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Title:

A species difference in the transport activities of H_2 receptor antagonists by rat and human renal organic anion and cation transporters.

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Running title:

Comparison of H_2-receptor antagonist uptake by OATs and OCTs

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Abbreviations used: hOAT, human organic anion transporter; rOat, rat organic anion transporter; hOCT, human organic cation transporter; rOct, rat organic cation transporter; CMD, cimetidine; FMD, famotidine; RND, ranitidine; TEA, tetraethylammonium

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Abstract

A clinical drug-drug interaction between famotidine (a H_2 receptor antagonist) and probenecid has not been reproduced in rats. The present study hypothesized that the species-dependent probenecid-sensitivity is due to a species-difference in the contribution of renal organic anion and cation transporters. The transport activities of the H_2 receptor antagonists (cimetidine, famotidine, ranitidine) by rat and human basolateral organic anion and cation transporters (hOAT1, hOAT2, r/hOAT3, rOct1, r/hOCT2) were compared using their cDNA transfectants. The transport activity (V_{max}/K_m) of famotidine by rOat3 was 8- and 15-fold lower than that of cimetidine (K_m: 91μM) and ranitidine (K_m: 155μM), respectively, while that by hOAT3 (K_m: 124μM) was 3-fold lower than that of cimetidine (K_m: 149μM), but similar to that of ranitidine (K_m: 234μM). Comparison of the relative transport activity with regard to that of cimetidine suggests that famotidine was more efficiently transported by hOAT3 than rOat3, and vice versa, for ranitidine. Only ranitidine was efficiently transported by hOAT2 (K_m: 396μM). rOct1 accepts all the H_2 receptor antagonists with a similar activity, while the transport activities of ranitidine (K_m: 61/56μM) and famotidine by r/hOCT2 were markedly lower than that of cimetidine (K_m: 69/73μM). Probenecid was a potent inhibitor of r/OAT3 (K_i: 2.6-5.8μM), whereas it did not interact with OCTs. These results suggest that, in addition to the absence of OCT1 in human kidney, a species difference in the transport activity by hOAT3 and rOat3 accounts, at least in part, for the species difference in the drug-drug interaction between famotidine and probenecid.

Introduction

The kidney plays important roles in the detoxification of xenobiotics and endogenous wastes as well as maintaining the correct balance of ions and nutrients in the body. Urinary excretion is the major detoxification mechanism in the kidney, and this is governed by glomerular filtration, tubular secretion across the proximal tubules and reabsorption. The renal uptake of organic anions and cations on the basolateral membrane of the proximal tubules has been characterized by multispecific organic anion and cation transporters (OAT/*SLC22*, OCT/*SLC22*), respectively (Lee and Kim, 2004; Shitara et al., 2004; Wright and Dantzler, 2004).

Molecular cloning of basolateral transporters from different species allows examination of differences in their substrate specificities and transport activities, leading to a better understanding of the molecular mechanisms of species differences in drug disposition. For OCTs, the isoform expressed in the kidney differs between rodents and humans. Both Oct1 (*Slc22a1*) and Oct2 (*Slc22a2*) are involved in the renal uptake of organic cations on the basolateral membrane of the proximal tubules in rodents, while OCT2 is the predominant isoform in the human kidney (Koepsell, 2004; Lee and Kim, 2004; Wright and Dantzler, 2004). As far as renal organic anion transporters are concerned, two isoforms (Oat1/*Slc22a6* and Oat3/*Slc22a8*) in rodents, and three isoforms (OAT1, OAT2/SLC22A7 and OAT3) in humans have been identified on the basolateral membrane of the proximal tubules (Lee and Kim, 2004; Miyazaki et al., 2004; Wright and Dantzler, 2004; Hasegawa et al., 2002). The mRNA expression level of hOAT3 quantified by real-time PCR, was the highest followed by hOAT1, while that of hOAT2 was quite low (Motohashi et al., 2002). We have previously reported that the transport activities of 9 substrates gave a poor correlation between rat and human OAT3 due to large species difference in the transport activities of estrone sulfate and indoxyl sulfate, while there was a minimal difference in the transport activities of 11 substrates between rat and human OAT1 (Tahara et al., 2005). Oat3/OAT3-mediated uptake may show a species difference, resulting in a difference in the contribution of transporters to the total uptake process, between rats and humans.

Histamine H_2-receptor antagonists have been widely and successfully used in the treatment of peptic ulcers and gastric acid hypertension. H_2-receptor antagonists are mainly eliminated by the kidney as the intact form by tubular secretion as well as glomerular filtration (Lin, 1991). Clinical drug-drug interaction studies in healthy subjects have reported that the renal secretion clearance of famotidine was considerably inhibited by oral coadministration of probenecid and, concomitantly, the plasma concentration of famotidine increased (Inotsume et al., 1990). However, this interaction has not been reproduced in rats even at higher plasma concentrations of probenecid (Lin et al., 1988). Unlike famotidine, probenecid only slightly affects the renal secretion clearance of cimetidine both in humans and rats (ca 20%) (Lin et al., 1988; Gisclon et al., 1989).

H_2-receptor antagonists are weak bases or organic cations at physiological pH, and substrates of organic cation transporters (Grundemann et al., 1999). They have been referred to as bisubstrates that are recognized by both renal organic cation and anion transporters (Ullrich et al., 1993). Indeed, Oat3 accepts H_2 receptor antagonists, such as cimetidine, ranitidine and famotidine, as substrates (Nagata et al., 2004) and cimetidine is a substrate of hOAT3 (Tahara et al., 2005). Although the transport activity is quite low, hOAT1 accepts cimetidine as a substrate whereas rOat1 does not interact with cimetidine (Nagata et al., 2002; Tahara et al., 2005). Both organic cation and anion transporters can be involved in the renal uptake of H_2 receptor antagonists. We hypothesized that the species-dependent effect of probenecid is ascribed to a difference in the contribution of organic anion and cation transporters between rats and humans. The transport activity of the uptake of H_2-receptor antagonists (cimetidine, famotidine and ranitidine) by rat and human organic anion and cation transporters was compared, and the effect of probenecid on the uptake of H_2-receptor antagonists by OCTs and OAT3 was also examined.

