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Note

Human cytosolic sulfotransferase SULT1C4 mediates the sulfation of doxorubicin and epirubicin

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ABSTRACT

Doxorubicin, an anthracycline, has been reported to be excreted in sulfate conjugated form. The current study aimed to identify the human cytosolic sulfotransferase(s) (SULT(s)) that is(are) capable of sulfating doxorubicin and its analog epirubicin, and to verify whether sulfation of doxorubicin and epirubicin may occur under metabolic conditions. A systematic analysis of thirteen known human SULTs, previously cloned, expressed, and purified, revealed SULT1C4 as the only human SULT capable of sulfating doxorubicin and epirubicin. Cultured HepG2 human hepatoma cells and Caco-2 human colon carcinoma cells were labeled with [^{35}S]sulfate in the presence of different concentrations of doxorubicin or epirubicin. Analysis of spent labeling media showed the generation and release of [^{35}S]sulfated doxorubicin and epirubicin by HepG2 cells and Caco-2 cells. Reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed the expression of SULT1C4 in both HepG2 cells and Caco-2 cells. These results provided a molecular basis underlying the previous finding that sulfate-conjugated doxorubicin was excreted in the urine of patients treated with doxorubicin.

1. Introduction

Doxorubicin and epirubicin are anthracyclines commonly used for the treatment of a wide range of hematologic malignancies as well as solid and soft tumors [1–4]. The use of doxorubicin, however, is associated with dose-dependent and cumulative cardiotoxicity that may lead to cardiac injury and congestive heart failure [5]. Epirubicin, an epimer of doxorubicin, differing in the orientation of the C-4 hydroxyl group of the sugar moiety [2], showed less cardiotoxicity than doxorubicin [6]. The modest structural change may account for the lesser cardiotoxicity of epirubicin since it may result in differential pharmacokinetics and metabolism of the drug [7]. Studies have shown that the plasma clearance of epirubicin appeared to be significantly faster than that of doxorubicin [7–9]. Doxorubicin metabolites excreted in human urine include those derived from *O*-demethylation, *O*-glucuronidation, and *O*-sulfation [10], while epirubicin has been shown to be subjected to glucuronidation [11,12]. In the use of doxorubicin and epirubicin in the treatment of pediatric malignancies, an important question is whether these drugs may be differentially metabolized at different stages during neonatal/child development. Importantly, studies have demonstrated that sulfation as mediated by the cytosolic sulfotransferases (SULTs) appears to be more important early during development for the detoxification of xenobiotics, since other conjugating enzymes such as the UDP-glucuronosyltransferases are not yet expressed at significant levels [13–16].

In humans, there are thirteen SULT isoforms, including three phenol SULTs (SULT1A1, SULT1A2 and SULT1A3), a thyroid hormone SULT (SULT1B1), three SULT1Cs (SULT1C2, SULT1C3 and SULT1C4), an estrogen SULT (SULT1E1), a dehydroepiandrosterone (DHEA) SULT (SULT2A1), two SULT2B1s (SULT2B1a and SULT2B1b), a neuronal SULT (SULT4A1) and a SULT6B1 [17]. The SULTs catalyze the transfer of a sulfonate group from the active sulfate, 3′-

Abbreviations: ATP, adenosine 5′-triphosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, cytosolic sulfotransferase.

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phosphoadenosine 5′-phosphosulfate (PAPS), to an acceptor substrate compound containing either hydroxyl or amino group(s) [18]. Sulfate conjugation by the SULT enzymes generally leads to the inactivation of biologically active compounds and/or the increase their water-solubility, thereby facilitating their removal from the body [18]. It is possible that sulfate conjugation of doxorubicin and epirubicin may help alleviate their adverse effects.

We report in this communication a systematic analysis of the sulfating activity of all known human SULTs toward doxorubicin and epirubicin. Moreover, metabolic labeling study was performed using cultured HepG2 and Caco-2 cells to investigate the generation and release of sulfated doxorubicin and epirubicin under metabolic conditions.

