Renal Secretion of the Antiviral Nucleoside Analog AM188 Is Inhibited by Probenecid, *p*-Aminohippuric Acid, and Cimetidine in the Isolated Perfused Rat Kidney

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Purpose. To investigate the effects of potential inhibitors of membrane transport on the tubular secretion of AM188, an antiviral guanosine analog, in the isolated perfused rat kidney (IPK).

Methods. AM188 was administered to the IPK perfusate as a bolus/infusion regimen. In inhibitor groups, probenecid, *p*-aminohippuric acid (PAH), cimetidine, or nitrobenzylthioinosine was added to the perfusing medium.

Results. In control IPKs, the ratio of renal clearance of AM188 (CL_R) to GFR was 7.7 ± 0.51 (mean ± SD). The CL_R/GFR ratio for AM188 was 6.20 ± 0.41*, 2.85 ± 0.20*, 1.45 ± 0.07*, and 0.80 ± 0.01* when the concentration of probenecid in perfusate was 10, 50, 100, and 1000 μM, respectively (*$p < 0.05$ compared to control group); the ratio was 7.71 ± 0.38, 6.02 ± 0.42*, 1.71 ± 0.15*, and 0.91 ± 0.07* for the PAH group and 6.42 ± 1.70*, 5.33 ± 1.53*, 3.16 ± 0.81*, and 1.21 ± 0.20* for the cimetidine group when the concentrations were 10, 100, 1000 and 10,000 μM, respectively; and the ratio was 5.33 ± 0.21* when the concentration of nitrobenzylthioinosine was 5 μM.

Conclusions. These results suggest that renal tubular secretion of AM188 involves organic anion and cation transport systems.

KEY WORDS: AM188; antiviral nucleoside analog; isolated perfused rat kidney; organic anion transporter; renal secretion.

INTRODUCTION

AM188 is a new antiviral guanosine analog (Fig. 1) that undergoes extensive renal excretion in humans and animals. The compound is subject to substantial net tubular secretion in the isolated perfused rat kidney with a relatively constant renal clearance (CL_R) to glomerular filtration rate (GFR) ratio of about 6 at perfusate concentrations from 1 to 25 μg/ml; saturation of renal excretion was observed at perfusate concentrations above 25 μg/ml (1). Extensive tubular secretion leads to relatively high concentrations of AM188 in tubular cells and tubular urine, which could potentially result in nephrotoxicity, as has been observed with acyclovir and ganciclovir (2,3). In addition, co-administration of compounds that compete for carrier-mediated transport systems involved in tubular secretion could lead to clinically important drug interactions. However, the cell membrane transport systems involved in the renal excretion of AM188 are currently unknown.

Possessing both acidic and basic properties [pK_{a1} = 9.40, pK_{a2} = 3.41, calculated by Advanced Chemistry Development (ACD) Software Solaris V4.67], AM188 exists as anionic, cationic, and neutral forms in aqueous solution at physiological pH (7.4). Therefore, the renal tubular secretion of the compound may involve multiple transport systems, including those responsible for the excretion of organic anions and organic cations. The organic anion transporter (the *p*-aminohippuric acid/dicarboxylate exchanger), which is involved in the cellular uptake of *p*-aminohippuric acid (PAH) and other organic anions against an electrochemical gradient, is located on the basolateral membrane of tubular cells (4). The acidic drug, probenecid, has been widely used as a prototypic inhibitor of the PAH transporter when investigating transport systems in the renal tubule (5,6). In the case of organic cations, a transporter is located on the basolateral membrane and involves an electrochemical gradient (7), while another is on the brush border membrane and works as a H^+/cation antiporter through which organic cations are extruded from the tubule cell into the tubular lumen (8). Both of these cation systems are inhibited by cimetidine, a typical cation transport inhibitor (9). Nucleoside transport systems in the kidney comprise equilibrative and concentrative transporters. An equilibrative transporter is located in both the basolateral and brush border membrane, whereas a concentrative transporter appears to be located on the brush border membrane (10). The equilibrative transporter can be inhibited by micromolar concentrations of nitrobenzylthioinosine (NBMPR) (11).

Because any one or more of the aforementioned transporters may be involved in the renal tubular handling of AM188, the following prototypic transport inhibitors were selected for investigation of their effects on its renal disposition: probenecid and PAH, as organic anions; cimetidine, as an organic cation; and NBMPR, as a nucleoside analog. The current study was designed to investigate the effect of these prototypic transport inhibitors on the disposition of AM188 in the isolated perfused rat kidney (IPK) to explore the mechanisms involved in its renal tubular handling. In addition, an understanding of the cell-membrane transport systems involved in the renal secretion of AM188 may provide a means to reduce the concentration of AM188 in tubular cells and tubular urine by inhibition of the relevant systems, thereby decreasing the potential for nephrotoxicity. Furthermore, because probenecid, cimetidine, and several nucleoside analogs are also clinically important drugs, the study would allow an assessment of the likelihood of interactions with AM188 in the clinical setting.

