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Interaction and transport characteristics of mycophenolic acid and its glucuronide via human organic anion transporters hOAT1 and hOAT3

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ABSTRACT

The immunosuppressant mycophenolate mofetil (MMF) is frequently administered with calcineurin inhibitors and corticosteroids to recipients of organ transplantations. However, the renal handling of the active metabolite mycophenolic acid (MPA) and 7-O-MPA-glucuronide (MPAG) has been unclear. The purpose of the present study was to assess the interaction of MPA and MPAG with the human renal organic anion transporters hOAT1 (SLC22A6) and hOAT3 (SLC22A8), by conducting uptake experiments using HEK293 cells stably expressing these transporters. MPA and MPAG inhibited the time-dependent uptake of *p*-[^{14}C]aminohippurate by hOAT1 and that of [^{3}H]estrone sulfate by hOAT3. The apparent 50% inhibitory concentration (IC_{50}) of MPA for hOAT1 and hOAT3 was estimated at 10.7 and 1.5 μM, respectively. In the case of MPAG, the IC_{50} values were calculated at 512.3 μM for hOAT1 and 69.1 μM for hOAT3. Eadie–Hofstee plot analyses showed that they inhibited hOAT1 noncompetitively and hOAT3 competitively. No inhibitory effects of tacrolimus, cyclosporin A and azathioprine on transport of *p*-[^{14}C]aminohippurate by hOAT1 and of [^{3}H]estrone sulfate by hOAT3 were observed. No transport of MPA by these transporters was observed. On the other hand, the uptake of MPAG into cells was stimulated by the expression of hOAT3, but not hOAT1. These findings propose the possibility that the administration of MMF decreases the renal clearance of drugs which are substrates of hOAT1 and hOAT3. Present data suggest that hOAT3 contributes to the renal tubular secretion of MPAG.

1. Introduction

An immunosuppressive agent, mycophenolate mofetil (MMF) is commonly prescribed with the calcineurin inhibitor tacrolimus or cyclosporin A and corticosteroids for patients who have received a solid organ transplantation. After orally administered and absorbed, MMF is converted to an active metabolite, mycophenolic acid (MPA; Fig. 1) by serum esterases. MPA is mainly excreted into urine after being metabolized to 7-O-MPA-glucuronide (MPAG) by the hepatic uridine diphosphate-glucuronosyltransferases [1,2]. The genetic variants of uridine diphosphate-glucuronosyltransferases contribute to the extensive variability in the pharmacokinetics of the immunosuppressant [3,4]. Furthermore, the enterohepatic circulation of MPAG/MPA exists, and tubular secretion as well as glomerular filtration is responsible for the

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Abbreviations: MMF, mycophenolate mofetil; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; OAT, organic anion transporter
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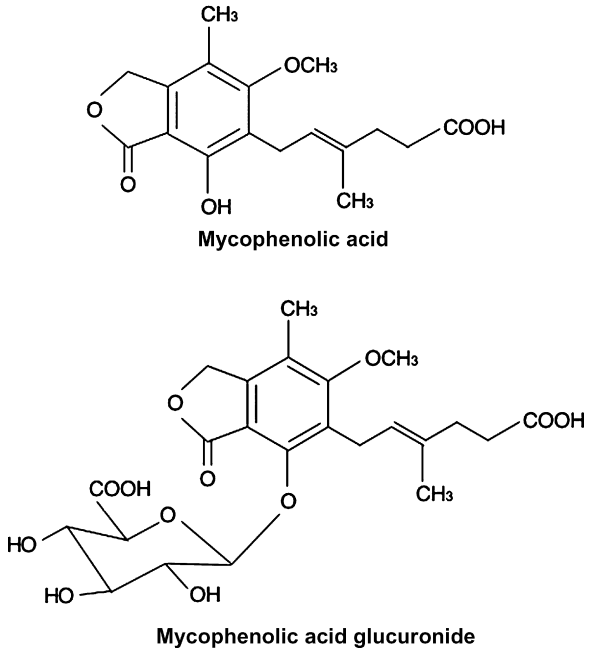

Fig. 1 – Chemical structure of mycophenolic acid and its glucuronide.

urinary excretion of MPAG [1]. Thus, the process by which MMF is eliminated, is very intricate.

