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TITLE PAGE

Selective tissue distribution of tibolone metabolites in mature ovariectomized female cynomolgus monkeys after multiple doses of tibolone

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Running title page

Short title: Tissue Distribution of Tibolone Metabolites in Monkeys

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Abbreviations: 3α/βOH-tib: 3α/β-hydroxytibolone; Δ^4-tib: Δ^4-isomer of tibolone; AKR1C: aldoketoreductase 1C family; mono-S/di-S: mono/di sulfates; LLE: liquid-liquid extraction; STEAR: selective tissue estrogen activity regulator. OH: hydroxy; SPE: solid phase extraction; GC-MS: gas chromatography with mass spectrometry; LC-MS/MS: liquid chromatography with tandem mass spectrometry.

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Abstract

Tibolone is a selective tissue estrogenic activity regulator (STEAR). In postmenopausal women, it acts as an estrogen on brain, vagina and bone, but not on endometrium and breast. Despite ample supporting *in vitro* data for tissue selective actions, confirmative tissue levels of tibolone metabolites are not available. Therefore, we analyzed tibolone and metabolites in plasma and tissues from six ovariectomized cynomolgus monkeys that received tibolone (0.5 mg/kg/day by gavage) for 36 days and were necropsied at 1, 1.25, 2.25, 4, 6 and 24 hours after the final dose. The plasma and tissue levels of active, non-sulfated (tibolone, 3α-hydroxytibolone, 3β-hydroxytibolone, Δ^4–tibolone), mono-sulfated (3α-sulfate,17β-hydroxytibolone, 3β-sulfate,17β-hydroxytibolone) and di-sulfated (3α,17β-di-sulfated-tibolone, 3β,17βS-di-sulfated-tibolone) metabolites were measured by validated gas chromatography with mass spectrometry and liquid chromatography with tandem mass spectrometry. Detection limits were 0.1-0.5 ng/mL (plasma) and 0.5-2 ng/g (tissues). In brain tissues, estrogenic 3α-hydroxytibolone was predominant with 3-8 times higher levels than in plasma; levels of sulfated metabolites were low. In vaginal tissues, major non-sulfated metabolites were 3α-hydroxytibolone and the androgenic/progestagenic Δ^4-tibolone; di-sulfated metabolites were predominant. Remarkably high levels of mono-sulfated metabolites were found in the proximal vagina. In endometrium, myometrium and mammary glands, levels of 3-hydroxymetabolites were low and those of sulfated metabolites high (about 98% di-sulfated). Δ^4-tibolone/3-hydroxytibolone ratios were 2-3 in endometrium, about equal in breast and proximal vagina, and 0.1 in plasma and brain. It is concluded that tibolone metabolites show a unique tissue-specific distribution pattern explaining the tissue effects in monkeys and the clinical effects in postmenopausal women.

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Tibolone (Livial®) is used for the management of postmenopausal symptoms: it relieves hot flushes and vaginal dryness, improves mood and libido, and prevents osteoporosis (Kenemans and Speroff, 2005; Kloosterboer, 2004; Landgren et al., 2005; Swanson et al., 2006). It does not act as an estrogen on endometrium, resulting in a low incidence of vaginal bleeding (Morris et al., 1999). It has little effects on breast as shown by no increase in mammographic density or tenderness (Lundström et al., 2002). It differs thus from estrogen-progestagen combinations. These effects of tibolone on different tissues are explained by the hypothesis that it is a selective tissue estrogenic activity regulator (STEAR).

