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Sulfation of tibolone and tibolone metabolites by expressed human cytosolic sulfotransferases

Josie L. Falany*, Nancy Macrina, Charles N. Falany

Department of Pharmacology and Toxicology, University of Alabama at Birmingham, 1670 University Boulevard, Volkder Hall G133M, Birmingham, AL 35294, USA

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Abstract

Tibolone is an important therapeutic agent used in the treatment of menopausal symptoms in many countries and has beneficial effects on menopausal and postmenopausal vasomotor, bone, vaginal and mood symptoms without affecting the endometrial, breast or cardiovascular systems. The rapid metabolism of tibolone to active metabolites including 3α-OH-tibolone, 3β-OH-tibolone and Δ^4-tibolone may be important in its tissue-specific effects. Sulfation also has a major role in the metabolism and regulation of the tissue-specific activity of tibolone and its metabolites. The ability of seven major expressed human sulfotransferase (SULT) isoforms to sulfate tibolone and its three metabolites was examined. Expressed human SULT2A1 was capable of sulfating tibolone and all three metabolites with the highest affinity for 3α-OH-tibolone. SULT1E1 conjugated both 3-OH-tibolone metabolites and tibolone itself slightly. SULT2B1b sulfated both 3-OH metabolites but not tibolone or Δ^4-tibolone. SULT isoforms 1A1, 1A3, 1B1 and 1C1 did not demonstrate detectable activity. Sulfation of tibolone and its metabolites by human tissue cytosols was analyzed to determine whether the pattern of tibolone sulfation corresponded to the known expression of SULT isoforms in each tissue. The tissue-specific effects of tibolone may be regulated in part by the inactivation of tibolone and its metabolites by specific human SULT isoforms.

Keywords: Tibolone; Hormone replacement therapy; Estrogen; Sulfation; Sulfotransferase

1. Introduction

Tibolone has been used for many years as a hormone replacement therapy and effectively prevents bone loss and relieves climacteric symptoms of menopause without stimulation of either the endometrium or the breast [1]. With the concerns involving the use of estrogens in estrogen-replacement therapy, there is a renewed interest in evaluating potential therapies that may be used in place of estrogens. The tissue-specific mode of action of tibolone makes it an attractive alternative to other therapies presently available. Tibolone has a unique mode of action as compared to estrogens and selective estrogen receptor modulators (SERMs) such as tamoxifen and raloxifene, and has recently been described as a selective, tissue estrogenic activity regulator (STEAR) [2].

Tibolone is a synthetic steroid possessing a 3-keto-Δ5-10 configuration, a 7α-methyl moiety and a 17α-ethinyl group. In humans, tibolone is quickly metabolized into its main active metabolites, 3α-OH and 3β-OH-tibolone, which are also substrates for sulfate conjugation; in addition, the Δ^4-metabolite of tibolone that is derived from 3β-OH-tibolone is found in the circulation. Fig. 1 shows the pathway for the generation of the major metabolites of tibolone in humans [1]. Sulfation is the major conjugation pathway involved in tibolone metabolism and may be significant in determining the tissue-specific effects of tibolone and its metabolites by modulating activity in situ [1,2]. In general, sulfation inhibits the biological activity of steroidal compounds by prevention of binding to hormone receptors.

Sulfation of tibolone and its metabolites is proposed to have an important role in regulating their tissue-specific effects [1,3]. Selective inhibition of sulfatase activity by tibolone and its metabolites has been proposed as a mechanism for the specific effects of tibolone in breast and bone cells [2,4]. Inhibition of sulfatase activity would decrease the conversion of the sulfates of tibolone and its metabolites to their unconjugated active forms. Also, the presence of specific sulfotransferase (SULT) isoforms in different human tissues may be involved in regulating tibolone activity in a tissue-specific manner.

Eleven isoforms of SULT that may be involved in the sulfation of tibolone and its metabolites are present in

* Corresponding author. Tel.: +1-205-934-9848; fax: +1-205-934-8240.
E-mail address: charles.falany@ccc.uab.edu (J.L. Falany).

