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Sulfation of 6-hydroxymelatonin, N-acetylserotonin and 4-hydroxyramelteon by the human cytosolic sulfotransferases (SULTs)

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Abstract

1. The present study aimed to investigate the involvement of sulfation in the metabolism of 6-hydroxymelatonin (6-OH-Mel), N-acetylserotonin (NAS) and 4-hydroxyramelteon (4-OH-Ram), and to identify and characterize the human cytosolic sulfotransferases (SULTs) capable of sulfating these drug compounds.

2. A systematic analysis using 13 known human SULTs revealed that SULT1A1 displayed the strongest activity in catalyzing the sulfation of 6-OH-Mel and 4-OH-Ram, whereas SULT1C4 exhibited the strongest sulfating-activity towards NAS. pH-dependence and kinetic parameters of these SULT enzymes in mediating the sulfation of respective drug compounds were determined. A metabolic labeling study showed the generation and release of [³⁵S]sulfated 6-OH-Mel, NAS, and 4-OH-Ram by HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells labeled with [³⁵S]sulfate in the presence of these drug compounds. Cytosols of human lung, liver, kidney and small intestine were examined to verify the presence of 6-OH-Mel-, NAS-, and 4-OH-Ram-sulfating activity *in vivo*. Of the four human organ samples tested, small intestine and liver cytosols displayed considerably higher 6-OH-Mel-, NAS-, and 4-OH-Ram-sulfating activities than those of lung and kidney.

3. Collectively, these results provided a molecular basis for the metabolism of 6-OH-Mel, NAS, and 4-OH-Ram through sulfation.

Keywords

6-Hydroxymelatonin; N-acetylserotonin; 4-hydroxyramelteon; sulfation; SULT

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Introduction

Insomnia is an increasingly common disorder among the general population due to the progressive aging and stressful life of the society (Silber, 2005). Melatonin (N-acetyl-5-methoxytryptamine), a neurohormone secreted primarily by the pineal gland (Maronde et al., 2011), is known to be involved in the regulation of circadian rhythms (Bubenik, 2002). As such, exogenous melatonin has been utilized for the management of circadian rhythm sleep disorders and insomnia (Costello et al., 2014). Ramelteon, an agonist of melatonin with selectivity for melatonin receptors MT1 and MT2 and higher binding affinities than melatonin, has been in use for the treatment of insomnia since 2005 (Uchikawa et al., 2002; Buysse et al., 2005; Kato et al., 2005; Slominski et al., 2012).

In regard to its pharmacokinetics, melatonin has been reported to be metabolized differentially in different tissues. In the liver, melatonin has been shown to be metabolized by CYP1A2 and CYP2C19 to form 6-hydroxymelatonin (6-OH-Mel) and N-acetylserotonin (NAS), with the former being a major metabolite (Härtter et al., 2001; Li et al., 2013). (It is noted that NAS is also a precursor of melatonin. Serotonin (5-HT) acetylation as catalyzed by arylalkylamine N-acetyltransferase leads to the formation of NAS (Coon et al., 1995). NAS is then methylated to form melatonin under the action of hydroxyindole-O-methyltransferase (Yu and Reiter, 1993; Bernard et al., 1995).) In the brain and skin, melatonin has been shown to be metabolized through indolic and/or kynuric pathways (Hirata et al., 1974; Hardeland et al., 1993; Tan et al., 2007; Slominski et al., 2008). The indolic pathway leads to the formation of 6-OH-Mel and 5-methoxytryptamine, as well as a number of downstream metabolites (Slominski et al., 2008). In contrast, the kynuric pathway generates nonenzymatically, among other metabolites, N¹-acetyl-N²-formyl-5-methoxykynuramine. Of these various metabolites, 6-OH-Mel and NAS have been shown to be further metabolized to form sulfate or glucuronide conjugates as final excreted products. That almost 80% of the conjugates detected in human urine were sulfated 6-OH-Mel implies that sulfation plays a major role in the metabolism of melatonin in humans (Cavallo and Dolan, 1996; Griefahn et al., 2003; Li et al., 2013). In a recent study, several human SULTs, including SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1E1 were found to be capable of sulfating 6-OH-Mel and NAS (Tian et al., 2015). Less is known about the metabolism of ramelteon. Using human liver microsomes, 4-hydroxyramelteon (4-OH-Ram) has been shown to be one of the metabolites of ramelteon (Obach and Ryder, 2010). Whether 4-OH-Ram may be subjected to conjugation by sulfation, however, has not been studied. **Figure 1** shows the chemical structures of 6-OH-Mel, NAS, and 4-OH-Ram.