Materials and Methods

Materials Famotidine and probenecid were purchased from Nacalai Tesque Inc. (Kyoto, Japan), and cimetidine and ranitidine were purchased from Sigma-Aldrich corporation (St Louis, MO). The purity of famotidine, cimetidine and ranitidine was more than 98%, and that of probenecid was more than 95%. All other chemicals were of analytical grade and commercially available.

Establishment of transfectants and Cell Culture The stable transfectants expressing human OAT1 and OAT3 (Tahara et al., 2005) and OCT (Goralski et al., 2002; Schlatter et al., 2002) were established previously. Construction of hOAT2- and rOat3-stable transfectants was carried out as follows. The full coding region of hOAT2 was amplified from human liver cDNA by reverse transcription-polymerase chain reaction following the reported sequence given by accession number NM 006672. Full-length hOAT2 was subcloned into mammalian expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, CA). The construct of pcDNA3.1 (+) containing rOat3 was established previously (Tahara et al., 2005). The vector constructs of hOAT2 and rOct3 were introduced into parental HEK293 cells by FuGENE6 (Roche Diagnostics) and Lipofect AMINE (Invitrogen) transfection reagent according to the manufacturer's protocol, respectively (hOAT2-HEK and rOat3-HEK). The stably transfected cells were selected by adding G418 sulfate to the culture medium. Two weeks after transfection, different clones were seeded on 12-well culture plates and the transport activity was tested for positive clones. The clone with the highest transport activity was used for the transport studies. hOAT1, hOAT2, hOAT3, hOCT2, rOat3, rOct1, or rOct2 expressing HEK293 cells were grown in D-MEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 400 µg/ml G418 (Invitrogen) at 37°C with 5% CO_2 and 95% humidity on the bottom of a dish and hOAT1-, hOCT2-, hOAT3-, rOat3- and rOct2- expressing cells were seeded in polylysine coated 12-well plates (Becton Dickinson, Franklin Lakes, NJ) at a density of 1.2×10^5 cells/well. Cell culture medium was replaced with culture medium supplemented with 5 mM sodium-butyrate 24 h before transport studies to induce the expression of those proteins.

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Transport Studies Transport studies were carried out as described previously (Tahara et al., 2005). Uptake was initiated by adding medium containing 10 µM of the compounds after cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 min. Probenecid was added to the uptake buffer simultaneously with the H_2-receptor antagonists. The Krebs-Henseleit buffer consisted of 118 mM NaCl, 23.8 mM $NaHCO_3$, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$ adjusted to pH 7.4. The uptake was terminated at a designated time by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml ice-cold Krebs-Henseleit buffer. For determination of the uptake of cimetidine, ranitidine, and famotidine, cells were dissolved in 300 µl 0.2 N NaOH and kept overnight. Aliquots (150 µl) were transferred to vials after adding 30 µl 1 N HCl. Aliquots (100 µl) were used for LC-MS quantification as described below. The remaining 10 µl of the aliquots of cell lysate were used to determine the protein concentration by the method of Lowry with bovine serum albumin as a standard. Ligand uptake was given as the cell-to-medium concentration ratio determined as the amount of ligand associated with cells divided by the medium concentration.

Quantification of H_2 receptor antagonists by LC-MS

The quantification of cimetidine, ranitidine and famotidine was performed by high-performance liquid chromatography (HPLC, Alliance 2690, Waters, Milford, MA, USA) connected to a mass spectrometer (ZQ, Micromass, Manchester, UK) (Nagata et al., 2004). Aliquots (100 µL) of samples containing H_2-receptor antagonists were precipitated by 100 µL methanol containing an internal standard (famotidine for cimetidine and ranitidine, cimetidine for ranitidine), mixed and centrifuged and then 25 µL of the supernatants was injected into the LC-MS. HPLC analysis was performed on a Capseul Pak MG column (3µm, 4.6 mm ID, 75 mm, Shiseido, Tokyo, Japan) at room temperature. Elution was performed with a 0% to 90% linear gradient of 10 mM ammonium acetate-methanol over 4 min at 0.8 ml/min. A portion of the eluent (split ratio = 1:3) was introduced to the MS via an electrospray interface. Detection was performed by selected ionization monitoring in positive ion mode (m/z: 253, m/z: 315, and m/z: 338 for

cimetidine, ranitidine and famotidine, respectively).

Kinetic analyses

Kinetic parameters were obtained using the Michaelis-Menten equation:

$v = V_{max} \times S / (K_m + S)$

where v is the uptake rate of the substrate (pmol/min/mg protein), S is the substrate concentration in the medium (μM), K_m is the Michaelis-Menten constant (μM) and V_{max} is the maximum uptake rate (pmol/min/mg protein). To obtain the kinetic parameters, the equation was fitted to the uptake velocity using a MULTI program (Yamaoka et al., 1981). The input data were weighted as the reciprocals of the observed values and the Damping Gauss Newton Method algorithm was used for fitting. Inhibition constants (K_i) of several compounds were calculated assuming competitive inhibition.

Inhibition constants (K_i) were calculated assuming competitive inhibition using the following equation since the substrate concentration was sufficiently low compared with their K_m values.

$CL_{+inh} = CL / (1 + I/K_i)$

where CL represents the uptake clearance and the subscript (+inh) represents the value in the presence of inhibitor. *I* represents the concentration of inhibitor (μM). The substrate concentration was low compared with the K_m value in the inhibition study. Fitting was performed by the nonlinear least-square method using a MULTI program and the Damping Gauss Newton Method algorithm was used for fitting.

