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Sulfation of tibolone metabolites by human postmenopausal liver and small intestinal sulfotransferases (SULTs)☆

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ABSTRACT

Sulfation is a major pathway in humans for the biotransformation of steroid hormones and structurally related therapeutic agents. Tibolone is a synthetic steroid used for the treatment for climacteric symptoms and postmenopausal osteoporosis. Sulfation inactivates the hydroxylated metabolites, 3α-hydroxytibolone (3α-OH-tibolone) and 3β-hydroxytibolone (3β-OH-tibolone), and contributes to the regulation of tissue responses to tibolone. We detected SULT1A1, SULT1A3, SULT1E1 and SULT2A1 mRNA expression by RT-PCR in postmenopausal liver and small intestine. Liver pool ($n=5$) SULT activities measured with tibolone substrates reflected COS-1 expressed SULT2A1 and SULT1E1 activities. Liver SULT2A1 activity (1.8 ± 0.3 units/mg protein, $n=8$, mean ± SEM), and activities with 3α-OH-tibolone (0.6 ± 0.1, $n=8$) and 3β-OH-tibolone (0.9 ± 0.2, $n=8$) were higher than SULT1E1 activities (<0.05, $n=10$). SULT1E1 activities were low or not detected in many samples. Mean small intestinal activities were 0.03 ± 0.01 with 3α-OH-tibolone and 0.04 ± 0.01 with 3β-OH-tibolone ($n=3$). In conclusion, SULT2A1 is the major endogenous enzyme responsible for sulfation of the tibolone metabolites in human postmenopausal tissues. The results support the occurrence of pre-receptor enzymatic regulation of hydroxytibolone metabolites and prompt further investigation of the tissue-selective regulation of tibolone effects.

1. Introduction

Tibolone (Livial) is a synthetic steroid that has been used extensively for the prevention of postmenopausal osteoporosis [1,2] and for the treatment of climacteric symptoms [3]. It is the prototype of a compound that acts as a selective tissue estrogen activity regulator (STEAR) [4]. A key element in this tissue specific regulation of estrogen activity is mediated through enzymatic mechanisms that activate or inactivate estrogenic compounds prior to their interaction with a steroid receptor [5]. After oral administration, tibolone is metabolized into three biologically active metabolites. The 3α-hydroxytibolone (3α-OH-tibolone) and 3β-hydroxytibolone (3β-OH-tibolone) metabolites have estrogen agonist properties, and the Δ^4-ketoisomer has progestogenic and androgenic effects. Sulfation of the hydroxytibolone metabolites by sulfotransferase enzymes (SULTs) renders these metabolites receptor-inactive and reduces their estrogenic effects. SULT activity may account for the clinical effects of tibolone treatment that include an unchanged mammographic breast density in most women, less symptoms related to the breast, and less vaginal bleeding when compared with usual hor-

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mone replacement therapy [6–10]. An additional protective effect of tibolone in these tissues is the inhibition of sulfatase activity by the tibolone metabolites in breast and endometrial cells [5,11]. By contrast, the lack of steroid SULT activity and minimal inhibition of sulfatase activity by tibolone in bone cells allow free hydroxytibolone metabolites to activate the estrogen receptor (α) in bone and to decrease bone resorption [5,11]. Thus, tibolone, as a STEAR, does not behave as a selective estrogen receptor modulator (SERM), but rather acts by enzymatic pre-receptor regulation of the estrogenic active metabolites.

Sulfated hydroxytibolone metabolites have been found in the circulation, supporting the involvement of SULTs in the regulation and metabolism of tibolone. In the cytosolic SULT superfamily there are at least 10 human SULT genes [12]. Fusion proteins of two steroid SULTs expressed in bacteria have been shown to use tibolone metabolites as substrates [13]. However, the exact SULT isozymes involved in the sulfation of the active tibolone metabolites in postmenopausal human liver and other tissues have yet to be fully characterized.

In this study we tested samples of human premenopausal and postmenopausal liver and small intestine for SULT activities with tibolone metabolites. We then used postmenopausal liver to establish biochemical properties and radiochemical assays with the substrates 3α-OH-tibolone and 3β-OH-tibolone. These tools allowed us to document the postmenopausal liver contribution to the sulfation of hydroxytibolone metabolites. Because the cytosolic liver preparation represented a composite of SULT activities, we also compared the kinetic parameters of cDNA expressed SULT activities to the liver SULT activities when tested with the tibolone metabolites.