Sulfate conjugation is a major Phase II metabolic pathway for the biotransformation and excretion of drugs/xenobiotics as well as for the homeostatic regulation of key endogenous compounds such as steroid and thyroid hormones, catecholamines, cholesterol, and bile acids (Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994). The SULTs are the responsible enzymes that 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).

We report in this communication a systematic analysis of the sulfating activity of the thirteen known human SULTs toward 6-OH-Mel, NAS, and 4-OH-Ram. pH-dependence and kinetic parameters of those SULTs that showed the strongest sulfating activity toward each of the tested drug compounds were determined. HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells were used to investigate whether sulfation of 6-OH-Mel, NAS, and 4-OH-Ram may take place in cultured cells. Moreover, cytosols of human lung, liver, kidney and small intestine were examined to verify the presence of 6-OH-Mel, NAS, and 4-OH-Ram-sulfating activity in *vivo*.

Materials and Methods

Materials

6-OH-Mel, NAS and 4-OH-Ram were products of Toronto Research Chemicals (Toronto, Ontario, Canada). Adenosine 5'-triphosphate (ATP), 3'-phosphoadenosine 5'-phosphosulfate (PAPS), 2-morpholinoethanesulfonic acid (MES), N-2-hydroxypiperazine-N'-2-ethanesulfonic acid (HEPES), 3-N-tris-hydroxymethyl methylamino-propanesulfonic acid (TAPS), 2-cyclohexylamino ethanesulfonic acid (CHES), 3-cyclohexylamino-1-propanesulfonic acid (CAPS), Trizma base, dithiothreitol (DTT), minimum essential medium (MEM), fetal bovine serum (FBS), penicillin G, streptomycin sulfate, and silica gel thin-layer chromatography (TLC) plates were from Sigma Chemical Company (St. Louis, MO, USA). Carrier-free sodium [³⁵S]sulfate was from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA); and Ecolume scintillation cocktail was from MP Biomedicals (Irvine, CA, USA). Ultra free-MC 5000 NMWL filter units were products of Millipore Corporation (Bedford, MA, USA). HepG2 human hepatoma cells (ATCC HB-8065) and Caco-2 human colon adenocarcinoma cells (ATCC HTB-37) were obtained from American Type Culture Collection (Manassas, VA, USA). Pooled human lung, liver, small intestine, and kidney cytosols were purchased from XenoTech, LLC (Lenexa, KS, USA). All other chemicals used were of the highest grade commercially available.

Metabolic labeling of HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells

HepG2 cells and Caco-2 cells were routinely maintained, under a 5% CO₂ atmosphere at 37°C, in MEM supplemented with 10% FBS, penicillin G (30 mg/mL) and streptomycin sulfate (50 mg/mL). Confluent cells grown in individual wells of a 24-well culture plate, pre-incubated in sulfate-free (prepared by omitting streptomycin sulfate and replacing magnesium sulfate with magnesium chloride) MEM without FBS for 4 hours, were labeled with 0.20 mL aliquots of the same medium containing [³⁵S]sulfate (0.3 mCi/mL) and different concentrations (0, 1, 5, 10, 25, and 50 μM) of 6-OH-Mel, NAS, or 4-OH-Ram. At the end of an 18-hour labeling period, the labeling media were collected, spin-filtered to remove high-molecular weight [³⁵S]sulfated macromolecules, and the supernatant was subjected to TLC for the analysis of [³⁵S]sulfated, using n-butanol/isopropanol/88% formic

acid/water in a ratio of 2:2:3:1 (by volume) as the solvent system. Upon completion of TLC, the TLC plate was air-dried and subjected to autoradiography by using X-ray film.