In postmenopausal women, 3α-hydroxytibolone (3αOH-tib) is the predominant non-sulfated, free metabolite in blood, followed by 3β-hydroxytibolone (3βOH-tib) (for structures, see Figure 1). Both 3-hydroxymetabolites bind to estrogen receptors (Kloosterboer, 2004; Gooyer et al., 2003) and are responsible for the estrogenic activity. Serum levels of tibolone and the Δ^4-isomer of tibolone (Δ^4-tib), which bind to progestagenic and androgenic receptors (Gooyer et al., 2003), are low and become undetectable after 4-6 hours (Timmer et al., 2002a,b). Over 75% of the metabolites in blood are sulfated (Vos et al., 2002).Tibolone and its metabolites have no antagonistic effect on estrogen receptors, rendering it different from selective estrogen receptor modulators (Kloosterboer, 2004). To explain why tibolone is estrogenic in some tissues and not in others, it was postulated that tibolone and its active metabolites are converted into sulfated metabolites and/or into the androgenic/progestagenic Δ^4-tib as has been shown in vitro (Kloosterboer, 2004): Tibolone can be converted to Δ^4-tib in endometrial explants (Tang et al., 1993); enzymes of the aldoketoreductase family (AKR1C) are able to reduce tibolone to 3-hydroxymetabolites (Steckelbroeck et al., 2006) and back again (Schatz et al., 2005. In addition, 3αOH-tib and 3βOH-tib are substrates for sulfotransferases expressed in different tissues (Chetrite et al., 1999a; Falany et al., 2004); SULT2A1 has been reported to be the major endogenous enzyme responsible for sulfation of tibolone metabolites in

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postmenopausal human tissues (Wang et al., 2006). Although sulfated metabolites are not active at receptor levels, sulfatases are able to readily convert 3-mono-sulfated, but not 17-sulfated steroids into active estrogenic metabolites (Gooyer et al., 2001). In breast cell lines, tibolone and its non-sulfated and sulfated metabolites inhibit sulfatases (Chetrite et al., 1997 and 1999a; Purohit et al., 2002). In addition, one study showed that tibolone and its metabolites inhibit sulfatase in endometrium derived cell lines, whereas no inhibition is found in a bone-derived cell line (Gooyer et al., 2001). These tissue-specific differences in the balance between sulfation and desulfation may contribute to tissue-selective effects of tibolone.

Although the mechanisms involved in the tissue-selective effects of tibolone have been demonstrated in vitro, it is not known whether these mechanisms play a role in vivo. Confirmation by measuring tissue levels of tibolone and its non-sulfated and sulfated metabolites is not yet available. In order to obtain these tissue levels, six ovariectomized cynomolgus monkeys were treated orally with 0.5 mg/kg/day tibolone and the tissue levels were analyzed. These monkeys have been selected in view of their marked similarity to humans, as found in a previous study with monkeys fed an atherogenic diet, demonstrating protection by tibolone against bone loss, without stimulation of breast and endometrium (Clarkson et al., 2002 and 2004; Cline et al., 2002). The dose of 0.5 mg/kg/day was chosen in order to be able to measure tissue levels of tibolone metabolites. This study presents the levels of tibolone, its non-sulfated, and its 3-mono-sulfated and 3 and 17β di-sulfated metabolites in efficacy (brain, vagina), safety (uterus, breast) and control (heart) tissues, and in plasma from ovariectomized monkeys treated with 0.5 mg/kg/day tibolone for 36 days.

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Materials and Methods

Study design

A study in mature, healthy, female, ovariectomized cynomolgus monkeys (n=6; 2.0-2.8 kg, supplied by Primate Products, Miami, USA) was approved by the Institutional Animal Care and Use Committee, performed at Huntingdon Life Sciences (USA and UK), and complied with the Animal Welfare Act Regulations and Good Laboratory Practice standards as described elsewhere (Verheul et al., 2007). After at least one month post-ovariectomy, the monkeys received a single dose, and after a washout of 1 week, they received repeated daily oral doses of tibolone (Org OD14; 0.5 mg/kg/d) from days 8-44 via nasogastric gavage. On day 1 and 36, plasma, urine and feces were collected for the assessment of the pharmacokinetic parameters of the sulfated and non-sulfated tibolone metabolites. These results are reported elsewhere (Verheul et al., 2007). At day 44, one monkey was necropsied at each of the following times: 1, 1.25, 2.25, 4, 6 and 24 hours after the final dose. This design was chosen to establish concentration-time curves in plasma and tissues, rather than to determine the concentrations at one time point in 6 animals. Blood was collected into tubes containing K_3EDTA, centrifuged for 10 minutes at 2000 x g within 1 hour, and plasma was stored below -20°C until analysis. The following tissues were washed free of blood and stored at -20 °C until further processing and analysis: brain (cortex, hypothalamus, brain stem, cerebellum, hippocampus, mid brain, corpus callosum, and corpus striatum); vagina (distal and proximal); uterus (endometrium and myometrium); breast (mammary glands and fat) and heart.

