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Short Communication

Inhibitory Effects of Various Beverages on Ritodrine Sulfation by Recombinant Human Sulfotransferase Isoforms SULT1A1 and SULT1A3

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Purpose. Ritodrine is known to undergo extensive presystemic sulfation in the intestinal mucosa, and its bioavailability is as low as 30%. Accordingly, inhibition of intestinal sulfation may lead to an increase in the bioavailability of ritodrine. In this study, we aimed to investigate the activities of ritodrine sulfation by SULT1A1, which is expressed predominantly in the liver, and SULT1A3, which is expressed predominantly in the intestine, as well as the inhibitory effects of beverages on their activities.
Methods. We investigated ritodrine sulfation by using recombinant human sulfotransferase (SULT) 1A1 and SULT1A3 in an *in vitro* study. Next, we investigated the inhibitory effects of grapefruit juice, orange juice, green tea, and black tea on ritodrine sulfation.
Results. Sulfation of ritodrine by SULT1A3 was much higher than that by SULT1A1, suggesting that the bioavailability of ritodrine may be limited by intestinal SULT1A3. The ritodrine sulfation activities of SULT1A1 and SULT1A3 were significantly inhibited by all beverages examined at a concentration of 10%. Green tea and black tea exhibited potent inhibition; even at a concentration of 5%, they both inhibited SULT1A1 by 100% and SULT1A3 by ≥95%.
Conclusion. Our results suggest that concomitant ingestion of beverages such as green tea and black tea may increase the bioavailability of orally administered ritodrine, and perhaps other β_2-agonists, and lead to an increase in the clinical effects or adverse reactions.

KEY WORDS: drug-food interaction; enzyme inhibitors; intestinal bioavailability; ritodrine; sulfotransferase.

INTRODUCTION

Ritodrine, a β_2-adrenergic agonist, is used as a tocolytic uterine relaxant, since it inhibits the contraction of uterine smooth muscle. However, ritodrine does not exclusively act on the uterine smooth muscle, and it may cause cardiovascular side effects owing to its β_1 adrenergic action (1).

Ritodrine does not undergo phase I metabolism, but is inactivated directly by conjugation (2). When orally administered, ritodrine is well absorbed from the intestine. It undergoes extensive sulfation, and the sulfate conjugate is the major metabolite of ritodrine excreted into urine (50%) (3). The ritodrine sulfation activity is higher in human intestinal cytosol than in human liver cytosol (4). Sulfotransferase (SULT) 1A1 is predominantly expressed in the liver, whereas SULT1A3 is predominantly expressed in the intestinal mucosa (5). Therefore, SULT1A1 and SULT1A3, especially the latter, are both considered to contribute to ritodrine sulfation. Sulfation of ritodrine has not been characterized by using recombinant human SULT1A1 and SULT1A3, however.

Bioavailability of ritodrine is as low as 30% (4), probably owing to presystemic sulfation in the intestinal mucosa. Therefore, inhibition of SULT1A3 may lead to an increase in the bioavailability of ritodrine.

Recently, we encountered a case of development of pulsation and tremor, typical adverse reactions to ritodrine, after concomitant ingestion of ritodrine and grapefruit (unpublished observation). Although we could not confirm a causal relationship between the ingestion of grapefruit and the adverse reactions, grapefruit might have inhibited the function of SULTs in the intestinal mucosa, leading to an increase in the blood ritodrine level. In addition, it has been reported that catechin gallates, a major group of tea constituents, potently inhibit the function of SULT1A1 and SULT1A3 (6,7). Tea is a very popular beverage that may be casually used as an aid to ingest drugs.

In this study, we aimed to investigate the activities of ritodrine sulfation by SULT1A1 and SULT1A3, and the inhibitory effects of beverages on their activities by using recombinant human SULT1A1 and SULT1A3.

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ABBREVIATIONS: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase.