Preparation of purified human SULTs

Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, the thyroid hormone SULT (SULT1B1), three SULT1Cs (SULT1C2, SULT1C3, and SULT1C4), the estrogen SULT (SULT1E1), the dehydroepiandrosterone (DHEA) SULT (SULT2A1), two SULT2B1s (SULT2B1a and SULT2B1b), a neuronal SULT (SULT4A1) and the SULT6B1, expressed using pGEX-2TK or pET23c prokaryotic expression system, were prepared as described previously (Sakakibara et al., 1998; Yanagisawa et al., 1998; Suiko et al., 2000; Pai et al., 2002; Sakakibara et al., 2002).

SULT assay

The sulfating activity of the recombinant human SULTs was assayed using PAP[³⁵S] as the sulfonate group donor. The standard assay mixture, in a final volume of 20 μ L, contained 50 mM of HEPES buffer at pH 7.0, 1 mM DTT, and 14 μ M PAP[³⁵S]. The substrate, at 10 times the final concentration (10 μ M) in the assay mixture, was added after HEPES buffer and PAP[³⁵S]. The reaction was started by the addition of the SULT enzyme, allowed to proceed for 10 min at 37°C and terminated by placing the thin-walled tube containing the assay mixture on a heating block, pre-heated to 100°C, for 3 min. The precipitates formed during heating, were cleared by centrifugation at 13,000 rpm for 3 min, and the supernatant was subjected to the analysis of [³⁵S]sulfated product using the TLC procedure with silica gel plates and n-butanol/acetonitrile (3:2; by volume) as the solvent system for NAS and 6-OH-Mel, or cellulose plates and n-butanol/isopropanol/88% formic acid/water(2:2:3:1) as the solvent system for 4-OH-Ram. 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, cut out and eluted in 0.5 mL water in a glass vial. 2 mL of Ecolume scintillation liquid was added to each vial, mixed thoroughly and the radioactivity therein was counted using a liquid scintillation counter. To examine the pH-dependence of the sulfation of 6-OH-Mel, NAS, and 4-OH-Ram by relevant human SULTs, different buffers (sodium acetate at 4.5, 5.0, or 5.5; MES at 6.0 or 6.5; HEPES at 7.0, 7.5, or 8.0; TAPS at 8.5; CHES at 9.0 or 10.0 and CAPS at 9.5, 10.5, 11.0, or 11.5), instead of 50 mM HEPES (pH 7.0), were used in individual reactions. Each experiment was performed in triplicate, together with two different controls, one without enzyme and the other without substrate. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme. To assay for the sulfating activity of human organ cytosols, the reaction mixture was supplemented with 50 mM NaF (a phosphatase inhibitor). The reaction was started by the addition of the cytosol (containing 10 μ g of proteins) and allowed to proceed for 30 min, followed by the TLC analysis for [³⁵S]sulfated compounds as described above.

Kinetic analysis

In the kinetic studies on the sulfation of 6-OH-Mel, NAS, and 4-OH-Ram, the sulfation assays were carried out using varying concentrations (1, 1.25, 1.67, 2.5, 5, and 10 μ M) of 6-OH-Mel, NAS, or 4-OH-Ram, with 50 mM HEPES at pH 7.0 according to the procedure

described above. Data obtained were analyzed based on Michaelis-Menten kinetics using KaleidaGraph 4.1 (Synergy Software Inc., PA, USA) and non-linear regression.

Miscellaneous methods

PAP[³⁵S] was synthesized from ATP and carrier-free [³⁵S]sulfate using the bifunctional human ATP sulfurylase/adenosine 5'-phosphosulfate kinase, and its purity was determined as described previously (Yanagisawa et al., 1998). The PAP [³⁵S] synthesized was adjusted to the required concentration and a specific activity of 15 Ci/mmol at 1.4 mM by the addition of cold PAPS. Protein determination was based on the method of Bradford with bovine serum albumin as the standard (Bradford, 1976).

Results

Identification of the human SULTs capable of sulfating 6-OH-Mel, NAS, and/or 4-OH-Ram

To identify the enzymes that are capable of sulfating 6-OH-Mel, NAS and/or 4-OH-Ram, thirteen known human SULTs previously cloned, expressed, and purified, were examined for sulfating activity with 6-OH-Mel, NAS, and 4-OH-Ram as substrates. Activity data obtained are compiled in **Table 1**. Results showed that seven (SULT1C2, SULT1C3, SULT2A1, SULT2B1a, SULT2B1b, SULT4A1 and SULT6B1) of the 13 SULTs displayed no detectable activities toward any of the three compounds. Of the other six SULTs (SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C4, and SULT1E1), SULT1A1 exhibited the strongest sulfating activities toward 6-OH-Mel and 4-OH-Ram, whereas SULT1C4 displayed the strongest sulfating activity toward NAS. These results implied that SULT1A1 is likely the major SULT involved in the sulfation of 6-OH-Mel and 4-OH-Ram, while SULT1C4 is a major enzyme responsible for the sulfation of NAS.

