Effects of Interactions Between Drugs on the Renal Excretion of Trientine in Rats—Acetazolamide and Furosemide Increase Trientine Excretion

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Purpose. To elucidate the effects of drug interactions on the urinary excretion of trientine in rats.

Method. Trientine and various other drugs were intravenously administered to rats and the urinary excretion of trientine was investigated. To clarify the mechanisms of drug-drug interactions, we also investigated the effects of various drugs on spermine uptake by rat renal brush-border membrane vesicles.

Results. Cimetidine, a substrate of the H^+/organic cation antiporter, and aminoglycoside antibiotics did not affect trientine excretion, while acetazolamide and furosemide, which increase the concentration of sodium ions in renal proximal tubules, increased the excretion of trientine. However, trichlormethiazide, which acts in renal distal tubules, did not affect trientine excretion. Acetazolamide and furosemide did not directly affect the Na^+/spermine transporter because these diuretics had no effect on the uptake of spermine into the rat renal brush-border membrane vesicles.

Conclusions. There is no interaction between trientine and the substrate of the H^+/organic cation antiporter or aminoglycoside antibiotics. However, drugs that change the concentration of sodium ions in renal proximal tubules, such as diuretics, can increase the trientine excretion since the increase in the luminal concentration of sodium ion accelerates the Na^+/spermine antiporter.

KEY WORDS: trientine; acetazolamide; furosemide; diuretics; drug interaction; renal excretion.

INTRODUCTION

Wilson's disease is a disorder characterized by chronic copper intoxication in the liver, brain, and other organs and tissues (1). D-penicillamine, a selective copper-chelating drug, is a "golden standard" for the treatment of Wilson's disease. However, D-penicillamine has severe adverse effects, such as significant marrow depression, dermatopathy, and autoimmune disease, and drug therapy should be stopped until these reactions have subsided (2). In 1982, Walshe used a new therapeutic drug, trientine, on a patient with Wilson's disease (3). Trientine is a selective chelating agent for copper, and it increases the urinary excretion of excess copper in patients with Wilson's disease. This drug has only slight adverse effects, and its efficacy is almost the same as that of D-penicillamine. Most patients require a long period of treatment with trientine; however, there is little information on the pharmacokinetic and pharmacodynamic interactions between trientine and other drugs.

Recently, we found that spermine, a physiological polyamine compound that has a chemical structure very similar to that of trientine, was actively secreted into urine by an Na^+/spermine antiporter (4,5) located in the brush-border membrane of cells in the rat renal proximal tubules. This transporter also recognized trientine and actively secreted trientine into urine. Therefore, it is probable that a drug that affects this transporter will also interact to induce the urinary excretion of trientine. In this study, we investigated the effects of interactions between drugs on the urinary excretion of trientine in rats.

MATERIALS AND METHODS

Materials

[^{3}H]Spermine tetrahydrochloride (1.48 TBq/mmol) was purchased from Amersham (Amersham, UK). Trientine dihydrochloride was kindly donated by Tsumura (Tokyo, Japan). Spermine tetrahydrochloride, amikacin, tobramicin, acetazolamide, furosemide and trichlormethiazide were purchased from Sigma (St Louis, MO). Other chemicals were of the highest grade available and were used without further purification.

Urinary Excretion Study

The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the "Guide for the Care and Use of Laboratory Animals" as adopted by the National Institutes of Health. All experiments were performed on three to five male Wistar rats (250–300 g). Rats were anaesthetized with sodium pentobarbitone (50 mg/kg, i.p.). Trientine · 2HCl saline solution was injected into the jugular vein at a dose of 2.5 mg/kg. Various drugs at a clinical doses (see figure captions) and/or the same moles of $CuSO_4 \cdot 5H_2O$ as of trientine were co-administered in order to investigate the effects of various drugs on the excretion of trientine or trientine-copper complex. Urine was collected from the bladder at 0.5, 1, 2, 3 and 4 hr after administration of the drugs. The concentration of trientine in urine was determined by HPLC.

Preparation of Renal Brush-Border Membrane Vesicles

Renal brush-border membrane vesicles (BBMVs) were prepared from the renal cortex of the rats by the Mg^{2+}/EGTA precipitation method according to Biber *et al.* (6) with several modifications described in our previous report (5). The BBMVs were washed and resuspended in a pre-loading buffer containing 100 mM D-mannitol, 100 mM NaCl and 20 mM N-2-hydroxymethylpiperazine-N′-2-ethansulphonic acid (HEPES) / tris (hydroxymethyl) aminomethane (Tris), pH 7.0. Enrichment of the BBMV fraction was routinely more than 10-fold greater than that of the homogenate, as revealed from the assessment of the specific activity of the membrane enzyme marker alkaline phosphatase.

