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Full paper

Sulfation of afimoxifene, endoxifen, raloxifene, and fulvestrant by the human cytosolic sulfotransferases (SULTs): A systematic analysis

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ABSTRACT

Previous studies demonstrated that sulfate conjugation is involved in the metabolism of three commonly used breast cancer drugs, tamoxifen, raloxifene and fulvestrant. The current study was designed to systematically identify the human cytosolic sulfotransferases (SULTs) that are capable of sulfating raloxifene, fulvestrant, and two active metabolites of tamoxifen, afimoxifene and endoxifen. A systematic analysis using 13 known human SULTs revealed SULT1A1 and SULT1C4 as the major SULTs responsible for the sulfation of afimoxifene, endoxifen, raloxifene and fulvestrant. Kinetic parameters of these two human SULTs in catalyzing the sulfation of these drug compounds were determined. Sulfation of afimoxifene, endoxifen, raloxifene and fulvestrant under metabolic conditions was examined using HepG2 human hepatoma cells and MCF-7 breast cancer cells. Moreover, human intestine, kidney, liver, and lung cytosols were examined to verify the presence of afimoxifene/endoxifen/raloxifene/fulvestrant-sulfating activity.

1. Introduction

Endocrine therapy, the most prevalent treatment for estrogen receptor-positive breast cancer, has been in use for more than a century (1). A great number of endocrine therapeutics have been developed in recent years, with selective estrogen receptor modulators (SERMs) and selective estrogen receptor down regulators (SERDs) being two major classes of these agents (1). Tamoxifen and raloxifene are both SERMs that act as antagonists of the estrogen receptor in breast tissue (1). Tamoxifen, the most extensively tested endocrine therapy drug, exerts its function via its active metabolites, 4-hydroxytamoxifen (afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen) (2–6). While tamoxifen antagonizes the effects of estrogen in breast tissue, it causes significant stimulation of uterine tissue (7). Raloxifene, on the other hand, lacks uterine stimulation and acts more selectively in antagonizing the effects of estrogens in the breast and endometrium (8). Fulvestrant (faslodex) is a SERD which is indicated for the treatment of estrogen receptor-positive metastatic breast cancer in postmenopausal women with disease progression following antiestrogen therapy (9). Both Phase I and Phase II enzymes have been reported to be involved in the metabolism of these drugs. Tamoxifen has been shown to be metabolized to N-desmethyltamoxifen by CYP3A enzymes (10) and to 4-hydroxytamoxifen and N-desmethyl-4-hydroxytamoxifen by CYP2D6 (11,12). These latter tamoxifen metabolites could be further subjected to sulfation and glucuronidation (13). Raloxifene has been reported to be metabolized by CYP3A4, and raloxifene glucuronides have been detected in human plasma (12,14). For fulvestrant, while CYP3A4-mediated

Abbreviations: ATP, adenosine 5′-triphosphate; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; DTT, dithiothreitol; FBS, fetal bovine serum; HEPES, N-2-hydroxylpiperazine-N′-2-ethanesulfonic acid; MEM, minimum essential medium; MES, morpholinoetha-nesulfonic acid; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, cytosolic sulfotransferase; TLC, thin-layer chromatography.

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metabolism was found using human liver microsomes, studies using human hepatocytes indicated that sulfate conjugation represented a more predominant pathway (15). Both sulfate and glucuronide metabolites of fulvestrant have been have been detected in feces of individuals administered with fulvestrant (16).

Sulfate conjugation is a major pathway operated in humans and other vertebrates for the biotransformation and excretion of drugs/xenobiotics as well as the homeostasis of key endogenous compounds such as steroid and thyroid hormones, catecholamine, cholesterol, and bile acids (17–19). 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 (20). 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 (17–19). Several human SULTs capable of sulfating afimoxifene, endoxifen, raloxifene and fulvestrant have been identified (13, 22–24; cf. Fig. 1 showing the chemical structures of these four drug compounds). To better understand the role of SULT-mediated sulfation in the pharmacokinetics of these drugs, however, a more systematic investigation is needed.

We report in this communication a systematic analysis of the sulfating activity of all known human SULTs toward afimoxifene, endoxifen, raloxifene and fulvestrant. The kinetic parameters of those SULTs that showed strongest sulfating activity toward the tested drugs were determined. A metabolic labeling study was performed using cultured HepG2 and MCF-7 cells. Moreover, the drug-sulfating activity of four major human organ specimens was evaluated.

