6β-hydroxycortisol is an endogenous probe for evaluation of drug–drug interactions involving a multispecific renal organic anion transporter, OAT3/*SLC22A8*, in healthy subjects

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Drug Metabolism and Disposition

Supplemental Table 1 Outlines of clinical studies the plasma and urine samples from which were used in this study

The specimens from the underlined study were used for the analysis in this study. Probenecid was given 2 h before the administration of the probe drugs (adefovir or benzylpenicillin), and pyrimethamine was given 1 h before the administration of metformin. Plasma and urine specimens used in this study were from underlined drug treatments.

Study	**Number of subjects**	**Phase number**	**Drug administered**	**Plasma specimens**	**Urine specimens**	**Reference**
1	6	Phase 1 Phase 2	Benzylpenicillin alone (400,000 U, po) :control group in this study	Plasma samples were collected at	Urine samples were collected in	Maeda K et al., submitted

		Phase 3 Phase 4	Benzylpenicillin+probenecid (500 mg, po) Benzylpenicillin+probenecid (750 mg, po) Benzylpenicillin+probenecid (1,500 mg, po)	0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after the administration of the probe drugs	the predose period and during the periods 0–4 h, 4–8 h, and 8–24 h after treatment with the probe drugs.	
2	6	Phase 1 Phase 2 Phase 3 Phase 4	Adefovir alone (10 mg, po) :control group in this study Adefovir + probenecid (500 mg, po) Adefovir + probenecid (750 mg, po) Adefovir + probenecid (1,500 mg, po)			
3	8	Phase 1 Phase 2	metformin alone (100 μg, po) :control group in this study metformin + pyrimethamine (50 mg,	Plasma samples were collected at 0.5, 1, 1.5, 2, 4, 6, 8,	Urine samples were collected in the predose	Kusuhara et al., 2011

			po)	and 12h after the administration of the probe drugs	period and during the periods 0–6 h, 6–12 h, and 12–24 h after treatment with the probe drugs.	

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Supplemental Table 2 The uptake of reference compounds in human kidney slices, and the effect of typical inhibitors

The uptake of 6β-OHF (20 μM) by human kidney slices from three different volunteers (Panel A; male 70 years, Panel B; female 51 years and Panel C male 75 years) for 20 min was determined at 37°C. Three slices were used in each batch of human kidney. Uptake represents the mean ± SE (n=3). **P* < 0.05, ***P* < 0.01.

Donor information	reference compounds	Uptake (ml/20 min/g kidney)	
		Control	+inhibitor[a]
male 70 years (panel A)	^{3}H-adefovir	5.47±0.57	0.68±0.01**
	^{14}C-benzylpenicillin	2.09±0.36	0.75±0.01*
	^{14}C-TEA	12.9±2.2	0.60±0.03**
female 51 years (panel B)	^{3}H-adefovir	3.97±0.26	0.46±0.06**
	^{14}C-benzylpenicillin	1.50±0.11	0.55±0.07**
	^{14}C-TEA	8.10±0.05	0.37±0.00**
male 70 years (panel C)	^{3}H-adefovir	8.33±0.09	0.60±0.05**
	^{14}C-benzylpenicillin	1.44±0.17	0.41±0.05**
	^{14}C-TEA	3.81±1.16	0.30±0.03*

[a] Probenecid (1mM) was used for the uptake of ^{3}H-adefovir and ^{14}C-benzylpenicillin, and MPP^{+} (1mM) was used for the uptake of ^{14}C-TEA.

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Supplemental Table 3 Comparison of apparent formation clearance of 6β-OHF ($CL_{6β\text{-}OHF}$)

$CL_{6β\text{-}OHF}$ was calculated using $X_{6β\text{-}OHF}$ and AUC of cortisol (AUC_F) following equation (7) in Materials and Methods.

