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Sulfation of benzyl alcohol by the human cytosolic sulfotransferases (SULTs): A systematic analysis

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Abstract

The current study aimed to identify human cytosolic sulfotransferases (SULTs) that are capable of sulfating benzyl alcohol and to examine whether benzyl alcohol sulfation may occur in cultured human cells as well as in human organ homogenates. A systematic analysis revealed that of the thirteen known human SULTs, SULT1A1, SULT1A2, SULTA3, and SULT1B1 were capable of mediating the sulfation of benzyl alcohol. The kinetic parameters of SULT1A1 that showed the strongest benzyl alcohol-sulfating activity were determined. HepG2 human hepatoma cells were used to demonstrate the generation and release of sulfated benzyl alcohol under the metabolic settings. Moreover, the cytosol or S9 fractions of human liver, lung, kidney, and small intestine were examined to verify the presence of benzyl alcohol sulfating activity *in vivo*.

Keywords

Benzyl alcohol; sulfation; cytosolic sulfotransferase; SULT; human

Introduction

Benzyl alcohol, an aromatic alcohol characterized by its polarity, relatively low toxicity and low vapor pressure, serves as a useful solvent for a variety of industrial applications. In the pharmaceutical industry, it is included as a bacteriostatic agent in many parenteral preparations, including multi-dose vials of bacteriostatic saline or water used to flush intravascular catheters or to reconstitute medications [Chang et al., 2008]. In the cosmetic industry, it is used as a fragrance component, preservative, solvent, and viscosity-decreasing agent [Nair, 2001]. Due to its various uses, exposure pathways may include oral consumption, dermal contact, intravenous injection, and inhalation. Upon oral exposure, benzyl alcohol is absorbed rapidly through the gastro-intestinal tract. Absorbed benzyl

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alcohol is oxidized to benzoic acid, followed by conjugation with glycine in the liver and the kidney to form hippuric acid, which is subsequently excreted in the urine [Gruber, 1923; Brandt, 1966]. While the above-mentioned detoxification pathway is operated in adults, it has been shown to be deficient in premature neonates [LeBel et al., 1988]. The question then is whether premature neonates, and possibly infants and young children as well, may be equipped with other pathway(s) capable of metabolizing/detoxifying benzyl alcohol. In regard to the Phase II drug-metabolizing/detoxifying enzymes, earlier human studies showed that sulfation as mediated by the SULTs appears to be more important for the detoxification of xenobiotics in fetal development [Barker et al., 1994; Darras et al., 1999; Richard et al., 2001; Duanmu et al., 2006], since other conjugating enzymes such as the UDP-glucuronosyltransferases are not yet expressed at significant levels [Barker et al., 1994; Darras et al., 1999; Richard et al., 2001; Duanmu et al., 2006].

Sulfate conjugation as mediated by the SULTs is known to be a major pathway for the biotransformation and excretion of drugs/xenobiotics in humans and other mammals [Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994]. The 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 cause the inactivation of the substrate compounds and/or the increase their water-solubility, thereby facilitating their removal from the body [Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994]. In humans, thirteen SULTs that are classified into four distinct gene families have been identified [Yamazoe et al., 1994; Blanchard et al., 2004]. Eight of the thirteen human SULTs that belong to the SULT1 gene family are SULT1A1 and SULT1A2 (both believed to be general detoxifying enzymes), SULT1A3 (a dopamine/catecholamine sulfotransferase), SULT1B1 (a thyroid hormone sulfotransferase), three SULT1Cs (SULT 1C2, SULT1C3, and SULT1C4) and SULT1E1 (an estrogen sulfotransferase). Three that belong to the SULT2 gene family are SULT2A1 (the dehydroepiandrosterone sulfotransferase), SULT2B1a (a pregnenolone sulfotransferase) and SULT2B1b (a cholesterol sulfotransferase). Of the two remaining SULTs, one (a neuronal/brain sulfotransferase) belongs to the SULT4 gene family and the other, which remains to be characterized, belongs to the SULT6 gene family.

We report in this communication a systematic analysis of the sulfating activity of all known human SULTs toward benzyl alcohol. The kinetic parameters of those SULTs that displayed strong sulfating activity toward benzyl alcohol were determined. A metabolic labeling study was performed using cultured HepG2 cells. Moreover, the homogenates of liver, kidney, lung, and intestine were evaluated to verify the presence of benzyl alcohol-sulfating activity *in vivo*.