Results

Uptake of the H_2-receptor antagonists by hOAT1-, hOAT2-, hOAT3-HEK and rOat3-HEK

Figures 1 and 2 show the time-profiles and concentration-dependence of the uptake of the H_2 receptor antagonists by hOAT1-, hOAT2-, hOAT3-, rOat-3 and vector-HEK cells respectively. As reported previously (Tahara et al., 2005), the uptake of cimetidine by hOAT1-HEK was slightly greater than that by mock cells (10.2±0.8 versus 6.12±1.71 μl/mg protein at 5 min). In addition, a slight increase was also observed in the uptake of ranitidine by hOAT1-HEK (12.7±1.15 versus 10.2 ± 0.1 μl/mg protein at 5 min), but no specific uptake was observed for famotidine (Figure 1-A). Like hOAT1, the uptake of cimetidine and ranitidine by hOAT2-HEK was significantly greater than that in vector-HEK (1.65 and 6.93 μL/min/mg protein), but no specific uptake was observed for famotidine (Figure 1-B). In contrast, the uptake of the cimetidine, famotidine and ranitidine by hOAT3-HEK was significantly greater than in vector-HEK at all time points (Figure 1-C). The uptake of the H_2 receptor antagonists by rOat3-HEK was also significantly greater than in vector-HEK at all time points (Figure 2-A). Since the uptake of the H_2-receptor antagonists by hOAT3- and rOat3-HEK increased linearly up to 2 and 5 min of incubation, respectively, the uptake of cimetidine and ranitidine by h/rOAT3 at 1 min, famotidine by hOAT3 at 2 min, and that by rOat3 at 5 min were used for further characterization.

The concentration-dependence of the uptake of ranitidine by hOAT2-, cimetidine, famotidine and ranitidine by r/hOAT3-HEK was examined (Figure 1-B, -C and Figure 2-B). The kinetic parameters are summarized in Table 1. The K_m values of ranitidine for hOAT2 and hOAT3 were almost identical, however, the intrinsic transport activity (V_{max}/K_m) by hOAT2 was greater than that by hOAT3. The K_m value of famotidine was almost 3-fold greater for rOat3 than for hOAT3, and those of cimetidine and ranitidine for rat and human OAT3 were similar. The intrinsic transport activities of cimetidine by hOAT3 were 3- and 4-times greater than those of famotidine and ranitidine, whereas that of ranitidine by rOat3 was almost 14- and 2-times greater than that of famotidine and cimetidine, respectively.

Uptake of the H_2-receptor antagonists and TEA by hOCT2-, rOct1- and rOct2-HEK

Figures 3 and 4 show the time-profiles and concentration-dependence of the uptake of the H_2

receptor antagonists by hOCT2-, rOct1- and rOct2- and vector-HEK. The uptake of the H_2 receptor antagonists by hOCT2-, rOct1- and rOct2-HEK was significantly greater than in vector-HEK at all time points although the specific uptake of ranitidine by rOct2 was too low for further characterization (Figure 3-A and 4-A). Since the uptake of the H_2-receptor antagonists by hOCT2-, rOct1- and rOct2-HEK increased linearly up to an incubation time of 2 min (hOCT2) and 3 min (rOcts), respectively, the uptake at 2 min (hOCT2), 1 min (rOct1) and 3 min (rOct2) was used for further characterization. In these OCTs-HEK, the uptake of TEA was significantly greater than in vector-HEK at all time points and the transport activities (μL/min/mg protein) by rOct1-HEK (8.81) and hOCT2-HEK (4.74) were 3.2 and 1.7-fold greater than that by rOct2-HEK (2.75).

The concentration-dependence of the uptake of cimetidine, famotidine and ranitidine by hOCT2-, rOct1- and rOct2-HEK was examined (Figure 3-B and 4-B), and the kinetic parameters were determined by non-linear regression analysis (Table 2). In rat Octs, rOct1 shows similar transport activities for all the H_2 receptor antagonists examined, while rOct2 preferentially transports cimetidine, and the transport activities of famotidine and ranitidine by rOct2 were fairly low compared with that of cimetidine. Ranitidine uptake by rOct2 was 4-fold smaller than that of famotidine. The transport activities of the H_2 receptor antagonists by hOCT2 showed a similar trend, but the transport activity of ranitidine by hOCT2 was similar to that of famotidine. The K_m values of the H_2 receptor antagonists were similar between rat and human OCTs (Table 2). Since the specific uptake of ranitidine by hOCT2 was too low to allow determination of the kinetic parameters, ranitidine inhibition of the uptake of cimetidine and famotidine was investigated. Ranitidine inhibited the hOCT2-mediated transport of cimetidine and famotidine in a concentration-dependent manner with Ki values of 79.0 ± 8.6 and 30.5 ± 8.0μM, respectively (Figure 5-A). Although ranitidine inhibited rOct2-mediated famotidine uptake with a Ki value of 34.8±7.2μM, it had no effect on the rOct2-mediated transport of cimetidine at the concentrations examined (0.1 to 1 mM) (Figure 5-A).

Effect of probenecid on the uptake of H_2 receptor antagonists (cimetidine and famotidine) by r/hOAT3, r/hOCT2 and rOct1-HEK

The inhibitory effect of probenecid on the r/hOAT3-, r/hOCT2- and rOct1-mediated transport of cimetidine and famotidine was examined (Figure 5-B and -C). Probenecid strongly inhibited the r/hOAT3-mediated transport of cimetidine and famotidine in a concentration-dependent manner whereas it had no inhibitory effect on the r/hOCT2 and rOct1-mediated transport of cimetidine and famotidine at the concentrations examined (0.1 to 1 mM). The K_i values of probenecid for cimetidine and famotidine uptake by r/hOAT3-HEK were found to be 5.77±0.97 / 3.37±0.47 μM and 2.55±0.31 / 4.17±1.10 μM, respectively.

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Discussion

In order to obtain an insight into the species-dependent effect of probenecid on the renal clearance of famotidine, the uptake of the H_2 receptor antagonists by basolateral organic ion transporters was examined.