Assay methods

The assay procedures for the determination of tibolone, its non-sulfated and sulfated metabolites in plasma and tissue homogenates have been described elsewhere (Verheul et al., 2007).

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After weighing, tissues were homogenized with internal standards in 70% ethanol for 5-15 minutes using an ultraturrax. The homogenates were divided into aliquots and stored for at least 24 hours prior to further sample processing. The aliquots were centrifugated; the supernatant was evaporated, redissolved in water and analyzed. In one aliquot, tibolone, 3αOH-tib and 3βOH-tib were determined by gas chromatographic and mass spectrometric (GC-MS) procedures (ABL, Assen, The Netherlands). The second aliquot was subjected to protein precipitation for analysis of the di-sulfated (di-S) metabolites: 3αS,17βS-tib and 3βS,17βS-tib. Liquid-liquid extraction (LLE) was performed on the third aliquot; Δ^4–tib was determined in the organic phase and the mono-sulfated (mono-S) metabolites [3αS,17βOH-tib and 3βS,17βOH-tib] were extracted by SPE from the water phase. All analytes were determined by liquid chromatography followed by tendem mass spectrometry (LC-MS/MS) procedures (Xendo, Groningen, The Netherlands). It was decided to develop assays for the 3-mono-S metabolites only, since (i) the supply of most tissues was very limited, (ii) the levels of the 17β-mono-S metabolites were demonstrated in a pilot experiment to be very low in myometrium and liver, and (iii) since the 17β-mono-S metabolites can not readily be re-converted to the non-sulfated metabolites again (Gooyer et al., 2001, Takanashi et al., 2003, Goldzieher et al., 1988, Simoncini et al., 2004). In a previous study (Vos et al., 2002) with radiolabelled tibolone, 17β-mono sulfated metabolites constituted less than 10% of the radiolabel administered. We have also determined the 3α- and 3β-disulfated metabolites because their levels were very high in the pilot study and these di-sulfated metabolites were considered as end-product for sulfation.

The GC-MS procedures have been validated for human serum, LC-MS/MS procedures for human serum, myometrium and breast tissue with regard to selectivity, sensitivity, callibration curves, accuracy, precision, stability, dilution and carry-over. The procedures have been used for monkey plasma and tissues without further validation, since no monkey

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control tissue was available for validation. Detection limits were 0.1-0.5 ng/mL (plasma) and 0.5-2 ng/g (tissues). Analytes in monkey plasma and tissues were determined with acceptable precision (Coefficient of Variation <20% for overall, within batch and between batch) and accuracy (bias <20%), except for the mono-S metabolites. Quality control samples for the mono-S metabolites showed that the bias was >20% for low and medium concentrations, resulting in a maximal 60% overestimation. Since mono-S metabolites contributed little to the metabolite patterns, potential overestimation at lower levels was accepted.

For metabolites with concentrations outside the calibration range, a "best estimate" of the concentration is given, provided that the peak exceeded the background by at least 3-fold; if lower, a best estimate of "0" was assigned.

Calculations

The area under the concentration-time curve from 0-24 hours (AUC_{0-24}), maximum plasma concentration (C_{max}) and time to maximum plasma concentration (t_{max}) were calculated using WinNonlin version 4.1 on SAS version 9.1.2. Missing values were estimated by intrapolation from adjacent time points. Plasma and tissue ratios and percentages of metabolites within tissues have been calculated using AUCs.

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Results

Plasma

C_{max}, t_{max} and AUC_{0-24} of tibolone and metabolites at necropsy are presented in Table 1 and plasma concentrations versus time plots in Figure 2 (panel A [non-sulfated metabolites] and B [sulfated metabolites]). Tibolone was not detectable. The predominant non-sulfated metabolite was the estrogenic 3αOH-tib; the AUC of the other estrogenic metabolite, 3βOH-tib was about 20-fold lower. The progestagenic/androgenic metabolite, Δ^4–tib, had about an 8-fold lower AUC than 3αOH-tib. The predominant sulfated metabolite was 3αS,17βS-tib, considerable levels of 3βS,17βS-tib were present; levels of mono-S metabolites were low. Peak levels of the free and di-S metabolites were reached after 1 and 1.25 hours, respectively. Plasma concentrations of all metabolites declined with time (Figure 2, panel A and B). The plasma pharmacokinetic parameters of tibolone metabolites at necropsy, based on one single monkey per timepoint, are comparable to the pharmacokinetic results obtained at day 36, based on 6 monkeys per timepoint (Verheul et al., 2007): e.g. the AUCs at day 36 were 0.1, 69, 5, 14, 127, 3922, 43, and 1801 for tib, 3αOH-tib, 3βOH-tib, Δ^4-tib, 3αS,17βOH-tib, 3αS,17βS-tib, 3βS17βOH-tib, 3βS,17βS-tib, respectively. The concentration-time curves at necropsy and at day 36 are superimposable. This indicates that the plasma results at necropsy obtained in a single monkey per time point are a good reflection of the results obtained in 6 animals.