2. Materials and methods

2.1. Materials

Afimoxifene, endoxifen, raloxifene, fulvestrant, 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, streptomycin sulfate and silica thin-layer chromatography (TLC) plates were products of Sigma Chemical Company (St. Louis, MO). Ultrafree-MC 5000 NMWL filter units and cellulose TLC plates were products of EMD Millipore (Bedford, MA). HepG2 human hepatoma cell line (ATCC HB-8065) and MCF-7 breast cancer cell line (ATCC HTB-22) were from American Type Culture Collection (Manassas, VA). Pooled human small intestine (duodenum and jejunum), kidney, liver, and lung cytosols were purchased from XenoTech, LLC (Lenexa, KS). Ecolume scintillation cocktail was from MP Biomedical, LLC, (Irvine, CA). All other chemicals were of the highest grade commercially available.

2.2. Preparation of purified human SULTs

Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, thyroid hormone SULT (SULT1B1), two SULT1Cs (SULT1C2 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 (24–28).

2.3. SULT assay

The sulfating activity of the recombinant human SULTs was assayed using PAP[^{35}S] 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[^{35}S]. Stock solutions of the substrates, prepared in dimethyl sulfoxide, were used in the enzymatic assay. The substrate, at 10 times the final concentration (10 μM) in the assay mixture, was added after HEPES buffer and PAP[^{35}S]. The reaction was started by the addition of 0.5 μg 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 [^{35}S] sulfated product using the TLC with n-butanol/acetonitrile (3:2; by volume) for afimoxifene, endoxifen or raloxifene, or n-butanol/isopropanol/88% formic acid/water (3:1:1:1; by volume) for fulvestrant as the solvent system. Upon completion of TLC, the TLC plate was air dried and autoradiographed using an X-ray film. The radioactive spots corresponding to the sulfated products were

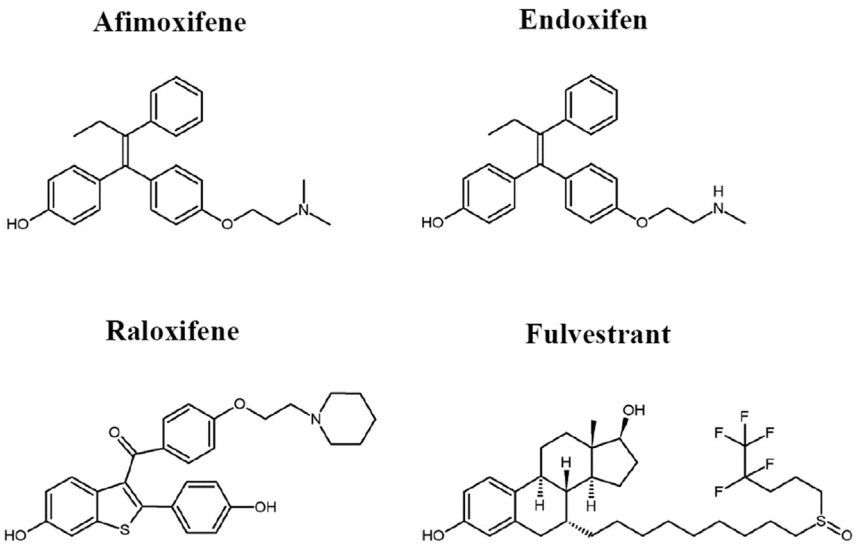

Fig. 1. Chemical structures of afimoxifene, endoxifen, raloxifene and fulvestrant.

located, cut out and eluted in 0.5 mL water in a glass vial. Afterwards, 4.5 mL of the Ecolume scintillation liquid was added to each vial, mixed thoroughly and the radioactivity therein was counted by using a liquid scintillation counter. Each assay was performed in triplicate, together with a control without enzyme. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme. To assay for afimoxifene, endoxifen, raloxifene or fulvestrant-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 and allowed to proceed for 20 min, followed by the TLC analysis for [^{35}S]sulfated product as described above.

2.4. Kinetic analysis

In the kinetic studies on the sulfation of afimoxifene, endoxifen and fulvestrant, the sulfation assays were carried out using varying concentrations of these substrate compounds and 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 software (Synergy Software Inc., PA) and non-linear regression.

