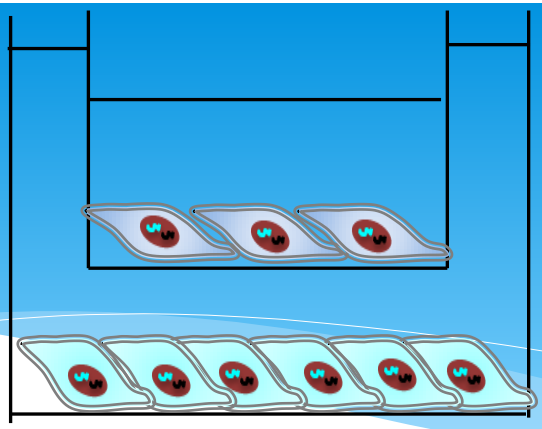
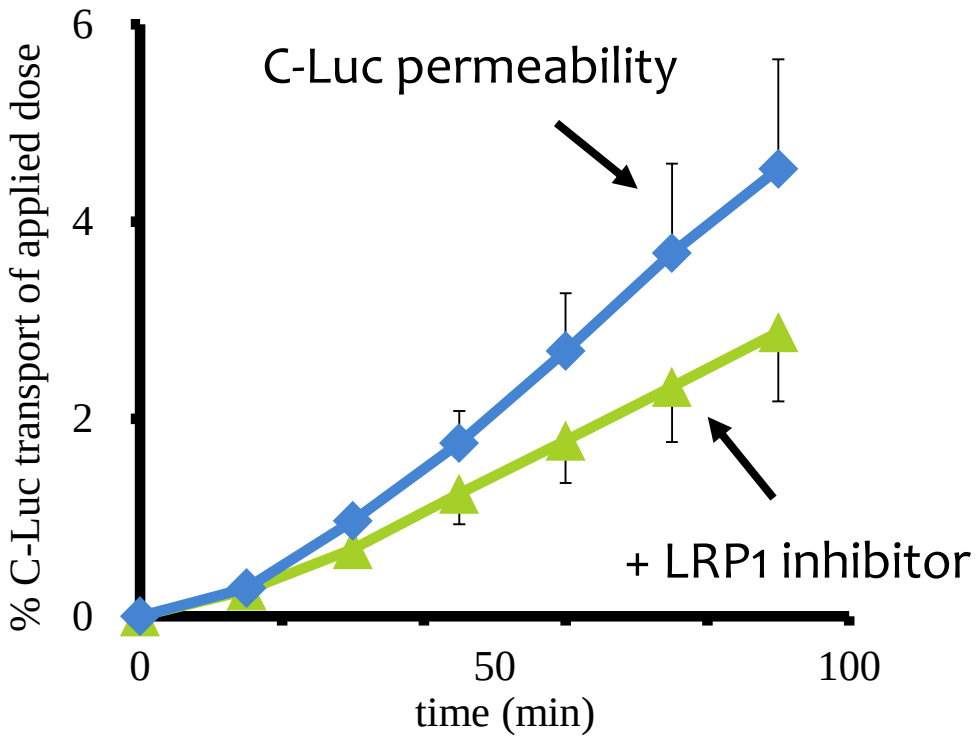
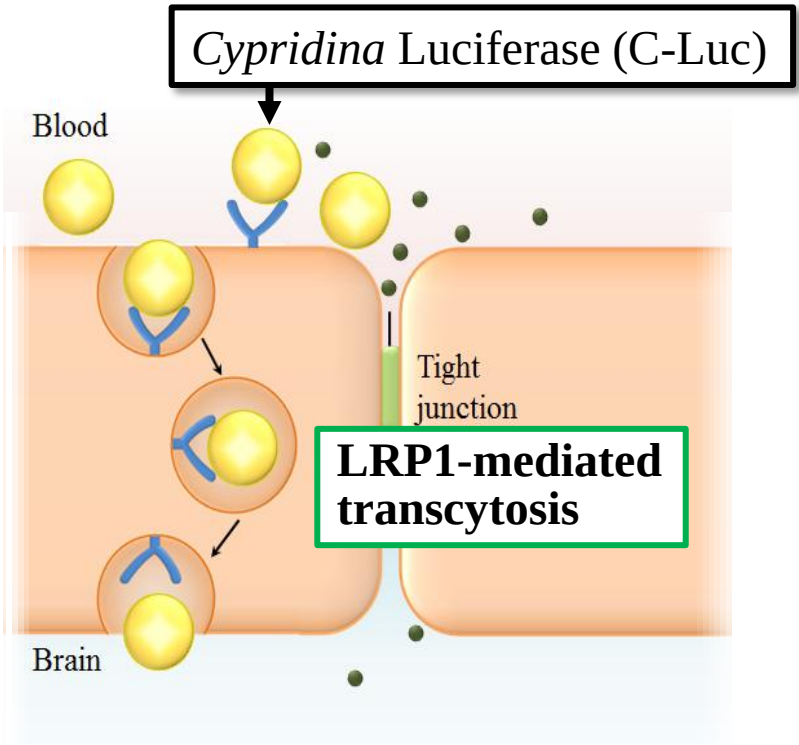


The BBB permeability screening tests for biopharmaceuticals





1-P-12

JP Pat. P.