Tissues

The AUCs of the metabolites in the heart muscle were <3% of those in plasma, indicating that the tissues had been adequately washed free of blood. Tibolone levels were undetectable in tissues and are not presented in tables and figures.

Efficacy tissues

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Brain

Tibolone metabolite patterns were qualitatively comparable in all brain tissues measured, but quantitative, regional differences were found as demonstrated by the AUCs (Table 2). The hypothalamic concentration-time curve is shown in Figure 3 (panel A) as a representative brain tissue. The predominant free metabolite in brain tissues was the estrogenic 3αOH-tib, with peak levels at 1-2.25 hours declining to 10-20% at 24 hours (Figure 3, panel A). The predominant sulfated metabolite in most brain regions was 3αS,17βS-tib, with peak levels later and lower (3-20x) than the corresponding 3αOH-tib. AUCs of 3βS,17βS-tib were lower than those of 3αOH-tib (Table 2), and AUCs of mono-S metabolites were relatively high. Levels of all sulfated metabolites declined with time and were below 1 ng/g at 24 hours (data not shown). Assuming that plasma levels (ng/mL) and tissue levels (ng/g) may be compared, AUCs in most brain areas were higher for 3αOH-tib, 3βOH-tib and Δ^4-tib than in plasma. In contrast, AUCs of di-S metabolites in most brain tissues were considerably lower, ranging from 1/25x in mid brain to <1/100x in other brain regions.

Vagina

The predominant free metabolite in distal vagina was 3αOH-tib. In proximal vagina the androgenic/progestagenic Δ^4–tib metabolite had the largest AUC of the three non-sulfated metabolites (Table 2), although the difference with 3αOH-tib is not large. In both distal and proximal vaginal tissues, 3αS,17βS-tib was the predominant inactive sulfated metabolite, followed by 3βS,17βS-tib (Table 2). T_{max} were 4 and 1-2.5 hours for di-S metabolites and for mono-S and free metabolites, respectively. AUCs of mono-S metabolites were considerably (6x) higher in proximal vagina. Compared with plasma, AUCs in vagina tissues were lower, except for 3βOH-tib and Δ^4-tib and for mono-S metabolites (proximal vagina only). Metabolite patterns in vaginal tissues are different from the metabolite patterns in brain and plasma.

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Safety tissues

Uterus

The AUC of the estrogenic metabolite, 3αOH-tib, in endometrium was 8-fold higher than that of 3βOH-tib (Table 3, Figure 3, panel B). After their peak at 1 hour, both declined rapidly. In contrast, Δ^4–tib levels initially increased for about 4 hours and became higher than those of 3αOH-tib and 3βOH tib (Figure 3, panel B). The predominant sulfated metabolite in endometrium was 3αS,17βS-tib, whereas the mono-S metabolites in endometrium were 40-50-fold lower than those of the di-S. The metabolite pattern in myometrium was comparable to the endometrial pattern. Compared with plasma, AUCs of free and sulfated metabolites were lower in uterine tissues, except those of Δ^4-tib which were considerably (8-10-fold) higher. Compared with brain and vagina, the AUCs of di-S and Δ^4-tib in uterus were higher. The metabolite pattern in uterus is different from those in plasma, brain and vagina.