2.5. Metabolic labeling of HepG2 human hepatoma cells and MCF-7 breast cancer cells

HepG2 cells and MCF-7 cells were routinely maintained, under a 5% CO_2 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 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 h, were labeled with 0.25 mL aliquots of the same medium containing [^{35}S]sulfate (0.3 mCi/mL) plus different concentrations (5, 10, 25 and 50 μM) of afimoxifene, endoxifen, raloxifene or fulvestrant. At the end of an 18-h labeling period, the labeling media were collected, spin filtered to remove high-molecular weight [^{35}S]sulfated macromolecules and subjected to thin-layer analysis for [^{35}S]sulfated afimoxifene, endoxifen, raloxifene or fulvestrant based on the procedure described above.

2.6. 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 (29). 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 non-radioactive PAPS. Protein determination was based on the method of Bradford with bovine serum albumin as the standard (30).

3. Results and discussion

SULT-mediated sulfation is known to play a critical role in the metabolism and inactivation of a diverse array of endogenous and xenobiotic compounds (17–19). Sulfation may result in the inactivation of these compounds and facilitate their removal from the body (17–19). Previous studies demonstrated that several breast cancer drugs and/or their metabolites may be subjected to sulfate conjugation (13,21–23). The current study aimed to systematically identify those human SULT enzymes capable of sulfating afimoxifene, endoxifen, raloxifene, and/or fulvestrant. Sulfation of these drug compounds under metabolic conditions was examined using cultured HepG2 human hepatoma cells and MCF-7 breast cancer cells. Moreover, human organ cytosols were evaluated for sulfating activities toward these drug compounds.

3.1. Differential sulfating activities of the human SULTs toward afimoxifene, endoxifen, raloxifene and fulvestrant

To identify the enzymes that are capable of sulfating afimoxifene, endoxifen, raloxifene and fulvestrant, 13 known human SULTs (SULT1A1, SULT1A2, SULT1A3, SULT1B2, SULT1C2, SULT1C3, SULT1C4, SULT1E1, SULT2A1, SULT2B1a, SULT2B1b, SULT4A1, SULT6B1), previously cloned, expressed, and purified, were examined for sulfating activity with 10 μM of afimoxifene, endoxifen, raloxifene and fulvestrant as substrates. Results obtained indicated that seven (SULT1B2, SULT1C2, SULT1C3, SULT2B1a, SULT2B1b, SULT4A1 and SULT6B1) of the 13 SULTs displayed no detectable activities toward any of the four drug compounds. Of the other six SULTs, SULT1A1 and SULT1C4 exhibited strong sulfating activities toward all four drug compounds, whereas SULT1A2, SULT1A3, SULT1E1, and SULT2A1 displayed weaker and differential sulfating activities toward (some of) the drug compounds tested. Overall, activity data compiled in Table 1A indicated that SULT1A1 is likely the major SULT enzyme involved in the sulfation of afimoxifene and fulvestrant, while SULT1C4 is a major enzyme responsible for the sulfation of endoxifen and raloxifene. SULT1A1 has been shown to be capable of mediating the sulfation of numerous drugs, particularly those that contain phenolic hydroxyl groups in their chemical structures (31). SULT1C4, initially reported to mediate the sulfation of N-hydroxy-2-acetylaminofluorene (24), has recently been shown to be capable of sulfating a wide range of drugs, including acetaminophen, phenylephrine, hydromorphone, oxymorphone, and naloxone that also contain phenolic hydroxyl groups in their chemical structures (32–34). From the structure-function standpoint, it has been reported that the crystal structure of SULT1A1 contains an L-shaped hydrophobic substrate-binding site where *p*-nitrophenol, a model substrate, is bound (31). This substrate-binding site displays plasticity so as to be able to interact with various substrate compounds. The catalysis has been shown to be via an SN2 in-line displacement mechanism, involving the conserved His108 residue which is coordinated to the phenolic group of the substrate (31). For SULT1C4, similar information is lacking and awaits the determination of its crystal structure. In regard to their tissue specificity of expression, SULT1A1 is known to be expressed at high levels in human liver, lung, brain, skin, platelets, gastrointestinal tissues and kidney (35–37); whereas SULT1C4 has been demonstrated to be expressed at high levels in human fetal lung, liver, small intestine and kidney, and at lower, but significant levels in adult kidney, ovary and spinal cord (24,38). These results, therefore, imply that the drug compounds tested may