Besides immunosuppressive agents, many drugs are given to recipients of organ transplantations, to prevent or treat infections, gastrointestinal ulcers, thrombus, ascites, pleural fluid, hypertension, diabetes, osteoporosis, gout, bronchitis and so on. Accordingly, side effects caused by drug interactions often occur. In addition to tacrolimus and cyclosporin A, MMF was shown to interact with various drugs. For instance, MMF reduced the renal clearance of acyclovir and ganciclovir, and the pharmacokinetics of MPA or MPAG were affected by cyclosporin A, glucocorticoids and non-steroidal anti-inflammatory drugs [1,5–8]. Taking these MPA and/or MPAG-mediated drug interactions into account, together with the complexity of the fate of MMF, it is important to identify the drug-metabolizing enzymes and drug transporters interacting with this immunosuppressive agent for a successful organ transplantation.

The human organic anion transporters (hOATs) mediate transport of clinically important drugs, such as diuretics, antibiotics, antivirals, histamine H_2 receptor antagonists, non-steroidal anti-inflammatory drugs and so on [9,10]. Among the family, hOAT1 (SLC22A6) and hOAT3 (SLC22A8) were shown to be predominantly expressed in the basolateral membrane of the renal proximal tubules [11], suggesting that they play main roles in the renal tubular uptake of organic compounds from blood. Furthermore, as previously reported [12], it is possible that hOAT1 and hOAT3 are targets of the interaction between methotrexate and non-steroidal anti-inflammatory drugs.

This background suggests that the renal organic anion transporters are concerned with the renal excretion of the metabolites of MMF and drug interaction with them, but to our knowledge, no report has examined the interaction of MPA and MPAG with the renal organic anion transporters at a molecular level. In the present study, the inhibitory effects of MPA and MPAG on hOAT1 and hOAT3 were assessed. In addition, the contribution of hOAT1 and hOAT3 to the renal tubular secretion of MPA and MPAG was investigated.

2. Materials and methods

2.1. Materials

p-[glycyl-1-^{14}C]Aminohippurate (1.9 GBq/mmol) and [6,7-^{3}H(N)]estrone sulfate, ammonium salt (2.1 TBq/mmol) were obtained from NEN™ Life Science Products Inc. (Boston, MA, USA) and Perkin-Elmer Life Sciences Inc. (Boston, MA, USA), respectively. MPA and its glucuronide were from Wako Pure Chemical Industries (Osaka, Japan) and Analytical Services International Ltd. (London, UK), respectively. Tacrolimus and cyclosporin A were kindly supplied by Fujisawa Pharmaceutical (Newly, Astellas Pharma Inc., Tokyo, Japan) and Novartis Pharma KK, Co. Ltd. (Tokyo, Japan), respectively. Azathioprine and unlabelled estrone sulfate, sodium salt and probenecid were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of the highest purity available.

2.2. Uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate by HEK293 cells stably expressing hOAT1 and hOAT3

According to our former report [13], experiments on the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate were performed using HEK293 cells stably transfected with a pBK-CMV vector containing hOAT1 cDNA, hOAT3 cDNA or no cDNA, named HEK-hOAT1, HEK-hOAT3 and HEK-pBK, respectively. In brief, 48 h after the cells were seeded on poly-D-lysine-coated 24-well plates at a density of 2×10^5 cells/well, the accumulation of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate by the cells was examined. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM HEPES (pH 7.4). After the preincubation of the cells with 0.2 ml of the incubation medium at 37 °C for 10 min, the medium was replaced with 0.2 ml of incubation medium containing test compounds. At the end of the incubation, the medium was aspirated and then the cells were washed twice with 1 ml of ice-cold incubation medium. The cells were lysed in 250 μl of 0.5N NaOH solution, and the radioactivity in aliquots was determined in 3 ml of ACSII (Amersham International, Buckinghamshire, UK). The protein contents of the solubilized cells were determined by the method of Bradford using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine γ-globulin as a standard.