Breast

The predominant metabolites in glandular breast tissue were di-S metabolites (Table 3) with peak levels at 4 hours. AUCs of 3αOH-tib and 3βOH-tib were 30x and >100x lower (Table 3). The AUC of Δ^4-tib was comparable to that of 3αOH-tib. As in the endometrium, levels of 3αOH-tib (t_{max} = 1 hour) in the breast rapidly declined, whereas those of Δ^4–tib (t_{max} = 2.25 hour) appeared to decline later in time. Compared to fat breast tissue, AUCs of mono-S and di-S metabolites in glandular tissue were higher (1.5-fold), whereas those of free metabolites were lower (0.5-fold). Compared with plasma, AUCs in glandular and fat tissue of S-metabolites were lower (3-fold), those of 3αOH-tib and 3βOH-tib were comparable, and those of Δ^4-tib were higher. Compared with vaginal tissue, in particular vagina proxima, the AUCs of the mono-S in breast tissues were lower. In both breast tissues and uterus, the AUCs of the di-S metabolites were large, but the AUCs of the Δ^4-tib were lower in mammary glands and

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higher in mammary fat tissues. The metabolite pattern in breast tissues is different from those in the efficacy tissues, brain and vagina and from that in uterus.

Ratios

We calculated various ratios and percentages to characterize and compare the qualitative metabolite profiles independent of the actual levels and to illustrate differences in metabolite patterns in different tissues (Table 2 and Table 3). In brain tissues, percentages of free, non-sulfated metabolites were high, in contrast to plasma and all non-brain tissues. Percentages of mono-S metabolites, which can be back converted to active estrogenic metabolites, were less than 10%, except in most brain tissues and proximal vagina. This indicates that sulfated metabolites in plasma, distal vagina, uterus and breast predominantly (>95%) occur in the di-S form. The difference in percentage of mono-S/total-S between proximal (39%) and distal (9%) vagina was remarkable. In all tissues and plasma, 3αOH-tib was predominant over 3βOH-tib; ratios ranging from >20 in brain to about 2 in vagina. The ratio [(tib+Δ^4-tib)/(3αOH-tib+3βOH-tib)] favored the estrogenic [3αOH-tib + 3βOH-tib] metabolites in plasma and all brain tissues, was about equal in the vagina (proximal and distal), breast (glands and fat) and myometrium, and was clearly towards the progestagenic and androgenic [tibolone + Δ^4-tib] metabolites in endometrium. These ratios illustrate that tibolone has unique metabolite patterns in plasma and tissues.

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Discussion

This report presents levels of tibolone, its free and sulfated metabolites in plasma and –for the first time- in tissues. The summary in Figure 4 shows that tissues have unique tibolone metabolite patterns which –in turn- are different from that in plasma.

As in humans, the predominant non-sulfated metabolite in monkeys is 3αOH-tib, whereas levels of tibolone and Δ^4-tib are low and rapidly decline over time. A quantitative difference from humans is the ratio of 3αOH-tib/3βOH-tib in plasma (2-35-fold versus a constant 3-fold in humans). This may be attributed to differences in species, formulation or design (e.g. dose). The 0.5 mg/kg/day dose was used to enhance the chance to detect metabolites in plasma and tissues, also at longer intervals. Based on body weight this is 10-15-fold, and on calorie intake 2-3-fold higher than the human dose (Clarkson et al., 2002; Cline et al., 2002).

Enzymes involved in tibolone's metabolism were previously described. Tibolone is rapidly hydrolyzed by AKR1C isoenzymes (Steckelbroeck et al., 2006). The liver-specific AKR1C4 preferentially forms 3αOH-tib, accounting for the predominance of 3αOH metabolites in plasma. AKR1C1 and AKR1C2 preferentially forming 3βOH-tib were demonstrated in tissues resulting in 3βOH-tib predominance in tissues in vitro. However, our data show that 3αOH-tib is predominant in monkey tissues, suggesting that in vitro data should be extrapolated with caution to in vivo. The high degree of sulfation in plasma may be explained by the SULT2A1 enzyme in the stomach, liver and intestine (Wang et al., 2006), which is able to convert tibolone and its metabolites to mono-sulfates and di-sulfates (Falany et al., 2004). Sulfation renders compounds inactive at receptors. Sulfation at the 17-position seems to be irreversible (Gooyer et al., 2001) as shown in rats with double-labeled estradiol-sulfate (Takanashi et al., 2003). Two studies suggest, however, that 17-sulfation may, to some degree, be reversible (Goldzieher et al., 1988; Simoncini et al., 2004).

