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Interactions of the human cytosolic sulfotransferases and steroid sulfatase in the metabolism of tibolone and raloxifene

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Abstract

Sulfation is important in the metabolism and inactivation of steroidal compounds and hormone replacement therapeutic (HRT) agents in human tissues. Although generally inactive, many steroid sulfates are hydrolyzed to their active forms by sulfatase activity. Therefore, the specific sulfotransferase (SULT) isoforms and the levels of steroid sulfatase (STS) activity in tissues are important in regulating the activity of steroidal and HRT compounds. Tibolone (Tib) is metabolized to three active metabolites and all four compounds are readily sulfated. Tib and the Δ4-isomer are sulfated at the 17β-OH group by SULT2A1 and the 17-sulfates are resistant to hydrolysis by human placental STS. 3α-OH and 3β-OH Tib can form both 3- and 17-monosulfates as well as disulfates. Only the 3β-sulfates are susceptible to STS hydrolysis. Raloxifene monosulfation was catalyzed by at least seven SULT isoforms and SULT1E1 also synthesizes raloxifene disulfate. SULT1E1 forms both monosulfates in a ratio of approximately 8:1 with the more abundant monosulfate migrating on HPLC identical to the SULT2A1 synthesized monosulfate. The raloxifene monosulfate formed by both SULT isoforms is sensitive to STS hydrolysis whereas the low abundance monosulfate formed by SULT1E1 is resistant. The benzothiophene sulfates of raloxifene and arzoxifene were hydrolyzed by STS whereas the raloxifene 4′-phenolic sulfate was resistant. These results indicate that tissue specific expression of SULT isoforms and STS could be important in the inactivation and regeneration of the active forms of HRT agents.

Keywords: Sulfation; Sulfotransferase; Tibolone; Raloxifene; Sulfatase; Arzoxifene; Sulfate hydrolysis

1. Introduction

Tissue-specific modulation of steroid activity is critical in regulating the effects of therapeutic compounds used to alleviate the climacteric symptoms of menopause as well as osteoporosis. Tibolone (Tib) has been used for many years in hormone replacement therapy (HRT), effectively preventing bone loss while relieving climacteric symptoms of menopause without estrogenic stimulation of either the endometrium or breast [1–4]. Raloxifene has been used more specifically in the treatment of osteoporosis and, similarly to Tib, antagonizes the effects of estrogens in the breast and endometrium [5]. To understand the specific mechanisms of action of these compounds, it is important to analyze the pathways involved in their activation and inactivation. Conjugation of estrogens, therapeutic estrogenic compounds as well as HRT agents with a sulfonate group is important in the regulation of their activity and disposition. Sulfation is essentially an inactivation reaction, inhibiting the biological activity of steroids by preventing binding to specific hormone receptors [6–8]. In humans, Tib is rapidly metabolized into its main active metabolites, 3α-OH Tib and 3β-OH Tib, as well as a Δ4-isomer [1,9]. Sulfation is the major phase II conjugation pathway involved in the metabolism and inactivation of Tib and its metabolites and is also involved in the metabolism of raloxifene in human tissues [10–12]. In conjunction with

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sulfation, desulfation of Tib and raloxifene sulfates by sulfatase (STS) activity may have a role in regulation of *in situ* activity via regeneration of the active compounds [13]. Therefore, the interactions between sulfation and desulfation in a particular tissue may be critical in regulation of the availability and activity of these therapeutic compounds, especially if the different sulfated forms of Tib and raloxifene are differentially sensitive to STS activity. The combination of sulfation and desulfation could result in either an increased or decreased availability of biologically active forms of these drugs in different tissues.

In human tissues, several members of the cytosolic sulfotransferase (SULT) family are responsible for the sulfation of estrogens, therapeutic estrogenic compounds and HRT agents [14–16]. As reported previously, SULT1E1 and SULT2A1 are the major isoforms involved in the sulfation of Tib and its metabolites [10]. Raloxifene is sulfated *in vitro* by at least seven expressed human SULT isoforms including SULTs 1E1 and 2A1 [12,17]. SULT1E1 is responsible for the high affinity sulfation of estrogens [14,18,19] while SULT2A1 sulfates hydroxysteroids, bile acids and several therapeutic drugs [10,15,20,21]. SULT1E1 and SULT2A1 are also of interest since SULT1E1 can form raloxifene disulfate and SULT2A1 can generate the 3-OH Tib disulfates [10,12]. SULT1E1 is expressed in hormonally responsive tissues including breast, endometrium and prostate as well as the liver and GI tract [8,14,22–24]. SULT2A1 is present in relatively large amounts in the liver and adrenals but is not detectable in the breast, endometrium and prostate [15,24,25].