Materials and Methods

Materials

Benzyl alcohol (98% in purity), adenosine 5'-triphosphate (ATP), 3'-phosphoadenosine-5'-phosphosulfate (PAPS), N-2-hydroxylpiperazine-N'-2-ethanesulfonic acid (HEPES), Trizma base, dithiothreitol (DTT), minimum essential medium (MEM), fetal

bovine serum (FBS), penicillin G, and streptomycin sulfate were products of Sigma Chemical Company (St. Louis, MO). Ultrafree-MC 5000 NMWL filter units and cellulose thin-layer chromatography (TLC) plates were from EMD Millipore (Billerica, MA). HepG2 human hepatoma cells (ATCC HB-8065) and Caco-2 human colon adenocarcinoma cells (ATCC HTB-37) from American Type Culture Collection (Manassas, VA). Pooled human lung S9 fraction from a mixed-gender group of 4 donors (Lot No. 0710281), liver cytosol from 50 donors (Lot No. 09103970), small intestine (duodenum and jejunum) S9 fraction from 18 donors (Lot No. 0710351), and kidney S9 fraction from 8 donors (Lot No. 0510093) were obtained from XenoTech, LLC (Lenexa, KS). Ecolume scintillation cocktail and carrier-free sodium [³⁵S]sulfate were from MP Biomedicals, LLC, (Irvine, CA, USA). All other chemicals 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 µg/ml) and streptomycin sulfate (50 µg/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 labelled with 0.25 ml aliquots of the same medium containing [³⁵S]sulfate (0.3 mCi/ml) and 0, 10, 25, 50, 75, 100, 250, 500, or 1000 µM of benzyl alcohol. 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 subjected to cellulose TLC for the analysis of [³⁵S]sulfated benzyl alcohol, using n-butanol/isopropanol/88% formic acid/water (3:1:1:1; by volume) as the solvent system.

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 a SULT6B1, expressed using the pGEX-2TK or pET23c prokaryotic expression system, were prepared as described previously [Sakakibara et al., 1998a; Sakakibara et al., 1998b; 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 sulfate group donor. The standard assay mixture, in a final volume of 20 µl, contained 50 mM of HEPES buffer at pH 7.0, 1 mM DTT and 14 µM PAP[³⁵S]. Stock solution of benzyl alcohol, at 10 times the final concentration (50 µM) 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 were cleared by centrifugation at 13,000 rpm for 3 min, and the supernatant was subjected to the analysis of [³⁵S]sulfated product by TLC with nbutanol/isopropanol/88%

formic acid/water (3:1:1:1; by volume) as the solvent system. On completion of TLC, the TLC plate was air dried and autoradiographed using X-ray film. The radioactive spot corresponding to [^{35}S]sulfated benzyl alcohol was located on the TLC plate, 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 using a liquid scintillation counter. Each experiment was performed in triplicate, together with a control lacking substrate. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme. To assay for benzyl alcohol-sulfating activity of human tissue cytosol or S9 fraction, the reaction mixture was supplemented with 50 mM NaF (a phosphatase inhibitor). The reaction was started by the addition of the cytosol or S9 fraction and allowed to proceed for 30 min, followed by the TLC analysis for [^{35}S]sulfated benzyl alcohol as described above.

Kinetic analysis

In the kinetic studies on the sulfation of benzyl alcohol, the sulfation assays were carried out using varying concentrations of benzyl alcohol and 50 mM HEPES at pH 7.0 according to the procedure described earlier. Data obtained were analyzed based on Michaelis-Menten kinetics using GraphPad Prism5 software and non-linear regression.

Miscellaneous methods

PAP[^{35}S] was synthesized from ATP and carrier-free [^{35}S]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[^{35}S] 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

Differential sulfating activities of the human SULTs toward benzyl alcohol

To identify the enzyme(s) that is (are) responsible for the sulfation of benzyl alcohol, a systematic survey of the benzyl alcohol-sulfating activity of the 13 known human SULTs was performed. Results obtained showed that nine (SULT1C2, SULT1C3, SULT1C4, SULT1E1, SULT2A1, SULT2B1a, SULT2B1b, SULT4A1 and SULT6B1) of the 13 SULTs displayed no detectable activities. Of the other four SULTs, SULT1A1 exhibited considerably stronger activities than the other three (SULT1A2, SULT1A3 and SULT1B1) toward benzyl alcohol (**Table 1**).

