Supplemental data

Drug Metabolism & Disposition

(Short Communication)

Title:

Comparative evaluation of dehydroepiandrosterone sulfate (DHEAS) potential to predict hepatic OATP transporter-based drug-drug interactions

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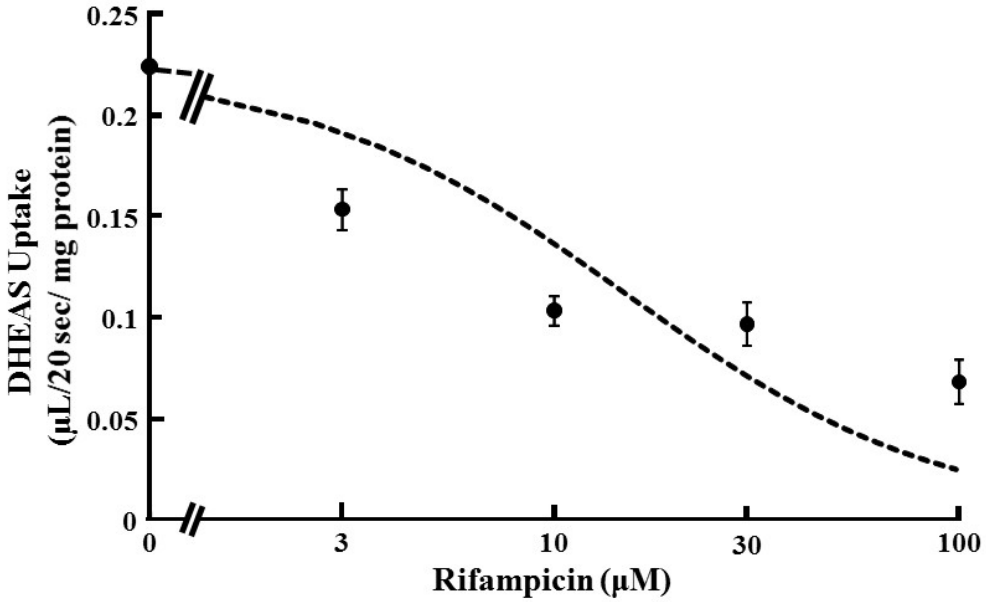
Supplemental Figure 1

[^{3}H]DHEAS uptake by freshly isolated rat hepatocytes in the presence of increasing concentrations of rifampicin



Method

Rat hepatocytes were isolated according to the collagenase perfusion method as described previously (Tamai and Tsuji, 1987). After pre-incubation of isolated hepatocytes for 5 min at 37˚C with Krebs-Henseleit buffer (KHB: 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $CaCl_2$, 25 mM $NaHCO_3$, 20 mM HEPES), the reaction was started by mixing an equal volume of KHB containing substrate with or without inhibitor. Final cell and substrate concentrations were 2×10^5 cells/mL and 2 nM (0.2 μCi/mL), respectively. The inhibitory effect of rifampicin was assessed in terms of the uptake for 20 sec (uptake activity was confirmed to increase linearly up to 20 sec). To terminate the reaction, 100 μL of reaction mixture was transferred into a micro centrifuge tube containing 100 μL silicone layer (silicone oil: liquid paraffin = 3:10) and 100 μL 2 N NaOH, and the samples were stored at room temperature overnight to dissolve the cells. Then, the centrifuge tubes was cut at the boundary of the silicone layer, and the cellular radioactivity was measured with a liquid scintillation counter (LCS-6100; Hitachi Aloka Medical, Tokyo, Japan) after addition of 100 μL 2 N HCl for neutralization and 1 mL Clear-sol scintillation fluid (Nacalai Tesque, Kyoto, Japan). The relative uptake of [^{3}H]DHEAS in the presence of increasing concentrations of

rifampicin [I] is given by the following equation, where IC_{50} is the 50% inhibitory concentration of rifampicin on DHEAS uptake by isolated rat hepatocytes.

$$\% \text{ of control uptake} = \frac{100 \times IC_{50}}{IC_{50}+[I]}$$

The value of IC_{50} was estimated by means of nonlinear least-squares analysis using the MULTI program (Yamaoka *et al*., 1981), and was obtained as 3.65 μM.
Protein concentration was determined with a protein assay kit (Bio-Rad Richmond, CA, USA) according to the manufacturer's instructions. The bovine serum albumin provided in the kit was used as a standard.