Transport studies using cDNA transfectants revealed that the H_2-receptor antagonists are substrates of r/hOAT3. The intrinsic transport activities (V_{max}/K_m) of the H_2-receptor antagonists by hOAT3 were similar, while famotidine showed a lower intrinsic transport activity by rOat3 than cimetidine and ranitidine (Figures 1 and 2, Table 1). According to our previous characterization, the uptake of cimetidine normalized by benzylpenicillin transport was comparable between rat and human OAT3 (Tahara et al., 2005). In contrast, the relative transport activity of famotidine was 3-fold greater in hOAT3 than rOat3, while that of ranitidine was 8-fold greater in rOat3 than in hOAT3. Since the K_m value of famotidine for hOAT3 was 3-fold smaller than for rOat3, the greater transport activity in hOAT3 is mainly accounted for by the smaller K_m value in hOAT3 (Table 1). Conversely, for ranitidine, the increased V_{max} value in rOat3 will account for the greater transport activity by rOat3 than hOAT3 since the K_m values were similar (Table 1). A significant uptake of cimetidine and ranitidine was observed in hOAT2-HEK. In particular, the intrinsic transport activity of ranitidine by hOAT2 was greater or similar to that of hOAT3 and hOCT2. Considering its low expression in the kidney compared with hOAT1, hOAT3 and hOCT2 (Motohashi et al., 2002), the contribution of hOAT2 to the total renal uptake may be limited. An inhibition study revealed that probenecid is a potent inhibitor of r/hOAT3 (Figure 5). Since the unbound plasma concentrations of probenecid at clinical doses [0.5-2.0 g, range 18-80 μM; (Selen et al., 1982)] were greater than its K_i value for hOAT3, inhibition of hOAT3 by probenecid could be clinically relevant.

The intrinsic transport activities of the H_2 receptor antagonists were compared between rOct1, and r/hOCT2. The K_m values of the H_2 receptor antagonists were similar between rOct1 and rOct2, however, it was found that there was a difference in the intrinsic transport activities. The intrinsic transport activities by rOct1 are similar among the H_2 receptor antagonists, while rOct2 preferentially transports cimetidine (Table 2). The same trend was observed for hOCT2, however, the absolute value of the intrinsic transport activity of ranitidine was greater than that by rOct2 (Table 2). Since the K_i value of

ranitidine for the uptake of famotidine by rOct2 was very close to its K_m value for hOCT2 (Table 2), this is likely accounted for by the difference in the V_{max} values. Interestingly, ranitidine had no effect on the uptake of cimetidine by rOct2 (Figure 5). This observation may be explained by a recent model involving the structure of the substrate binding region of the polyspecific organic cation transporters (Popp et al., 2005). This model suggests that the organic cation transporters contain large binding regions with different binding sites for structurally different cations and explains why two transported cations may not inhibit each other. Motohashi et al could not detect any specific uptake of famotidine by hOCT2 in cRNA injected *Xenopus laevis* oocytes (Motohashi et al., 2004). Furthermore, the IC_{50} value of famotidine for the uptake of TEA by hOCT2 was reported to be 1.8 mM (Motohashi et al., 2004), greater than its own K_m value for hOCT2 determined in this study. This may partly be due to the difference in the method of detection and/or host cells between *Xenopus laevis* oocytes and HEK293 cells. Due to the difference in the detection limit between LC-MS (1nM) and HPLC-UV (200nM) analysis, the substrate concentration used in our and their transport experiments was 10 and 1000μM famotidine, respectively. Taking the K_m value of famotidine for hOCT2 determined in this study into consideration, it is possible that saturation of hOCT2-mediated transport makes it difficult to detect the specific transport of famotidine by hOCT2. However, the discrepancy involving the K_m and IC_{50} values remains to be resolved. Ciarimboli et al (2004) reported that the IC_{50} value of TEA for hOCT1 was different in CHO and HEK293 cells. Since the IC_{50} value was modified by protein kinase activation (Mehrens et al., 2000; Ciarimboli et al., 2004), the host- dependent IC_{50} value might be explained by the difference in the basal regulation of hOCT1. It is possible that such a difference in basal regulation of hOCT2 in *Xenopus-laevis* oocytes and HEK293 cells accounts for the discrepancy in the uptake of famotidine by hOCT2, and its Ki and IC_{50} values.

Boom and Russel et al (1993) demonstrated that the major fraction of cimetidine uptake (approximately 50%) by freshly isolated rat proximal tubular cells was inhibited by TEA, suggesting a major role of OCTs. Probenecid was only a weak inhibitor with an IC_{50} value (700μM) greater than the plasma unbound concentration employed in drug-drug interaction studies (Emanuelsson and Paalzow, 1988; Lin et al., 1988). Indeed, probenecid had a weak effect against OCTs. Therefore, the absence of an inhibitory effect of probenecid on the renal elimination of cimetidine in rodents is rational. Both Oct1

and Oct2 can mediate the renal uptake of the H_2 receptor antagonists in rodent kidney. Their contribution was estimated based on the relative transport activities of the H_2 receptor antagonists with regard to the uptake of TEA to the renal uptake to which Oct1 and Oct2 apparently make identical contributions (Jonker et al., 2003). The normalized uptake data for TEA transport indicated that the V_{max}/K_m ratio (rOct1/rOct2) is 4 for famotidine and 0.4 for cimetidine, suggesting that rOct1 plays a relatively more important role in famotidine uptake than rOct2, and vice versa, as far as cimetidine uptake is concerned. This estimation is in good agreement with a previous report using Oct1 knockout mice in which the knockout of mOct1 resulted in a only a small reduction in cimetidine accumulation in the kidney (Jonker et al., 2001). Lin et al (1988) found that quinine inhibited the renal elimination of famotidine, but did not affect the renal clearance of cimetidine. Since quinine is a more potent inhibitor of rOct1 than rOct2 (6-fold) (Arndt et al., 2001), the difference in the inhibition potency also supports our speculation.