Kinetics of the sulfation of benzyl alcohol by the relevant human SULTs

The kinetics of the sulfation of benzyl alcohol by SULT1A1 was further analyzed by performing sulfotransferase assays using varying concentrations of benzyl alcohol as the substrate. To obtain the kinetic parameters, saturation curve analyses were performed using non-linear regression. The sulfation of benzyl alcohol by SULT1A1 was fitted to hyperbolic kinetic curves (Michaelis-Menten kinetics; cf. **Figure 1**), and was further confirmed by a

linear Eadie–Hofstee plot. **Table 2** shows the calculated Michaelis-Menten kinetic constants of the sulfation of benzyl alcohol by human SULT1A1.

Generation and release of [³⁵S]sulfated benzyl alcohol by HepG2 cells and Caco-2 cells labeled with [³⁵S]sulfate in the presence of benzyl alcohol

HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells were used to investigate whether sulfation of benzyl alcohol may occur under metabolic conditions. Confluent HepG2 cells grown in individual wells of a 24-well plate respectively were labeled with [³⁵S]sulfate in sulfate-free medium containing different concentrations (0, 10, 25, 50, 75, 100, 250, 500, and 1000 μ M) of benzyl alcohol. TLC analysis of the labeling media collected at the end of an 18-hour labeling period revealed the presence of [³⁵S]sulfated benzyl alcohol in a concentration-dependent manner (**Figure 2**). A similar metabolic labeling experiment was performed using Caco-2 cells. TLC analysis of the labeling media collected at the end of the labeling period, however, failed to show the presence of [³⁵S]sulfated benzyl alcohol (figure not shown). These results, therefore, indicated differential metabolism of benzyl alcohol through sulfation in the two types of cells tested.

Sulfation of benzyl alcohol by human organ samples

To obtain evidence for the presence of benzyl alcohol-sulfating activity in human organs, enzymatic assays were performed using cytosol or S9 fraction prepared from human liver, kidney, lung or small intestine. Activity data obtained are compiled in **Table 3**. All four human organ samples displayed sulfating activity toward benzyl alcohol with 50 μ M of benzyl alcohol as substrate. Among the four, small intestine cytosol displayed the strongest benzyl alcohol-sulfating activity, followed by the liver cytosol. In contrast, kidney and lung samples showed considerably weaker benzyl alcohol-sulfating activity.

Discussion

The current study represents part of an overall effort to investigate whether SULT-mediated sulfation, which is known to be an important Phase II detoxification pathway during early life, may constitute an alternative route for the metabolism and detoxification of benzyl alcohol in neonates, infants, and young children.

In a systematic analysis of the thirteen known human SULTs, four (SULT1A1, SULT1A2, SULT1A3, and SULT1B1) were found to be capable of sulfating benzyl alcohol, with SULT1A1 displaying much stronger sulfating activity than the other three SULTs. These results indicated that SULT1A1 is likely the major enzyme responsible for sulfating benzyl alcohol in the body. It is noted that previous studies have demonstrated the expression of SULT1A1 in the human liver throughout the prenatal period [Duanmu et al., 2006], as well as in postnatal liver (albeit at a lower level) [Richard et al., 2001]. Moreover, SULT1A1 was shown to be present in human placenta during 13–42 weeks of gestation [Stanley et al., 2001]. These findings indicate that SULT1A1 is in place in both the developing fetus and the placenta for metabolizing benzyl alcohol which may be used by the pregnant mother for medical and/or cosmetic purposes and may pose a potential threat for the developing fetus. It

is interesting to note that the K_m of SULT1A1 with benzyl alcohol (68.5 μM) is much lower than that with ethyl alcohol (previously estimated to be in the mM range [Ko et al., 2012]). Moreover, the specific activity of SULT1A1 with benzyl alcohol (10.52 nmol/min/mg enzyme) is more than 2.5 times that (4.07 nmol/min/mg enzyme) with ethyl alcohol [Ko et al., 2012]. Sulfation of benzyl alcohol therefore appears to be much more catalytically efficient than the sulfation of ethyl alcohol. In relation to this latter point, SULT1A1 used to be referred to as a phenol sulfotransferase [Mulder and Jakoby, 1990; Falany and Roth, 1993; Weinshilboum and Otterness, 1994], displaying preference for aryl hydroxyl group over alkyl hydroxyl group. Previous studies have shown that SULT1A1 displayed a K_m ranging 0.6 - 4.0 μM and a V_{max} ranging 1.35 - 74 nmol/min/mg enzyme toward *p*-nitrophenol, a prototype substrate [Veronese et al., 1994; Wang et al., 2006; Riches et al., 2007]. It therefore seems that benzyl alcohol, while being a better substrate than ethyl alcohol, may not be as good a substrate as *p*-nitrophenol for SULT1A1.