MATERIALS AND METHODS

Materials

AM188 was obtained from Amrad Corporation Limited (Victoria, Australia), and ^{3}H-AM188, in a solution of ethanol and water (1:1), 1 mCi/ml, 3.8 Ci/mmol, with a radiochemical

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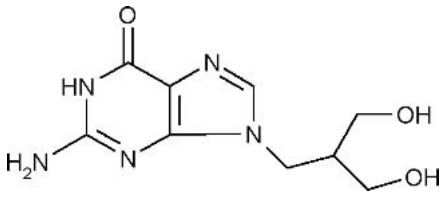

Fig. 1. Structure of a new antiviral guanosine analog, AM188.

purity of 97.6%, was obtained from Moravek Biochemicals (Brea, CA, USA). Probenecid, PAH, cimetidine, NBMPR, dextran (MW = 60,000-90,000), L-cysteine, glycine, L-glutamic acid, and mannitol were purchased from Sigma Chemical Co. (St Louis, MO, USA), and Fraction V bovine serum albumin (BSA) was from ICN Biomedicals Inc. (Aurora, OH, USA). [^{14}C-Carboxyl]-inulin was purchased from NEN products (Boston, MA, USA). Aqueous counting scintillant (ACS), NCS-II tissue solubilizer and BCS-NA organic counting scintillant were purchased from Amersham (Arlington Heights, IL, USA) and D-glucose monohydrate of analytical grade was obtained from AJAX Chemicals (Auburn, NSW, Australia). All other chemicals were of analytical grade or equivalent.

Isolated Perfused Rat Kidney Model

The study was approved by the Institute of Medical and Veterinary Science Animal Ethics Committee. Male Sprague-Dawley rats (320–400 g) from the Gilles Plains Animal Resource Center (Adelaide, Australia) were maintained at approximately 21°C on a 12 h light/dark cycle with free access to food and water. The IPK preparation was based on methods described previously (12).

The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing BSA (6.5 g/L), dextran (36 g/L), D-glucose (5 mmol/L), L-cysteine (0.5 mmol/L), glycine (2.3 mmol/L) and L-glutamic acid (0.5 mmol/L). Prior to inclusion in the perfusion medium, BSA was purified by dialyzing against three exchanges of protein-free buffer over 3 days at 4°C, as described by Shanahan *et al.* (12). The perfusion medium was filtered successively through 1.2- and 0.45-μm filters (Millipore, Bedford, MA, USA) prior to use and was equilibrated with a mixture of 95% O_2 and 5% CO_2 for 1 h before and throughout the perfusion.

Rats were anesthetized by an intraperitoneal injection of sodium pentobarbitone (60 mg/kg). A midline laparotomy incision was made from pelvis to sternum in the animal. Next, the right ureter was ligated immediately proximal to the bladder. A solution of mannitol (150 mg) and heparin (100 U) in 1 ml normal saline was injected into the penile vein. The right ureter was then cannulated by the introduction of a cannula consisting of a 200-mm length of tubing (o.d. 0.61 mm, i.d. 0.28 mm, Paton Scientific, Victor Harbour, Australia). The area around the anastomosis of the superior mesenteric artery, renal artery, and aorta was cleared of connective tissue and loose ligatures placed around the superior mesenteric artery and renal artery. After removing the capsule of the kidney, a right-angled glass cannula was inserted into the superior mesenteric artery via a small incision and passed proximally along the superior mesenteric artery, across the aorta, and into the renal artery where it was tied in place. Perfusate flow was commenced immediately. The cannulated kidney was then excised from the body of the rat and suspended within a thermostatically controlled cabinet at 34–37°C. The recirculating perfusion medium (160 ml) was pumped at 25–45 ml/min, which maintained perfusion pressure at 100 ± 20 mmHg. The functional viability of each kidney was assessed by the glomerular filtration rate (GFR), which was determined as the renal clearance of [^{14}C]-inulin and the percentage tubular reabsorption of water, glucose, and sodium. The function of a kidney was considered acceptable if GFR was greater than 0.5 ml/min and the percentage tubular reabsorption of water, glucose, and sodium was greater than 60, 90, and 70%, respectively.