pH-dependence of the sulfation of 6-OH-Mel, NAS and 4-OH-Ram by relevant human SULTs

pH-dependence of the sulfation of 6-OH-Mel and 4-OH-Ram by SULT1A1, and that of the sulfation of NAS SULT1C4 were analyzed. As shown in **Figure 2**, for all three compounds tested, the responsible SULTs showed sulfating activity over a broad pH range (pH 6.5 - 10.5 or 11). It appeared that the pH profiles conformed to bell-shaped curves for all three pairs of substrate/SULT tested.

Kinetics of the sulfation of 6-OH-Mel, NAS and 4-OH-Ram by relevant human SULTs

The kinetics of the the sulfation of 6-OH-Mel and 4-OH-Ram by SULT1A1 and that of the sulfation of NAS by SULT1C4 were analyzed using varying concentrations of these three drug compounds as substrates. Saturation curve analyses were examined using non-linear regression. Data on the sulfation of 6-OH-Mel, NAS and 4-OH-Ram by the SULTs tested were fitted to hyperbolic kinetic curves (Michaelis-Menten kinetics), which were further verified by linear Eadie-Hofstee plots. **Figure 3** shows the Lineweaver-Burk double-reciprocal plots of the sulfation of each of the three compounds by respective SULT enzymes. **Table 2** shows the kinetic constants derived from these experiments.

Generation and release of [³⁵S]sulfated products by HepG2 and Caco-2 cells labeled with [³⁵S]sulfate in the presence of 6-OH-Mel, NAS, or 4-OH-Ram

HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells were used to investigate whether sulfation of 6-OH-Mel, NAS and 4-OH-Ram may occur under the metabolic conditions. As shown in **Figure 4**, the autoradiographs taken from the TLC plates used for the analysis of spent media of HepG2 cells or Caco-2 cells labeled with [³⁵S]sulfate in the presence of increasing concentrations (0, 5, 10, 25, and 50 μM) of 6-OH-Mel, NAS, or 4-OH-Ram showed clearly the generation and release of their [³⁵S]sulfated derivatives in a concentration-dependent manner.

Sulfation of 6-OH-Mel, NAS, and 4-OH-Ram by human organ samples

To verify the presence of 6-OH-Mel-, NAS-, and 4-OH-Ram-sulfating activity in human tissues, enzymatic assays were performed using cytosols prepared from human lung, liver, kidney or small intestine. Activity data obtained are compiled in **Table 3**. Of the four human organ samples tested, small intestine and liver cytosols exhibited much stronger sulfating activities than lung and kidney cytosols toward all three compounds.

Discussion

Previous studies have revealed that sulfation represents an important pathway involved in the metabolism of melatonin in humans (Cavallo and Dolan, 1996; Härtter et al., 2001; Griefahn et al., 2003; Li et al., 2013; Tian et al., 2015). For ramelteon, while a hydroxylated metabolite, 4-hydroxyramelteon, has been reported (Obach and Ryder, 2010), the involvement of sulfation has not yet been investigated. The current study was designed to systematically analyze the human SULT(s) for sulfating activity toward 6-OH-Mel, NAS, and 4-OH-Ram and to verify the presence of 6-OH-Mel-, NAS-, and 4-OH-Ram-sulfating activity in cultured cells and in cytosols prepared from human organs.