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Uptake Study

Uptake studies were performed at 37°C using a rapid filtration technique reported previously (5). The reaction was started by mixing a BBMV suspension (20 μL) with an incubation medium (100 μL) containing of 100 mM D-mannitol, 100 mM NaCl or KCl, 20 mM HEPES/Tris (pH 7.0), and substrates. At a predetermined time, the reaction was terminated by diluting the reaction mixture with ice-cold stop buffer (150 mM KCl, 10 mM HEPES/Tris, pH 7.0; 4 mL) followed by filtration through a Millipore filter (HAWP, 0.45 μm, 2.5 cm diameter). The filter was then washed once with ice-cold stop buffer (4 mL). When the [^{3}H]spermine was used as a substrate, radioactivity was measured by liquid scintillation counting. In experiments conducted to measure trientine uptake, trientine trapped on the filter was extracted with a phosphate buffer (5 mM, pH 7.5; 0.5 mL) containing KCl (500 mM). Trientine was completely extracted with this buffer, and the concentration of trientine was determined by HPLC.

Analytical Method

The detection of trientine in urine and BBMVs was performed by HPLC (Hitachi L-6000, Hitachi, Tokyo) with fluorometric detection (820-FP, Jasco, Tokyo) as described previously (7,8). The [^{3}H]-labeled spermine was measured by liquid scintillation counting. Protein concentration was determined by the method of Lowry *et al.* (9) with bovine serum albumin as the standard. Analysis of variance (ANOVA) and unpaired Student's t-test, where appropriate, were used to determine the statistical significance of differences between experimental groups, and a value of $P < 0.05$ was considered significant.

RESULTS

Effect of Various Drugs on the Trientine Excretion *In Vivo*

We previously reported that Na^+/spermine antiporter recognized polyamine compounds such as spermine and free-trientine (not the copper-chelated form), which has more than 4 amino-groups in its molecule, in rat renal BBMVs (5). Moreover, the carrier-mediated transport of spermine was not inhibited by cimetidine and aminoglycoside antibiotics. To clarify the substrate specificity of the Na^+/spermine antiporter that was expressed *in vivo*, the effect of various drugs on the urinary excretion of trientine in rats was determined. As shown in Table 1, spermidine, which is a physiological polyamine with 3 amino-groups, did not affect the urinary excretion of trientine. However, spermine, which is a physiological polyamine with 4 amino-groups, reduced the urinary excretion of trientine. When copper was co-administered with trientine, the urinary excretion of trientine was also reduced. Cimetidine, amikacin and tobramicin did not have any effects on the urinary excretion of trientine.

It is well known that a diuretic drug such as furosemide increases the excretion of Na^+ ions in the urine (10). Therefore, it is probable that the increase in Na^+ concentration in urine produced by a diuretic drug affects the activity of carrier-mediated trientine secretion. To confirm this point, the diuretic drugs acetazolamide (ACZ), furosemide (FSM) and trichlormethiazide (TCM) were co-administered with trientine to rats. These diuretics vary in terms of their active site and the mechanism of their pharmacological action (Table 2). As shown in Table 1, ACZ and FSM significantly increased the urinary excretion of trientine, while TCM had no effect.

Since the effect of FSM was not so remarkable under the experimental conditions used, we further investigated the effect of FSM using various doses of trientine (0.5 to 10 mg/kg). Figure 1 shows that the urinary excretion of trientine decreased with increases in the dose of trientine in the presence and absence of FMS. Furthermore, the acceleration effect of FSM at a low dose of trientine was greater than that at a high dose. This result suggests that the trientine-excreted transporter is saturable under this condition.

Moreover, it is also possible that diuretics change the glomerulus filtration rate or blood flow, which would affect the trientine excretion. Thus, we investigated the effects of these diuretics on the excretion of trientine-copper complex *in-vivo*. Figure 2 shows that the initial excretion of trientine was stimulated by co-administration with ACZ and FSM. However, the stimulation of the trientine excretion significantly decreased when copper was co-administered. These results strongly suggest that the diuretic drugs ACZ and FSM stimulate the excretion of trientine but not that of trientine-copper complex.

