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Sulfation of ritodrine by the human cytosolic sulfotransferases (SULTs): Effects of SULT1A3 genetic polymorphism

Ying Hui [a,b], Ming-Cheh Liu [a,*]

[a] Department of Pharmacology, College of Pharmacy and Pharmaceutical Sciences, University of Toledo Health Science Campus, 3000 Arlington Avenue, Toledo, OH 43614, USA

[b] Department of Obstetrics and Gynecology, Beijing Hospital, Beijing 100730, China

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ABSTRACT

Previous studies have demonstrated the metabolism of ritodrine through sulfation. The current study was designed to identify the human SULTs that are capable of sulfating ritodrine and to investigate how genetic polymorphism of the major ritodrine-sulfating SULT, SULT1A3, may affect its sulfating activity. A systematic analysis revealed that of the 13 known human SULTs, SULT1A1, SULT1A3, and SULT1C4, were capable of mediating the sulfation of ritodrine, with SULT1A3 displaying the strongest sulfating activity. Effects of genetic polymorphism on the sulfating activity of SULT1A3 were examined. By employing site-directed mutagenesis, 4 SULT1A3 allozymes were generated, expressed, and purified. Purified SULT1A3 allozymes were shown to exhibit differential sulfating activity toward ritodrine. Kinetic studies further demonstrated differential substrate affinity and catalytic efficiency among the SULT1A3 allozymes. Collectively, these results provided useful information concerning the differential metabolism of ritodrine through sulfation in different individuals.

1. Introduction

Preterm labor is known to be a major cause of perinatal mortality and morbidity (Neilson et al., 2014). Ritodrine is a tocolytic agent used for treating preterm labor (Neilson et al., 2014). Ritodrine stimulates the β_2-adrenergic receptor in the body, causing an attenuation of uterine contractility (Neilson et al., 2014). Due to the lack of uterine selectivity, however, it may lead to a number of adverse effects for the mother and the fetus (Yaju and Nakayama, 2006; Kimura et al., 2013; Driul et al., 2014). Cardiac side effects, including increased heart rate and systolic blood pressure, myocardial ischemia, and pulmonary edema, are most common for the mother (Yaju and Nakayama, 2006; Kimura et al., 2013; Driul et al., 2014). Since ritodrine can cross the placental barrier, it may produce similar side effects in the fetus. The therapy with ritodrine is thus associated with these adverse effects which may vary among patients. To better understand its therapeutic effects as well as the adverse effects in different individuals, it is important to clarify the mechanism underlying the metabolism of ritodrine. Previous studies indicated that ritodrine was eliminated primarily through sulfation and glucuronidation (Pacifici et al., 1993; Pacifici, 2005), and both the mother treated with ritodrine and neonate delivered by treated mother were found to excrete sulfate and glucuronide conjugates of ritodrine in urine (Brashear et al., 1988).

Sulfate conjugation is a major pathway operated in humans and other vertebrates for the biotransformation and excretion of a diverse array of xenobiotics including drugs (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994). The responsible enzymes, called the cytosolic sulfotransferases (SULTs), catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to an acceptor substrate compound containing a hydroxyl or an amino group (Lipmann, 1958). Sulfate conjugation by these enzymes may result in the inactivation of the substrate compounds and/or increase their water-solubility, thereby facilitating their removal from the body (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994). Previous studies have demonstrated the sulfation of ritodrine by human liver and duodenum cytosols (Pacifici et al., 1998), and several human SULTs have been shown to display ritodrine-sulfating activity (Nishimuta et al., 2005). To clarify further the involvement of SULT-mediated sulfation in the metabolism of ritodrine, it is prudent to identify all human SULTs that are capable of mediating the sulfation of ritodrine. Moreover, in view of the individual differences in susceptibility to the adverse effects of ritodrine, it is an intriguing issue whether SULT genetic polymorphism may affect the metabolism of ritodrine through sulfation. Like with many other genes, single nucleotide polymorphisms (SNPs) of SULT genes have been

Abbreviations: SULT, cytosolic sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

* Corresponding author. Tel.: +1 419 383 1918; fax: +1 419 3831909.
E-mail address: ming.liu@utoledo.edu (M.-C. Liu).

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reported (Glatt et al., 2000; Lindsay et al., 2008; Daniels and Kadlubar, 2013). For example, four non-synonymous coding SNPs (cSNPs) for the SULT1A3 gene were detected by sequencing DNA samples from African–American and Caucasian–American subjects (Thomae et al., 2003; Hildebrandt et al., 2004). Since SULT1A3 has been shown to be capable of sulfating ritodrine (Nishimuta et al., 2005), it is important to find out whether its genetic polymorphism may have a significant impact on the metabolism of ritodrine through sulfation, thereby influencing the efficacy and side effects of the drug in different individuals.

In this communication, we report a systematic analysis of the sulfating activity of all known human SULTs toward ritodrine. Different allozymes of SULT1A3, a major ritodrine-sulfating SULT, were generated, expressed, purified, and characterized with respect to their kinetic parameters in mediating ritodrine sulfation.