As mentioned earlier, a previous study had demonstrated that several human SULTs, including SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1E1 were found to be capable of sulfating 6-OH-Mel and NAS (Tian et al., 2015). In the current study, we showed that while SULT1C4 was also capable of sulfating 6-OH-Mel and NAS. While SULT1A1 showed the strongest sulfating activity toward 6-OH-Mel, SULT1C4 exhibited the strongest activity toward NAS. For 4-OH-Ram, SULT1A1 appeared to be the major enzyme responsible for its sulfation. SULT1A1 is known to be expressed in human liver, lung, kidney, brain, skin, platelets, and gastrointestinal tract (Sundaram et al., 1989; Cappiello et al., 1990; Hempel et al., 2005); whereas SULT1C4 has been shown to be expressed at higher levels in human fetal lung, liver, small intestine and kidney, and at lower levels in adult kidney, ovary and spinal cord (Sakakibara et al., 1998; Sakakibara et al., 2002). It is conceivable that SULT1A1 and SULT1C4, as well as some other SULTs, may act on 6-OH-Mel, NAS, and 4-OH-Ram to convert them to sulfated derivatives in different human tissues/organs in which they are expressed. Upon further characterization of the 6-OH-Mel-, NAS-, and/or 4-OH-Ram-sulfating activity of SULT1A1 and SULT1C4, it was noted that both enzymes were active over a wide range of pH, implying that these enzymes may be active toward the three compounds in cells under different metabolic states with varying pH

environment. Kinetic experiments were performed to gain insight into the mechanism of the sulfation of 6-OH-Mel, NAS, and 4-OH-Ram by relevant human SULTs. It appeared that both SULT1A1 and SULT1C4 followed Michaelis-Menten kinetics in catalyzing the sulfation of 6-OH-Mel, NAS, and/or 4-OH-Ram. Based on V_{max}/K_m , the catalytic efficiency of SULT1A1 in mediating the sulfation of 6-OH-Mel was a order of magnitude higher than the SULT1A1-mediated sulfation of 4-OH-Ram or SULT1C4-mediated sulfation of NAS.

HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells were used to investigate whether sulfation of 6-OH-Mel, NAS and 4-OH-Ram may occur under the metabolic conditions. It was worth point out HepG2 human hepatoma cells are known to express several human SULTs including SULT1A1, SULT1A2, SULT1A3, SULT1E1 and SULT2A1 (Shwed et al., 1992; Dooley et al., 2000; Chen et al., 2005; Miyano et al., 2005; Stanley et al., 2005; Li et al., 2007; Westerink and Schoonen, 2007); whereas Caco-2 human colon adenocarcinoma cells are known to express SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C2, SULT1C4 and SULT2A1 (Dooley et al., 2000; Teubner et al., 2007; Riches et al., 2009). A metabolic labeling study initially performed showed indeed the generation and release of sulfated 6-OH-Mel, NAS, and 4-OH-Ram, indicating that all of them can be taken up by HepG2 cells and Caco-2 cells, and then subjected to the sulfation by the SULT enzyme(s) therein. An ensuing question is whether sulfation of 6-OH-Mel, NAS, and 4-OH-Ram may occur in the liver and intestine, as well as other organs in the body. Enzymatic assays using the cytosols prepared from the human lung, liver, kidney and small intestine showed indeed the presence of 6-OH-Mel-/NAS-/4-OH-Ram-sulfating activity in lung, liver, and small intestine. Interestingly, the small intestine and liver sample showed higher sulfating activity towards all of these three compounds. These latter results are in agreement with previous studies showing the high levels of expression of SULT enzymes in the small intestine and the liver, whereas the kidney and lungs contained only low levels of SULTs (Riches et al., 2009).

In short, the current study demonstrated that among the thirteen known human SULTs, SULT1A1 displayed the strongest sulfating activity towards 6-OH-Mel and 4-OH-Ram, while SULT1C4 exhibited strongest sulfating activity towards NAS. Metabolic labeling experiments showed that the drug compounds tested could be sulfated by HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells. Of the four human organ specimens tested, liver and intestine cytosols showed strong sulfating activity toward these drug compounds. Collectively, these results imply that SULT-mediated sulfation plays an important role in the metabolism of melatonin and ramelteon, the melatonin receptor agonist.

Abbreviations

6-OH-Mel	6-Hydroxymelatonin
NAS	N-acetylserotonin
4-OH-Ram	4-hydroxyramelteon
PAPS	3'-phosphoadenosine 5'-phosphosulfate

CAPS	3-cyclohexylamino-1-propanesulfonic acid
HEPES	N-2-hydroxylpiperazine-N'-2-ethanesulfonic acid
TAPS	3-N-tris-hydroxymethyl methylamino-propanesulfonic acid
CHES	2-cyclohexylamino ethanesulfonic acid
MES	morpholinoethanesulfonic acid
SULT	cytosolic sulfotransferase
TLC	thin-layer chromatography

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A) 6-OH-Mel **B) NAS** **C) 4-OH-Ram**

Figure 1.
Chemical structures of 6-OH-Mel, NAS, and 4-OH-Ram.