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Supplemental Table 1

Species	Markers		Rifampicin			AUC	AUC	non treated	rifampicin tretaed	AUCR	Reference	Symbol
	Compounds	Administration roots	Dose (mg/kg)	Dose (mg/m2)	Administration roots		unit					
Monkey	Pitavastatin	p.o.	2	24	p.o.	AUC_{last}	ng・h/mL	67.1	225	3.4	1	A
	Pitavastatin	p.o.	10	120	p.o.	AUC_{last}	ng・h/mL	67.1	685	10.2	1	A
	Pitavastatin	p.o.	20	240	p.o.	AUC_{∞}	ng・h/mL	72	1089	15.1	2	A
	Rosuvastatin	p.o.	2	24	p.o.	AUC_{last}	ng・h/mL	213	641	3.0	1	B
	Rosuvastatin	p.o.	10	120	p.o.	AUC_{last}	ng・h/mL	213	2942	13.8	1	B
	Rosuvastatin	p.o.	18	216	p.o.	AUC_{∞}	μM・h	0.56	2.72	4.9	3	B
	Rosuvastatin	p.o.	15	180	p.o.	AUC_{∞} (cross over)	nM・h	176	439	2.9	4	B
	Pitavastatin	i.v.	20	240	p.o.	AUC_{∞}	ng・h/mL	412	1578	3.8	2	A
	Rosuvastatin	i.v.	18	216	p.o.	AUC_{∞}	μM・h	1.88	4.99	2.7	3	B
Human	Pitavastatin	p.o.	10.0	370	p.o.	AUC_{∞} (cross over)	ng・h/mL	376	1989	6.4	5	A
	Pitavastatin	p.o.	10.0	370	p.o.	AUC_{∞}	ng・h/mL	29	157	5.4	6	A
	Rosuvastatin	p.o.	10.0	370	p.o.	AUC_{∞}	ng・h/mL	23.5	96.1	4.1	6	B
	Pravastatin	p.o.	8	296	p.o.	AUC_{∞}	ng・h/mL	234.1	603.2	2.6	7	C
	Pravastatin	p.o.	10.0	370	p.o.	$AUC_{0\text{-}8}$	pg・h/mL/μL	5.97	27.7	4.6	8	C
	Pitavastatin	p.o.	10.0	370	i.v.	AUC_{∞}	ng・h/mL	29	193	6.7	6	A
	Rosuvastatin	p.o.	10.0	370	i.v.	AUC_{∞}	ng・h/mL	23.5	71.3	3.0	6	B
Rat	Pitavastatin	i.v.	30	180	p.o.	AUC_{∞}	μM・h	4.99	28.3	5.7	9	A
	Rosuvastatin	i.v.	30	180	p.o.	AUC_{∞}	μM・h	1.17	3.69	3.2	9	B
	Pravastatin	i.v.	30	180	p.o.	AUC_{∞}	μM・h	1.77	4.5	2.5	9	C
Monkey	DHEAS	-	2	24	p.o.	$AUC_{0\text{-}8}$	ng・h/mL	530	609	1.1	1	D
	DHEAS	-	10	120	p.o.	$AUC_{0\text{-}8}$	ng・h/mL	530	1030	1.9	1	D
	Total Bilirubin	-	18	216	p.o.	$AUC_{0\text{-}24}$		Fold change		2.8	3	E
	CP I	-	15	180	p.o.	$AUC_{0\text{-}48}$	nM・h	14.2	37.6	2.6	10	G
	CP III	-	15	180	p.o.	$AUC_{0\text{-}48}$	nM・h	5.8	20.6	3.6	10	H
Human	Total Bilirubin	-	10.0	370.0	p.o.	plasma concentration	mg/100 mL	0.87	1.51	1.7	11	E
	Bile Acids	-	12.9	477.3	p.o.	AUC	μM・h	21.17	66.94	3.2	12	F
	CP I	-	10.0	370.0	p.o.	$AUC_{0\text{-}24}$(+RSV)	nM・h	20.9	84.2	4.0	13	G
	CP III	-	10.0	370.0			nM・h	3.77	12.6	3.3	13	H
Rat	DHEAS	-	30	180	i.v.	AUC_{∞}	μg・h/mL	11.38	47.96	4.2	x	D
	Total Bilirubin	-	20	120	i.v.	$AUC_{0\text{-}t}$	mg・h/dL	1.36	3.24	2.4	14	E
	Total Bilirubin	-	5	30	i.v.	$AUC_{0\text{-}t}$	mg・h/dL	1.36	1.88	1.4	14	F

This table summarizes the values plotted in Fig. 2, which were obtained from the cited sources. Reference numbers in Figure 2 and Supplemental Table 1 refer to the following references.

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