MATERIALS AND METHODS

Chemicals

Unlabeled 3′-phosphoadenosine 5′-phosphosulfate (PAPS) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). [^{35}S]PAPS (3.0 Ci/mmol) was purchased from Perkin-Elmer Life Sciences, Inc. (Boston, MA, USA). Rito- drine hydrochloride was kindly provided by Kissei Pharmaceutical Co., Ltd. (Tokyo, Japan). pET-14b vector and His-binding metal chelation resin were purchased from Novagen (Madison, WI, USA). Other reagents used were of analytical grade.

Beverage Samples

Grapefruit (white) (*Ocean Spray*) (Florida), orange (*Sunkist*) (navel), green tea (Itoen, Ltd., Tokyo, Japan), and Lipton brand (Thomas J. Lipton, Englewood Cliffs, NJ, USA) black tea packaged in tea bags (2.0 g/tea bag) were obtained from local commercial sources. A portion of leaves of green tea (5 g) or a tea bag of black tea was extracted with 200 ml of hot water (98°C) for 3 min. Fruit samples were hand-squeezed to obtain juice. These samples were filtered through a 0.22-μm membrane filter (Millex GV25; Millipore Corp., Bedford, MA, USA) to eliminate insoluble materials and the filtrates were designated as 100% beverages. In the sulfation assay, all beverage samples were adjusted to pH 6.8 with 1 N NaOH and were diluted to target concentration by incubation buffer [50 mM sodium phosphate buffer containing 8 mM dithiothreitol and 0.0625% (w/v) BSA, pH adjusted to 6.8].

Preparation of Recombinant Human SULTs

Construction of *Escherichia coli* (*E. coli*) expression plasmids for SULT1A1 and SULT1A3 was performed as described previously (8). In brief, the coding regions of human SULTs were amplified by polymerase chain reaction (PCR) from a human cDNA library, subcloned into the *Nde*I/*Xho*I sites (SULT1A1 and SULT1A3) of the pET-14b expression vector, and introduced into *E. coli* BL21 (DE3) pLysS (Novagen, Madison, WI, USA). The subcloned cDNA sequences determined by using an ABI Prism™ 377 DNA sequencer (Perkin-Elmer, Foster, CA, USA) were in agreement with published sequences of SULT1A1 (Genbank accession no. X78283) and SULT1A3 (Genbank accession no. L25275) cDNAs.

Bacterial Expression and Purification of Recombinant Human SULTs

Bacterial expression and purification of His-tagged recombinant human SULTs by one-step column chromatography were performed as described previously (9): a bacterial lysate (25 mg protein/ml) containing 0.025% (w/v) Tween-20 and 10% (w/v) glycerol was loaded onto a His-binding metal chelation resin column (1 × 5 cm) preequilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 5 mM imidazole, 500 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.025% (w/v) glycerol (buffer I). The column was washed with 60 ml of the same buffer, and the recombinant protein was eluted with a stepwise gradient of 15 ml each of 100, 200, 400, and 1000 mM imidazole in buffer I. Fractions containing the homogeneous enzyme protein, appearing as a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, were pooled as described previously (9).

Enzyme Assay

The sulfating activity of purified recombinant human SULTs toward ritodrine was determined as described previously with the following modifications (10). The reaction mixture, containing substrates [ritodrine hydrochloride, dopamine, or *p*-nitrophenol), [^{35}S]PAPS (10 μM, 100 mCi/mmol), and enzyme (18.75 ng) in a final volume of 50 μl of incubation buffer (8 mM dithiothreitol and 0.0625% (w/v) BSA in 50 mM sodium phosphate buffer pH adjusted to 6.8], was incubated for 10 min at 37°C. In the experiments on inhibition by the beverages, the reaction solution in a final volume of 50 μl contained the indicated final concentration of beverages (pH 6.8). After a 2-min preincubation, the reaction was started by the addition of ritodrine hydrochloride (and beverages in the inhibition experiments) and terminated by placing the reaction vessel in an ice bath. An aliquot (5 μl) of the reaction mixture was applied to a polyethyleneimine cellulose thin-layer plate (Marcherey-Nagel GmbH & Co. KG, Dueren, Germany) and developed with 1-propanol-30% NH_3 aq-H_2O (6:3:1, v/v/v). The radioactivity of the sulfate ester was determined by radioluminography with a BAS 2500 bioimaging analyzer (Fuji Photo Film, Tokyo, Japan). To avoid the saturation of SULTs activity and substrate inhibition, we chose 3000 μM and 100 μM ritodrine as substrate for SULT1A1 and SULT1A3, respectively. The extent of substrate inhibition in SULT1A3 at 100 μM ritodrine was estimated to be less than 5% as assessed by the K_{is} value (Fig. 1B).