To investigate the occurrence of benzyl alcohol sulfation under metabolic conditions, HepG2 human hepatoma cells and Caco-2 human colon adenocarcinoma cells, known to express human SULTs including SULT1A1, SULT1A2, and SULT1A3 [Westerink and Schoonen, 2007; Meinel et al., 2008], were labeled with [^{35}S]sulfate and challenged with benzyl alcohol. The results clearly indicated that HepG2 cells could take up benzyl alcohol, and the SULT enzymes inside the cells could mediate the sulfation of benzyl alcohol. No sulfation of benzyl alcohol by Caco-2 cells, however, was detected. This latter finding might have been due to the alternative metabolic pathways employed by Caco-2 cells or the poor penetration of benzyl alcohol into Caco-2 cells in order to be sulfated by the SULT enzymes therein. In conjunction with the cell culture study, the cytosol or S9 fractions prepared from human organs were tested for benzyl alcohol-sulfating activity. Results obtained indicated that of the four human organs samples tested, small intestine cytosol displayed the strongest benzyl alcohol-sulfating activity, followed by the liver cytosol. In contrast, kidney and lung samples showed considerably weaker benzyl alcohol-sulfating activity. These results thus indicated that human small intestine and liver are likely the major human organs involved in the metabolism of benzyl alcohol through sulfation.

In conclusion, the present study demonstrated that benzyl alcohol could be sulfated under the action of SULT enzymes, particularly SULT1A1. Kinetic analysis indicated that SULT1A1-mediated sulfation of benzyl alcohol appeared to be much more efficient than that of ethyl alcohol. Moreover, sulfation of benzyl alcohol was shown to occur in HepG2 cells under the metabolic setting as well as in the cytosol or S9 fractions of human liver, lung, kidney, and small intestine. That the major benzyl alcohol-sulfating SULT, SULT1A1, is widely expressed in fetal and neonatal liver [Duanmu et al., 2006] implies that fetuses and neonates are capable of metabolizing the benzyl alcohol through sulfation. In line with this latter notion, it has been reported that based on enzymatic assays, the level of SULT1A1 appeared to be lower in postnatal liver and lung than in fetal tissues [Richard et al., 2001]. Since the commonly known detoxification pathway involving the oxidation of benzyl alcohol to benzoic acid followed by conjugation with glycine [Gruber, 1923; Brandt, 1966] has been shown to be deficient in premature neonates [LeBel et al., 1988], it is possible that sulfation may indeed represent an important alternative for the detoxification of benzyl alcohol in neonates and during fetal development.

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Abbreviations

PAPS	3'-phosphoadenosine 5'-phosphosulfate
SULT	cytosolic sulfotransferase
TLC	thin-layer chromatography