Study	Groups	AUC_F [1)] (μg • h/ml)	$X_{6β\text{-}OHF}$ [1)] (μg)	$CL_{6β\text{-}OHF}$ (ml/min)
1[¶]	control	8.66 ± 0.60	995 ± 108	1.90 ± 0.12
	+probenecid (750 mg)	8.75 ± 0.41	917 ± 91	1.76 ± 0.18
2[†]	control	7.94 ± 0.73	990 ± 130	2.10 ± 0.27
	+probenecid (750 mg)	8.86 ± 0.45	1072 ± 53	2.04 ± 0.13
3[‡]	control	4.45 ± 0.34	480 ± 30	1.90 ± 0.24
	+pyrimethamine (50 mg)	4.59 ± 0.37	387 ± 42	1.44 ± 0.15

Healthy subjected received oral dose of [¶]benzylpenicillin (400,000 U), [†]adefovir (10 mg), and [‡]metformin (100 μg). Each parameter represents mean ± S.E (n=6 or 8).

AUC: area under the plasma concentration-time curve from 0 to 24 (Study 1 and 2) or to 12 hours (Study No 3). $X_{6β\text{-}OHF}$: cumulative amount of 6β-OHF in the urine up to 24 (Study 1 and 2) or 12 hours (Study No 3) after administration of probe drug

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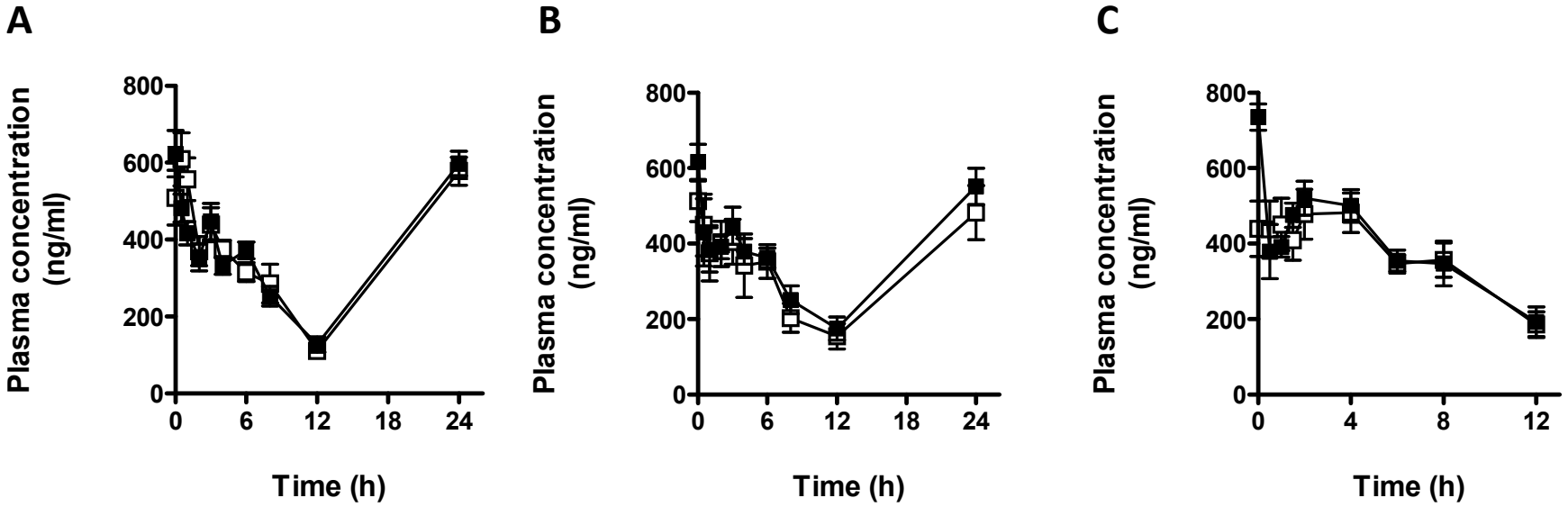

Supplemental Figure 1 Effect of probenecid and pyrimethamine on the plasma concentrations of cortisol in healthy subjects.

Plasma concentrations of cortisol were determined in healthy subjects at designated time points. Open and closed symbols represent control or inhibitor treated groups (panel A; Study 1, panel B Study 2 and panel C Study 3). Each point and bar represent the mean±SE (panel A and B n = 6 and panel C n = 8).