Table 1A
Specific activity of human SULTs with afimoxifene, endoxifen, raloxifene and fulvestrant as substrates.[a]

Specific Activity (nmol/min/mg)				
SULTs	Afimoxifene	Endoxifen	Raloxifene	Fulvestrant
1A1	3.24 ± 0.37	2.07 ± 0.22	3.75 ± 0.01	7.77 ± 0.43
1A2	ND[b]	ND	ND	3.32 ± 0.30
1A3	ND	ND	1.42 ± 0.16	ND
1C4	1.24 ± 0.10	6.51 ± 0.67	35.91 ± 1.47	0.56 ± 0.18
1E1	ND	ND	ND	1.59 ± 0.77
2A1	0.01 ± 0.01	ND	0.02 ± 0.02	ND

[a] Specific activity refers to nmol substrate sulfated/min/mg purified enzyme. Data represent means ± SD derived from three experiments.
[b] ND refers to activity not detected.

be differentially metabolized through sulfation under the action of respective SULT enzymes that are differentially expressed in the above-mentioned human organs.

To investigate further their sulfation, the kinetics of the sulfation of afimoxifene, endoxifen and fulvestrant by relevant human SULTs 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 afimoxifene, endoxifen and fulvestrant by relevant human SULTs were fitted to hyperbolic kinetic curves (Michaelis–Menten kinetics), which were further verified by linear Eadie–Hofstee plots. Fig. 2 shows a representative set of Lineweaver–Burk double reciprocal plots that were used for the calculation of K_m and V_{max} values. The kinetic constants obtained are compiled in Table 1B. It should be pointed out that the kinetics of the sulfation of raloxifen was not examined, since the two phenolic hydroxyl groups of this drug may both be subjected to sulfate conjugation, rendering the data difficult to interpret. In regard to this latter issue, our enzymatic assays using raloxifen as the substrate yielded indeed two [^{35}S]sulfated products of raloxifen (figure not shown). Whereas in the case of fulvestrant, while there are two hydroxyl groups in its chemical structure, only the phenolic 3-hydroxyl group, but not the alkyl 17-hydroxyl group, could be subjected to SULT-mediated sulfation (9).

Table 1B
Kinetic constants of the human SULTs responsible for the sulfation of afimoxifene, endoxifen and fulvestrant.[a]

Substrate/SULT	K_m (μM)	V_{max} (nmol/min/mg)	V_{max}/K_m
Afimoxifene/SULT1A1	22.45 ± 1.31	16.36 ± 2.49	0.73
Endoxifen/SULT1C4	50.41 ± 7.08	32.58 ± 5.72	0.65
Fulvestrant/SULT1A1	4.02 ± 0.31	13.40 ± 1.55	3.33

[a] Data shown represent means ± SD derived from three determinations.

3.2. *Generation and release of [^{35}S]sulfated products by HepG2 and MCF-7 cells labeled with [^{35}S]sulfate in the presence of afimoxifene, endoxifen, raloxifene or fulvestrant*

HepG2 human hepatoma cells and MCF-7 breast cancer cells were used to investigate whether sulfation of afimoxifene, endoxifen, raloxifene and fulvestrant may occur under metabolic conditions. As shown in Fig. 3A, autoradiograph of the TLC plate used for the analysis of spent media of HepG2 cells labeled with [^{35}S]sulfate in the presence of increasing concentrations (0, 5, 10, 25, and 50 μM) of afimoxifene, endoxifen, or raloxifene revealed the generation and release of [^{35}S]sulfated afimoxifene, endoxifen, and raloxifene in a concentration-dependent manner. It was noted that a minor [^{35}S]sulfated species migrating at the same position as [^{35}S]sulfated endoxifen was detected in labeling medium samples of HepG2 cells incubated in the presence of afimoxifene. In the case of raloxifen, a minor, slower migrating [^{35}S]sulfated species was also detected in the spent medium samples. Whether the two [^{35}S]sulfated derivatives of raloxifen correspond to mono- and di-sulfated raloxifen or whether the slower migrating [^{35}S]sulfated species corresponds to a metabolite of raloxifen which was subsequently [^{35}S]sulfated remains to be clarified. Intriguingly, no [^{35}S]sulfated derivative of fulvestrant was detected in spent labeling medium samples of HepG2 cells incubated in the presence of fulvestrant. It will be important to clarify whether in HepG2 cells, fulvestrant is differentially metabolized via other pathways, e.g., glucuronidation, or whether there was little uptake of fulvestrant by HepG2 cells, resulting in little or no production of [^{35}S]sulfated fulvestrant. In the case of MCF-7 cells (Fig. 3B), [^{35}S]sulfated derivatives of afimoxifene, raloxifene and fulvestrant were generated and released in a concentration-dependent manner. For endoxifen,