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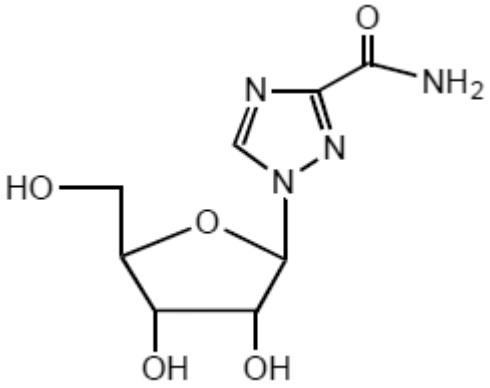
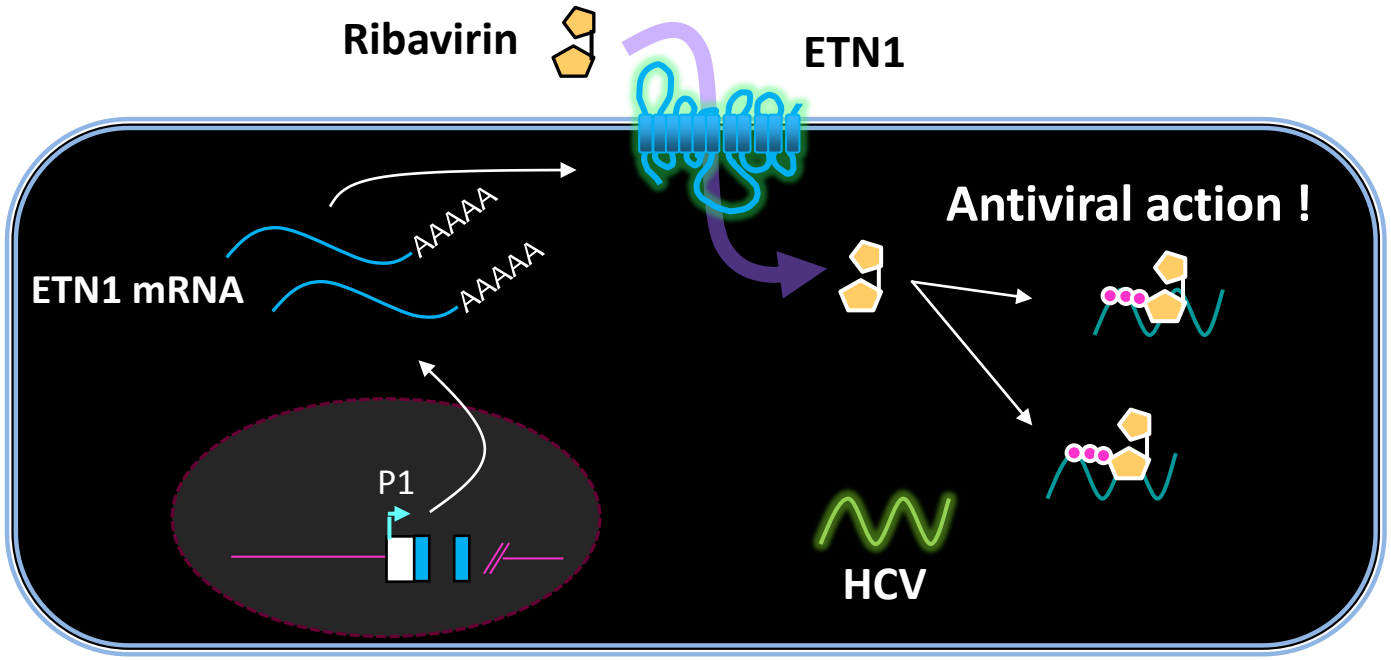


Ribavirin is a nucleoside analogue that is essential to the treatment of hepatitis C virus (HCV) infection. Since it is a hydrophilic molecule, uptake process into human hepatocytes is a prerequisite step for the action of ribavirin. We recently identified that equilibrative nucleoside transporter 1 (ENT1) is the primary ribavirin uptake transporter in human hepatocytes . Thus we studied the role of ENT1 in ribavirin's antiviral action using OR6 cells,