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Both AUCs and concentration-time curves of tibolone metabolites in plasma at necropsy from one monkey per time-point were similar to AUCs and concentration-time curves obtained on day 37, based on the mean of 6 monkeys. It would thus appear that –despite single measurements at necropsy- these results are a good reflection of the actual situation. The time-dependent concentration-time curves in tissues also support this conclusion.

Our results show that tibolone metabolites have unique patterns in various tissues. Brain tissues contain high levels of 3-hydroxymetabolites and low levels of di-sulfated metabolites and a considerable percentage of mono-S metabolites (Figure 4). One explanation may be that only more lipophilic, non-sulfated metabolites can pass the blood-brain barrier. However, this would not explain the higher levels of free metabolites in brain compared with plasma. Recently, organic anion transporter proteins (OATP) have been found in various tissues, including the brain (Kullak-Ublick et al., 1998; Steckelbroeck et al., 2004). These proteins can transport 3-sulfated steroids, such as estrone-sulfate and dehydroepiandrosterone-sulfate, across membranes. Assuming that these OATPs described in human temporal lobe are expressed in monkey brain and that they act on other 3-sulfated steroids, it would explain the relatively high levels of the 3-mono-S tibolone metabolites in brain tissues. Mono-S metabolites entering the brain can then readily be desulfated by sulfatases (Gooyer et al., 2001; Takanashi et al., 2003; Goldzieher et al., 1988) leading to receptor-active 3αOH-tib and 3βOH-tib. The 3-mono-S metabolites can thus serve as a reservoir for estrogenic activity. These two mechanisms, transporters for 3-mono-S steroids and local presence of sulfatases, can explain high levels of free and mono-S metabolites in brain tissues. The regional differences in brain mono-S and di-S metabolite levels may be explained by a site-specific balance between sulfotransferase and sulfatase activity; for example, the lack of activity and mRNA expression of SULTs in the temporal lobe (cortex) of human brain (Kullak-Ublick et al., 1998) may account for the low di-S levels in the cortex, whereas different SULT activities

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as demonstrated in different brain tissues in rats (Aldred and Waring, 1999) may explain regional differences in sulfation. The region-specific expression of SULT4A1 in human brain further supports this notion (Liyou et al., 2003), when SULT4A1 can also sulfate tibolone and its metabolites. Irrespective of these mechanisms, our data show that estrogenic 3αOH-tib and 3βOH-tib are predominant in brain, including the hypothalamus, which is primarily involved in control of hot flushes (Stearns et al., 2002). This estrogenic metabolite pattern is in line with the observed estrogenic effects on the brain, i.e. a reduction in hot flushes as shown in ovariectomized monkeys (Jelinek et al., 1984) and postmenopausal women (Landgren et al., 2005). However, estrogenic effects of tibolone on the pituitary seem not to be very strong since tibolone only partly reduces follicle stimulating hormone levels in postmenopausal women (Doeren et al., 2001) and cynomolgus monkeys (Gibbs et al., 2002), in contrast to continuous combined regimens resulting in near complete suppression. Unfortunately, the pituitary was too small for assessment of hormone levels.

Compared to the brain, the proximal vagina has a different metabolite pattern (Figure 4) with higher progestagen/estrogen ratios. The vaginal balance is only slightly towards estrogenic metabolites, but this is apparently sufficient for favorable vaginal effects of tibolone (Kloosterboer, 2004). Interestingly, in the proximal vagina, the percentage of mono-S metabolites is high (39%) compared with the distal vagina. These high levels of 3-mono-S metabolites in the proximal vagina can be desulfated to active estrogenic hydroxy metabolites and may thus serve as an estrogenic reservoir. The androgenic/progestagenic Δ^4-tib is likely not to interfere with tibolone's estrogenic effects on the vagina: in rats (Gooyer et al., 2003) progestagens do not interfere with estrogenic responses in vagina and in early postmenopausal women, addition of medroxyprogesterone acetate does not prevent estrogenic effects of conjugated equine estrogens on the vagina maturation index (Utian et al., 2001). The androgenic effects of Δ^4-tib may also contribute to vaginal lubrication (Traish et al., 2002).