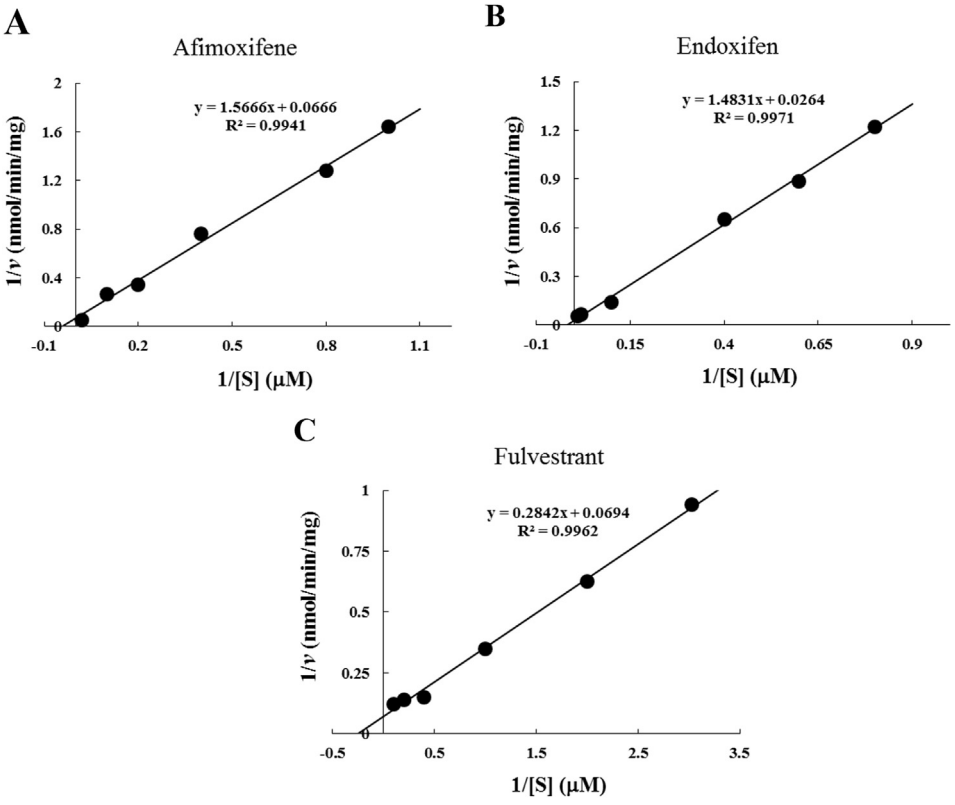

Fig. 2. Lineweaver–Burk double-reciprocal plots of the sulfation of A) afimoxifene, B) endoxifen, and C) fulvestrant by human SULT1A1, SULT1C4, and SULT1A1, respectively.