Experimental Design and Sample Collection

After successful cannulation of the renal artery, the kidney was perfused with blank (drug-free) perfusate for an equilibration period of approximately 5 min. Following the initial 5-min equilibration period, 5 μCi [^{14}C]-inulin, 0.8 μCi [^{3}H]-AM188, and 0.16 mg of unlabeled AM188 were added to the perfusion medium (160 ml) as a bolus dose. The bolus dose was followed by a constant-rate infusion at a rate of 1.5 μCi/h for [^{3}H]-AM188 and 5 μg/min for unlabeled AM188. This dosage regimen was selected, from preliminary experiments, to achieve nominal perfusate concentrations of 5 nCi/ml for [^{3}H]-AM188 and 1 μg/ml (4.2 μM) for unlabeled AM188. A total of 25 perfusions were performed, five perfusions for each experimental group. Each experimental group (n = 5) was perfused with AM188 alone or in the presence of increasing concentrations of either PAH, probenecid, cimetidine, or NBMPR. Each perfusion consisted of five 20-min periods. The first period started 10 min after adding the bolus dose of AM188 to the reservoir. The potential inhibitors were added as bolus doses at 30, 50, 70, and 90 min to achieve low, medium, high, and very-high concentrations, respectively, according to Table I. A 5-min equilibration was allowed after the addition of each dose of inhibitor. Urine samples were collected during three 5-min intervals for each experimental period, as shown in Table I. Urine volume was measured gravimetrically in preweighed collection vials. Perfusate samples (0.8 ml) were collected from the reservoir at the midpoint of each urine collection interval. Aliquots of perfusate (100 μl) and urine (50 μl) were taken into scintillation vials immediately after the samples were collected. At the end of perfusion, the perfused kidney was removed from the perfusion system, and the left kidney (unperfused) was excised from the rat carcass. The perfusate or blood on the kidney surface was absorbed by tissue towel. The kidneys and the remaining perfusate and urine samples were stored at –20°C.

Analytical Methods

The concentrations of [^{3}H]-AM188 and [^{14}C]-inulin in perfusate and urine were determined by mixing 100 μl perfusate or 50 μl urine with 3 ml aqueous counting scintillant. Samples were subjected to differential radiochemical analysis for [^{3}H] and [^{14}C] using a liquid scintillation analyser (Packard Model 2200CA, Canberra, Australia).

The content of [^{3}H]-AM188 in the perfused kidney tissue (3 cross sections from each kidney weighing about 150 mg) and in the perfusate (150 μl) collected at the end of perfusion was determined after digestion over about 16 h with NCS-II

Table I. Experimental Groups (n = 5 in Each Group) for 25 Isolated Perfused Rat Kidneys Administered AM188 at 0 min, and the Concentrations of Each Potential Inhibitor (Probenecid, PAH, Cimetidine, and NBMPR) in Perfusate After Addition of the Low Dose at 30 min, Medium Dose at 50 min, the High Dose at 70 min, and Very High Dose at 90 min

Treatment group	Concentration of potential inhibitor (μM)				
AM 188 (4.2 μM) in each group (n = 5)	Period 1 (10–30 min) No inhibitor	Period 2 (30–50 min) Low dose	Period 3 (50–70 min) Medium dose	Period 4 (70–90 min) High dose	Period 5 (90–110 min) Very high dose
Control	0	0	0	0	0
Probenecid	0	10	50	100	1000
PAH	0	10	100	1000	10000
Cimetidine	0	10	100	1000	10000
NBMPR	0	0.005	0.05	0.5	5
Urine collection intervals (min)	15–20 20–25 25–30	35–40 40–45 45–50	55–60 60–65 65–70	75–80 80–85 85–90	95–100 100–105 105–110

PAH, *p*-aminohippuric acid; NBMPR, nitrobenzylthioinosine.

tissue solubilizer and calculated and expressed as DPM per gram of kidney tissue or per milliliter of perfusate.

Glucose concentrations in perfusate and urine were determined by the glucose oxidase method using a diagnostic glucose kit (Sigma Chemical Co., procedure no. 510-A). Sodium concentrations in perfusate and urine samples were determined using atomic absorbance flame spectrophotometry (Varian Techtron, model no. AA6, Melbourne, Australia).

Pharmacokinetic and Statistical Analyses

The renal excretory clearance (CL_R) of AM188 in each 5-min urine collection interval was determined as the rate of excretion into urine divided by the perfusate concentration of AM188 at the midpoint of the urine collection interval, according to Eq. 1:

$$CL_R = ([\text{Urine}] \times \text{UFR})/[\text{Perfusate}] \quad (1)$$

where [Urine] and [Perfusate] are the concentrations of analyte in the urine and perfusate, respectively, and UFR is the urine flow rate. The renal excretory clearance of [^{14}C]-inulin, glucose, and sodium was determined in an analogous manner.