Data Analysis

Kinetic parameters were obtained by nonlinear regression analysis using MLAB (Civilized Software, Bethesda, MD, USA). The Hill equation [Eq. (1)] or an equation based on substrate inhibition [Eq. (2)] was fitted to the data from the kinetic studies on ritodrine sulfation by SULT1A1 or SULT1A3, respectively, to obtain kinetic parameters such as the maximum velocity (V_{max}), Michaelis–Menten constant (K_m), Hill coefficient (n), and substrate inhibition constant (K_{is}).

$$V = \frac{V_{max} \bullet S^n}{K_m{}^n + S^n} \quad (1)$$

$$V = \frac{V_{max}}{\left(1 + \frac{K_m}{S}\right) \bullet \left(1 + \frac{S}{K_{is}}\right)} \quad (2)$$

In the inhibition experiment, the activity of ritodrine sulfation in the presence of beverages was represented as a percentage of the control (in the absence of beverages).

Statistical significance was determined by one-way analysis of variance followed by Dunnett's test and p-values <0.05 (two-tailed) were considered statistically significant.

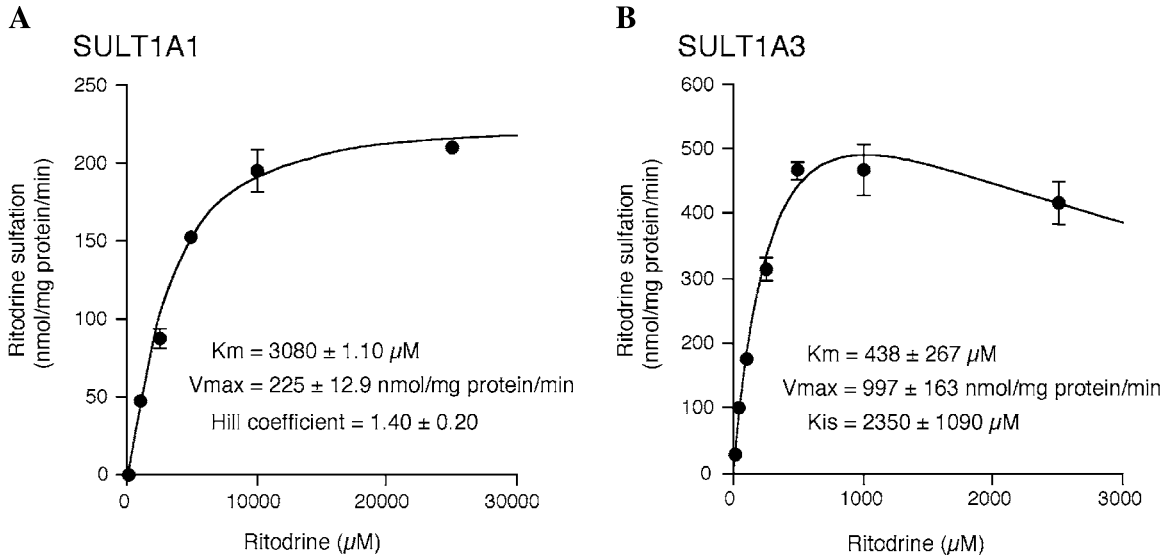

Fig. 1. Concentration dependency of ritodrine sulfation by recombinant human SULT1A1 (A) and SULT1A3 (B). SULTs were incubated with various concentrations of ritodrine at 37°C and pH 6.8. Each point represents the mean ± SEM of three determinations. The solid line represents the fitting line described in the text. Each parameter is given as the estimate ± SD.