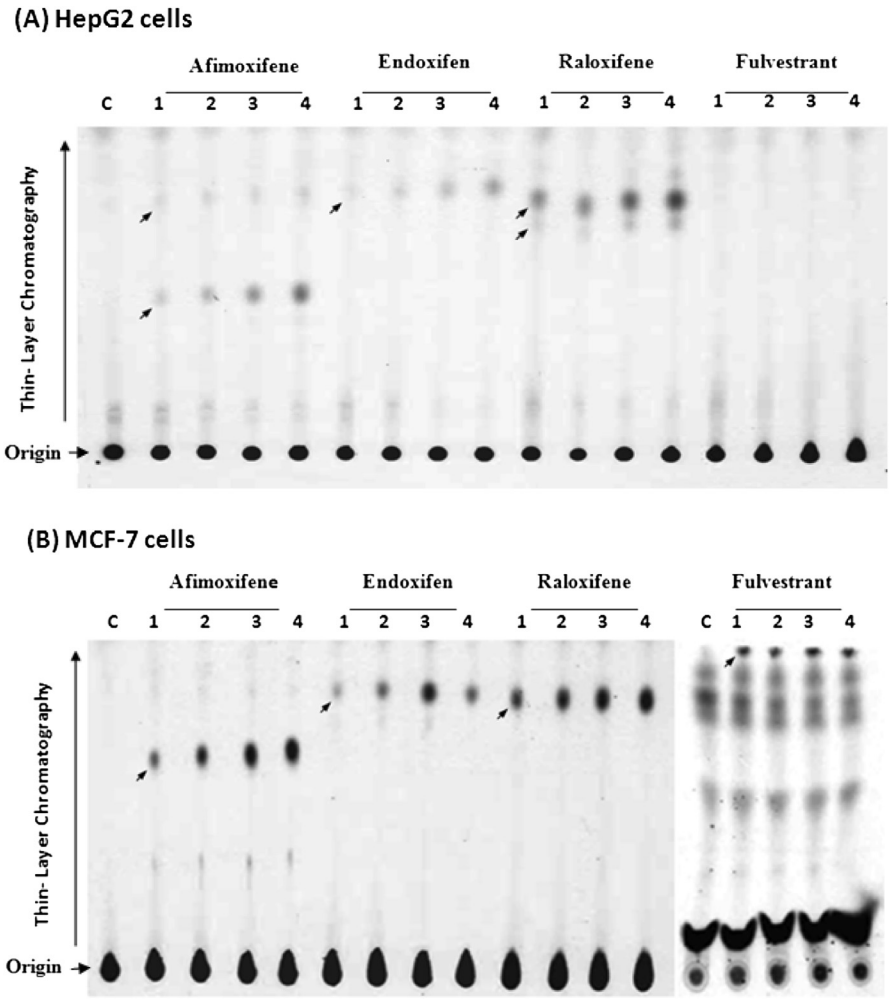

Fig. 3. Analysis of [^{35}S]sulfated products generated and released by (A) HepG2 human hepatoma cells and (B) MCF -7 human breast cancer cells labeled with [^{35}S]sulfate in the presence of different drug compounds. The figure shows the autoradiographs taken from the plates at the end of the TLC analysis. Confluent HepG2 or MCF-7 cells were incubated in labeling media containing, respectively, 5, 10, 25, and 50 μM (corresponding to lanes 1–4) of afimoxifene, endoxifen, raloxifene and fulvestrant for 18 h. C refers to the control labeling medium without added drug compounds. The arrows indicate the sulfated derivatives of each of the four drug compounds tested.

there was also a concentration-dependent generation and release of its [^{35}S]sulfated derivative by cells incubated with 5, 10, and 25 μM of endoxifen. A dramatic decrease in [^{35}S]sulfated endoxifen, however, was noted in spent labeling medium of cells incubated with 50 μM of endoxifen. It is possible that at elevated levels, endoxifen may become cytotoxic, causing cell death and therefore decreased sulfating capacity. Collectively, these results indicated that all four drug compounds tested could be metabolized through sulfation by HepG2 and/or MCF-7 cells.

3.3. *Sulfation of afimoxifene, endoxifen, raloxifene and fulvestrant by human organ samples*

To verify the presence of afimoxifene, endoxifen, raloxifene and fulvestrant-sulfating activity in human tissues, enzymatic assays were performed using cytosols prepared from human intestine, liver, lung, or kidney. Activity data obtained are compiled in Table 2. Of the four human organ samples tested, intestine and liver cytosols exhibited much stronger sulfating activities than cytosols prepared from lung and kidney toward all four tested drug compounds. These results indicated that liver and intestine are better equipped with SULT enzymes capable of sulfating these drug compounds.

Table 2
Sulfating activities of human lung, liver, kidney, and small intestine cytosols with afimoxifene, endoxifen, raloxifene, and fulvestrant as substrates.[a]

Substrate	Specific activity (pmol/min/mg)			
	Lung	Liver	Kidney	Small intestine
Afimoxifene	9.54 ± 1.67	64.67 ± 9.41	7.44 ± 0.27	63.24 ± 2.54
Endoxifen	ND[b]	25.04 ± 2.02	ND	29.46 ± 2.69
Raloxifene	4.93 ± 0.07	63.18 ± 4.46	3.62 ± 0.09	100.50 ± 6.45
Fulvestrant	2.68 ± 0.68	77.62 ± 2.36	3.02 ± 0.50	93.01 ± 3.27

[a] Specific activity refers to pmol substrate sulfated/min/mg protein. Data represent mean ± SD derived from three determinations. The concentration of the substrate used in the assay mixture was 50 μM.
[b] ND refers to activity not detected.

