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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

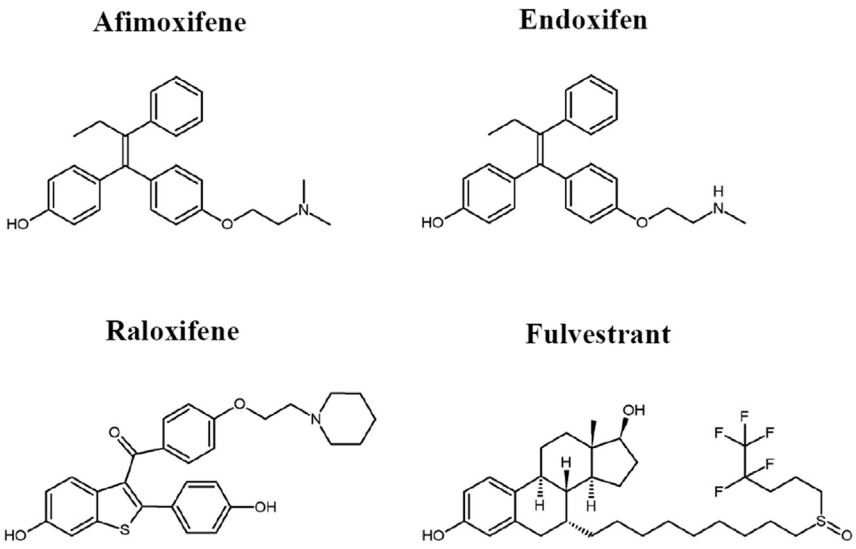

Fig. 1. Chemical structures of afimoxifene, endoxifen, raloxifene and fulvestrant.