2.3. Uptake of MPA and MPAG by HEK293 cells stably expressing hOAT1 and hOAT3

Forty-eight hours after the cells were seeded on poly-D-lysine-coated 12-well plates at a density of 4×10^5 cells/well, the amounts of MPA and MPAG taken up by the cells were

examined. After the uptake, experiments were performed as described above, the cells were scraped off with a rubber policeman into 300 μl of 50% acetonitrile in 20 mM phosphate buffer (pH 3.0) and maintained for 30 min at room temperature. The extract solution was centrifuged at 14,000 rpm (Centrifuge 5417C, eppendorf, Hamburg, Germany) for 20 min. The supernatant was filtered through a Cosmonice Filter W (0.45 μm; Nacalai Tesque, Kyoto, Japan) and analyzed by high-performance liquid chromatography. The protein contents of the cells solubilized in 200 μl of 0.5N NaOH were determined.

2.4. Analytical method for MPA and MPAG

The amounts of MPA and MPAG in the extract solution were measured using a high-performance liquid chromatograph (LC-10AS, Shimadzu Co., Kyoto, Japan) equipped with a UV spectrophotometric detector (SPD-10AV, Shimadzu Co.) and an integrator (Chromatopac C-R6A, Shimadzu Co.) under the following conditions: column, TSK-GEL ODS-80^{TM}, 4.6 mm × 250 mm (TOSOH Co., Tokyo, Japan); flow rate, 0.8 ml/min; temperature, 40 °C; wavelength, 254 nm for both compounds; mobile phase, 40% acetonitrile in 20 mM phosphate buffer (pH 3.0) for MPA, 30% acetonitrile in 20 mM phosphate buffer (pH 3.0) for MPAG. When the calibration curve of MPA was depicted from 50 to 500 nM, the linearity was observed. In the case of MPAG, the standard curve showed the linearity ranged from 40 to 400 nM. The concentration of all samples was within the range.

2.5. Statistical analysis

Data were statistically analyzed with a one-way analysis of variance followed by Scheffe's test using StatView (SAS Institute Inc., NC, USA).

3. Results

3.1. Inhibitory effects of MPA and MPAG on hOAT1 and hOAT3

First, to examine whether MPA and MPAG interact with hOAT1 and hOAT3, the effects of MPA and MPAG on the time-dependent uptake of *p*-[^{14}C]aminohippurate by HEK-hOAT1 and of [^{3}H]estrone sulfate by HEK-hOAT3 were investigated. As shown in Fig. 2A, the amount of *p*-[^{14}C]aminohippurate taken up by HEK-hOAT1 increased linearly for 2 min. MPA at 300 μM completely inhibited the hOAT1-mediated transport of *p*-[^{14}C]aminohippurate. MPAG at 300 μM also inhibited the uptake of *p*-[^{14}C]aminohippurate by hOAT1, but the inhibitory effect was weaker. A similar phenomenon was observed for their inhibitory effects on hOAT3 (Fig. 2B). No linearity in the uptake of [^{3}H]estrone sulfate by hOAT3 was detected. Subsequent uptake experiments using *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate were performed with an incubation time of 2 min for hOAT1 and 1 min for hOAT3.

Next, the concentration-dependence of the inhibitory effects of MPA and MPAG on hOAT1 and hOAT3 was investigated. Fig. 3 is representative of three independent