To summarize, the current study demonstrated that among the thirteen known human SULTs, SULT1A1 displayed strongest sulfating activity toward afimoxifene and fulvestrant, while SULT1C4 exhibited strongest sulfating activity toward endoxifen and raloxifene. Metabolic labeling experiments showed that the drug compounds tested could be sulfated by HepG2 human hepatoma cells and/or MCF-7 breast cancer cells. Of the four human organ specimens tested, liver and intestine cytosols showed strong sulfating activity toward these drug compounds. Collectively, these results

provided useful information concerning SULT-mediated sulfation in the metabolism of above-mentioned breast cancer drugs. The question whether sulfated metabolites of these drugs are still pharmacologically active or are simply destined to be excreted from the body, nevertheless, remains. More work is warranted in order to find an answer to this important issue.

Conflict of interest

The authors declare no conflict of interest.

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References

(1) Lumachi F, Luisetto G, Basso SM, Basso U, Brunello A, Camozzi V. Endocrine therapy of breast cancer. Curr Med Chem. 2011;18:513–522.
(2) Heath DD, Flat SW, Wu AH, Pruitt MA, Rock CL. Evaluation of tamoxifen and metabolites by LC-MS/MS and HPLC methods. J Biomed Sci. 2014;71:33–39.
(3) Li XF, Carter S, Dovichi NJ, Zhao JY, Kovarik P, Sakuma T. Analysis of tamoxifen and its metabolites in synthetic gastric fluid digests and urine samples using high-performance liquid chromatography with electrospray mass spectrometry. J Chromatogr A. 2001;914:5–12.
(4) De Vos F, Van de Wiele C, Vandecapelle M, Dierckx RA, Slegers G. High performance liquid chromatographic determination of 123-I labeled tamoxifen metabolites in human plasma. Nucl Med Biol. 2001;28:335–338.
(5) Carter SJ, Li XF, Mackey JR, Modi S, Hanson J, Dovichi NJ. Biomonitoring of urinary tamoxifen and its metabolites from breast cancer patients using nonaqueous capillary electrophoresis with electrospray mass spectrometry. Electrophoresis. 2001;22:2730–2736.
(6) MacCallum J, Cummings J, Dixon JM, Miller WR. Concentrations of tamoxifen and its major metabolites in hormone responsive and resistant breast tumours. Br J Cancer. 2000;82:1629–1635.
(7) Jordan VC, Morrow M. Raloxifene as a multifunctional medicine? Current trials will show whether it is effective in both osteoporosis and breast cancer. BMJ. 1999;319:331–332.
(8) Nicholson RI, Gee JM, Manning DL, Wakeling AE, Montano MM, Katzenellenbogen BS. Responses to pure antiestrogens (ICI 164384, ICI 182780) in estrogen-sensitive and -resistant experimental and clinical breast cancer. Ann N Y Acad Sci. 1995;761:148–163.
(9) Edavana VK, Yu X, Dhakal IB, Williams S, Ning B, Cook IT, et al. Sulfation of fulvestrant by human liver cytosols and recombinant SULT1A1 and SULT1E1. Pharmgenomics Pers Med. 2011;4:137–145.
(10) Crewe HK, Notley LM, Wunsch RM, et al. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4′-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. Drug Metab Dispos. 2002;30:869–874.
(11) Crewe HK, Ellis SW, Lennard MS, Tucker GT. Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. Biochem Pharmacol. 1997;53:171–178.
(12) Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther. 2004;310:1062–1075.
(13) Nishiyama T, Ogura K, Nakano H, et al. Reverse geometrical selectivity in glucuronidation and sulfation of cis- and trans-4-hydroxytamoxifens by human liver UDP-glucuronosyltransferases and sulfotransferases. Biochem Pharmacol. 2002;63:1817–1830.
(14) Chen Q, Ngui JS, Doss GA, Wang RW, Cai X, DiNinno FP, et al. Cytochrome P450 3A4-mediated bioactivation of raloxifene: irreversible enzyme inhibition and thiol adduct formation. Chem Res Toxicol. 2002;15:907–914.