Characteristics of Na^+/Spermine Antiporter in BBMVs

We previously reported that trientine inhibited the Na^+-dependent uptake of spermine by rat renal BBMVs (5). However, the kinetic parameters for this inhibitory effect of trientine remained unclear. Therefore, we performed a Dixon plot analysis of the inhibitory effect of trientine on the Na^+-dependent uptake of spermine. As shown in Fig. 3, trientine competitively inhibited the Na^+-dependent uptake of spermine, and the Ki value was 30.6 μM.

In Table 1, the excretion of trientine was significantly increased with co-administration of ACZ or FSM. However, the possibility that ACZ and FSM directly affect the Na^+/spermine antiporter and stimulate the excretion of trientine can not be ruled out. To clarify whether this result was induced by the increase in Na^+ ion concentration in urine or the direct effect of the diuretics on the Na^+/spermine transporter, we first investigated the effect of the degree of the Na^+ gradient on the uptake of spermine by BBMVs. As shown in Fig. 4, the uptake of spermine increased as the degree of the Na^+ gradient increased. On the other hand, Fig. 5 shows that none of the diuretics affected the uptake of spermine either in the presence or absence of an outward Na^+ gradient. This result suggests that diuretics do not affect the spermine transporter protein in the rat renal brush-border membrane.

DISCUSSION

Wilson's disease is a disorder characterized by chronic copper intoxication and it is inherited as a pair of abnormal alleles of an autosomal recessive gene (1). The symptoms of Wilson's disease usually appear in childhood, and drug therapy should be started as soon as possible. Since drug therapy is not a radical treatment for Wilson's disease, drugs must continue to be administered over a long period of time. However, there is little information on the interaction of trientine with other drugs.

We previously reported that the Na^+/spermine antiporter recognized straight-chain polyamine compounds that have more

Table 1. Effects of Various Drugs on Cumulative Urinary Excretion of Trientine After Intravenous Injection

Co-administered drug	Time (hr)				
	0.5	1	2	3	4
No additive	32.6 ± 1.7	57.4 ± 1.7	68.7 ± 2.1	71.2 ± 2.1	72.0 ± 2.1
Spermidine	34.7 ± 3.3	56.0 ± 2.7	70.0 ± 2.0	71.4 ± 1.1	72.8 ± 1.8
Spermine	30.5 ± 1.1	52.4 ± 2.5**	62.7 ± 3.4*	65.5 ± 2.2**	66.3 ± 2.1**
Cimetidine	30.0 ± 2.9	58.0 ± 4.4	69.9 ± 2.0	70.7 ± 2.0	71.4 ± 2.5
Tobramicin	30.0 ± 2.0	53.3 ± 2.6	68.3 ± 0.7	71.4 ± 1.9	72.7 ± 1.4
Amikacin	30.5 ± 3.3	57.0 ± 3.7	71.4 ± 2.1	74.6 ± 2.3	74.9 ± 1.7
$CuSO_4$	26.9 ± 2.0**	50.1 ± 3.6**	58.1 ± 3.6**	61.2 ± 4.9**	62.8 ± 4.2**
Acetazolamide	49.4 ± 4.6**	89.4 ± 10.5**	121.4 ± 14.4**	139.4 ± 12.1**	144.4 ± 13.0**
Furosemide	42.9 ± 1.6**	77.3 ± 5.8**	86.1 ± 4.7**	88.9 ± 4.8**	89.1 ± 3.9**
Trichlormethiazide	35.1 ± 3.9	55.3 ± 3.4	64.5 ± 5.2	70.5 ± 1.9	72.6 ± 1.8

Note: Trientine • 2HCl (2.5 mg/kg) was administered by intravenous injection with or without spermidine (2.5 mg/kg), spermine (2.5 mg/kg), cimetidine (3.3 mg/kg), tobramicin (1.2 mg/kg), amikacin (1.5mg/kg), CuSO4 • $5H_20$ (2.8 mg/kg), acetazolamide (8 mg/kg), furosemide (0.3 mg/kg) and trichlormethiazide (0.1mg/kg). Each value represents the mean ± SD of 3 to 5 rats.
* Significantly different from the value of "No additive" at $P<0.05$, **$P<0.01$.