The present findings highlight the importance of OAT3 as the site of drug-drug interactions involving probenecid. It is suggested that the species-dependent effect of probenecid is due to the following two factors : (1) the intrinsic transport activity of famotidine by OAT3 is greater in humans than in rats and (2), unlike rodents, the renal uptake of organic cations in humans is solely accounted for by hOCT2 which preferentially transports cimetidine. These factors increase the contribution of OAT3 to the renal uptake of famotidine in humans. Although there are no clinical implications at present, it is possible that the renal clearance of ranitidine is also probenecid-inhibited in humans. Transport studies using cortical slices as well as isolated proximal tubules from human kidney will support this speculation. Because of the limited availability of human materials for transport studies, animals whose transporters have similar transport properties to human orthologs will be helpful. Rats or mice are not appropriate animal models for this purpose due to the inevitable species difference. Recently, we found that monkey OATs show similar transport properties to human OATs (Tahara et al., 2005). Therefore, the monkey will be a better animal model than rodents for predicting the incidence of drug-drug interactions in humans as far as basolateral uptake is concerned. This should be further confirmed by comparing in vivo pharmacokinetics in monkeys and humans.

In addition to famotidine, inhibition of renal elimination by probenecid has been reported for

benzylpenicillin (Overbosch et al., 1988), cephalosporins (Shitara et al., 2004), oseltamivir (its active metabolite Ro 64-0802) (Hill et al., 2002), furosemide (Vree et al., 1995), bumetanide (Lau et al., 1983), ciprofloxacin (Jaehde et al., 1995), enalapril/enalaprilat (Noormohamed et al., 1990) and fexofenadine (Yasui-Furukori et al., 2005). Among these drugs, benzylpenicillin (Tahara et al., 2005), cephalosporins (Jung et al., 2002; Uwai et al., 2002), oseltamivir (its active metabolite Ro 64-0802) (Hill et al., 2002), furosemide and bumetanide (Hasannejad et al., 2004) have been reported to be substrates of OAT1 and/or OAT3. Thus, these interactions are likely to involve inhibition of basolateral uptake in the kidney. Further studies are necessary to determine the importance of OAT1 and/or OAT3 in these drug-drug interactions. In addition to the kidney, Nagata et al (2004) found that probenecid, given by an intravenous constant infusion, increased the cerebrospinal fluid concentrations of H_2 receptor antagonists (also given by an intravenous constant infusion) by inhibiting OAT3-mediated efflux at the choroid plexus (Nagata et al., 2004). In humans, probenecid will synergetically increase the accumulation of OAT3 substrates in the cerebrospinal fluid by inhibiting renal elimination as well as efflux transport at the choroid plexus.

In conclusion, the results of the present study suggest that the species difference in the drug-drug interaction between famotidine and probenecid may be ascribed to the difference in transport activity of famotidine between rat and human OAT3 and the absence of OCT1 in the human kidney.

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Legends to figures

Figure 1. Time-profile and concentration-dependence of the uptake of H2-receptor antagonists by hOAT1-, hOAT2- and hOAT3-HEK

The time-dependent uptake of cimetidine (CMD), famotidine (FMD) and ranitidine (RND) (10 μM) by hOAT1-, hOAT2- and hOAT3-HEK was examined at 37°C. Closed and open circles represent the uptake by OATs-HEK and vector-HEK, respectively (A). The concentration-dependence of OAT-mediated cimetidine, famotidine and ranitidine uptake is shown as Eadie-Hofstee plots (B and C). The hOAT3-mediated uptake of cimetidine for 1 min, famotidine for 2 min, ranitidine for 1 min and hOAT2 mediated uptake of ranitidine for 3 min were determined at various concentrations (3 to 1000 μmol/L, range of concentration used). The hOAT-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in hOATs-HEK. Each point represents the mean ± S.E. (n=3). Statistical differences in the uptake of h/rOAT3-HEK were compared with vector-HEK by a two-tailed unpaired *t*-test with $p<0.05$ as the limit of significance (*$p<0.05$; **$p<0.01$). Each point represents the mean ± S.E. (n=3).

Figure 2. Time-profile and concentration-dependence of the uptake of H2-receptor antagonists by rOat3-HEK

The time-dependent uptake of cimetidine (CMD), famotidine (FMD) and ranitidine (RND) (10 μM) by rOat3-HEK was examined at 37°C. Closed and open circles represent the uptake by rOat3-HEK and vector-HEK, respectively (A). The concentration-dependence of rOat3-mediated cimetidine, famotidine and ranitidine uptake is shown as Eadie-Hofstee plots (B). The concentration-dependence of rOAT3-mediated cimetidine, famotidine and ranitidine uptake is shown as Eadie-Hofstee plots. The rOAT3-mediated uptake of cimetidine for 1 min, famotidine for 5 min, and ranitidine for 1 min was determined at various concentrations (3 to 1000 μmol/L, range of

concentration used). The rOAT3-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in rOAT3-HEK. Each point represents the mean ± S.E. (n=3).

Figure 3. Time-profile and concentration-dependence of the uptake of H2-receptor antagonists by hOCT2-HEK

The time-dependent uptake of cimetidine (CMD), famotidine (FMD) and ranitidine (RND) (10 μM) by hOCT2-HEK was examined at 37°C. Closed and open circles represent the uptake by hOCT2-HEK and vector-HEK, respectively (A). The concentration-dependence of hOCT2-mediated cimetidine, famotidine and ranitidine uptake is shown as Eadie-Hofstee plots (B). The hOCT2-mediated uptake of cimetidine for 1 min, famotidine for 2 min, and ranitidine for 1 min was determined at various concentrations (3 to 1000 μmol/L, range of concentration used). The hOCT2-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in rOAT3-HEK. Each point represents the mean ± S.E. (n=3). Statistical differences in the uptake of hOCT2-HEK were compared with vector-HEK by a two-tailed unpaired *t*-test with $p<0.05$ as the limit of significance (*$p<0.05$; **$p<0.01$). Each point represents the mean ± S.E. (n=3).

