Sulfate Homeostasis. IV. Probenecid-Induced Alterations of Inorganic Sulfate in Rats

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Received June 5, 1990; accepted September 10, 1990

Homeostasis of inorganic sulfate is maintained by the capacity-limited renal reabsorption of sulfate in the proximal tubule. The purpose of the present investigation was to determine if probenecid, the classical inhibitor of renal organic anion secretion, may affect sulfate renal clearance. Two groups of rats were administered in a randomized crossover design, an i.v. bolus dose (20.6 or 92.4 mg/kg) and 4-hr infusion (0.28 or 0.59 mg/min/kg) of probenecid or vehicle, and blood and urine samples were collected. At a steady-state serum concentration of 0.45 m*M*, probenecid had no significant effect on the serum concentrations or renal clearance of inorganic sulfate, whereas at a serum concentration of 1.4 m*M*, probenecid treatment caused a significant decrease in serum sulfate concentrations (0.57 ± 0.11 vs 0.96 ± 0.19 m*M* in controls, mean ± SD, $n = 6$, $P < 0.001$) due to an increase in the renal clearance of sulfate (3.88 ± 1.18 vs 2.13 ± 0.84 ml/min/kg in controls, $P < 0.01$). The fraction of the filtered sulfate that was reabsorbed was significantly decreased (0.38 ± 0.23, vs 0.74 ± 0.09 in controls, $P < 0.01$). Therefore, probenecid treatment results in the inhibition of the renal reabsorption of inorganic sulfate in rats *in vivo*.

KEY WORDS: probenecid; sulfate; renal clearance.

INTRODUCTION

Inorganic sulfate is an important physiological anion that is involved in the conjugation of exogenous compounds (such as acetaminophen, isoproterenol, and α-methyldopa) and many endogenous compounds (such as glycosaminoglycans, cerebrosides, steroids, and catecholamines) (1). As such, inorganic sulfate is important in both detoxification and biosynthetic metabolic pathways. The renal clearance of inorganic sulfate in man and animals (dogs, rats) is approximately 10–35% of the glomerular filtration rate (GFR) under normal physiological conditions and increases to a rate approximately equal to the GFR when serum sulfate concentrations are increased (2–4), suggesting saturable reabsorption and little, if any, tubular secretion. Sulfate reabsorption occurs predominantly in the proximal tubule (5) and the luminal uptake in rats and rabbits has been demonstrated to be sodium-dependent and electroneutral both in brush border membrane vesicles (6) and in intact tubules (7). There is also active transport across the contraluminal membrane by carrier-mediated anion exchange (7–9).

Probenecid, the classical inhibitor of the active renal tubular secretion of organic anions (10), has been reported to inhibit (7–9,11) or have no effect on (12,13) sulfate renal transport. In rat renal basolateral membrane vesicles, probenecid (at a concentration of 5 m*M*) inhibits bicarbonate-, sodium-, and pH-driven inorganic sulfate transport, as well as the trans-stimulation of sulfate transport by inorganic and organic anions (8,9). Further, in isolated proximal tubules probenecid inhibits sulfate (11) and thiosulfate secretion (7); thiosulfate is a competitive inhibitor of both renal luminal and contraluminal transport of sulfate, which suggests that thiosulfate shares common renal transport systems with sulfate (6,7). However, in rat kidney cortex slices probenecid, at concentrations of 10 m*M*, causes no inhibition of sulfate transport (12). In addition, probenecid, at concentrations of 0.53 or 0.68 m*M*, does not affect the secretion of thiosulfate in the dog *in vivo* (13).

The purpose of the present investigation was to examine the influence of probenecid, at two different serum concentrations, on the renal clearance of inorganic sulfate in rats. The lower plasma concentration of probenecid examined in this investigation (0.45 m*M*) is approximately equivalent to the maximum plasma concentration obtained in humans after the administration of 2 g of probenecid (14,15). Due to the paucity of information in the literature on the effect of probenecid on electrolyte disposition, we additionally examined the serum concentrations and renal clearance of sodium, potassium, magnesium, calcium, and inorganic phosphorus.