RESULTS AND DISCUSSION

The activities of ritodrine sulfation were investigated by using recombinant human SULT1A1 and SULT1A3 (Fig. 1). Although these recombinant enzymes have His-tags at their N-termini, our previous kinetic study showed that these His-tagged SULTs showed a slight difference in K_m values for the sulfations of typical substrates, *p*-nitrophenol by SULT1A1 and dopamine by SULT1A3, from those of the previously reported SULTs purified from human tissues (8). Therefore, the presence of His-tag is unlikely to have affected their sulfating activities to ritodrine. The concentration dependencies of ritodrine sulfation by SULT1A1 and SULT1A3 were adequately explained by the Hill equation [Eq. (1)] and an equation based on substrate inhibition [Eq. (2)], respectively. Substrate inhibition of SULTs has also been demonstrated with dopamine or 1-hydroxypyrene (11,12).

The K_m values of ritodrine sulfation by SULT1A1 and SULT1A3 were 3080 and 438 μM, respectively, showing that ritodrine is a relatively poor substrate for both SULT1A1 and SULT1A3. Other SULTs isoforms such as SULT1E1, SULT2A1, and SULT1B1 have been shown to be expressed in human intestine (13,14), so that these isoforms may be possibly involved in ritodrine sulfation. On the other hand, Pacifici *et al.* reported that the K_m values of ritodrine sulfation by human liver cytosol and human duodenum cytosol were 3.41 mM and 0.66 mM, respectively (4). These values are in good accordance with the K_m values in recombinant human SULT1A1 (3.08 mM) and SULT1A3 (0.44 mM) in our study. Taken together, these results suggest that ritodrine sulfation in human intestinal mucosa is mediated by SULT1A3, but not by other SULT isoforms with higher affinity.

The values of intrinsic clearance (V_{max}/K_m) of ritodrine by SULT1A1 and SULT1A3 were 0.07 and 2.28 ml/mg protein/min, respectively; that is, sulfation of ritodrine by SULT1A3 is 30 times higher than that by SULT1A1. Because SULT1A1 and SULT1A3 are predominantly expressed in the

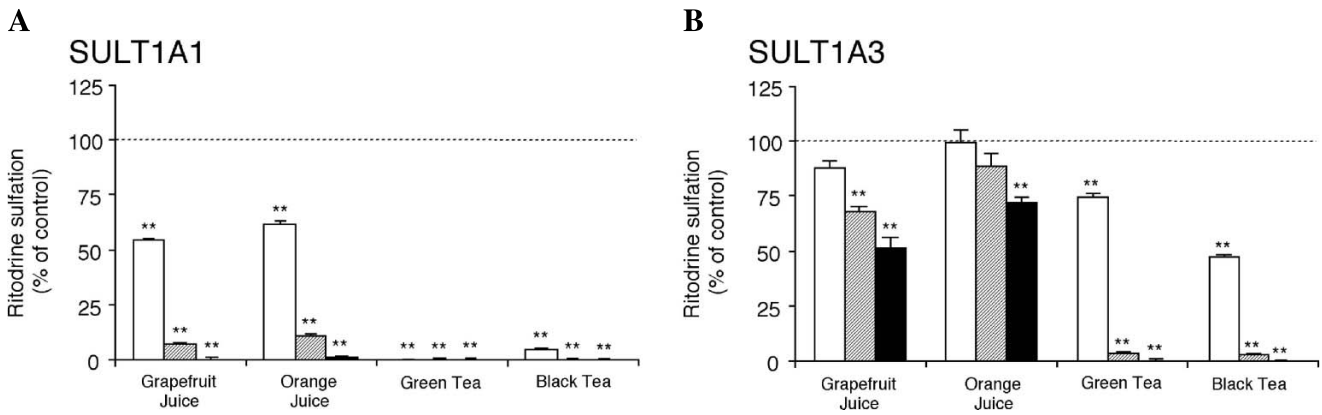

Fig. 2. Inhibitory effects of various beverages on the sulfation of ritodrine by recombinant human SULT1A1 (A) and SULT1A3 (B). SULTs-mediated ritodrine sulfation (nmol/mg protein/min) was assessed at a concentration of 3000 μM (A) or 100 μM (B) in the absence or presence of 1% (open column), 5% (hatched column), or 10% (closed column) beverages at 37°C and pH 6.8. Each column represents the mean ± SEM of three determinations. Significance of differences from the control was determined by ANOVA followed by Dunnett's test (**$p < 0.01$).