The ratio of the CL_R of AM188 to GFR (CL_R/GFR) was calculated for each 5-min urine collection interval, and the mean of the ratios from the three intervals in each period was used as an index of the extent of renal tubular secretion of AM188. The ratio was not corrected for unbound fraction of AM188 in perfusate because this fraction was approximately equal to unity (0.98~0.99) (1).

For all IPK experiments, the percentage tubular reabsorption (%TR) of water, glucose and sodium was calculated using Eq. 2:

$$\%TR = (1 - X/GFR) \times 100 \quad (2)$$

where X is either the UFR for water or renal clearance for glucose and sodium.

The kidney tissue-to-perfusate concentration ratio ($K_{T/P}$) was taken to be the concentration of [^{3}H]-AM188 per gram of tissue, relative to the concentration in perfusate (per milliliter) collected at the end of each experiment. The urine-to-kidney tissue concentration ratio ($K_{U/T}$) at the end of perfusion was also calculated.

For each IPK, the value for a parameter within a period was calculated as the average for the three 5-min intervals. Group data are presented as mean ± SD. For each period, parameters across the treatment groups were compared using ANOVA, with Dunnett's test used for post-hoc comparison of inhibitor groups against the data for the control group in the respective period. Where indicated, a one-sample *t* test was used to determine if CL_R/GFR was different from unity.

RESULTS

The functional viability of the IPKs, as assessed by GFR and the %TR of water, glucose and sodium, is presented in Figure 2. Each parameter was within acceptable limits and remained relatively constant with time.

The regimen for administration of AM188 comprising a bolus dose and constant-rate infusion into the perfusate reservoir produced relatively stable AM188 concentrations in perfusate during the five experimental periods (15–110 min) for the control and NBMPR groups. However, the infusion regimen produced progressively increasing AM188 concentrations in the probenecid, PAH and cimetidine groups (Fig. 3).

In the control group, the CL_R/GFR ratio for AM188 was substantially greater than unity (mean 7.7) during the five perfusion periods, over 110 min (Fig. 4). The CL_R/GFR ratio was also uniformly high (mean 7.2) in period 1 in the five groups when no potential inhibitor was present in perfusate in any of the groups. The addition of probenecid to the perfusion medium to achieve increasing concentrations across periods 2 to 5 resulted in a progressive decrease in the CL_R/GFR ratio. In each of periods 2 to 5, the excretory ratio for AM188 in the presence of probenecid was significantly lower ($p < 0.01$) than observed for the control group (Fig. 4). For the PAH group, the ratio for AM188 was significantly lower ($p < 0.001$) than for the control group across periods 3 to 5. As with probenecid, the excretory ratio for AM188 was significantly lower ($p < 0.05$) in the presence of cimetidine compared with the corresponding data in the control group for each of periods 2 to 5 (Fig. 4). For the NBMPR group, there was only a modest effect on the CL_R/GFR ratio for AM188 with the ratio being lower than for the control group in period 5 only ($p < 0.001$).

The distribution of AM188 between kidney tissue and perfusate ($K_{T/P}$) at the end of perfusion is shown in Fig. 5A.