In contrast to sulfation, desulfation hydrolyzes sulfated inactive compounds to their unconjugated active forms. The enzyme responsible for the majority of steroid desulfation in human tissues is human STS or aryl sulfatase C, and is known to hydrolyze both 17β-estradiol (E2) and dehydroepiandrosterone (DHEA) sulfates to generate biologically active hormones from their sulfated precursors [26]. STS is a membrane-bound, hydrophobic enzyme that is difficult to solubilize, and thus attempts to clone and express the full-length active protein have been relatively unsuccessful [26,27]. Therefore, the potential contribution of STS in the metabolism of sulfated therapeutic drugs to render them biologically active has been inadequately examined. In this report purified human placental STS was utilized to characterize the desulfation of sulfated Tib and its metabolites as well as the different raloxifene sulfates. Both the Tib 3-OH metabolites and raloxifene possess two hydroxyl groups and can form two monosulfates as well as disulfates [10,12]. The ability of purified placental STS to desulfate the different raloxifene and Tib monosulfates and disulfates was analyzed to understand the possible role of desulfation in the regulation of the tissue specific activity of these therapeutic reagents. Preliminary studies of the sulfation/desulfation of arzoxifene, a recently described SERM related to raloxifene, were also carried out to clarify the results of the raloxifene studies.

2. Materials and methods

2.1. Materials

Tib, 3β-OH Tib, 3α-OH Tib, Δ4-Tib and arzoxifene were provided by N.V. Organon (the Netherlands). Raloxifene hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). 3′-Phosphoadenosine 5′-phosphosulfate (PAPS) was purchased from Dr. Sanford Singer (University of Dayton, Dayton, OH). [^{35}S]-PAPS (2.2 Ci/mmol) and [1,2,6,7-^{3}H(N)] DHEA-sulfate (60 Ci/mmol) were purchased from New England Nuclear (Boston, MA). LK6DF 60 Å silica gel thin layer chromatography (TLC) plates with a layer thickness of 250 μm were obtained from Whatman Inc. (Clifton, NJ). Con-A sepharose was purchased from GE Healthcare (Pistcataway, NJ). All other chemicals used were of reagent grade from Fisher Scientific (Norcross, GA).

2.2. Methods

2.2.1. Preparation of human SULT1E1, SULT2A1 and STS

SULT1E1 and SULT2A1 were expressed in *Escherichia coli* using the pKK233-2 vector to generate the native form of the enzymes and purified by DEAE-Sepharose chromatography to obtain a preparation suitable for enzymatic characterization as described previously [14,28,29].

For use in activity assays, STS was purified from human full-term placenta based upon the method of Hernandez-Guzman et al. [26]. Fresh placental tissue was obtained from the Tissue Procurement Service of the UAB Comprehensive Cancer Center with Institutional Review Board approval. The placental tissue was homogenized in 67 mM phosphate buffer, pH 7.4 containing 0.5 mM DTT and 0.24 M sucrose in a volume of approximately 1.5 l and stored at −80 °C. STS activity was determined using reactions (125 μl) containing 20 μM [^{3}H]-DHEA-sulfate as substrate in 0.1 M Tris–maleate buffer, pH 7.4, and 25 μl enzyme. Reactions were stopped by the addition of 200 μl 0.25 M Tris–HCl, pH 8.7 to alkalinize the reaction and extracted with 800 μl ethyl acetate. Radioactivity was determined in 500 μl of the ethyl acetate phase to quantify desulfated [^{3}H]-DHEA. For STS purification, 100 ml of placental homogenate was thawed on ice and diluted with 200 ml of buffer containing 10 mM potassium phosphate, pH 7.4, 20% glycerol, 0.5 μM androstenedione and 0.1 mM EDTA, then centrifuged at 105,000 × *g* for 60 min. The pellet was homogenized in the same buffer, adjusted to 0.3% Na cholate and 0.3% Emulgen 911, and stirred at 4 °C for 30 min then centrifuged at 105,000 × *g* for 1 h. The supernatant fraction was diluted with an equal volume of 10 mM potassium phosphate, pH 7.4, 20% glycerol, 0.5 μM androstenedione and 0.1 mM EDTA then loaded on a 60 ml DE52 column equilibrated in the same buffer. The column was washed with buffer containing 0.15% Emulgen 911 then with 20 mM Tris–HCl, pH 7.4 containing 0.1% Triton X-100. STS activity was eluted with 0.15 M