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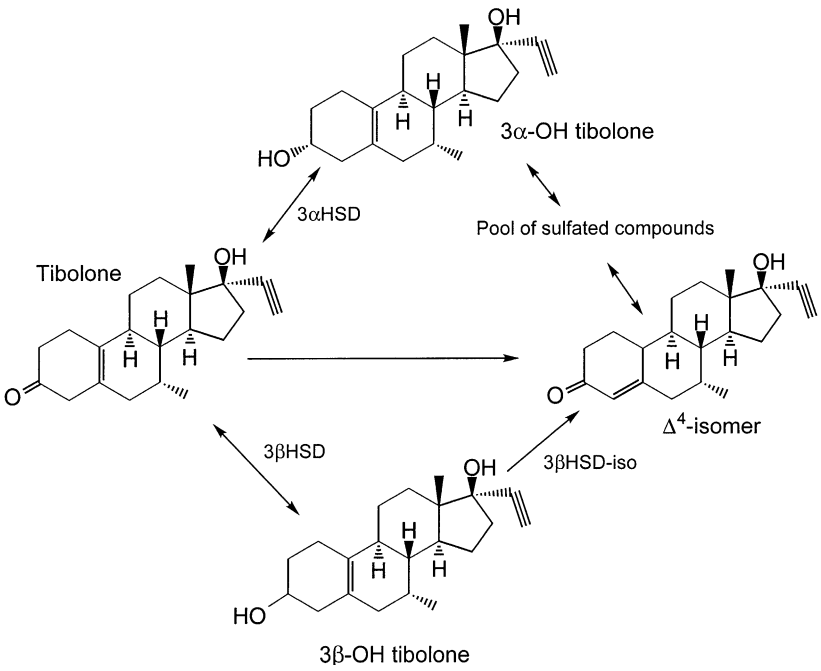

Fig. 1. Major pathways of tibolone metabolism in humans. 3α/β HSD, 3α/β-hydroxysteriod dehydrogenase; 3βHSD-iso, 3β-hydroxysteroid dehydrogenase/isomerase.

human tissues. The cytosolic SULTs are Phase II metabolic enzymes and play an important role in the sulfation of xenobiotics, drugs and small endogenous compounds such as steroids and monoamine neurotransmitters leading to a decrease in the biological activity of these compounds [5–7]. The SULT isoforms can be grouped into three families (1) the phenol SULTs comprised of P-PST-1 (SULT1A1), P-PST-2 (SULT 1A2), M-PST (SULT1A3), EST (SULT1E1), ST1B2 (SULT1B2), ST1C1 (SULT1C1) and ST1C2 (SULT1C2); (2) the hydroxysteroid SULTs, comprised of DHEA-ST (SULT2A1) and ST2B1a and ST2B1b (SULT2B1a and SULT2B1b) [8], the third family of human SULTs consists of SULT4A1 which is highly expressed in brain and highly conserved among mammalian species [9]. The cytosolic SULTs are present in many tissues; therefore, the metabolism and biological activity of tibolone and its metabolites may be differentially regulated in responsive tissues partly as a result of the levels and activity of the SULT isoforms expressed in a specific tissue [10].

This study evaluates the sulfation of tibolone and its metabolites by the different human SULT isoforms. Following an initial analysis to determine which SULT isoforms were active in sulfating the different tibolone compounds, kinetic analysis for sulfation of the four tibolone compounds by the human SULTs was performed and kinetic parameters were determined. Additionally, a panel of representative human tissues was analyzed to characterize the tissue-specific sulfation pattern for tibolone and its metabolites.

2. Materials and methods

2.1. Materials

Tibolone, 3β-OH-tibolone, 3α-OH-tibolone and Δ^4-tibolone were a gift from N.V. Organon (The Netherlands). 3′-Phosphoadenosine, 5′-phosphosulfate (PAPS) was purchased from Dr. Sanford Singer (Dayton, OH). [^{35}S]PAPS (2.2 Ci/mmol) was purchased from New England Nuclear (Boston, MA). LK6DF silica gel thin layer chromotography (TLC) plates were obtained from Whatman International Ltd. (Madison, England). Zirconia/silica beads (1.0 mm) were obtained from BioSpec Products Inc. (Bartlesville, OK).

2.2. Sulfation of tibolone and its metabolites by expressed human SULTs

Sulfation activity was determined using tibolone and its metabolites as substrates with each of the bacterially-expressed human SULT isoforms. With the exception of ST2B1b, the SULTs were expressed in *E. coli* using the pKK233-2 vector to generate the native form of the enzyme and purified by DEAE-Sepharose chromatography to obtain a preparation suitable for enzymatic characterization [6,11–13]. Because the native ST2B1b enzyme is very unstable, ST2B1b assays were performed with a His-tagged construct that confers stability to this particular enzyme [13]. Sulfation assays using tibolone and its metabolites as substrates were performed with each

of the expressed human SULTs (SULT2A1, SULT1E1, SULT2B1b, SULT1A1, SULT1A3, SULT1B1, SULT1C1) with the reaction and isolation procedure that is used for non-radiolabeled steroids. Sulfation activity is assayed using the appropriate non-radiolabeled tibolone substrate and [^{35}S]PAPS with the subsequent resolution of [^{35}S]tibolone sulfates by TLC [14]. Reactions contained the appropriate substrate dissolved in ethanol, 7 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.4 and 10 μM [^{35}S]PAPS in a final volume of 62.5 μl. Control reactions were run with no substrate but containing the appropriate volume of the ethanol vehicle. Reactions were incubated for appropriate times at 37 °C then terminated by spotting a 50 μl aliquot of each reaction on a silica gel TLC plate. The plate was developed in methylene chloride:MeOH:ammonium hydroxide (85:15:5 by volume) and the radiolabeled sulfated products were localized by autoradiography. The sulfated products were scraped into scintillation fluid and radioactivity was determined by scintillation spectroscopy. Initially, substrate concentration curves were performed to determine the substrate concentration generating maximal activity. For comparative purposes, SULT isoforms were assayed at the substrate concentration allowing maximal activity. For determination of apparent Km values, reactions were monitored for linearity with respect to both time and protein concentration. Km values were calculated using the Enzyme Kinetics program (Trinity Software).