MATERIALS AND METHODS

Chemicals. Probenecid (*p*[dipropylsulfamoyl]benzoic acid) was purchased as anhydrous crystals from Sigma Chemical Co. (St. Louis, MO), and sulfamethazine from ICN Pharmaceuticals Inc. (Cleveland, OH). All other reagents and solvents were obtained from Baker Chemical Co. (Phillipsburg, NJ).

Experimental. Female Lewis rats weighing between 172 and 206 g (Charles River, Wilmington, MA) were used in the low- and high-dose probenecid studies. The rats had right jugular vein and bladder cannulas implanted under light ether anesthesia 1 day prior to the first study day. For the bladder cannulation, PE-50 tubing (Clay Adams, Parsippany, NJ) was inserted into the bladder and exteriorized at the back of the neck. A randomized crossover study design was used, with all studies performed over the same time period during the day. In the first study rats received either an i.v. bolus of probenecid, 20.6 mg/kg (as 5.02 mg/ml in dilute NaOH solution, pH 8.7), over a period of 1 min followed by a constant-rate infusion of approximately 0.28 mg/min/kg (as a 2.61-mg/ml solution) for 4 hr, or a bolus dose andinfusion of the same volume of the vehicle. This dosage regimen was designed to achieve a steady-state serum concentration of about 100 μg/ml (0.35 m*M*) based on a mean clearance (Cl) of 0.17 liter/hr/kg and a volume of distribution at steady state (V_{ss}) of 0.21 liter/kg. These pharmacokinetic parameters were determined in a preliminary study in two rats which were administered 50 mg/kg i.v. probenecid. In the second study, steady-state serum concentrations of approximately 425 μg/ml (1.5 m*M*) were desired, and the dosage regimen was based on the pharmacokinetic parameters for a 100-mg/kg dose (16). Rats received either an i.v. bolus dose of probenecid, 92.4 mg/kg (as a 10-mg/ml solution in

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0724-8741/91/0300-0376$06.50/0

dilute NaOH, pH 7.7), followed by a constant rate infusion of 0.59 mg/min/kg (as a 5.8-mg/ml solution) for 4 hr, or a bolus dose and infusion of the same volume of the vehicle. Blood samples were collected via the right jugular vein at 2 and 4 hr (0.15 ml in low-dose and 0.25 ml in high-dose study) and at 3 hr (0.6 ml in low-dose and 0.75 ml in high-dose study). Urine samples were collected between 2 and 4 hr. Three 1-ml aliquots of distilled water were used to slowly flush the bladder at the beginning and end of the urine collection period. Preliminary studies demonstrated no contamination of blood samples with infusion solution when sampling from the right jugular vein after flushing the cannula with 1 ml of saline, as opposed to sampling from the femoral vein. The crossover leg for each study was carried out 5 days (low-dose study) or 4 days (high-dose study) later.

An additional study was carried out in five rats to examine the influence of the 0.1 *N* NaOH solution, pH 8.7 (the vehicle used in the low-dose probenecid study), on systemic and urinary pH. Right jugular vein and carotid artery cannulas were implanted in rats under light ether anesthesia on the day prior to the first study day. Using a randomized crossover study design rats received either the NaOH solution or a NaCl solution containing equimolar concentrations of sodium. The solutions were administered as a bolus injection via the jugular vein followed by an infusion over 4 hr using the same volumes of the vehicle as in the low-dose probenecid study. Arterial blood pH was monitored using a pH/blood gas analyzer (Micro 13, Instrumentation Laboratory, Lexington, MA).