References

- Barker EV, Hume R, Hallas A, Coughtrie MWH. Dehydroandrosterone sulfotransferase in the developing human fetus: Quantitative biochemical and immunological characterization of the hepatic, renal, and adrenal enzymes. *Endocrinology*. 1994; 134:982–989. [PubMed: 8299591]
- Blanchard RL, Freimuth RR, Buck J, Weinshilboum RM, Coughtrie MW. A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics*. 2004; 14:199–211. [PubMed: 15167709]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 1976; 72:248–254. [PubMed: 942051]
- Brandt IK. Glycine acyltransferase activity in developing rat liver. *Biochem. Pharmacol*. 1966; 15:994–995. [PubMed: 5967912]
- Chang YS, Wu CL, Tseng SH, Kuo PY, Tseng SY. *In vitro* benzyl alcohol cytotoxicity: implications for intravitreal use of triamcinolone acetonide. *Exp. Eye Res*. 2008; 86:942–950. [PubMed: 18420195]
- Darras VM, Hume R, Visser TJ. Regulation of thyroid hormone metabolism during fetal development. *Mol. Cell. Endocrinol*. 1999; 151:37–47. [PubMed: 10411318]
- Duanmu Z, Weckle A, Koukouritaki SB, Hines RN, Falany JL, Falany CN, Kocarek TA, Runge-Morris M. Developmental expression of aryl, estrogen, and hydroxysteroid sulfotransferases in pre- and postnatal human liver. *J. Pharmacol. Exp. Ther*. 2006; 316:1310–1317. [PubMed: 16339912]
- Falany, CN.; Roth, JA. Properties of human cytosolic sulfotransferases involved in the drug metabolism. In: Jeffery, HE., editor. *Human Drug Metabolism; From Molecular Biology to Man*. CRC Press; Boca Raton: 1993. p. 101-115.
- Gruber GM. The pharmacology of benzyl alcohol and its esters. *J. Lab. Clin. Med*. 1923; 9:92–112.
- Ko K, Kurogi K, Davidson G, Liu MY, Sakakibara Y, Suiko M, Liu MC. Sulfation of ractopamine and salbutamol by the human cytosolic sulfotransferases. *J. Biochem*. 2012; 152:275–283. [PubMed: 22763752]
- LeBel M, Ferron L, Masson M, Pichette J, Carrier C. Benzyl alcohol metabolism and elimination in neonates. *Dev. Pharmacol. Ther*. 1988; 11:347–356. [PubMed: 3229281]
- Lipmann F. Biological sulfate activation and transfer. *Science*. 1958; 128:575–580. [PubMed: 13580219]
- Meinl W, Ebert B, Glatt H, Lampen A. Sulfotransferase forms expressed in human intestinal Caco-2 and TC7 cells at varying stages of differentiation and role in benzo[a]pyrene metabolism. *Drug Metab Dispos*. 2008; 36:276–283. [PubMed: 17967930]
- Mulder, GJ.; Jakoby, WB. Sulfation in conjugation reactions. In: Mulder, GJ.; Jakoby, WB., editors. *Drug Metabolism*. Taylor and Francis; London: 1990. p. 107-161.
- Nair B. Final report on the safety assessment of benzyl alcohol, benzoic acid, and sodium benzoate. *Int. J. Toxicol*. 2001; 20(Suppl 3):23–50. [PubMed: 11766131]
- Pai TG, Sugahara T, Suiko M, Sakakibara Y, Xu F, Liu MC. Differential xenoestrogen-sulfating activities of the human cytosolic sulfotransferases: molecular cloning, expression, and purification of human SULT2B1a and SULT2B1b sulfotransferases. *Biochim. Biophys. Acta*. 2002; 1573:165–170. [PubMed: 12399026]