2.3. Identification of tibolone sulfates

The UAB Mass Spectroscopy Shared Facility was used for the identification of sulfated tibolone reaction products by MS/MS. To this end, reactions were carried out with low trace amounts of [^{35}S]PAPS to allow localization of reaction products by TLC and autoradiography as described above. Sulfated reaction products were scraped from the TLC plates then extracted from the TLC powder with methylene chloride and water (85:15). Samples were dried and resolubilized in methanol for analysis by LC/MS or MS/MS.

2.4. Sulfation of tibolone and its metabolites by human tissue cytosols

Human tissue specimens were obtained from the Tissue Procurement Service of the UAB Comprehensive Cancer Center. Frozen human tissues were weighed (50–300 mg) and five volumes of 5 mM phosphate buffer, pH 7.4, containing 10% glycerol was added to the tissue in a 1.5 ml screw-cap microcentrifuge tube containing 1 g zirconia/silica gel tissue disruption beads. The tissue was lysed at 4 °C with the Fast Prep Lysis machine using 3 × 20 s bursts and 1 min cooling between bursts. Samples were centrifuged at 10,000 × *g* at 4 °C for 20 min to remove tissue fragments and beads. The tissue lysate supernatant fractions were recovered and centrifuged for 1 h at 100,000 × *g* at 4 °C to recover the cytosolic fraction which was then assayed for protein by the Bradford method [15], aliquoted and stored at −80 °C until use. Sulfation assays were carried out using the TLC method described above for each cytosol.

2.5. Sulfation of tibolone at two sites by DHEA-ST

When reactions were run with either 3α-OH-tibolone or 3β-OH-tibolone as substrate and SULT2A1, a second radioactive reaction product was detected slightly above the origin of the TLC plate. This band became clearly visible only when the TLC plates were developed, dried and developed for a second time in the same solvent system. To ascertain whether this lower Rf band was tibolone disulfate, the higher Rf monosulfate reaction product was purified from the TLC plate by scraping the powder and eluting the tibolone products in ethanol. The purified monosulfate (identified by MS) was then used as substrate in a reaction with [^{35}S]PAPS and SULT2A1 then analyzed by TLC.

3. Results

3.1. Sulfation of tibolone and its metabolites by human SULTs

To ascertain the ability of seven major human SULT isoforms to sulfate tibolone and its metabolites, reactions were run with tibolone and its metabolites as substrates with the expressed human SULTs. Fig. 2 is the autoradiograph of a TLC plate demonstrating the sulfation of tibolone and its metabolites by three of the SULT isoforms tested. SULT2A1 is capable of sulfating tibolone as well as all three of its metabolites with the highest activity towards the 3α-OH and 3β-OH-tibolone metabolites. SULT1E1 sulfates tibolone as well as the 3α-OH and 3β-OH metabolites but shows no detectable activity with the Δ^4-isomer. SULT2B1b sulfates the 3α-OH and 3β-OH-tibolone metabolites with no demonstrable activity when either tibolone or the Δ^4-tibolone metabolite is used as substrate. None of the other SULTs evaluated (SULT1A1, SULT1A3, SULT1B1 or SULT1C1) sulfated any of the tibolone compounds tested. Additionally, when the TLC plates containing tibolone reaction products are dried and redeveloped in the same solvent system, it becomes apparent that the sulfates of 3α-OH and 3β-OH-tibolone migrate more slowly than those generated from tibolone and Δ^4-tibolone. This correlates with the structures of the tibolone-derived compounds. The 3α-OH and 3β-OH-tibolone compounds are preferentially sulfated at the 3-position whereas tibolone and Δ^4-tibolone can be sulfated only on the 17-OH group.

3.2. Identification of tibolone sulfates by MS/MS

For tibolone and each of the tibolone metabolite and SULT combinations generating a [^{35}S]labeled reaction product, the identity of the major reaction products as tibolone-derived