2. Materials and methods

2.1. Materials

Ritodrine was a product of Santa Cruz Biotechnology Inc. (Dallas, TX). Adenosine 5′-triphosphate (ATP), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), N-2-hydroxylpiperazine-N′-2-ethanesulfonic acid (HEPES), dimethyl sulfoxide (DMSO), Trizma base, dithiothreitol (DTT), and silica gel thin-layer chromatography (TLC) plates were from Sigma Chemical Company (St. Louis, MO, USA). Ultrafree-MC 5000 NMWL filter units were products of Millipore (Bedford, MA, USA). Carrier-free sodium [^{35}S]sulfate was a product of Perkin-Elmer (Waltham, MA, USA). Ecolume scintillation cocktail was purchased from MP Biomedicals, LLC. (Irvine, CA, USA). Recombinant human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase was prepared as previously described (Yanagisawa et al., 1998). EX *Taq* DNA polymerase was a product of Takara Bio (Mountain View, CA, USA). Protein molecular weight markers were from New England Biolabs, Inc. (Ipswich, MA, USA). Oligonucleotide primers were synthesized by MWG Biotech (Huntsville, AL, USA). X-ray films were purchased from BioExpress (Kaysville, UT, USA). All other chemicals were of the highest grade commercially available.

2.2. Preparation of the human SULTs

Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, thyroid hormone SULT (SULT1B1), two SULT1Cs (SULT1C2, SULT1C3, and SULT1C4), estrogen SULT (SULT1E1), dehydroepiandrosterone (DHEA) SULT (SULT2A1), two SULT2B1s (SULT2B1a and SULT2B1b), a neuronal SULT (SULT4A1) and SULT6B1, expressed using pGEX-2TK or pET23c prokaryotic expression system, were prepared as described previously (Sakakibara et al., 1998a, 1998b, 2002; Pai et al., 2002; Suiko et al., 2002).

2.3. Generation, expression, and purification of SULT1A3 allozymes

The QuikChange site-directed mutagenesis kit from Stratagene was used for the generation of cDNAs encoding SULT1A3 allozymes. Briefly, wild-type SULT1A3 cDNA packaged in pGEX-2TK prokaryotic expression vector was used as the template in conjunction with specific mutagenic primers (see Table 1 for the mutagenic primers used). The amplification conditions were 12 cycles of 30 s at 95 °C, 1 min at 55 °C, and 6 min at 68 °C. The "mutated" SULT1A3 sequences were verified by nucleotide sequencing (Sanger et al., 1977). pGEX-2TK vector harboring individual mutated SULT1A3 cDNA was transformed into competent XL1-Blue *E. coli* cells. The transformed cells, grown to $A_{600\ nm} = \sim 0.5$ in 1 l of LB medium supplemented with 100 μg/ml ampicillin and induced with 0.1 mM IPTG overnight at room temperature, were collected by centrifugation and homogenized in 20 ml of an ice-cold lysis buffer (10 mM Tris–HCl, pH 8.0, 150 mm NaCl, and 1 mM EDTA) using an Aminco French press. The crude homogenate thus prepared was subjected to centrifugation at 10,000*g* for 30 min at 4 °C. The supernatant collected was fractionated using 0.5 ml of glutathione-Sepharose, and the bound fusion protein was treated with 2 ml of a thrombin digestion buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 2.5 mM $CaCl_2$) containing 5 units/ml bovine thrombin. Following a 1-h incubation at room temperature with constant agitation, the preparation was subjected to centrifugation. The recombinant enzyme present in the supernatant collected was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to enzymatic characterization as described below.

Table 1
Primer sets used for the site-directed mutagenesis of human SULT1A3.

SULT1A3 allozyme	Amino acid substitution	Mutagenic primer set
SULT1A3*2	Lys234Asn	5′-ttcaaggagatgaagaacaaccctatgaccaactac-3′ 5′-gtagttggtcatagggttgttcttcatctccttgaa-3′
SULT1A3*3	Pro101Leu	5′-actctgaaagacacaccgctcccacggctcatcaagtca-3′ 5′-tgacttgatgagccgtgggagcggtgtgtctttcagagt-3′
SULT1A3*4	Pro101His	5′-actctgaaagacacaccgcacccacggctcatcaagtca-3′ 5′-tgacttgatgagccgtgggtgcggtgtgtctttcagagt-3′
SULT1A3*5	Arg144Cys	5′-tcctactaccatttccactgtatggaaaaggcgcaccct-3′ 5′-agggtgcgccttttccatacagtggaaatggtagtagga-3′

2.4. Sulfotransferase assay

The sulfating activity of the recombinant human SULTs was determined using PAP[^{35}S] as the sulfonate donor. The reaction mixture for the standard enzymatic assay, prepared in a final volume of 20 μl, contained, 50 mM MOPS at pH 7.0, 14 μM of PAP [^{35}S], 1 mM DTT, and 50 μM substrate. Stock solutions of the substrates, prepared in DMSO, were used in the enzymatic assay. Controls with water or DMSO replacing substrate were also included. The reaction was started by the addition of the enzyme, allowed to continue at 37 °C for 10 min (5 min in case of the kinetic assays), and terminated by placing the tube containing the reaction mixture on a heating block at 100 °C for 3 min. The precipitates were cleared by centrifugation at 15,000*g* for 3 min and the supernatant was subjected to the analysis of [^{35}S]sulfated product. Afterwards, 1 μl of the reaction mixture was spotted on a silica TLC plate and the spotted TLC plate was subjected to TLC analysis using a solvent system containing n-butanol: acetonitrile in a ratio of 3:2 (by volume). Upon completion of TLC, the TLC plate was air-dried and autoradiographed by using an X-ray film. The radioactive spot corresponding to the sulfated product was located and cut out and eluted in 0.5 ml water in a glass vial. 4.5 ml of Ecolume scintillation liquid was added to each vial, mixed thoroughly, and the radioactivity therein was counted by using a liquid