Table 2. Active Site and Mechanism of Diuresis of Acetazolamide, Furosemide, and Trichlormethiazide in the Mammalian Kidney

	Acetazolamide	Furosemide	Trichlormethiazide
Active site	Proximal tubule	Loop of Henle (proximal tubule)	Distal tubule
Mechanism	Inhibits carbonic anhydrase	Inhibits Na^+-K^+-$2Cl^-$ symporter	Inhibits Na^+-Cl^- symporter

Note: This Table is the summary of reference No. 10.

than 4 amino-groups in rat renal BBMVs (5). In that study, cimetidine, a substrate of the H^+/organic cation antiporter (11), and aminoglycoside antibiotics that have more than 4 amino-groups in their molecules did not affect the uptake of spermine. In this study, we found that there was no interaction between trientine and cimetidine or aminoglycoside *in vivo*. Spermine, a physiological polyamine, inhibited trientine excretion *in vivo*, but this compound is not used as a therapeutic drug. Although urinary excretion of spermine is elevated in patients with certain kinds of cancer (12–14), this is not considered to be clinically relevant for the majority of patients with Wilson's disease.

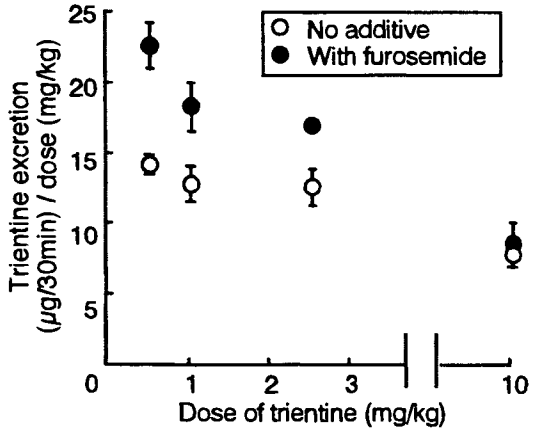

Fig. 1. Dose dependence of the urinary excretion of trientine (30 min) with or without furosemide in rats. Trientine • 2HCl (0.5 to 10 mg/kg) was administered with or without furosemide (0.3 mg/kg) by intravenous injection. Each point represents the mean ± SD of 4 rats.

When we co-administered ACZ or FSM with trientine to rats, the urinary excretion of trientine was significantly stimulated. This was due to the increase in Na^+ concentration in urine caused by the diuretics because the extravesicular Na^+ concentration strongly affected the activity of the Na^+/spermine transporter (Fig. 4). Since these diuretics were used at clinical doses in this study, it is possible that these diuretics will decrease the efficacy of trientine in patients with Wilson's disease.

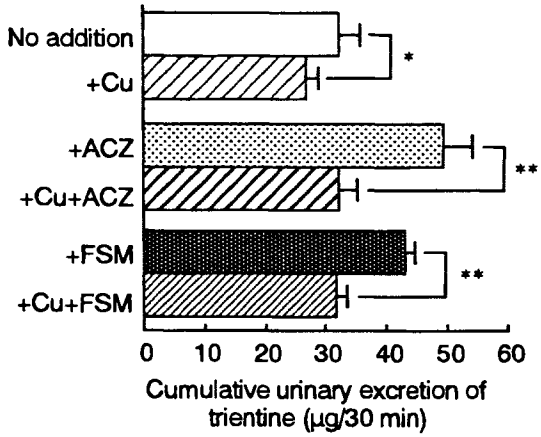

Fig. 2. Comparison of the effects of diuretics on the urinary excretion of trientine and trientine-copper complex in rats. Trientine • 2HCl (2.5 mg/kg) or trientine-copper complex (trientine • 2HCl; 2.5 mg/kg, $CuSO_4$ • $5H_2O$; 2.8 mg/kg) was administered by intravenous injection with or without acetazolamide (8 mg/kg) and furosemide (0.3 mg/kg). Each column represents the mean with SD of 4 rats. * Significantly different at $P < 0.05$, ** $P < 0.01$.

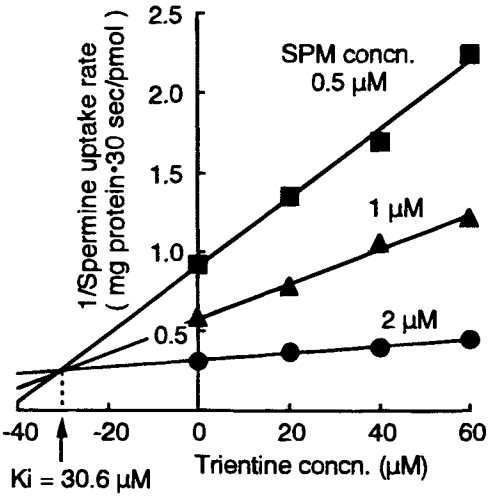

Fig. 3. Dixon plot analysis of the inhibitory effect of trientine on the uptake of spermine by rat renal BBMVs. The data were calculated by subtracting the uptake value at Na^+_{out}/Na^+_{in} from the uptake at K^+_{out}/Na^+_{in}.