Figure 2. pH-dependence of 6-OH-Mel-, NAS-, and 4-OH-Ram-sulfating activity of relevant human SULTs

Enzymatic assays were carried out under standard assay conditions as described in the **Materials and Methods** section using different buffer systems as indicated. Data shown represent calculated mean $\pm$ standard deviation derived from three independent experiments.

Figure 3.
Lineweaver-Burk double-reciprocal plots of the sulfation of A) 6-OH-Mel, B) NAS, and C) 4-OH-Ram by, respectively, human SULT1A1, SULT1C4, and SULT1A1.

Figure 4. Analysis of the [^{35}S]sulfated product generated and released by HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells labeled with [^{35}S]sulfate in the presence of different concentrations of 6-OH-Mel, NAS, or 4-OH-Ram

Confluent HepG2 cells and Caco-2 cells were incubated in labeling media containing, respectively, 10, 25, 50, and 100 μM (corresponding to lanes 1-4) of 6-OH-Mel, NAS or 4-OH-Ram. At the end of an 18-hour labeling, spent labeling media were collected and analyzed by thin-layer chromatography. The figure shows the autoradiographs taken from the plates at the end of the TLC analysis. C refers to the control labeling medium without

tested compounds. The arrows indicate radioactive spots corresponding to [^{35}S]sulfated 6-OH-Mel, NAS or 4-OH-Ram.

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Table 1

Specific activities of human SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C4, and SULT1E1 with 6-OH-Mel, NAS and 4-OH-Ram as substrates^a

Substrate	Specific Activity (nmol/min/mg)					
	SULT1A1	SULT1A2	SULT1A3	SULT1B1	SULT1C4	SULT1E1
6-OH-Mel	25.10±0.53	1.33±0.04	11.97±0.49	0.32±0.02	3.94±0.07	1.65±0.20
NAS	2.86±0.01	ND ^b	0.27±0.01	0.59±0.05	5.87±0.22	ND
4-OH-Ram	2.82±0.02	ND	0.17±0.04	ND	0.27±0.01	0.56±0.02

^a Specific activity refers to nmol substrate sulfated/min/mg purified enzyme. Data shown represent means ± SD derived from three experiments. The concentration of the substrate used in the assay mixture was 10 µM.

^b ND refers to activity not detected. Specific activity determined was lower than the detection limit (estimated to be less than 0.01 nmol/min/mg protein)

Table 2Kinetic constants of relevant SULTs in mediating the sulfation of 6-OH-Mel, NAS, and 4-OH-Ram^a

Substrate/SULT	K_m (μM)	V_{max} (nmol/min/mg)	V_{max}/K_m
6-OH-Mel/SULT1A1	8.20 $\pm$ 0.38	39.53 $\pm$ 1.85	4.82
NAS/SULT1C4	45.68 $\pm$ 6.23	32.36 $\pm$ 4.63	0.71
4-OH-Ram/SULT1A1	19.15 $\pm$ 1.72	7.86 $\pm$ 0.76	0.41

^aData shown represent means $\pm$ SD derived from three determinations.

Table 3

Sulfating activities of human lung, liver, kidney, and small intestine cytosol or S9 fractions with 6-OH-Mel, NAS, and 4-OH-Ram as substrates^a

Substrate	Specific Activity (pmol/min/mg)			
	Lung	Liver	Kidney	Small Intestine
6-OH-Mel	18.00±2.18	128.31±6.13	10.26±0.33	180.93±8.64
NAS	1.29±0.52	25.40±1.30	3.56±0.13	24.04±4.09
4-OH-Ram	1.30±0.26	13.42±0.81	0.80±0.01	23.94±0.13

Specific activity refers to pmol substrate sulfated/min/mg purified enzyme. Data represent mean ± SD derived from three determinations. The concentration of the substrate used in the assay mixture was 50 µM.

^aND refers to activity not detected. Specific activity determined was lower than the detection limit (estimated to be ~0.01 pmol/min/mg protein)