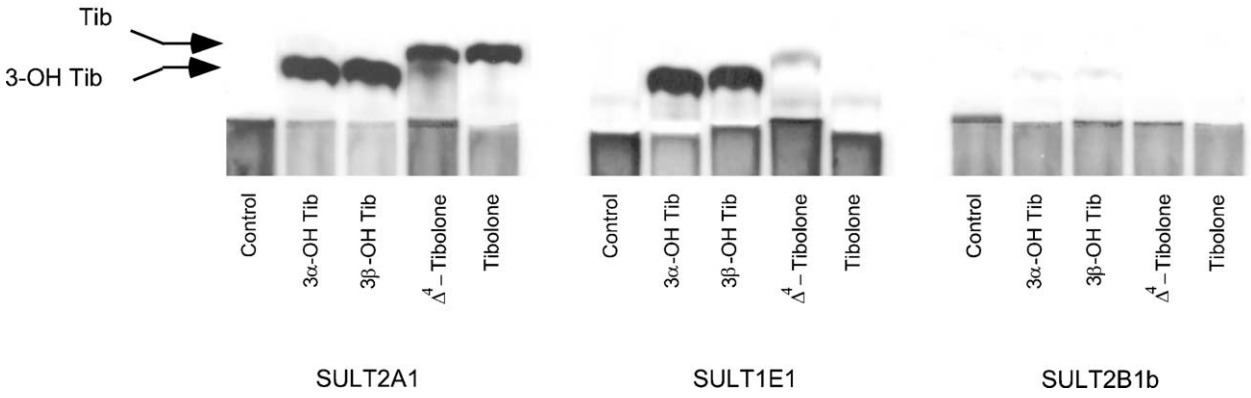

Fig. 2. Sulfation of tibolone and its metabolites by expressed human SULT isoforms. Reactions were run for 20 min with a substrate concentration of 10 μM and 10 μM [^{35}S]PAPS; control reactions were run with no substrate. A 50 μl aliquot of each reaction was spotted on a channeled TLC plate that was developed as described in Section 2 and visualized by autoradiography.

monosulfates was confirmed by MS/MS (data not shown). Although the different Rf values of the 3-OH-tibolone sulfates and the tibolone and Δ^4-tibolone sulfates during TLC suggest that SULT2A1 and SULT1E1 sulfate the tibolone compounds at different sites, the site of sulfation was not confirmed because fragmentation of the tibolone sulfates by MS/MS is difficult due to the stability of the steroid nucleus.

4. Kinetics of sulfation

The ability of the expressed human SULT isoforms to sulfate a range of concentrations of tibolone and its metabolites was tested. Fig. 3 shows concentration curves for the sulfation of tibolone and each of its metabolites using expressed SULT2A1. At concentrations up to 10 μM, 3β-OH-tibolone is sulfated the most rapidly while tibolone itself shows the lowest amount of sulfation. The Km values determined for the sulfation of tibolone and its metabolites using SULT2A1 are shown in Table 1. For Km calculations, reactions were run in duplicate at substrate concentrations in the lower linear portion of each curve to avoid substrate inhibition which is frequently observed with the SULTs [6,11].

Table 1
Km values for the sulfation of tibolone and metabolites using expressed human SULT isoforms

	SULT2A1 (μM)	SULT1E1 (μM)	SULT2B1b (μM)
Tibolone	4.8 + 0.2	19.5 + 2.8	N.D.
3β-OH-tibolone	4.5 + 2.2	6.6 + 2.2	7.9 + 3.1
3a-OH-tibolone	1.4 + 0.1	2.1 + 0.5	3.9 + 1.5
Δ^4-Tibolone	6.4 + 0.8	N.D.	N.D.

The SULTs were expressed in *E. coli* as described in Section 2. The Km values represent the average + S.D. of three separate determinations. Km values were calculated using the Enzyme Kinetics program (Trinity Software). N.D. refers to no detectable activity.

Kinetic analysis of the combinations of individual SULT isoforms and tibolone and metabolites showed that

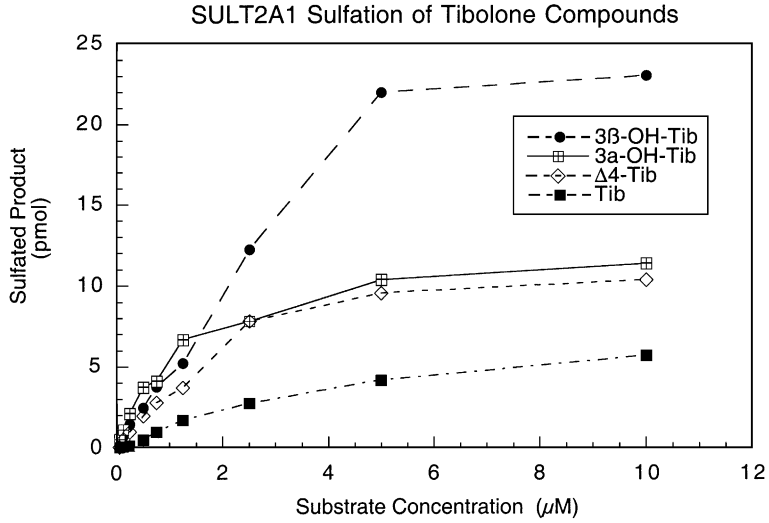

Fig. 3. Concentration curves for sulfation of tibolone and metabolites by expressed human SULT2A1. Reactions were run and analyzed by TLC as described in Section 2 at substrate concentrations from 0 to 10 μM.