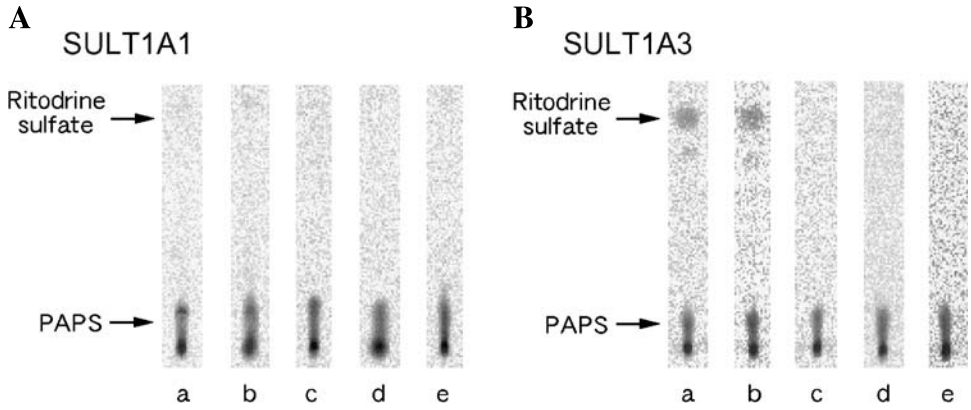

Fig. 3. Typical autoradiograph of TLC plates in the sulfation assay of ritodrine in the presence of 10% grapefruit juice (a), orange juice (b), green tea (c), and black tea (d) by recombinant human SULT1A1 (A) and SULT1A3 (B). Concentrations of ritodrine were 3000 μM for SULT1A1 study or 100 μM for SULT1A3 study, respectively. The autograph for blank experiment in the absence of beverage and substrate is also shown in lane (e).

liver and in the intestinal mucosa, respectively (5), it is conceivable that SULT1A3 in the intestinal mucosa may mainly limit the bioavailability of ritodrine.

Next, we investigated the inhibitory effects of grapefruit juice, orange juice, green tea, and black tea on ritodrine sulfation by using recombinant human SULT1A1 and SULT1A3. The activities of ritodrine sulfation by both SULT1A1 and SULT1A3 were significantly inhibited by all the beverages investigated at a concentration of 10%. Even at a concentration of 5%, green tea and black tea both inhibited SULT1A1 by 100% and SULT1A3 by ≥95% (Fig. 2). The ethyl acetate extracts of grapefruit juice and orange juice also inhibited the function of SULTs (data not shown), suggesting that the inhibitory effects of these juice are not likely to be attributable to hydrophilic matrix such as carbohydrates or electrolytes. Moreover, similar results were observed in the preliminary experiments using other lots or brands of beverages, suggesting that the inhibitory effects of these beverages are reproducible.

Figure 3 represents a typical autoradiograph of thin-layer chromatography (TLC) plates. The intense spot of unreacted PAPS was observed in each lane, suggesting that PAPS was not depleted under the current experimental condition. Moreover, no spot was detected to indicate sulfation reaction on TLC plate in the blank study (Fig. 3e).

In addition, we examined the inhibitory effects of various beverages on the sulfation of typical substrates, *p*-nitrophenol and dopamine, by SULT1A1 and SULT1A3, respectively (Fig. 4). The inhibitory effects similar to those for ritodrine sulfation were observed, suggesting that the inhibitory effects of these beverages on SULT1A1 and SULT1A3 are not substrate specific.