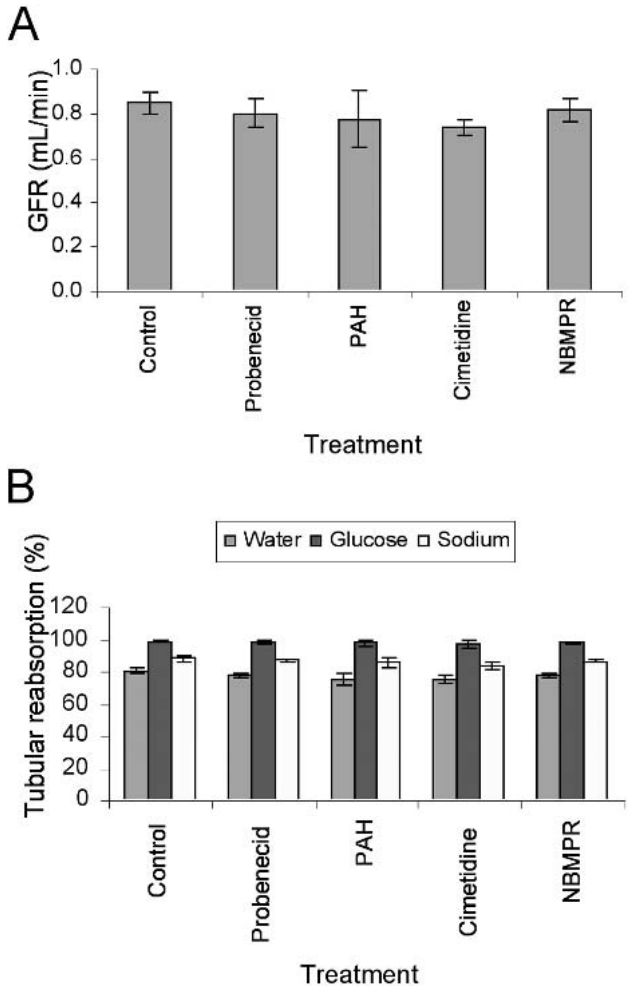

Fig. 2. Functional parameters, (A) GFR and (B) percentage tubular reabsorption of water, glucose, and sodium for the rat isolated perfused kidney in the five treatment groups. Data are presented as the mean ± SD.

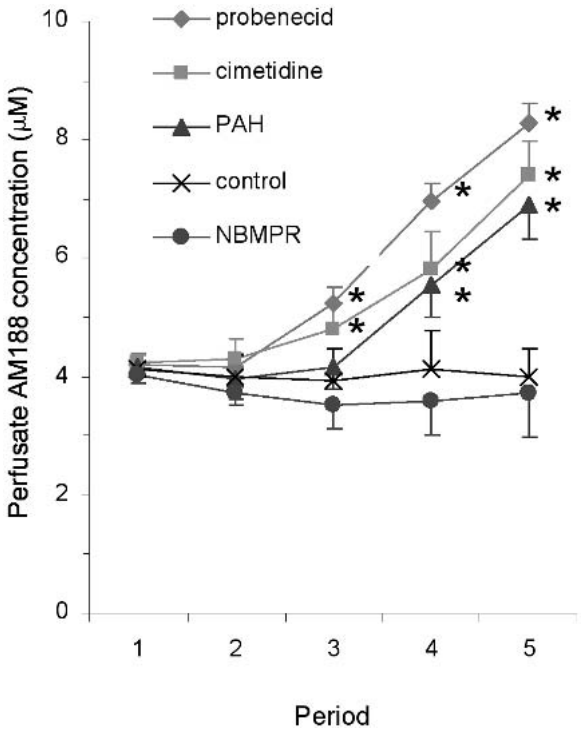

Fig. 3. AM188 concentration in perfusate in the five periods (period 1, 15–30 min; period 2, 35–50 min; period 3, 55–70 min; period 4, 75–90 min; period 5, 95–110 min) in the five experimental groups (control; probenecid; PAH; cimetidine; NBMPR). Data are shown as mean ± SD ($n = 5$ in each group). *$p < 0.05$ as compared with AM188 concentration in the same period of the control group.

In the control group, the distribution ratio was substantially higher than unity with a value of 18.6 ± 3.2. The distribution ratio was significantly lower ($p < 0.001$) in the probenecid, PAH, and cimetidine groups compared with the control group. The urine-to-kidney tissue concentration ratios for AM188 ($K_{U/T}$) at the end of perfusion are shown in Fig. 5B. The ratios were significantly lower in the probenecid ($p < 0.05$), cimetidine ($p < 0.001$), and NBMPR ($p < 0.01$) groups compared with the control group.

DISCUSSION

Consistent with our previous findings (1), the current study demonstrated that the nucleoside analog AM188 undergoes extensive net tubular secretion in the isolated perfused kidney of the rat. The CL_R/GFR ratio when the nucleoside was present alone in perfusate was approximately seven (Fig. 4), a value similar to that observed in a phase 1 study in humans (Cox and Nation, unpublished data). Though the plasma concentrations required to elicit an antiviral effect in humans remain to be determined, the initial perfusate AM188 concentration of 1 μg/ml used in the current study is comparable to the concentration required for *in vitro* anti-hepatitis B virus effects (EC_{90} 0.455 μg/ml) (13). Several other nucleoside analogs have also been shown to undergo substantial net tubular secretion. These include acyclovir (6), penciclovir (14), ganciclovir (2), zidovudine (5,15), and lamivudine (16). Interestingly, in the current study, the CL_R/GFR ratios for AM188 were statistically smaller than unity in the presence of the very-high concentrations of probenecid and PAH, which blocked the secretion of the nucleoside analog, suggesting the possibility of a modest component of reabsorption in the renal handling of AM188.