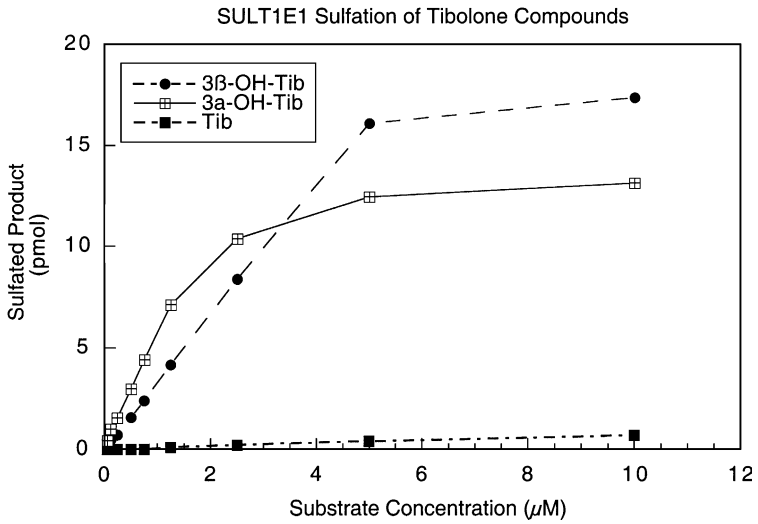

Fig. 4. Concentration curves for sulfation of tibolone and metabolites by expressed human SULT1E1. Reactions were run and analyzed by TLC as described in Section 2 at substrate concentrations from 0 to 10 μM.

the highest affinity was observed for the sulfation of 3α-OH-tibolone and SULT2A1 (Table 1). The lowest affinity was detected for tibolone sulfation by SULT1E1. The remainder of the SULT isoform substrate combinations that demonstrated activity generated apparent Km values in the range of 4–8 μM. Although 3α-OH-tibolone showed the greatest affinity for SULT2A1, higher rates of sulfation were observed with 3β-OH-tibolone.

Fig. 4 shows concentration curves for the sulfation of tibolone, 3α-OH-tibolone and 3β-OH-tibolone by SULT1E1. 3β-OH-tibolone is sulfated at a slightly more rapid rate than 3α-OH-tibolone at concentrations greater than 5 μM. As observed with SULT2A1, 3α-OH-tibolone has a lower Km for sulfation by SULT1E1; however, 3β-OH-tibolone displays a higher sulfation rate at high concentrations. Significantly less sulfation activity was detected with tibolone as a substrate. The high Km value determined for tibolone sulfation by SULT1E1 correlates with the low level of tibolone sulfation (Table 1).

SULT2B1b demonstrates selectivity for the sulfation of 3β-hydroxysteroids such as DHEA and pregnenolone [13]. Consistent with these reports, ST2B1b sulfates only 3α-OH and 3β-OH-tibolone and at much slower rates than either SULT2A1 or SULT1E1 (Fig. 5). Although the sulfation rates for 3α-OH and 3β-OH-tibolone with SULT2B1b were low compared to those of SULT2A1 and SULT1E1, the Km values were similar (Table 1). Again, 3β-OH-tibolone has a lower affinity for sulfation by SULT2B1b but displays the higher rate of sulfation with increasing concentrations.

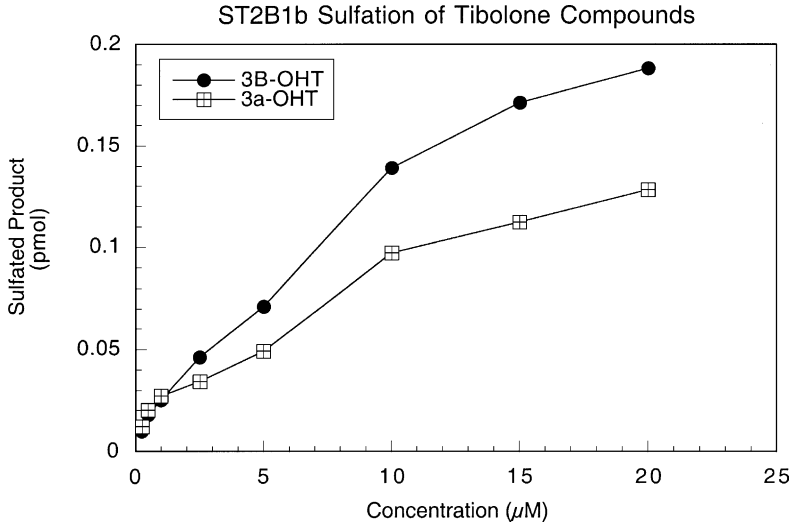

Fig. 5. Concentration curves for sulfation of tibolone and metabolites by expressed human SULT2B1b. Reactions were run and analyzed by TLC as described in Section 2 at substrate concentrations from 0 to 20 μM.