(15) Trontelj J, Bogataj M, Marc J, Mrhar A. Development and validation of a liquid chromatography-tandem mass spectrometry assay for determination of raloxifene and its metabolites in human plasma. J Chromatogr B Anal Technol Biomed Life Sci. 2007;855:220–227.
(16) Robertson JF, Harrison M. Fulvestrant: pharmacokinetics and pharmacology. Br J Cancer. 2004;1:S7–S10.
(17) Mulder GJ, Jakoby WB. Conjugation reactions. In: Mulder GJ, Jakoby WB, editors. Drug metabolism. London: Taylor & Francis Ltd; 1990. p. 107–161.
(18) Falany C, Roth JA. In: Jeffery EH, editor. Human drug metabolism; from molecular biology to man. Boca Raton: CRC Press; 1993. p. 101–115.
(19) Weinshilboum R, Otterness D. In: Kaufmann FC, editor. Conjugation–deconjugation reactions in drug metabolism and toxicity. Berlin: Springer-Verlag; 1994. p. 45–78.
(20) Lipmann F. Biological sulfate activation and transfer. Science. 1958;128:575–580.
(21) Chen G, Yin S, Maiti S, Shao X. 4-Hydroxytamoxifen sulfation metabolism. J Biochem Mol Toxicol. 2002;16:279–285.
(22) Gjerde J, Hauglid M, Breilid H, Lundgren S, Varhaug JE, Kisanga ER, et al. Effects of CYP2D6 and SULT1A1 genotypes including SULT1A1 gene copy number on tamoxifen metabolism. Ann Oncol. 2008;19:56–61.
(23) Falany JL, Pilloff DE, Leyh TS, Falany CN. Sulfation of raloxifene and 4-hydroxytamoxifen by human cytosolic sulfotransferases. Drug Metab Dispos. 2006;34:361–368.
(24) Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, et al. Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. J Biol Chem. 1998;273:33929–33935.
(25) 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. 1998;273:6242–6247.
(26) Sakakibara Y, Suiko M, Pai TG, Nakayama T, Takami Y, Katafuchi J, et al. Highly conserved mouse and human brain sulfotransferases: molecular cloning, expression, and functional characterization. Gene. 2002;285:39–47.
(27) 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.
(28) Suiko M, Sakakibara Y, Liu MC. Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. Biochem Biophys Res Commun. 2000;267:80–84.
(29) Yanagisawa K, Sakakibara Y, Suiko M, Takami Y, Nakayama T, Nakajima H, et al. cDNA cloning, expression, and characterization of the human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase enzyme. Biosci Biotechnol Biochem. 1998;62:1037–1040.
(30) 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.
(31) Hempel N, Gamage N, Martin JL, McManus ME. Human cytosolic sulfotransferase SULT1A1. Int J Biochem Cell Biol. 2007;39:685–689.
(32) Kurogi K, Chen M, Lee Y, Shi B, Yan T, Liu MY, et al. Sulfation of buprenorphine, pentazocine, and naloxone by human cytosolic sulfotransferases. Drug Metab Lett. 2012;6:109–115.
(33) Kurogi K, Chepak A, Hanrahan MT, Liu MY, Sakakibara Y, Suiko M, et al. Sulfation of opioid drugs by human cytosolic sulfotransferases: metabolic labeling study and enzymatic analysis. Eur J Pharm Sci. 2014;62:40–48.
(34) Yamamoto A, Kim J, Liu MY, Kurogi K, Sakakibara Y, Suiko M, et al. Sulfation of phenylephrine by the human cytosolic sulfotransferases. Drug Metab Lett. 2014;8:96–100.
(35) Sundaram RS, Szumlanski C, Otterness D, van Loon JA, Weinshilboum RM. Human intestinal phenol sulfotransferase: assay conditions, activity levels and partial purification of the thermolabile form. Drug Metab Dispos. 1989;17:255–264.
(36) Cappiello M, Giuliani L, Pacifici GM. Differential distribution of phenol and catechol sulphotransferases in human liver and intestinal mucosa. Pharmacology. 1990;40:69–76.
(37) Hempel N, Barnett AC, Duggleby RG, Martin J, Windmill KF, McManus ME. Human SULT1A sulfotransferases. In: Pacifici GM, Coughtrie MWH, editors. Human cytosolic sulfotransferases. London: CRC Press; 2005. p. 181–232.
(38) Stanley EL, Hume R, Coughtrie MW. Expression profiling of human fetal cytosolic sulfotransferases involved in steroid and thyroid hormone metabolism and in detoxification. Mol Cell Endocrinol. 2005;240:32–42.