Figure 4. Time-profile and concentration-dependence of the uptake of H2-receptor antagonists by rOct1- and rOct2-HEK

The time-dependent uptake of cimetidine (CMD), famotidinen (FMD) and ranitidine (RND) (10 μM) by rOct1- and rOct2-HEK was examined at 37°C. Closed and open circles represent the uptake by rOcts-HEK and vector-HEK, respectively (A). The concentration-dependence of rOct1- and rOct2-mediated cimetidine, famotidine and ranitidine uptake is shown as Eadie-Hofstee plots (B). The uptake of cimetidine by rOct1 and rOct2 for 1 min, that of famotidine by rOct1, 2 for 3 min, and that of ranitidine by rOct1 for 1 min, was determined at various concentrations (3 to 1000

μmol/L, range of concentration used). The rOct1- and rOct2-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in rOct1- and rOct2-HEK. Each point represents the mean ± SE. (n=3).

Figure 5. Inhibitory effect of ranitidine and probenecid on the uptake of cimetidine and famotidine by r/hOAT3-, r/hOCT2- and rOct1-HEK.

The uptake of cimetidine (CMD) and famotidine (FMD) (10 μM) by h/rOCT2 for 1 min was determined in the absence or presence of ranitidine (RND) at the designated concentrations. Closed and open circles represent the uptake by hOCT2 and rOct2, respectively (A). The uptake of cimetidine and famotidine (10 μM) by hOCT2 and hOAT3 for 1 min was determined in the absence or presence of probenecid at the designated concentrations. Closed and open circles represent the uptake by hOCT2 and hOAT3, respectively (B). The uptake of cimetidine (10 μM) by rOat3, rOct1 and rOct2 for 1 min, that of famotidine by rOat3, rOct1 and rOct2 for 5, 1 and 3 min was determined in the absence or presence of probenecid at the designated concentrations. Open circles, triangles and closed circles represent the uptake by rOat3, rOct1 and rOct2, respectively (C). The values were expressed as a percentage of cimetidine or famotidine transport by OAT3- or OCTs-HEK in the presence of inhibitors *versus* that in the absence of inhibitors. Each point represents the mean ± SE. (n=3).

Table 1. Kinetic parameters of the uptake of H_2-receptor antagonists by organic anion transporters

K_m and V_{max} were determined by nonlinear regression analysis as described under *Experimental Procedures*. Data are taken from Figures 1 and 2. Each value represents the mean ±computer-calculated S.D. The value in parenthesis represents the relative transport activity with regard to cimetidine transport.

Isoform	Cimetidine			Famotidine			Ranitidine		
	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein
hOAT2	n.d.	n.d.	1.65 (1)	-	-	-	396±41	4320±358	10.9 (6.61)
hOAT3	149±35	1470±230	9.86 (1)	124±4	448±10	3.61 (0.37)	234±49	551±49	2.35 (0.24)
rOat3	90.7±4.8	512±17	5.64 (1)	345±22	252±12	0.73 (0.13)	155±9	1660±63	10.7 (1.90)

n.d. not determined

Table 2. Kinetic parameters of the uptake of H_2-receptor antagonists by organic cation transporters

K_m and V_{max} were determined by nonlinear regression analysis as described under *Experimental procedures.* Data are taken from Figures 3 and 4. Each value represents the mean ±computer-calculated S.D. The value in parenthesis represents the relative transport activity with regard to cimetidine transport.

Isoform	Cimetidine			Famotidine			Ranitidine		
	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein	K_m μmol/L	V_{max} pmol/min/mg of protein	V_{max}/K_m μL/min/mg of protein
hOCT2	72.6±13.9	2170±240	29.9 (1)	56.1±4.0	204±8	3.63 (0.12)	65.2±8.1	265±20	4.06 (0.14)
rOct1	71.5±10.7	2210±190	30.9 (1)	86.7±6.2	2020±90	23.3 (0.75)	39.2±1.9	1050±30	26.8 (0.87)
rOct2	68.8±8.0	1490±100	21.7 (1)	60.6±4.1	117±4	1.93 (0.09)	n.d.	n.d.	0.50 (0.02)