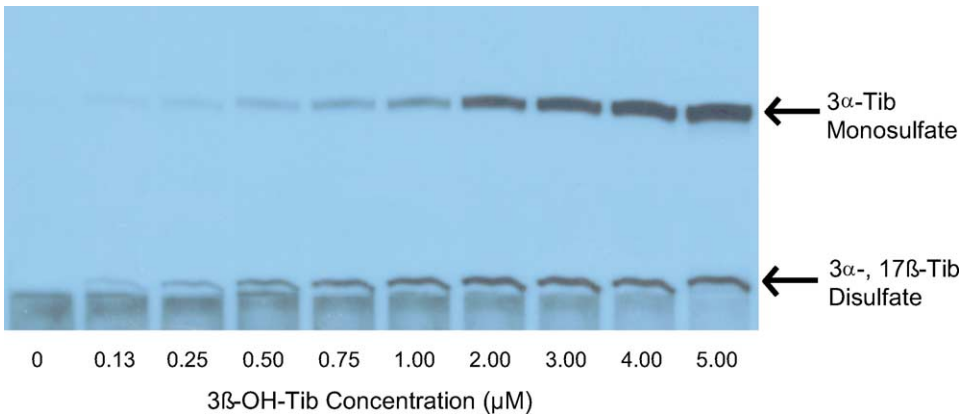

Fig. 6. Generation of two sulfated reaction products from 3α-OH-tibolone by SULT2A1. Reactions were run as described in Section 2 at 3α-OH-tibolone concentrations from 0 to 5 μM with SULT2A1. The TLC plate was developed once in the methylene chloride:methanol:ammonium hydroxide solvent (85:15:5), dried and developed again in the same solvent to ensure visualization of low Rf bands. Two reaction products of different Rfs are generated in a concentration-dependent manner.

4.1. 3α-OH-tibolone and 3β-OH-tibolone disulfates

Previous investigators have reported the presence of tibolone disulfate metabolites in human plasma [16]. Two [^{35}S]-sulfated reaction products were detected in reactions with either 3α-OH or 3β-OH-tibolone and SULT2A1 (Fig. 6). These two [^{35}S]-products were potentially either (1) the 3- and 17-monosulfates or (2) a monosulfate and a disulfate. Based upon the different Rf values, it was anticipated that the higher Rf product was a monosulfate and the more hydrophilic lower Rf product was a disulfate. To determine whether this was the case, each [^{35}S]-labeled product was purified from the TLC plate. Reactions were then run with each radioactive product as substrate, 10 μM [^{35}S]PAPS and SULT2A1 and analyzed by TLC. Fig. 7 demonstrates that the reaction with the higher Rf product as substrate yields the product with the lower Rf, indicating that SULT2A1 is converting a tibolone monosulfate to a disulfate. No new reaction product is formed when the lower Rf compound is used as substrate. No formation of tibolone disulfate was observed when utilizing SULT1E1 or SULT2B1b in these reactions (data not shown).

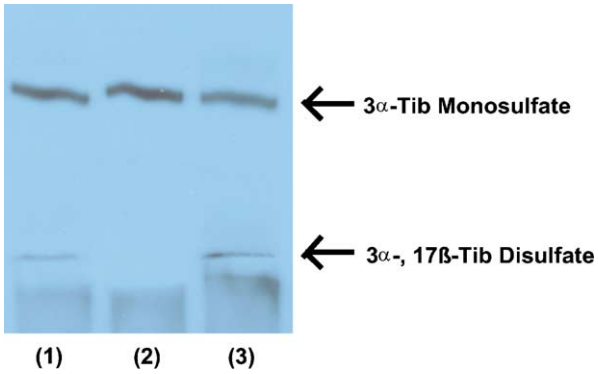

Fig. 7. Production of a disulfated 3α-OH-tibolone reaction product by SULT2A1. From the reactions visualized in Fig. 6, the higher Rf reaction product was purified from the TLC powder. This monosulfate was then used as substrate in a reaction with [^{35}S]PAPS and SULT2A1. Lane (1) aliquot of reaction with SULT2A1 and 3α-OH-tibolone substrate to demonstrate monosulfate and disulfate reaction products; lane (2) reaction run with purified monosulfated reaction product and [^{35}S]PAPS but no SULT2A1; lane (3) reaction run with purified monosulfated reaction product, [^{35}S]PAPS and SULT2A1. A second, lower Rf reaction product presumed to be 3α-, 17β-tibolone disulfate is produced from the monosulfated reaction product in the presence of [^{35}S]PAPS and SULT2A1.