2. Experimental

2.1. Materials

[^{35}S] Phosphoadenosine-5-phosphosulfate ([^{35}S]-PAPS, specific activity from 2.4 to 3.0 Ci/mmol) was purchased from Perkin-Elmer LAS, Shelton, CT. 2-Difluoro-methyloestrone-3-O-sulphamate (EMATE), the hydroxytibolone metabolites and their respective sulfated compounds were provided by Dr. H.J. Kloosterboer (Organon, Oss, The Netherlands). Dithiothreitol (DTT) was purchased from CalBiochem, La Jolla, CA. Bovine serum albumin (BSA), sodium chloride (NaCl), 2,6-dichloro-4-nitrophenol (DCNP) and magnesium chloride ($MgCl_2$) were obtained from Sigma Chemical Company, St. Louis, MO. Protein assay reagent was obtained from Bio-Rad Laboratories, Richmond, CA and Bio-Safe II scintillation fluor was purchased from Research Products International Corp., Mount Prospect, IL. COS-1 cells were obtained from the American Type Culture Collection (ATCC), Rockville, MD. The human liver SULT1A1, SULT1A2, SULT1A3, SULT1E1, SULT1C2, SULT2A1, SULT2B1_v1 and SULT2B1_v2 cDNAs were gifts from Dr. Weinshilboum [14–18]. Trizol reagents, Superscript First-Strand Synthesis kit, Lipofectamine, Platinum PCR Supermix kit were purchased from Invitrogen Corp., Carlsbad, CA.

2.2. Tissue samples and cytosol preparations

Both the IRB and the Research and Development Committee of the VA-Nebraska Western Iowa Health Care System approved these studies. Frozen human liver premenopausal (37.8 ± 6.3 years, mean ± S.D., $n = 5$) and postmenopausal (69.1 ± 7.4 years, mean ± S.D., $n = 10$) samples, and small intestine premenopausal (39.2 ± 5.4 years, mean ± S.D., $n = 5$) and postmenopausal (71 ± 10 years, mean ± S.D., $n = 11$) samples were obtained from the National Disease Research Interchange (NDRI). All samples were obtained from Caucasian subjects at autopsy except for one premenopausal liver surgical sample and two premenopausal small intestine surgical samples. Tissue was stored at −80 °C until preparation.

Tissue samples were homogenized for 15–30 s in four volumes of 2.5 mM DTT, 1.25 mM disodium EDTA in 5 mM potassium phosphate buffer, pH 7.5, with a Polytron Tissue Homogenizer (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at 12,900 × g for 10 min at 4 °C. The supernatant was removed and centrifuged at 100,000 × g for 1 h at 4 °C. Aliquots of the high speed supernatant (HSS) were stored at −80 °C until assay. Equal volumes of each HSS were pooled for each tissue. COS-1 cells were transfected with SULT cDNA and processed by our previously published procedures [19–22].

2.3. RNA extraction and RT-PCR

Total RNA was extracted from tissue using Trizol reagent according to manufacturer's instructions (Invitrogen). First strand cDNA synthesis was performed using Superscript II RT with oligo dT priming for 1 μg total RNA by manufacturer's instructions. Each liver cDNA (87 ng), SULT1A1, SULT1E1 and SULT2A1 plasmid cDNAs (1 ng) were used as the templates for PCR with 0.1 nmol each SULT specific primers [21,23,24]. PCR was performed using Platinum PCR Supermix (Invitrogen) and an Eppendorf thermal cycler. Transcripts were amplified by an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C (SULTs 1E1 and 2A1) or 65 °C (SULT1A1) for 1 min 15 s and 72 °C for 2 min. The PCR products were resolved by 2% agarose gel electrophoresis and detected with ethidium bromide.

2.4. Sulfotransferase (SULT) and protein assays

SULT activities were measured by the method of Foldes and Meek [25], as modified by Anderson and Liebentritt [26]. Optimal conditions with hydroxytibolone as the substrate were established with regard to pH, protein and substrate concentrations. Assays with the hydroxytibolone substrates were performed with 2.5 and 3.5 μg protein per assay tube for the liver pool HSS and the small intestinal HSS, respectively. SULT1E1 and SULT2A1 were assayed with 1.1 and 0.7 μg protein per assay tube, respectively. The final concentrations used in the assays were 1 μM 3α-OH-tibolone and 4 μM 3β-OH-tibolone with liver and small intestinal HSS and 1 μM hydroxytibolone substrates for the activity screen of expressed SULT enzymes. The pH values for potassium phosphate buffers at a final concentration of 8.3 mM were 7.5 for the liver and small intestine, 6.5 for expressed SULT1E1 with hydroxytibolone, and 6.0 for expressed SULT2A1 with