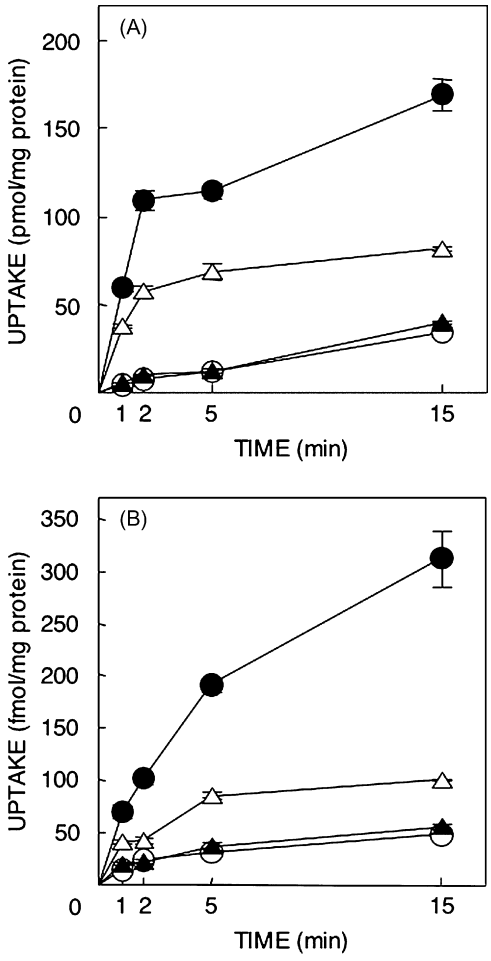

Fig. 2 – Time-dependent uptake of *p*-[^{14}C]aminohippurate by hOAT1 (A) and [^{3}H]estrone sulfate by hOAT3 (B) in the presence of MPA and MPAG. (A) HEK-pBK (open circle) and HEK-hOAT1 were incubated with 5 μM *p*-[^{14}C]aminohippurate for the indicated period in the absence (closed circle) or presence of MPA (closed triangle) and MPAG (open triangle) at 300 μM. (B) HEK-pBK (open circle) and HEK-hOAT3 were incubated with 17.5 nM [^{3}H]estrone sulfate for the indicated period in the absence (closed circle) or presence of MPA (closed triangle) and MPAG (open triangle) at 300 μM. Each point represents the mean ± S.E. of the uptake of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate in three monolayers.

experiments. The apparent 50% inhibitory concentration (IC_{50}) of MPA and MPAG were estimated to be 10.7 and 512.3 μM for hOAT1, and 1.5 and 69.1 μM for hOAT3, respectively (Table 1). It was elucidated that MPA inhibited hOAT1 and hOAT3 to a greater extent than MPAG, and that the inhibitory effects of MPA and MPAG on hOAT3 were more potent than those on hOAT1.

In order to clarify the inhibition modes of MPA and MPAG, effects of MPA and MPAG on concentration-dependent uptake of *p*-[^{14}C]aminohippurate by hOAT1 and of [^{3}H]estrone sulfate by hOAT3 were examined and Eadie–Hofstee plot analysis was conducted (Fig. 4). Based on the

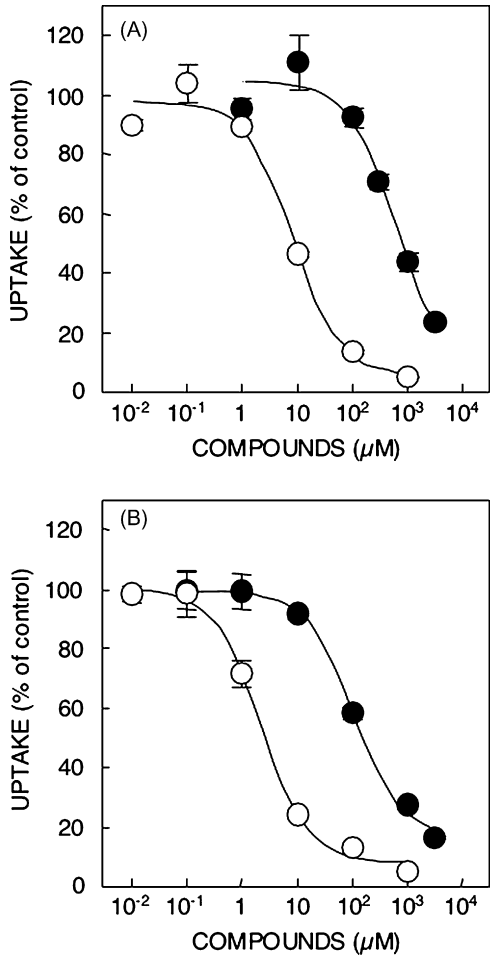

Fig. 3 – Dose-dependent effect of MPA and MPAG on hOAT1 (A) and hOAT3 (B). (A) HEK-hOAT1 was incubated with 5 μM *p*-[^{14}C]aminohippurate for 2 min in the absence (control) or presence of MPA (open circle) and MPAG (closed circle) at various concentrations. (B) HEK-hOAT3 was incubated with 17.5 nM [^{3}H]estrone sulfate for 1 min in the absence (control) or presence of MPA (open circle) and MPAG (closed circle) at various concentrations. Each point represents the mean ± S.E. of the uptake of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate in three monolayers.