4.2. Sulfation of tibolone by tissue cytosols

Sulfation of tibolone and its metabolites by several representative human tissue cytosols was evaluated and results are shown in Table 2. At least three different specimens of each tissue were assayed using 20 μM tibolone or metabolite as substrate. The human liver specimens sulfate tibolone as well as all three tibolone metabolites. The highest level of activity was also observed with the liver tissue. Small intestine and adrenal cytosols sulfated the 3-OH-tibolone metabolites but not tibolone. The small intestine cytosols also sulfated Δ^4-tibolone to a small extent whereas no activity was detected with the adrenal cytosols. No activity was detected

Table 2
Sulfation of tibolone and metabolites by human tissue cytosols

	Tibolone	3β-OH-tibolone	3α-OH-tibolone	Δ^4-tibolone
Liver	8.5 + 4.1	21.0 ± 5.8	17.6 ± 3.9	6.4 ± 4.2
Adrenal	N.D.	7.9 ± 2.9	8.2 ± 5.2	N.D.
Intestine	N.D.	N.D.	1.6 ± 1.8	1.0 ± 1.0

Cytosol was prepared from human tissue specimens as described in Section 2. Rates are expressed as pmol/min mg protein and represent the means ± S.D. of the assay of at least three different tissue samples. Adrenal specimens were from 52-, 59-, 62- and 66-year-old women. Liver specimens were from a 14-year-old girl and two 56-year-old women. Intestinal specimens were from 44-, 59-, 60- and 65-year-old women.

with tibolone using the small intestine cytosols. No demonstrable activity towards tibolone or its metabolites was found with human kidney, human breast or post-menopausal human endometrium.

5. Discussion

Tibolone is widely used in hormone replacement therapy due to its beneficial effects on bone density, climacteric events and libido with no breast or endometrial effects [1,2,17]. Tibolone is capable of being directly converted to three major metabolites, 3α-OH-tibolone, 3β-OH-tibolone and Δ^4-tibolone, then the further conversion of 3β-OH-tibolone to Δ^4-tibolone can occur (Fig. 1). Sulfation of tibolone and these metabolites is believed to have an important role in regulating the steroidal activity and particularly the tissue-specific effects of tibolone. Therefore, tissue-specific expression of the SULTs may regulate the intracellular activity of the tibolone and its metabolites [18].

Previous investigators [1] have established that tibolone binds to the estrogen receptor (ER) as well as the progesterone receptor (PR) and the androgen receptor (AR), whereas 3α-OH-tibolone and 3β-OH-tibolone bind only to the ER; Δ^4-tibolone binds to both the PR and the AR. Although tibolone itself can theoretically have estrogenic, progestagenic and androgenic actions, it is so rapidly metabolized to 3α-OH and 3β-OH-tibolone that it is more likely that these metabolites as well as Δ^4-tibolone are the primary active compounds in vivo [1,2,17]. Thus the ability of a tissue to respond to tibolone or its metabolites is delineated in part by its receptor status; the receptor-mediated responses may be greatly regulated by the SULT isoforms expressed in the given tissue. Correlation of the receptor status with the known SULT activities in a given tissue will provide a prediction for why certain tissues may be more responsive to tibolone than other tissues.

In healthy postmenopausal women, tibolone is rapidly metabolized to the 3α-OH and 3β-OH isomers. Only 2–3% of an oral dose of tibolone was present as tibolone in the plasma of post-menopausal women and over 75% of tibolone and its metabolites were present in a sulfated form. The three major tibolone metabolites in plasma were 3α-OH 17β-sulfate tibolone, 3α-,17β-disulfate tibolone and 3α-sulfate tibolone. No 3β-sulfates of tibolone metabolites were reported [16]. Following oral administration of tibolone, SULT activity in the liver and intestines may initially modulate tibolone activity via generation of a pool of sulfated tibolone metabolites in the plasma. These sulfated tibolone metabolites may be desulfated in peripheral tissues by sulfatase activity; however, tibolone, its metabolites and both the 3-sulfates have been reported to inhibit sulfatase activity in breast cancer cells [3]. Inhibition of sulfatase activity may aid in maintaining the high levels of tibolone sulfates in the plasma. Also, the profile of SULT isoforms expressed in a tissue may be critical in modulating the intracellular level of active tibolone metabolites in that tissue [17].

SULT2A1, SULT1E1 and SULT2B1b, the three major human steroid SULTs, were responsible for tibolone sulfation [5]. No sulfation activity was detectable with the phenol SULTs; SULT1A1, SULT1A3, SULT1B1 or SULT1C1. SULT2A1, SULT1E1 and SULT2B1b possessed sulfation activity towards tibolone and/or its metabolites but with different Kms, different substrate preferences and, in the case of 3α-OH and 3β-OH-tibolone, with sulfation at different sites on the steroid nucleus (Figs. 3–5). That the major products of these reactions were monosulfates was confirmed via MS/MS. SULT1E1 sulfates the 3-OH position of 3α-OH-tibolone and 3β-OH-tibolone. This is consistent with the Rf value of 3-OH-tibolone sulfates and the preference of SULT1E1 for the 3β-OH of estradiol [11]. The high sulfation activity observed with 3α-OH-tibolone and 3β-OH-tibolone may be due to the presence of the unsaturated bond in the A-ring giving estrogenic structural characteristics to tibolone. This may also explain the formation of small amounts of 17β-tibolone sulfate. The position of the unsaturated bond in the A-ring allows sulfation of the 17-OH group since tibolone is sulfated but Δ^4-tibolone is not.