Analytical. Serum samples were analyzed for probenecid by HPLC using a modification of a previously described assay (17). Inorganic sulfate concentrations in serum and urine were determined by single-column anion chromatography (18). The intraday coefficients of variation of the assay at a serum concentration of 0.79 m*M* or urine concentrations of 1.2 and 2.7 m*M* are 4.1% (n = 6), 2.0% (n = 10), and 1.6%, respectively. Serum and urine concentrations of magnesium, calcium, and potassium were determined by atomic absorption spectrophotometry (Model 603, Perkin-Elmer, Norwalk, CT). Concentrations of creatinine, uric acid, and phosphorus were determined using commercially available kits (kit numbers 555A, 685, and 670, respectively, Sigma Chemical Co.).

Data Analysis. Initial pharmacokinetic parameters were calculated from the preliminary study data using noncompartmental techniques. Cl was calculated as dose divided by AUC. Since probenecid exhibits nonlinear kinetics (16), V_{ss} was calculated by dose/C_o, where C_o is the plasma concentration at time 0 (19).

For the infusion studies, renal clearances of electrolytes and creatinine were calculated as the urinary excretion rate (from 2 to 4 hr) divided by the midpoint serum concentration (3 hr). Since sulfate in serum is not bound to serum proteins (3) and therefore is completely ultrafiltrable, the renal filtration rate of inorganic sulfate was calculated as the product of serum sulfate concentration and creatinine renal clearance (GFR). The renal tubular reabsorption rate was estimated as the difference between the renal filtration and the urinary excretion rates, assuming negligible renal tubular secretion of sulfate (2). The fraction of filtered sulfate that was reabsorbed (fraction reabsorbed) was calculated as the reabsorption rate divided by the filtration rate.

Data for each infusion study were analyzed using paired t tests, with $P < 0.05$ regarded as significant. All results are expressed as mean ± SD.

RESULTS

Following the low-dose probenecid infusion, animals obtained mean steady-state serum concentrations of 127 ± 19 μg/ml (mean ± SD). Probenecid administration did not significantly alter the serum concentrations, renal clearance, or fraction reabsorbed of inorganic sulfate. Additionally, there were no alterations in the disposition of creatinine, uric acid, potassium, magnesium, calcium, of phosphorus. The vehicle administered to the control animals in this low-dose probenecid study consisted of 0.1 *N* NaOH solution, pH 8.7. The possibility of alterations in sulfate clearance in the control animals, due to systemic pH changes following the infusion of the drug vehicle, was examined in a further study. pH, pO_2, pCO_2, and bicarbonate concentrations remained constant throughout the vehicle infusion and did not differ from the saline control (data not presented).

Administration of the high-dose probenecid infusion produced serum concentrations at 2, 3, and 4 hr of 363 ± 34, 394 ± 34, and 398 ± 30 μg/ml. Serum sulfate concentrations were significantly decreased, the renal clearance significantly increased, and the fraction of the filtered load that was reabsorbed significantly decreased (0.38 ± 0.23 vs 0.74 ± 0.09 in controls, $P < 0.01$) (Fig. 1, Table I). At these higher serum concentrations, probenecid produced significant decreases in the urinary excretion rate and renal clearance of creatinine (Table I). In additional studies, it was shown that these changes do not reflect an alteration in GFR (as reflected by inulin clearance) but, instead, are due to the inhibition of the renal tubular secretion of creatinine (I. M. Darling and M. E. Morris, submitted). The change in creatinine clearance noted in the probenecid-treated animals would influence the determination of the fraction reabsorbed: however, assuming that there was no change in GFR between the control and the treated periods (i.e., the creatinine clearance value for each animal's control period can be used to calculate the fraction reabsorbed during the treatment period), the fraction reabsorbed following probenecid treatment is still significantly decreased compared with control values (0.51 ± 0.17, $P < 0.02$ compared with the control