three separate experiments, V_{max} values of *p*-[^{14}C]aminohippurate uptake by hOAT1 were significantly reduced in the presence of MPA and MPAG (control: 750.9 ± 44.9 pmol/mg protein/2 min; with MPA: 416.1 ± 28.7 pmol/mg protein/2 min; with MPAG: 476.5 ± 51.8 pmol/mg protein/2 min, mean ± S.E.) and K_m values were not changed (control: 34.2 ± 2.4 μM; with MPA: 34.8 ± 9.0 μM; with MPAG: 50.7 ± 12.6 μM). On the other hand, they significantly increased K_m values of hOAT3-mediated transport of [^{3}H]estrone sulfate (control: 13.2 ± 2.5 μM; with MPA: 54.0 ± 9.6 μM; with MPAG: 61.3 ± 9.6 μM, mean ± S.E.) and did not affect V_{max} values (control: 76.1 ± 9.8 pmol/mg protein/min; with MPA: 79.3 ± 11.6 pmol/mg protein/min; with MPAG: 63.7 ± 6.1 pmol/mg protein/min). These findings indicate that the inhibition manner of MPA and MPAG is noncompetitive for hOAT1 and competitive for hOAT3.

Table 1 – The IC_{50} values of MPA and MPAG for the uptake of *p*-[^{14}C]aminohippurate by hOAT1 and [^{3}H]estrone sulfate by hOAT3

	IC_{50} value (μM)	
	MPA	MPAG
hOAT1	10.7 ± 1.7	512.3 ± 109.7
hOAT3	1.5 ± 0.4	69.1 ± 23.2

HEK-hOAT1 and HEK-hOAT3 were incubated with 5 μM *p*-[^{14}C]aminohippurate for 2 min and 17.5 nM [^{3}H]estrone sulfate for 1 min, respectively, in the absence or presence of MPA or MPAG at various concentrations. The IC_{50} values were estimated by a nonlinear regression analysis of competition curves with one compartment with the following equation: uptake amount = (uptake amount without MPA and MPAG) × IC_{50}/(IC_{50} + [concentration of MPA or MPAG]) + (hOAT-independent uptake amount). The values represent the mean ± S.E. of three separate experiments.

3.2. *Effect of other immunosuppressive drugs on hOAT1 and hOAT3*

In addition to MPA and MPAG, the influences of other immunosuppressants, such as tacrolimus, cyclosporin A and azathioprine on hOAT1 and hOAT3 were assessed. Significant inhibition by these immunosuppressive agents was not observed (Table 2). These results indicate that tacrolimus, cyclosporin A and azathioprine do not interact with hOAT1 and hOAT3. The residue uptake amounts of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate in the presence of MPA at 300 μM were considered to be hOAT-independent uptake.

Table 2 – Effect of various immunosuppressants on the uptake of *p*-[^{14}C]aminohippurate by hOAT1 and [^{3}H]estrone sulfate by hOAT3

	Uptake (% of control)	
	hOAT1	hOAT3
HEK-pBK	6.7 ± 0.2*	11.3 ± 0.9*
Control	100 ± 10.6	100 ± 6.0
DMSO (0.1%)	86.4 ± 5.7	99.7 ± 0.9
Tacrolimus (1 μM)	92.7 ± 2.3	87.4 ± 2.5
Cyclosporin A (1 μM)	96.4 ± 1.2	105.2 ± 3.4
Azathioprine (10 μM)	95.4 ± 4.3	81.6 ± 3.6
MPA (300 μM)	10.8 ± 0.5*	14.6 ± 1.8*
MPAG (300 μM)	44.5 ± 2.4*	24.0 ± 1.9*

HEK-hOAT1 and HEK-hOAT3 were incubated with 5 μM *p*-[^{14}C]aminohippurate for 2 min and 17.5 nM [^{3}H]estrone sulfate for 1 min, respectively, in the absence (control) or presence of dimethylsulphoxide (DMSO), tacrolimus, cyclosporin A, azathioprine, MPA or MPAG at the indicated concentrations. Each value represents the mean ± S.E. of the uptake of *p*-[^{14}C]aminohippurate by hOAT1 and [^{3}H]estrone sulfate by hOAT3 in three monolayers.
Test solutions with tacrolimus, cyclosporine A and azathioprine contained DMSO at less than 0.1%.
* $p < 0.0001$, significantly different from control.