SULT2A1 sulfated tibolone and the three metabolites with the greatest activity towards 3α-OH and 3β-OH-tibolone (Fig. 3). Tibolone and Δ^4-tibolone can be sulfated only at the 17β-OH position which is reflected in their migration during TLC. SULT2A1 is also capable of sulfating the 17β-OH of testosterone [19] which is consistent with the sulfation of tibolone and Δ^4-tibolone. In reactions with SULT2A1 and either 3α-OH and 3β-OH-tibolone, 3,17β-tibolone disulfate was also formed. Significant amounts of tibolone disulfates are present in the plasma of patients receiving oral tibolone [16] and only SULT2A1 was capable of forming tibolone disulfate metabolites. These results suggest that SULT2A1 in the liver and GI tract is responsible for the formation of the majority of the sulfates of tibolone and metabolites in plasma. Interestingly, Vos et al. [16] did not detect the presence of any 3β-sulfate containing tibolone metabolites in the plasma of postmenopausal women. Since 3β-OH-tibolone is readily sulfated in vitro, the lack of 3β-tibolone sulfate detection in vivo may be due to its conversion to Δ^4-tibolone, its desulfation by sulfatase activity or lack of sensitivity of the assay.

SULT2B1b sulfated only 3α-OH and 3β-OH-tibolone, and at significantly lower levels than either SULT2A1 or SULT1E1 (Fig. 5). SULT2B1b is generally specific for the sulfation of 3β-OH steroids [13]; however, structural analysis of the 3α-OH and 3β-OH-tibolone metabolites suggests that they are more similar in structure than most 3α-OH and 3β-OH steroids which is reflected in the reactivity of ST2B1b.

The role of the SULTs in modulating the effects of these tibolone compounds should also be considered with respect to their tissue-specific expression. SULT2A1 is found

in liver, intestine, stomach, the reticular layer of the adult adrenal cortex and fetal adrenal [6,20] but it is not detectable in endometrium, breast, prostate or kidney [6,21,22]. SULT1E1 expression is limited in tissues but has a major role in regulating the activity of β-estradiol in endometrium where it is cyclically expressed [22,23] and in the breast [14,24]. SULT2B1b is expressed in prostate, skin, placenta, and normal and cancerous breast tissue but not in endometrial tissue [13]. Although SULT2A1 may be responsible for generation of plasma tibolone sulfates, the effects of SULT1E1 and SULT2B1b are probably expressed via the intracellular inactivation of steroids [25,26] rather than by affecting plasma steroid levels.

The sulfation pattern for tibolone and its metabolites in several representative human tissues was elucidated. Liver cytosols sulfated, tibolone and all three metabolites at significantly higher levels than other tissues studied due to the high levels of SULT2A1 in liver. However, only high levels of sulfated tibolone metabolites derived from 3α-OH-tibolone or Δ^4-tibolone have been reported in plasma [16]. Although human adrenal cytosol did not sulfate tibolone or Δ^4-tibolone, significant sulfation activity was detected with both of the 3-OH-tibolone isomers. Adult human adrenal tissue possesses high levels of SULT2A1 involved in the synthesis of DHEA-sulfate [21]. The reason for the lack of detectable tibolone and Δ^4-tibolone sulfation in adrenal tissue is not known. Intestinal cytosol sulfates both of the 3-OH isomers but at a significantly lower rates than either liver or adrenal; additionally, intestine possesses some activity towards Δ^4-tibolone, adding to the pool of plasma sulfates produced by the liver. Tibolone sulfation was not detectable in any of the intestinal cytosols.

Tissue cytosols with no detectable sulfation activity towards tibolone or its metabolites include kidney, postmenopausal endometrium and breast. Endometrial and breast cytosols were included because the effects of tibolone on these hormonally-responsive tissues are of particular interest. Previous studies have established that SULT1E1 is present in human endometrium but in a cyclical manner dependent upon a progesterone surge; thus, in post-menopausal tissues SULT1E1 expression would not be anticipated [22]. Although SULT1E1 has previously been described in normal breast [27], it is present only at low levels which function well in situ but are below detection levels of our TLC assay.

Thus, tibolone acts as a tissue-specific compound whose effects are mediated by steroid receptors and enzymatic pathways. The activity of tibolone and its metabolites may be tightly regulated by the interactions of SULTs and sulfatase; local enzyme activity confers the tissue-specific action of these tibolone compounds that is thus dependent upon the local SULT/sulfatase milieu of each tissue. Ultimately, this translates to the clinical value of tibolone as a compound with desirable effects on bone loss and the climacteric symptoms of menopause but lacking the less desirable stimulatory effects on endometrium and breast.

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