Although the inhibitory effects of these beverages on SULT1A3 were weaker than those on SULT1A1, the inhibitory effects of green tea and black tea on SULT1A3 were potent. Since the volume of gastric fluid is assumed to be 250–1000 ml, the concentration of these teas in the intestinal lumen may readily exceed 10% even after taking one cup of tea. Therefore, it is likely that SULT1A3 in the intestinal mucosa will be potently inhibited after ingestion of a usual volume of green or black tea. Taken together, these results suggest that concomitant ingestion of tea with ritodrine is very likely to lead to an increase in the bioavailability of ritodrine via the inhibition of intestinal

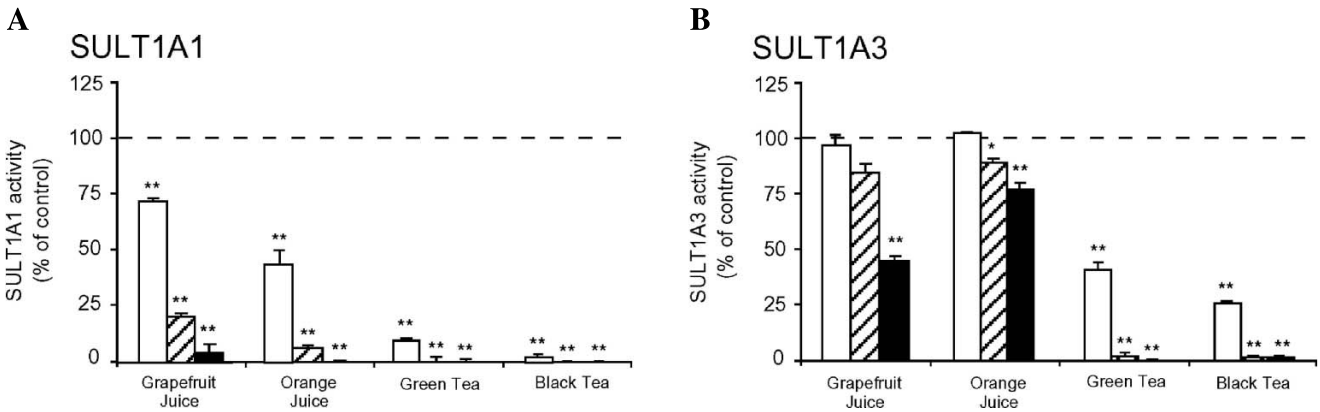

Fig. 4. Inhibitory effects of various beverages on the sulfation of typical substrates by human recombinant SULT1A1 (A) and SULT1A3 (B). SULT1A1-mediated sulfation of *p*-nitrophenol (A) and SULT1A3-mediated sulfation of dopamine (B) were assessed at substrate concentrations of 1 μM (A) and 5 μM (B), respectively, in the absence or presence of 1% (open column), 5% (hatched column), or 10% (closed column) beverages at 37°C and pH 6.8. Each column represents the mean ± SEM of three determinations. Significance of differences from the control was determined by ANOVA followed by Dunnett's test (*$p < 0.05$; **$p < 0.01$).

sulfation. Grapefruit juice and orange juice also significantly inhibited the activity of SULT1A3, although their inhibitory effects were weaker than those of green tea and black tea. The aforementioned case of adverse reaction to ritodrine associated with the ingestion of grapefruit juice may possibly have been caused by the inhibition of intestinal SULT1A3 by grapefruit. However, we did not determine the blood concentration of ritodrine in that case, so we cannot show a causal relationship between the inhibition of SULT1A3 by grapefruit ingestion and an increase in ritodrine bioavailability.

There are many β_2-adrenergic agonists with limited bioavailability besides ritodrine, including fenoterol [1.5%; (15)] and terbutaline [14%; (16)]. For these β_2-agonists, sulfation is the primary metabolic route after oral administration, as in the case of ritodrine (17,18). Therefore, the bioavailability of these β_2-agonists may be also increased by some of the beverages examined in this study.

In conclusion, our results suggest that concomitant ingestion of beverages such as green tea and black tea may increase the bioavailability of orally administered ritodrine, and perhaps other β_2-agonists, and lead to an increase in the clinical effects or adverse reactions.

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