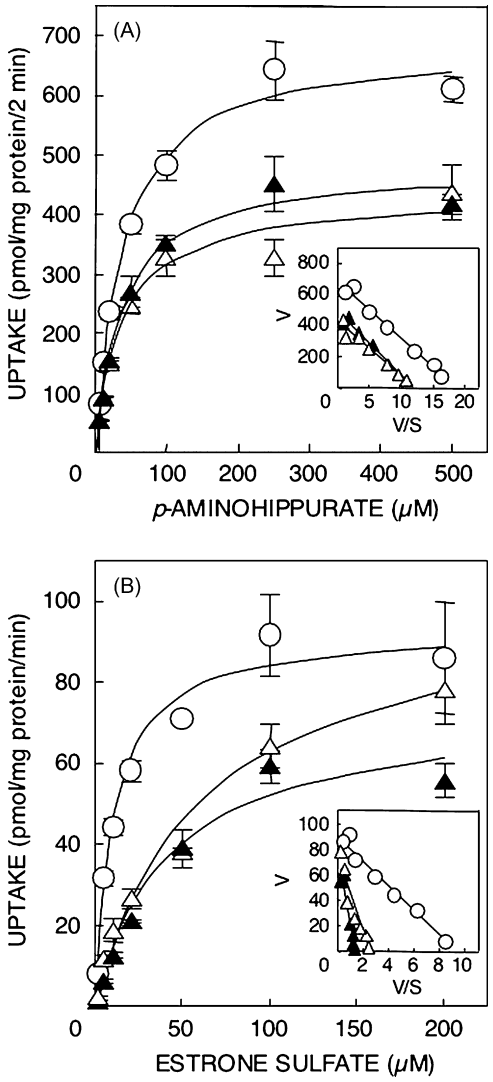

Fig. 4 – Effect of MPA and MPAG on kinetic parameters of *p*-[^{14}C]aminohippurate uptake by hOAT1 (A) and [^{3}H]estrone sulfate uptake by hOAT3 (B). (A) HEK-pBK and HEK-hOAT1 were incubated with *p*-[^{14}C]aminohippurate at various concentrations for 2 min in the absence or presence of 5 μM MPA and 200 μM MPAG and the hOAT1-mediated uptake of *p*-[^{14}C]aminohippurate was plotted in the figure (control: open circle; with MPA: open triangle; with MPAG: closed triangle). (B) HEK-pBK and HEK-hOAT3 were incubated with [^{3}H]estrone sulfate at various concentrations for 1 min in the absence or presence of 5 μM MPA and 200 μM MPAG and the hOAT3-mediated uptake of [^{3}H]estrone sulfate was plotted in the figure (control: open circle; with MPA: open triangle; with MPAG: closed triangle). Each point represents the mean ± S.E. of the uptake of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate in a typical experiments with three determinations. Insets represent Eadie–Hofstee plots of the uptake; V, uptake rate (pmol/mg protein/2 min for *p*-[^{14}C]aminohippurate uptake by hOAT1 and pmol/mg protein/min for [^{3}H]estrone sulfate uptake by hOAT3); S, concentration of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate (μM).

Table 3 – Amounts of MPA and MPAG taken up by HEK-hOAT1 and HEK-hOAT3

	Uptake (pmol/mg protein/h)		
	HEK-pBK	HEK-hOAT1	HEK-hOAT3
MPA	75.3 ± 4.1	81.2 ± 3.2	69.8 ± 5.6
MPAG	108.3 ± 9.7	165.3 ± 2.9	429.9 ± 30.7*

HEK-pBK, HEK-hOAT1 and HEK-hOAT3 were incubated with 10 μM MPA or 400 μM MPAG for 1 h. The values represent the mean ± S.E. of the amounts of MPA and MPAG taken up by three monolayers.
* $p < 0.001$, significantly different from HEK-pBK.

3.3. *Uptake of MPA and MPAG by HEK-hOAT1 and HEK-hOAT3*

Finally, to examine whether hOAT1 and hOAT3 transport MPA and MPAG, the amounts of MPA and MPAG taken up by HEK-pBK, HEK-hOAT1 and HEK-hOAT3 were evaluated. As represented in Table 3, significant increases in the uptake of MPA with the expression of hOAT1 or hOAT3 were not detected. On the other hand, the amount of MPAG taken up by HEK-hOAT3 was significantly larger than that by HEK-pBK. The hOAT3-mediated uptake of MPAG was decreased to 61.1 and 62.8% in the presence of estrone sulfate and probenecid, respectively, at 100 μM. Although, the accumulation of MPAG by HEK-hOAT1 was greater than that by the control cells, the difference was not significant. These findings indicate that MPAG is a substrate of hOAT3.