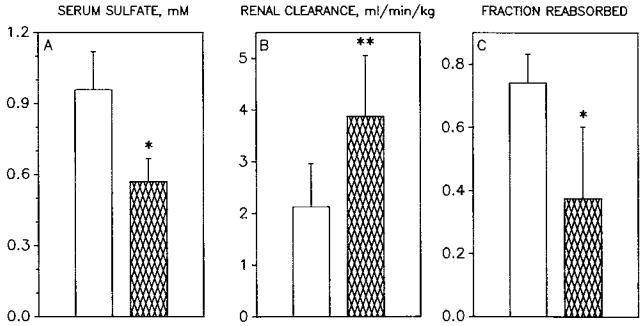

Fig. 1. Effect of the high probenecid dose on (A) serum sulfate, (B) sulfate renal clearance, and (C) fraction reabsorbed. Data are presented as the mean values for control (□) and probenecid-treated (▨) animals with the error bars representing standard deviations; n = 6 for all groups. (*) $P < 0.01$; (**) $P < 0.001$.

Table I. Effect of Probenecid (C_{ss} of 1.4 m*M*) on Creatinine and Various Electrolytes[a]

	Control	Treated	Significance
Creatinine			
Serum conc., mg/dl	0.40 ± 0.02	0.36 ± 0.06	NS
Excretion rate, mg/min ($\times 10^{-3}$)	5.96 ± 2.12	4.31 ± 2.39	$P < 0.002$
Clearance, ml/min/kg	8.38 ± 3.13	6.45 ± 2.79	$P < 0.02$
Inorganic sulfate			
Serum conc., m*M*	0.96 ± 0.19	0.57 ± 0.11	$P < 0.001$
Excretion rate, mmol/min ($\times 10^{-4}$)	3.69 ± 1.72	4.00 ± 1.48	NS
Clearance, ml/min/kg	2.13 ± 0.84	3.88 ± 1.18	$P < 0.01$
Potassium			
Serum conc., mEq/L	3.62 ± 0.11	3.00 ± 0.30	$P < 0.01$
Excretion rate, mEq/min ($\times 10^{-3}$)	1.50 ± 0.78	1.07 ± 0.53	$P < 0.05$
Clearance, ml/min/kg	2.31 ± 1.18	1.97 ± 0.98	NS
Sodium			
Serum conc., mEq/L	146 ± 7	132 ± 8	$P < 0.05$
Excretion rate, mEq/min ($\times 10^{-3}$)	5.44 ± 1.47	3.91 ± 1.25	NS
Clearance, ml/min/kg	0.21 ± 0.05	0.17 ± 0.06	NS

[a] Results expressed as mean ± SD; $n = 6$. No alterations were observed in the serum concentration, urinary excretion rates or renal clearance values for either uric acid, magnesium, calcium, or inorganic phosphorus.

values). Significant decrements in the serum concentrations of sodium and potassium and in the urinary excretion rate of potassium were also noted (Table I). There were no changes in the disposition of uric acid, magnesium, calcium, or inorganic phosphorus.

The variability in serum sulfate concentrations observed in control animals in the high- and low-dose studies in this investigation is similar to the variability encountered in our laboratory when analyzing serum concentrations in different shipments of Lewis female rats. The timing of the studies relative to when the animals were shipped may also contribute to the differences in serum sulfate concentrations.

DISCUSSION

Probenecid inhibited the renal transport of inorganic sulfate *in vivo* in rats at the higher probenecid serum concentration (~1.4 m*M*) in this investigation. Our results suggest that decreased serum sulfate concentrations occur as a consequence of the increased renal clearance and decreased reabsorption of sulfate. If the primary effect of probenecid were to decrease the serum concentrations of sulfate, then one would expect a significant decrease in sulfate renal clearance due to its concentration-dependent renal reabsorption. Such a decrease in sulfate renal clearance, following the lowering of serum sulfate concentrations due to the administration of acetaminophen, has been demonstrated in both rats (4) and humans (20).