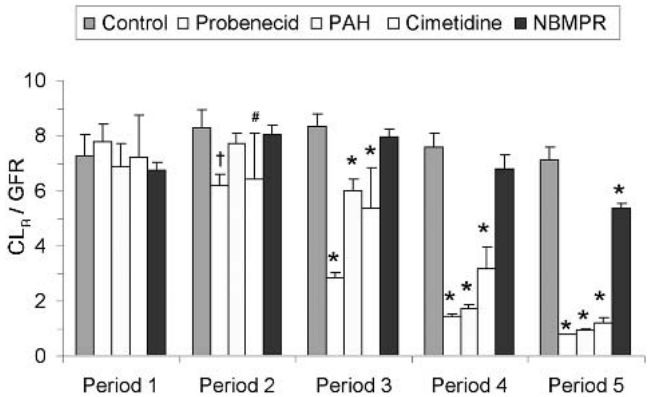

Fig. 4. CL_R/GFR ratio for AM188 in the five perfusion periods in the five treatment groups. The inhibitor concentration in each period is contained in Table I. #$p < 0.05$, †$p < 0.01$, *$p < 0.001$ compared with the value for the control group within the same period.

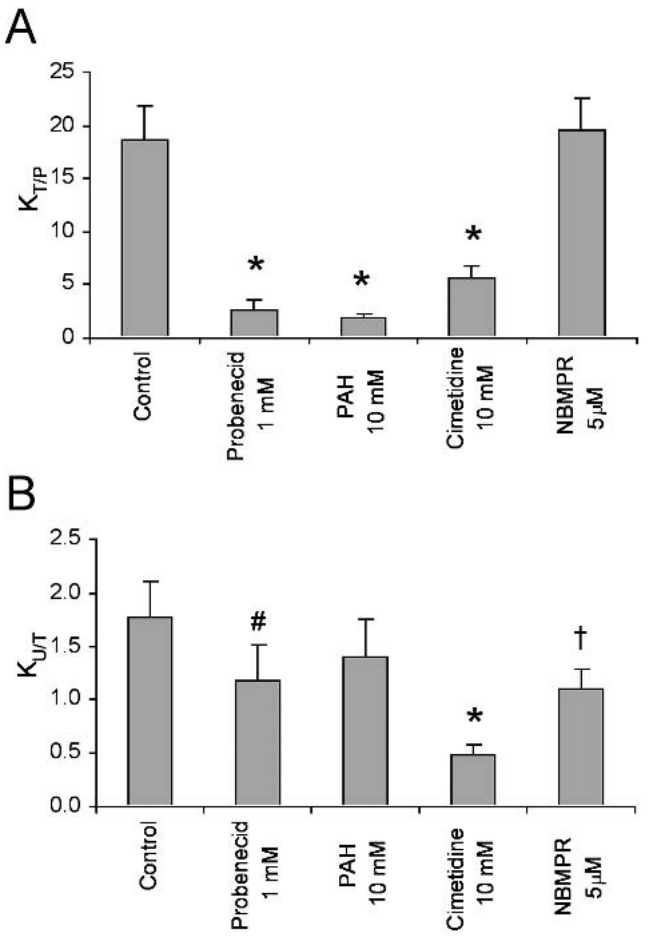

Fig. 5. Kidney tissue-to-perfusate concentration ratio ($K_{T/P}$, A) and urine-to-kidney tissue concentration ratio ($K_{U/T}$, B) for AM188 in the five treatment groups. The inhibitor concentrations shown in the figure were those used in period 5 (90–110 min). #$p < 0.05$, †$p < 0.01$, *$p < 0.001$ compared with control group.

In the control group of the current study, the distribution ratio for AM188 between kidney tissue and perfusate at the end of perfusion ($K_{T/P}$) was substantially greater than unity (approximately 18, Fig. 5A), whereas the mean $K_{U/T}$ ratio was approximately 1.8 (Fig. 5B). Because the tubular reabsorption of water would have served to elevate the experimentally determined $K_{U/T}$ ratios, the concentration ratio between tubular urine and cells is likely to have been lower than 1.8. Thus, from the relative magnitudes of $K_{T/P}$ and $K_{U/T}$ in the control group it is apparent that the transport of AM188 across the basolateral membrane is a highly concentrative step in comparison with the movement from cell to urine across the brush border membrane. The $K_{T/P}$ value of approximately 18 observed in the control group may be due to either tissue binding/partitioning or entrapment of AM188 within tubular cells. AM188 does not bind to BSA (1) or to macromolecules in human plasma (Cox and Nation, unpublished data) and it has very low lipophilicity ($logD_7 = -1.44$, calculated by ACD Software Solaris V4.67). Thus, it is unlikely that the very high $K_{T/P}$ value for AM188 was the result of binding to cellular macromolecules or partitioning into lipid components of tubular cells. The tissue-to-perfusate concentration ratio is consistent with intracellular entrapment of AM188 arising from cellular efflux being less efficient than cellular uptake. It is apparent, therefore, that the efflux of AM188 across the brush border membrane is the rate-limiting step in the overall net secretion of the drug.