4. Discussion

In organ transplantation, many drugs, including immunosuppressants, antibiotics, antivirals, antifungals, diuretics, histamine H_2 receptor antagonists, proton pump inhibitors, anticoagulants, bronchodilators and hypouricemic agents are administered to recipients. Accordingly, drugs should be prescribed with predictions of drug interactions [14]. To avoid adverse effects via drug interactions, information on the routes of elimination of a drug and its inhibitory effects on drug-metabolizing enzymes and drug transporters is required. The internal use of MMF causes a delay in the elimination of acyclovir [5]. Renal organic anion transporters are involved in the urinary excretion of acyclovir [15], suggesting that the interaction occurs via the renal organic anion transporters. However, the interaction between acyclovir and MMF has not been examined in detail. In addition, although many studies regarding uridine diphosphate-glucuronosyltransferases involved in the glucuronidation of MPA have been performed, there is no report showing the renal handling of MPA and MPAG. Against this background, we evaluated the inhibitory effects of MPA and MPAG on hOAT1 and hOAT3, which play important roles in the renal tubular secretion of various drugs, including acyclovir [16,17]. In addition, the contribution of hOAT1 and hOAT3 to the tubular secretion of MPA and MPAG was investigated.

The present study shows that MPA and MPAG inhibited hOAT1 and hOAT3 (Fig. 2), and that the inhibitory effects of MPA on both transporters were much stronger than those of

MPAG (Fig. 3). The IC_{50} values of MPA for hOAT1 and hOAT3 were estimated to be 10.7 and 1.5 μM, respectively. According to Bullingham et al. [18], the maximum plasma concentration of MPA reached 34.0 μg/ml (106 μM) when 1.5 g of MMF was administered in healthy volunteers. MPA strongly binds to serum albumin, and the free fraction of MPA in blood is 1–3% at normal albumin levels [1]. Taking these findings into consideration, together with the IC_{50} values of MPA for hOAT1 and hOAT3, these transporters should be moderately inhibited by unbound MPA in patients taking MMF. Furthermore, because MPA plasma profiles exhibit a sharp peak after the administration of MMF, it is speculated that the MPA-mediated inhibition of hOAT1 and hOAT3 would disappear 6 h after the dosing of MMF. However, Nowak and Shaw [19] reported that the free fraction of MPA was dramatically increased when albumin concentrations were below 20 g/l in vitro. There is a case report representing that the free level of MPA was enhanced up to 18.3% in a renal transplant recipient whose serum albumin level fell to less than 20 g/l, leading to myelosuppression [20]. Because the inhibition of hOAT1 and hOAT3 by free MPA is predicted to be marked in such a severe case of hypoalbuminemia, MPA-related drug interaction via hOAT1 and hOAT3 also should be considered.

One of the most important findings of the present study is that MPAG is a substrate of hOAT3, and no transport of MPA by hOAT1 and hOAT3 was observed (Table 3). The results are consistent with the disposition of MPA and MPAG. MPA is little excreted into urine, and the renal tubular secretion of MPAG is involved in its urinary excretion [1]. Our previous study showed that among the hOAT family, hOAT3 had the highest levels of mRNA in the kidney cortex [11]. Therefore, hOAT3 is considered to play a major role in the renal secretion of MPAG. In contrast, hardly any MPA is excreted into the urine. MPA is little filtrated through the glomerulus because of its extensive protein binding and is not transported by either hOAT1 or hOAT3. Therefore, MPA would not be transferred from blood to urine.