n.d: not determined

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Figure 1

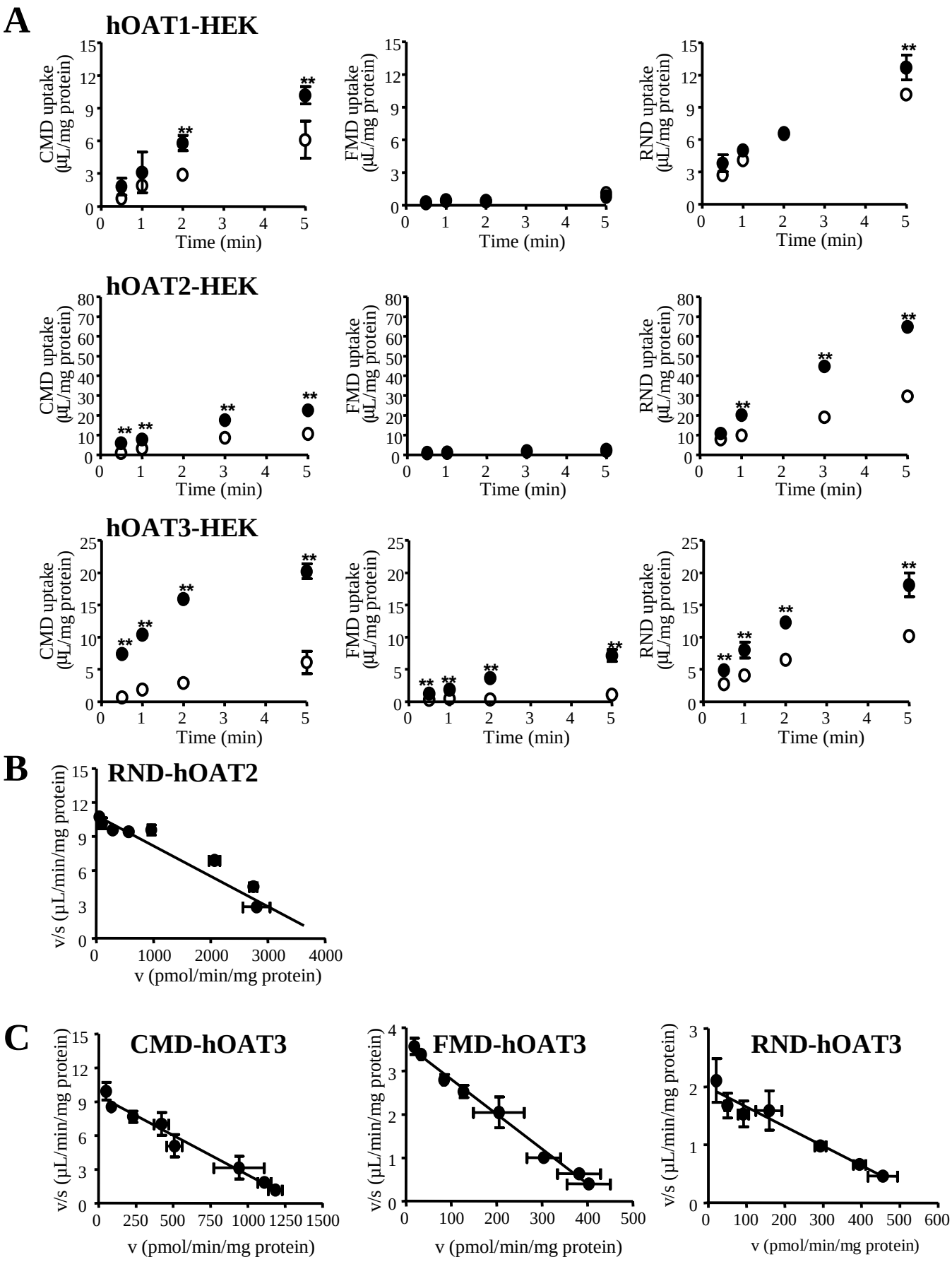

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Figure 2

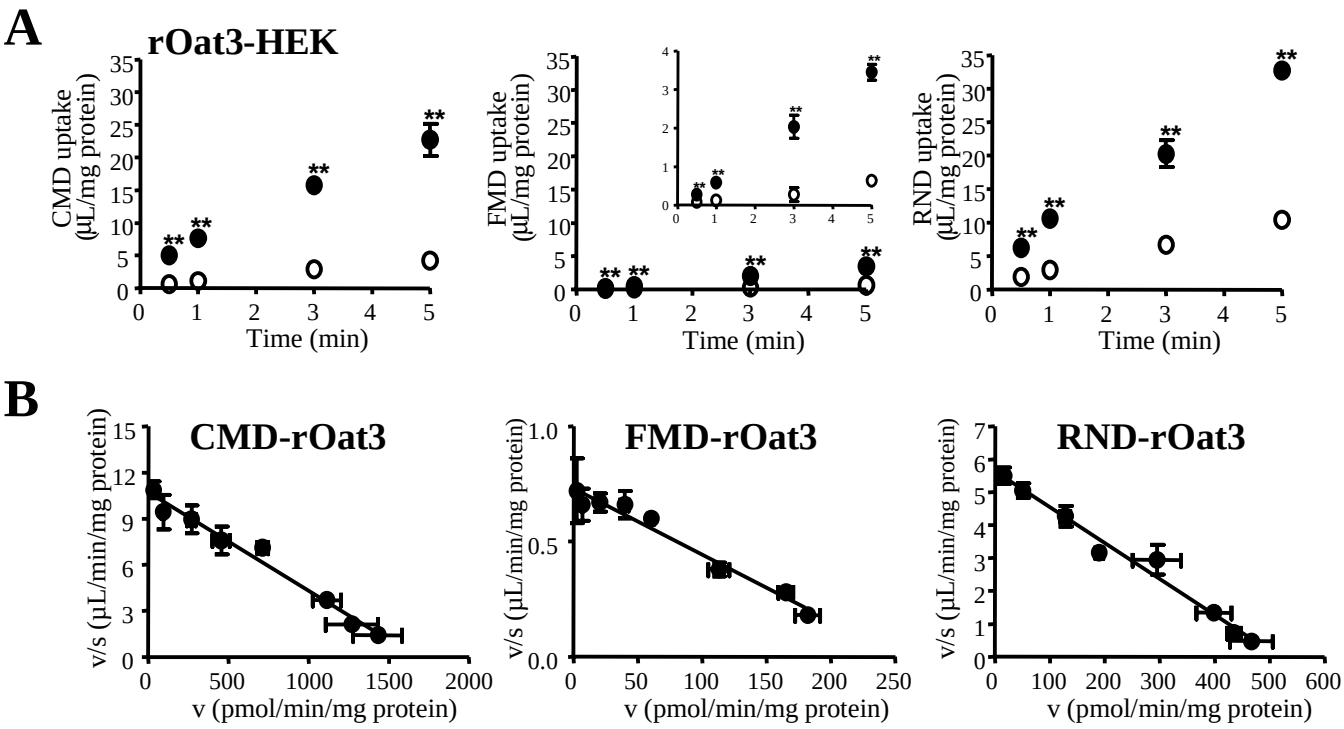