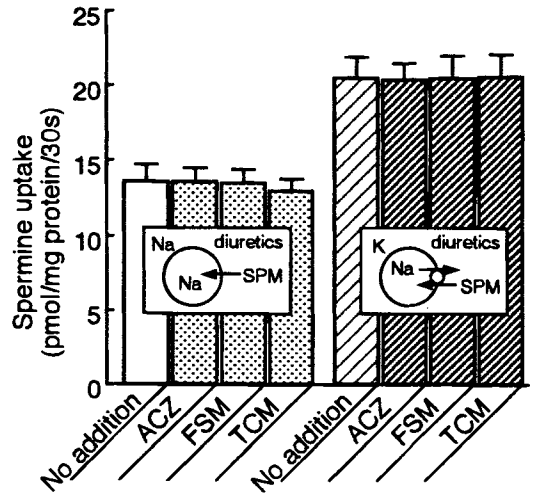

Fig. 5. Effects of diuretics on the initial uptake (30 sec) of spermine by rat renal BBMVs. Final concentration of spermine was 2 μM, and the concentration of each diuretic was 1 mM. Each column represents the mean with SD of 3 preparations.

Recently, Silbernagl *et al.* reported that FSM inhibited the reabsorption of taurine in Henle's loops (15). They were concerned that the concentration of intracellular Cl^- ions was decreased, since FMS inhibited the $Na^+/2Cl^-/K^+$ symporter, and that this decrease of Cl^- ions decelerated the Cl^--dependent taurine transporter. The results of the study also suggested that ACZ and FSM increased the concentration of luminal Na^+ ions, causing trientine secretion to increase.

On the other hand, TCM did not affect trientine excretion *in vivo*. This was thought to be due to the different sites of action of the diuretics. It is well known that ACZ acts in the proximal tubule and that it stimulates urinary Na^+ excretion. FMS acts in both the proximal tubule and the loop of Henle (10,16,17). On the other hand, TCM mainly acts in the distal tubule of the kidney. It was therefore thought that an Na^+/spermine antiporter does not exist in the distal tubule. However, this is still unclear because our investigations have only used brush-border membranes prepared from rat renal proximal tubules (4,5).

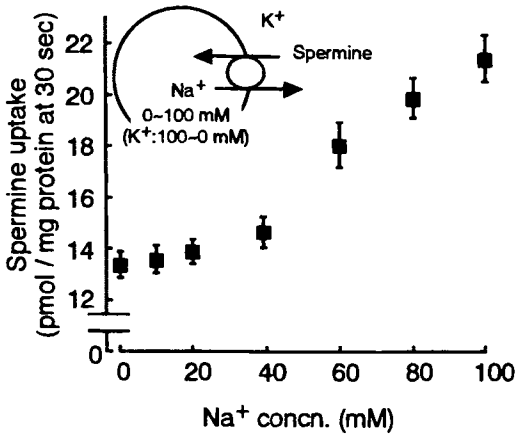

Fig. 4. Effect of intra vesicular Na^+ concentration on the uptake of spermine by rat renal BBMVs. Membrane vesicles (20 μL) were suspended in 0 to 100 mM NaCl, 100 to 0 mM KCl, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7.0). Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7.0), 2.4 μM spermine and 100 mM KCl. Each point represents the mean ± SD of 3 preparations.

The Km value for trientine transport can not be determined due to the limitations in trientine detection by HPLC. However, it is well known that if a compound inhibits carrier-mediated transport competitively, the Ki value of the compound is almost the same as the Km value of its transport. Thus, the Km value of trientine transport can be assumed to be about 30 μM (about 4.4 μg/mL). This value is very close to that of the maximum plasma concentration (C_{max}) of trientine in patients with Wilson's disease (7).

In conclusion, a substrate of the H^+/organic cation antiporter and aminoglycoside did not interact to induce the urinary excretion of trientine. However, drugs that change the concentration of sodium ions in renal proximal tubules, such as diuretics, can increase the trientine excretion since the increase in the luminal concentration of sodium ion accelerates the Na^+/spermine antiporter.

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