So far, MPAG has been regarded as the causative compound in acyclovir–MMF interaction, because MPAG as well as acyclovir is secreted into urine in the renal proximal tubules [5]. Acyclovir is a substrate of hOAT1 [16,17], suggesting that hOAT1 is, at least in part, involved in the drug interaction. The present study indicates that MPAG is an inhibitor of hOAT1 and hOAT3, but its inhibitory effects are not very strong (Fig. 3). It was reported that the maximum plasma concentration of MPAG in healthy subjects was 43.1 μg/ml (87 μM) when 1.5 g of MMF was administered [18]. In addition, the fact that 80% of the MPAG in blood binds to serum albumin suggests that the inhibition of hOAT1 and hOAT3 by MPAG would not be so prominent. However, a good correlation between the renal clearance of MPAG and glomerular filtration rate was observed, and the AUC of MPAG was increased in patients with impaired renal function [21]. Accordingly, MPAG-involved drug interaction via hOAT1 and hOAT3 would be frequently recognized in recipients with renal dysfunction. In addition, MPAG competes with MPA to bind proteins [19]. The accumulation of MPAG would lead to the enhancement of unbound MPA levels, and inhibition of hOAT1 and hOAT3 by MPA would be more potent in such a case. Furthermore, Kaplan et al. [22] suggested an unknown influence on the protein binding of MPA in patients with renal dysfunction besides the accumulation of MPAG. Renal disease is considered an important factor for MMF-related drug interaction via hOAT1 and hOAT3.

As represented in Fig. 4, it has been found that MPA and MPAG inhibit hOAT1 and hOAT3 in different manners. The noncompetitive inhibition of hOAT1 by MPA and MPAG may lead to no transport of these compounds by hOAT1 (Table 2). On the other hand, MPA and MPAG inhibited hOAT3 competitively, and only MPAG was transported by hOAT3. It is interesting that MPA is a potent competitive inhibiter of hOAT3 and is not transported by the transporter. Like MPA, several compounds, including probenecid, glibenclamide, ibuprofen and ketoprofen are shown to inhibit OAT1 strongly and not to be transported by OAT1 [23–25].

It seems that there is inter-individual variation in the interaction between acyclovir and MMF. Royer et al. [26] reported neutropenia in a patient taking valacyclovir and MMF. However, in the report of Gimenez et al. [5], a significant increase in the concentration of acyclovir was not observed in healthy volunteers using valacyclovir and MMF. Recently, we found extensive variability in mRNA levels of hOATs in the human kidney, and a correlation between the elimination rates of cefazolin and hOAT3 mRNA levels in renal biopsy specimens was recognized [27,28]. In addition to the hypoalbuminemia and renal failure described above, mRNA levels of hOATs are suggested to be related to the inter-individual variability of acyclovir–MMF interaction. At present, the application of therapeutic drug monitoring, when MMF is administered, is argued. In terms of the MMF-induced drug interaction via hOAT1 and hOAT3, the free levels of MPA also should be monitored in patients with hypoalbuminemia or renal failure.

Present study represented that other immunosuppressants, including tacrolimus, cyclosporin A and azathioprine did not inhibit hOAT1 and hOAT3 (Table 2). The clinical unbound ranges of tacrolimus, cyclosporin A and azathioprine were less than the concentrations used in this study. Accordingly, it is suggested that tacrolimus, cyclosporin A and azathioprin do not interfere with hOAT1 and hOAT3 in patients. They are extensively metabolized in the body [29–31]. We did not examine effects of their metabolites on hOAT1 and hOAT3, and the possibility that the metabolites inhibit hOAT1 and hOAT3 has remained.

In summary, the present study shows that MPA and MPAG inhibit hOAT1 and hOAT3, and that the inhibitory effects of MPA on the transporters were much stronger than those of MPAG. From their IC_{50} values for these transporters and normal free levels of MPA and MPAG in plasma, it is considered that there is no need to pay too much attention to the inhibition of hOAT1 and hOAT3 by MPA and MPAG. However, in patients with hypoalbuminemia or renal impairment, care should be taken to avoid MMF-related drug interaction via hOAT1 and hOAT3, because there is a possibility that plasma concentrations of unbound MPA and MPAG will reach levels inhibitory to the transporters. Among drugs frequently administered to recipients of organ transplantations, ganciclovir and famotidine as well as acyclovir are substrates of hOAT1 or hOAT3 [16,17,32] and are myelosuppressive in addition to MPA. Furthermore, the present study shows that

MPAG is a substrate of hOAT3, suggesting that hOAT3 contributes to the renal tubular secretion of MPAG. These findings are useful for optimizing treatments for organ transplantation.

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