The probenecid-induced inhibition of the renal reabsorption of sulfate *in vivo* agrees with the *in vitro* findings that probenecid at high concentrations can inhibit the transport of sulfate in basolateral membrane vesicles (8,9) and can inhibit the transport of sulfate in intact renal proximal tubules (11). The *in vitro* interaction between probenecid and the sulfate transporter in the contraluminal membrane appears to be weak, with a K_i in isolated tubule preparations of approximately 7.5 m*M* (11). In the present *in vivo* investigation, only the free (unbound) probenecid would be subject to renal secretion. Assuming a free fraction of about 38% at this serum probenecid concentration in rats (16), the free concentration of probenecid would be 0.53 m*M*, which is much lower than the estimated K_i in isolated tubules. Therefore, although an interaction between probenecid and sulfate at the basolateral membrane appears likely, there may be other mechanisms involved in this interaction. The effect of probenecid on sulfate transport at the brush border membrane has not been examined, so an additional interaction at this site remains possible. Also, it is possible that the putative inhibitor of sulfate renal transport *in vivo* may not be probenecid itself but, instead, a probenecid metabolite or, alternatively, an endogenous substrate which is present at higher concentrations in plasma or in the renal epithelial cell due to a probenecid-induced inhibition of this substrate's renal transport.

The renal interaction between probenecid and inorganic sulfate is most likely of little clinical significance. Following a 2-g dose of probenecid to humans, peak concentrations of approximately 150 μg/ml (0.53 m*M*) are attained clinically (14,15). This corresponds to serum concentrations obtained in the low-probenecid dose study, where no significant alterations in sulfate homeostasis were observed. The finding of such an interaction between probenecid and sulfate suggests that other organic anions may also influence the renal clearance of sulfate. *In vitro* investigations have demonstrated that some sulfate conjugates and various sulfonate and hydroxybenzoate compounds can inhibit the contraluminal membrane transport of sulfate (21,22). Furosemide and 3-sulfamoyl-4-phenoxybenzoate inhibit sulfate renal transport at low concentrations in isolated tubule preparations (mean K_i's of 0.87 and 0.78 m*M*, respectively) (11). In *in vivo* studies, the administration of salicylic acid (23,24) and various nonsteroidal anti-inflammatory drugs (all of which are organic anionic compounds) (25) produce lowered serum sulfate concentrations. Decreased sulfate availability has clinically significant consequences: it can limit the sulfate conjugation of both xenobiotics and endogenous sub-

strates (25–27). Inorganic sulfate is an important substrate not only for detoxification of exogenous and endogenous compounds but also for reactions essential for normal growth and development; these reactions include the formation of sulfated glycosaminoglycans in connective tissue (28) and sulfated tyrosine residues of a number of enzymes and proteins such as cholecystokinin and gastrin (29).

Additional findings in this study were alterations in creatinine clearance and decreased serum concentrations of sodium and potassium following probenecid treatment. Although probenecid has uricosuric action in humans (10), no alterations in uric acid disposition were noted after either the low- or the high-dose probenecid treatment. The decreased urinary excretion and clearance of creatinine have been found, in subsequent studies in our laboratory, to reflect a probenecid-induced inhibition of the active tubular secretion of creatinine. Probenecid had no effect on GFR as assessed by inulin clearance (I. M. Darling and M. E. Morris, submitted).

In summary, probenecid increases the renal clearance of inorganic sulfate, which consequently results in lower serum sulfate concentrations. No alteration in sulfate homeostasis was observed at the low dose of probenecid used in this investigation; since the serum concentrations achieved following this low dose correspond to probenecid concentrations obtained clinically, the therapeutic importance of this interaction may be minimal. However, this renal interaction may occur with other organic anions subject to renal tubular secretion, thereby resulting in a decreased availability of inorganic sulfate for metabolic biotransformation reactions.

ACKNOWLEDGMENTS

This work was supported by a Merck Faculty Development Grant, a Pharmaceutical Manufacturers Association Foundation Research Starter grant, and a grant from the National Institutes of Health (GM 40551). I.M.D. was the recipient of a National Institutes of Health Predoctoral Training Grant (GM 7145-13) and an American Foundation for Pharmaceutical Education Fellowship. We would like to thank Kimberly Emerson, who was a Minority High School Student Research Apprentice at SUNY/Buffalo.

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