The perfusate AM188 concentration increased in the presence of probenecid, PAH and cimetidine (Fig. 3) because AM188 renal excretion was decreased by these inhibitors. The elevated concentrations (the highest concentration was 8.74 μM, Fig. 3) would not have induced saturation of AM188 renal excretion, as our previous study indicated that saturation did not occur until the perfusate AM188 concentration exceeded 25 μg/ml (105 μM) (1).

Probenecid, PAH, and cimetidine reduced the CL_R/GFR ratio for AM188 in a concentration-dependent manner (Fig. 4), and all three of the transport inhibitors caused a decrease in the $K_{T/P}$ ratio for AM188 (Fig. 5A). It was notable that the pattern of reduction in the $K_{T/P}$ ratios across the inhibitor groups, relative to the control group, mirrored the reduction in CL_R/GFR in period 5 (Fig. 4). Based on the decreases in CL_R/GFR and $K_{T/P}$ among the three inhibitors, the relative potencies of the compounds were: probenecid > PAH > cimetidine (Figs. 4 and 5A). For example, the mean CL_R/GFR ratios were approximately 0.8, 1.7, and 3.2 in the presence of 1 mM probenecid, PAH, and cimetidine, respectively, and $K_{T/P}$ values were 2.0 and 5.7 in the presence of 10 mM PAH and cimetidine, and 2.7 in the presence of 1 mM probenecid. These findings suggest that the anionic compounds probenecid and PAH blocked the renal secretion of AM188 (and reduced its tissue uptake) more strongly than the cation transport inhibitor, indicating that AM188 may be principally transported by organic anion transporters at the basolateral membrane.

As noted earlier, AM188 possesses both weak acidic and weak basic characteristics with pK_a values of 9.40 and 3.41, respectively. Therefore, in aqueous solution at physiological pH (7.4), AM188 would exist predominantly as a neutral unionized molecule with small proportions in anionic (approximately 1%) and cationic (approximately 0.01%) forms, in an analogous manner to acyclovir (13,17). Therefore, it is possible that AM188 interacts with, and is primarily transported by, organic anion transport systems. Hence, it is not unexpected that the renal tubular secretion for AM188 was inhibited strongly by classical organic anion transport inhibitors—probenecid and PAH. The impact of cimetidine on the CL_R/GFR ratio for AM188 suggests that it may rely partially on an organic cation transporter or, as discussed below, that cimetidine inhibited the secretion of AM188 via an organic anion transport system.

The $K_{T/P}$ values for AM188 in the probenecid and PAH groups were reduced by more than 80% when compared to the value for the control group (Fig. 5A). In contrast, the $K_{U/T}$ values for the probenecid and PAH groups were only slightly reduced (Fig. 5B), suggesting that the transport of AM188 was principally blocked at the basolateral membrane. This is consistent with the fact that the classical PAH transporter (equivalent to the cloned transporter OAT1 and rOAT1) was reported to be present on the basolateral membrane (18). As noted above, probenecid was a more potent inhibitor of the renal secretion of AM188 possibly because probenecid not only has a higher affinity than PAH for the organic anion transporter (19), but is also an inhibitor for organic cation transport (20).

Cimetidine has been shown to act as a substrate and inhibitor for both the facilitated cation transport system at the basolateral membrane (9) and the electroneutral H^+/organic cation antiport system at the brush border membrane of rat renal tubule cells (20). In the current study, cimetidine (0.01–10 mM) significantly inhibited the tubular secretion of AM188. At the very-high concentration (10 mM), cimetidine reduced both $K_{T/P}$ and $K_{U/T}$ to approximately one third of the corresponding values for the control group (Fig. 5), suggesting that cimetidine altered AM188 transport at both the basolateral membrane and brush border membrane. Studies have shown that cimetidine can be transported not only by several cloned organic cation transporters, such as rat organic cation transporter1 (rOCT1), rat organic cation transporter 2 (rOCT2), human organic cation transporter 2 (hOCT2), and human organic cation transporter 3 (hOCT3) (9,21), but also by cloned organic anion transporters, including flounder renal organic anion transporter (fROAT), rat organic anion transporter 3 (rOAT3), human organic anion transporter 1 (hOAT1), and human organic anion transporter 3 (hOAT3) (22). Cimetidine inhibited the uptake of organic anions by human and rat OAT3, and a K_i of 46.8 μM was determined for rOAT3 (23). Because cimetidine can act as a bisubstrate, it could conceivably inhibit both the organic cation and anion transporters (24). Accordingly, the cimetidine-mediated inhibition of AM188 secretion in this study might have been mediated via an interaction with organic anion and cation transporters located within the basolateral and brush border membrane domains of kidney tubular cells.