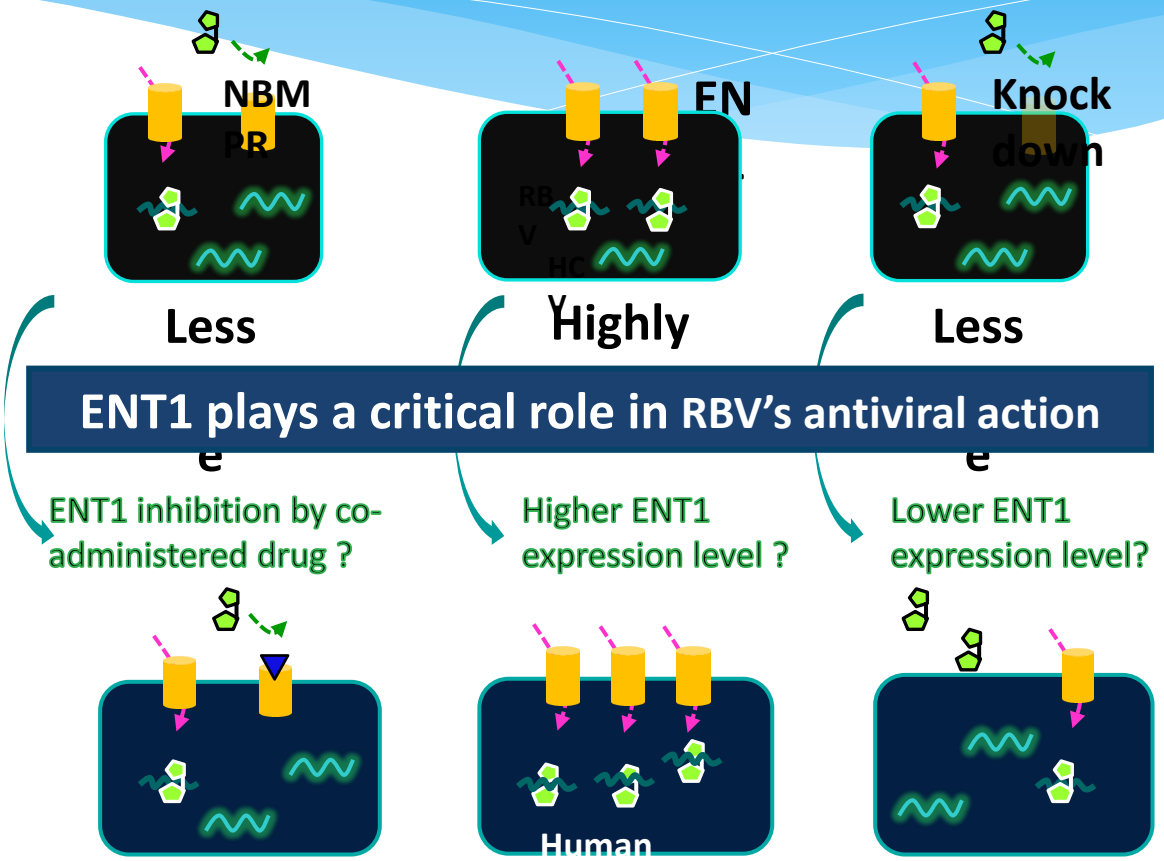


ENT1 plays a determinant role in the antiviral activity of ribavillin in OR6 cells

OR6 cells

OR6 cell is a HCV replication cell system with no ability of infection

Functional disturbance of ENT1 in OR6 cells significantly attenuated antiviral activity of ribavilin



Iikura, Furihata, et al. Antimicrob Agent Chemother 2012;56:1407-13.

Acknowledgements

Memorable work

- Mechanism of neurotoxic effect of MPTP which causes Parkinsonism in human
  - **Dr. Trevor** (UCSF)
  - **Dr. Castagnoli** (VergiaTec)
- Metabolism of omeprazole by CY2C19 which shows genetic polymorphism in human
  - **Dr. Ishizaki** (Kumamoto Univ)
  - **Dr. Sohn** (Soonchunhyang Univ)
- *SLCO1B1*15* as a genetic marker predisposed to statin-induced rhabdomyolysis
  - **Dr. Morimoto** (Takasaki Univ of Health & Welfare)
  - **Dr. Kameyama** (Nippon Kayaku)

Recent work

- Trans-chromosomic mice for the assessment of drug disposition and toxicity in human
  - **Dr. Kobayashi** (Chiba Univ)
  - **Dr. Kazuki** (Tottori Univ)
  - **Dr. Oshimura** (Tottori Univ)
- Development of in vitro blood-brain barrier model for the assessment of drug penetration into the brain in human
- ENT1 as a determinant factor for antiviral efficacy of ribavirin in human
  - **Dr. Furihata** (Chiba Univ)

Acknowledgements

* **I deeply appreciate**
  * **All the coworkers**
  * **Past and present stuff members and students of my laboratory**