2. Materials and methods

2.1. Materials

Doxorubicin and epirubicin were products of Cayman Chemical Company (Ann Arbor, MI, USA). Adenosine 5′-triphosphate (ATP), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), N-2-hydroxylpiperazine-N-2-ethanesulfonic acid (HEPES), 3-N-tris-hydroxymethyl methylamino-propanesulfonic acid (TAPS), 2-cyclohexylamino ethanesulfonic acid (CHES), 3-cyclohexylamino-1-propanesulfonic acid (CAPS), dithiothreitol (DTT), minimum essential medium (MEM), fetal bovine serum (FBS), penicillin G, streptomycin sulfate and were products of Sigma Chemical Company (St. Louis, MO, USA). Cellulose thin-layer chromatography (TLC) plates and Ecolume scintillation cocktail were from MP Biomedicals (Irvine, CA, USA). Carrier-free sodium [^{35}S]sulfate was from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). 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 [19]. Ultra free-MC 5000 NMWL filter units were products of Millipore (Bedford, MA, USA). HepG2 human hepatoma cell line (ATCC HB-8065) and Caco2 human colon carcinoma cell line (ATCC HTB-37) were from American Type Culture Collection (Manassas, VA, USA). Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, a thyroid hormone SULT (SULT1B1), three SULT1Cs (SULT1C2 SULT1C3 and SULT1C4), an estrogen SULT (SULT1E1), a 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 [20]. All other chemicals used were of the highest grade commercially available.

2.2. Metabolic labeling of HepG2 human hepatoma cells and Caco-2 human colon carcinoma cells

HepG2 cells and Caco-2 cells were 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 respectively, pre-incubated in sulfate-free (prepared by omitting streptomycin sulfate and replacing magnesium sulfate with magnesium chloride) MEM without FBS for 4 h, labeled with 0.2 ml aliquots of the same medium containing [^{35}S]sulfate (0.3 mCi/ml) and different concentrations (0, 100, 200, 300, 400, and 500 μM) of doxorubicin or different concentrations (0, 50, 100, 150, 200 and 250 μM) of epirubicin. At the end of an 18-hr labeling period, the labeling media were collected, spin-filtered to remove high-molecular weight [^{35}S] sulfated macromolecules and subjected to cellulose TLC plate for the analysis of [^{35}S]sulfated doxorubicin and epirubicin, using n-butanol/isopropanol/88% formic acid/water (2:2:3:1; by volume) as the solvent system.

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]. Substrate (doxorubicin or epirubicin) at 10 times the final concentration (100 μM) in the assay mixture, were added after HEPES buffer and PAP[^{35}S]. 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 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 procedure with n-butanol/isopropanol/88% formic acid/water (2:2:3:1; by volume) as the solvent system. On 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. A total of 4.5 ml of Ecolume scintillation liquid was added to each vial, mixed thoroughly and the radioactivity therein was counted by using a liquid scintillation counter. Each experiment was performed in triplicate, together with two kinds of control, one without enzyme and the other is without substrate. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme.

2.4. RT-PCR analysis for the expression of SULT1C4 mRNA in HepG2 cells and Caco-2 cells

Total RNAs from HepG2 cells and Caco-2 cells were isolated using the TRI Reagent based on manufacturer's instructions. First-strand cDNAs were reverse-transcribed from the total RNA samples using the First-strand cDNA Synthesis Kit (Amersham Biosciences). PCRs in 20 μl reaction mixtures were carried out using One*Taq* DNA polymerase with the first-strand cDNAs prepared as templates, in conjunction with gene-specific sense (5′-ATGGCCTTACACGACATGGAGGATTTTACATTTGATGGAA-3′) and antisense (5′-CTATAGGAACTGGAAGTGGAAAGTTAGTCTGGTATCAGTCATT-3′) oligonucleotide primers designed based on the reported human SULT1C4 nucleotide sequence (GenBank Accession # AF186263). Amplification conditions were 2 min at 94 °C followed by 39 cycles of 30 s at 94 °C, 30 s at 60 °C, and 1.5 min at 68 °C. The final reaction mixtures were applied onto a 1.5% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining. As a control, PCR amplification of the sequence encoding human β-actin was concomitantly performed using the above-mentioned first-strand cDNAs as templates, in conjunction with gene-specific sense (5′-CCGCCCGTCCACACCCGCCGCCAGCTCACC-3′) and antisense (5′-AGGGTGTAACGCAACTAAGTCATAGTCCGC-3′) oligonucleotide primers designed based on the reported human β-actin nucleotide sequence (GenBank Accession # AK304552).

3. Results and discussion

3.1. Differential sulfating activities of the human SULTs toward doxorubicin and epirubicin

To identify the enzyme(s) that is (are) responsible for the sulfation of doxorubicin and epirubicin, 13 known human SULTs,