Our results are consistent with those of others, who have demonstrated that acyclovir, ganciclovir, and zidovudine were transported by both cation and anion transport systems. For example, it was reported that acyclovir renal clearance is 3-fold greater than the glomerular filtration rate in healthy human subjects and that co-administration with probenecid or cimetidine could decrease acyclovir renal clearance (6). In rats, the renal secretion of zidovudine was mediated by organic anion and cation transporters (5), and probenecid and cimetidine inhibited zidovudine transport in rat renal basolateral membrane vesicles (15). The cloned hOAT1 and hOCT1 were shown to mediate acyclovir and ganciclovir uptake, whereas rOCT1, rOAT1, rOAT2, hOAT1, hOAT2, hOAT3, and hOAT4 mediated zidovudine uptake (25). Acyclovir, zalcitabine, didanosine, lamivudine, stavudine, and trifluridine are transported via rOAT1 (26); and lamivudine was also transported by rOCT1 (27).

It has been suggested that concentrative nucleoside transporters are located within the brush border membrane and actively transport endogenous nucleosides from lumenal urine into tubule cells as one step of the overall reabsorption of endogenous nucleosides (10,11). Therefore, the concentrative nucleoside transporter is unlikely to play a part in the renal secretion of nucleoside analogs such as AM188. The equilibrative nucleoside transporters appear to be located on both the brush border and basolateral membrane domains and mediate both influx and efflux transport of nucleosides (10,28). Because the equilibrative sensitive (es) nucleoside transporter is sensitive to nanomolar concentrations of NBMPR, whereas the equilibrative insensitive (ei) nucleoside transporter is inhibited by micromolar concentrations of NBMPR (10), the perfusate NBMPR concentrations in the current study spanned the range of 5 nM to 5 μM. Perfusate concentrations of NBMPR in the range 5–500 nM had no effect on the renal secretion of AM188, suggesting that the es nucleoside transporter was not involved in the secretion process. The fact that NBMPR at 5 μM modestly reduced CL_R/GFR suggests a potential role for an ei nucleoside transporter. In mice, NBMPR reduced the tubular secretion of the nucleoside analogs deoxyadenosine and 5′-deoxy-5′-fluorouridine (29). The nucleoside analog lamivudine also has been shown to undergo extensive renal secretion in the isolated perfused rat kidney (30) and in rat *in vivo* (16). It was recently reported that in rat renal brush border membrane vesicles, the pH–driven uptake of lamivudine was inhibited by 10 μM of NBMPR (31). In the current study, the $K_{T/P}$ for AM188 was not reduced, but the $K_{U/T}$ for AM188 was significantly lower in the presence of 5 μM of NBMPR, suggesting that NBMPR at 5 μM interfered with AM188 transport across the brush border membrane. The effect of NBMPR on $K_{U/T}$ was similar to that of cimetidine in the current study, suggesting that NBMPR could interact with the H^+/organic cation antiporter on the brush border membrane, as also indicated by Takubo *et al.* (31). However, at this stage, we cannot definitely exclude the possibility that NBMPR could also inhibit an ei nucleoside transporter associated with the brush border membrane.

In conclusion, the high renal excretory ratio and kidney tissue-to-perfusate distribution ratio in the control group indicates that AM188 is substantially taken up by kidney tissue and undergoes extensive tubular secretion. The relative magnitudes of the tissue:perfusate and urine:tissue ratios indicates that the major concentrative step in the overall secretion process occurs at the basolateral membrane. The renal tubular secretion of AM188 is inhibited by both the organic anions probenecid and PAH and by the organic cation cimetidine, whereas NBMPR caused a modest inhibition at only the highest concentration tested. Probenecid, PAH, and cimetidine decreased not only the excretion of AM188, but also its distribution ratio between kidney tissue and perfusate, suggesting that these inhibitors may reduce potential renal tubular cell accumulation of AM188. The concurrent and similar percentage reductions in CL_R/GFR and $K_{T/P}$ in the probenecid, PAH, and cimetidine groups suggests that the AM188 transporters that are inhibited are those (probably both anion and cation systems) located on the basolateral membrane of the renal tubular cells. At the brush border membrane, the transport of AM188 possibly involves a nucleoside and/or cation transport system.

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