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Figure 3

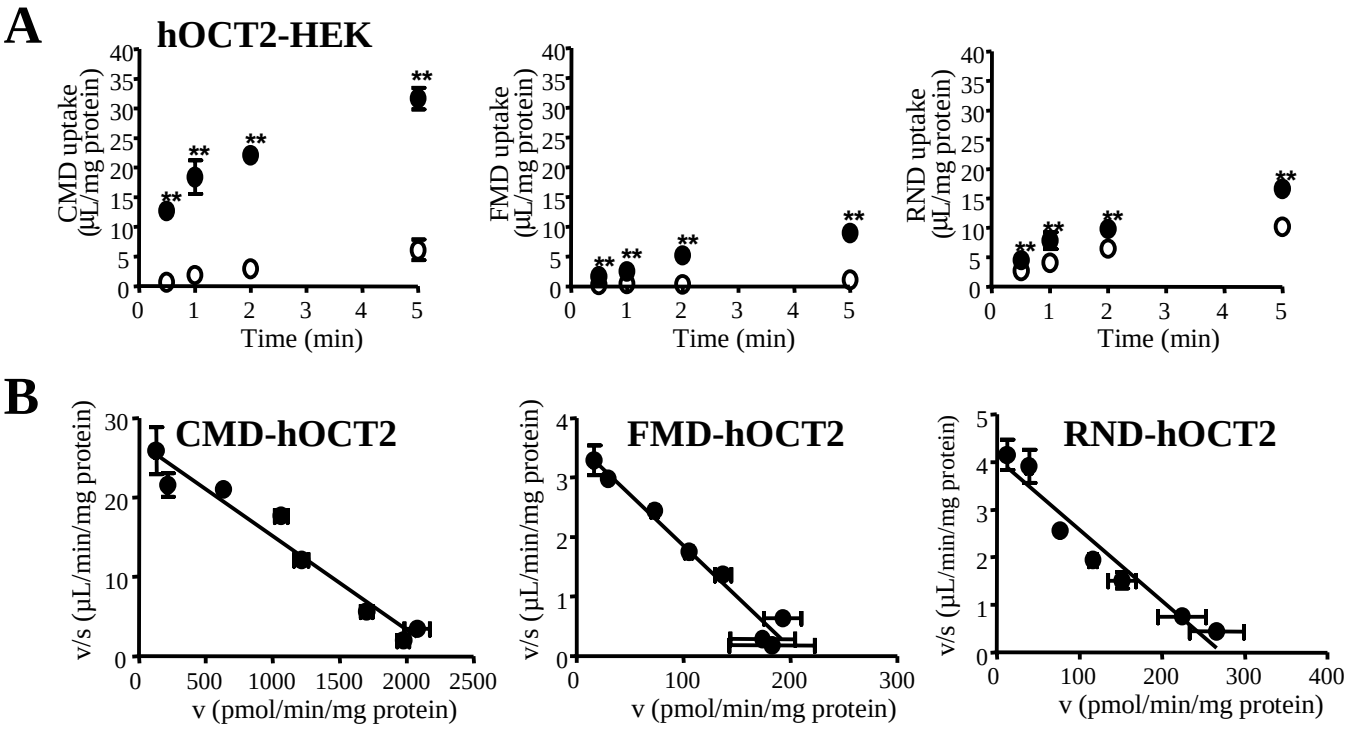

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Figure 4

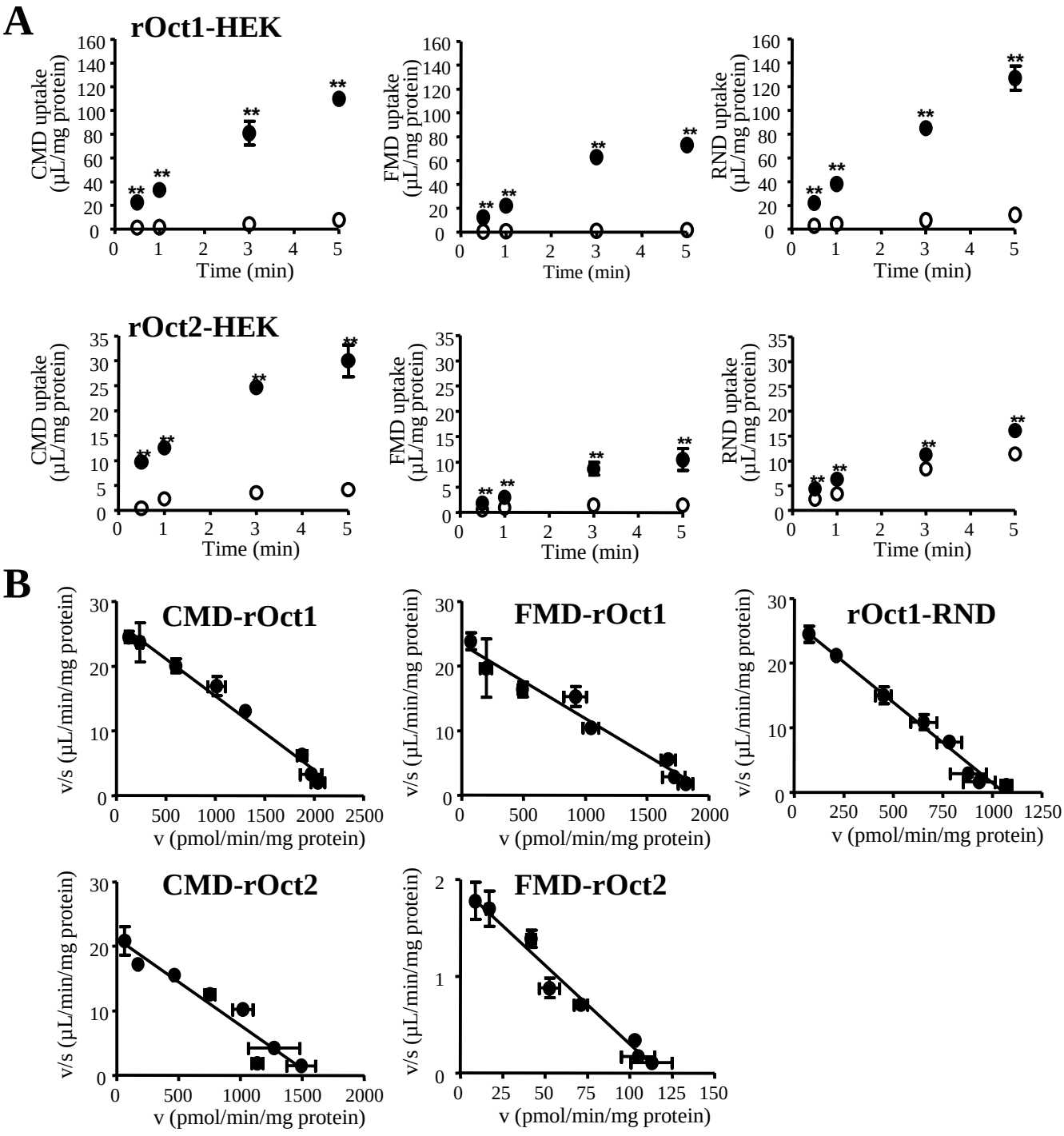

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Figure 5