- Richard K, Hume R, Kaptein E, Stanley EL, Visser TJ, Coughtrie MWH. Sulfation of thyroid hormone and dopamine during human development: Ontogeny of phenol sulfotransferases and arylsulfatase in liver, lung, and brain. *J. Clin. Endocrinol. Metab.* 2001; 86:2734–2742. [PubMed: 11397879]
- Riches Z, Bloomer JC, Coughtrie MW. Comparison of 2-aminophenol and 4-nitrophenol as in vitro probe substrates for the major human hepatic sulfotransferase, SULT1A1, demonstrates improved selectivity with 2-aminophenol. *Biochem Pharmacol.* 2007; 74:352–358. [PubMed: 17506995]
- Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, Suiko M, Liu MC. Molecular cloning, expression, and characterization of novel mouse sulfotransferase that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. *J. Biol. Chem.* 1998a; 273:33929–33935. [PubMed: 9852044]
- Sakakibara Y, Takami Y, Nakayama T, Suiko M, Liu MC. Localization and functional analysis of the substrate specificity/catalytic domains of human M-form and P-form phenol sulfotransferases. *J. Biol. Chem.* 1998b; 273:6242–6247. [PubMed: 9497349]
- Sakakibara Y, Suiko M, Pai TG, Nakayama T, Takami Y, Katafuchi J, Liu MC. Highly conserved mouse and human brain sulfotransferases: molecular cloning, expression, and functional characterization. *Gene.* 2002; 285:39–47. [PubMed: 12039030]
- Stanley EL, Hume R, Visser TJ, Coughtrie MW. Differential Expression of Sulfotransferase Enzymes Involved in Thyroid Hormone Metabolism during Human Placental Development. *J. Clin. Endocrinol. Metab.* 2001; 86:5944–5955. [PubMed: 11739469]
- Suiko M, Sakakibara Y, Liu MC. Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. *Biochem. Biophys. Res. Commun.* 2000; 267:80–84. [PubMed: 10623578]
- Veronese ME, Burgess W, Zhu X, McManus ME. Functional characterization of two human sulphotransferase cDNAs that encode monoamine- and phenol-sulphating forms of phenol sulphotransferase: substrate kinetics, thermal-stability and inhibitor-sensitivity studies. *Biochem J.* 1994; 302:497–502. [PubMed: 8093002]
- Wang LQ, Lehmler HJ, Robertson LW, James MO. Polychlorobiphenyls are selective inhibitors of human phenol sulfotransferase 1A1 with 4-nitrophenol as a substrate. *Chem Biol Interact.* 2006; 159:235–246. [PubMed: 16413005]
- Weinshilboum, RM.; Otterness, DM. Sulfotransferase enzymes in conjugation-deconjugation reactions. In: Kaufman, FC., editor. *Drug Metabolism and Toxicity*. Springer-Verlag; Berlin: 1994. p. 45-78.
- Westerink WM, Schoonen WG. Phase II enzyme levels in HepG2 cells and cryopreserved primary human hepatocytes and their induction in HepG2 cells. *Toxicol. In Vitro.* 2007; 21:1592–1602. [PubMed: 17716855]
- Yamazoe Y, Nagata K, Ozawa S, Kato R. Structural similarity and diversity of sulfotransferases. *Chem. Biol. Interact.* 1994; 92:107–117. [PubMed: 8033246]
- Yanagisawa K, Sakakibara Y, Suiko M, Takami Y, Nakayama T, Nakajima H, Takayanagi K, Natori Y, Liu MC. cDNA cloning, expression, and characterization of the human bifunctional ATP sulfurylase/adenosine 5'-phosphosulfate kinase enzyme. *Biosci. Biotech. Biochem.* 1998; 62:1037–1040.

Figure 1.
Kinetic analysis of the sulfation of benzyl alcohol by human SULT1A1. The figure shows the saturation curve of the sulfation of benzyl alcohol by SULT1A1.

Figure 2.

Analysis of [^{35}S]Sulfated Product Generated and Released by HepG2 Human Hepatoma Cells Labeled with [^{35}S]Sulfate in the presence of Benzyl Alcohol. The figure shows the autoradiograph taken from the plate used for the TLC analysis of spent labeling media. Confluent cells were incubated in labeling media containing, respectively, 10, 25, 50, 75, 100, 250, 500, or 1000 μM (lanes 1-8) of benzyl alcohol for 18 hours. C refers to the control labeling medium without benzyl alcohol. E refers to [^{35}S]sulfated benzyl alcohol generated enzymatically using human SULT1A1. The arrows indicate the position of sulfated benzyl alcohol.

Table 1

Specific activities of human SULT1A1, SULT1A2, SULT1A3 and SULT1B1 with benzyl alcohol as a substrate^a

Substrate	Specific activity (nmol/min/mg)			
	SULT1A1	SULT1A2	SULT1A3	SULT1B1
Benzyl Alcohol	10.52 ± 0.36	1.88 ± 0.04	0.69 ± 0.07	0.08 ± 0.01

^aSpecific activity refers to nmol substrate sulfated/min/mg purified enzyme. Data represent means ± standard deviation derived from three experiments. The final concentration of the substrate used in the assay mixture was 50 µM.

Table 2

Kinetic constants of the sulfation of benzyl alcohol by human SULT1A1

	K_m (μM)	V_{max} (nmol/min/mg)	k_{cat} (min^{-1})	k_{cat}/K_m ($\text{min}^{-1}\text{mM}^{-1}$)
SULT1A1	68.5 ± 2.1	22.5 ± 0.8	0.77 ± 0.03	11.2

^a Data 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 towards benzyl alcohol as a substrate^a

Substrate	Specific Activity ((pmol/min/mg)			
	Lung	Liver	Kidney	Small Intestine
Benzyl alcohol	2.72 ± 0.11	15.45 ± 0.29	1.36 ± 0.07	17.74 ± 1.10

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