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These observations provide an explanation for favorable effects of tibolone on vaginal atrophy and dryness observed in postmenopausal women (Kenemans and Speroff, 2005; Kloosterboer, 2004; Morris et al., 1999).

Compared to efficacy tissues, metabolite patterns in safety tissues were different. In contrast to the brain, tibolone metabolites in the mammary glands were almost completely (98%) in the di-S form (Figure 4), implying that the balance is towards sulfation. This is supported by in vitro data. Falany et al., (2004) have shown that of the three major human sulfotransferases, SULT2B1b is expressed in breast tissue, and that tibolone, 3αOH-tib and 3βOH-tib are substrates. In addition, tibolone and its metabolites inhibit sulfatase activity in breast cell lines (Gooyer et al., 2001). These combined mechanisms shift the system towards sulfation. The AUCs of the androgenic/progestagenic Δ^4–tib metabolite are higher in breast tissues than in plasma and the brain and the AUC-based Δ^4-tib/3hydroxy-tibolone ratios in mammary glands and fat are approximately 1, suggesting no androgenic/progestagenic or estrogenic predominance. Recently, Stute et al., (2006) showed that in monkeys the amount of breast sulfatase activity is lower after 2-years tibolone treatment in tissues with a high fat content. The potential stimulatory estrogenic effects of tibolone on the breast may be attenuated by the androgenic effects of Δ^4-tib, since physiological doses of testosterone inhibit estrogen-induced mammary epithelial proliferation in ovariectomized monkeys (Dimitrakakis et al., 2003). These observations suggest anti-estrogenic effects of tibolone on breast tissue, in line with its neutral effects on mammographic density and its low incidence of breast tenderness in postmenopausal women (Lundström et al., 2002; Hofling et al., 2005).

In the endometrium, the percentage of sulfated metabolites is also high (>97%) (Figure 4) indicating that comparable mechanisms may be operational to those in the breast. SULT1E1 is expressed in endometrium and modulated by prostagenic activity (Chetrite et al., 1999a,b; Falany and Falany, 1996) and sulfatase activity in endometrial tissue-derived cell lines is

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inhibited by tibolone, and its non-sulfated and sulfated metabolites (Gooyer et al., 2001). In addition to the high sulfation rate, levels of Δ^4–tib in endometrium increase after about 1 hour and remain high, exceeding levels of 3αOH-tib and 3βOH-tib after about 4 hours. This rise in Δ^4–tib at times when plasma Δ^4–tib is undetectable strongly suggests local formation from the 3-hydroxy metabolites, possibly by AKR1C enzymes, as demonstrated in vitro (Steckelbroek, 2006; Schatz et al., 2005). The (tib+Δ^4-tib)/(3αOH+3βOH-tib) ratios in the endometrium are towards the androgenic/progestagenic, Δ^4-tib. The presence of Δ^4-tib is supported by the generation of progestagen-sensitive factors in endometrial cells (Tang et al., 1993; Schatz et al., 2005). It appears that at least two mechanisms preventing stimulation are operational in the endometrium: a high degree of sulfation and the presence of the progestagenic metabolite Δ^4-tib. This corroberates with the lack of endometrial stimulation after tibolone treatment observed in cynomolgus monkeys (Cline et al., 2002) and in clinical studies (Kloosterboer, 2004).

In conclusion, the different tibolone metabolite patterns in plasma and tissues of cynomolgus monkeys are the result of multiple mechanisms including metabolism, enzymatic inactivation or activation, confirming that tibolone is a STEAR. The observed patterns explain the clinical effects in postmenopausal women.

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References

Aldred S, Waring RH (1999) Localisation of dehydroepiandrosterone sulphotransferase in adult brain. *Brain Res Bull* 48:291-296.

Chetrite G, Kloosterboer HJ, Pasqualini JR (1997) Effect of tibolone (Org OD14) and its metabolites on estrone sulfatase activity in MCF-7 and T47D mammary cancer cells. *Anticancer Res* 17:135-140.

Chetrite GS, Kloosterboer HJ, Philippe J-C, Pasqualini JR (1999a) Effect of Org OD14 (Livial) and its metabolites on human estrogen sulfotransferase activity in the hormone-dependent MCF-7 and T47D, and the hormone-independent MDA-MB-231 breast cancer cell lines. *Anticancer Res* 19:269-276.

Chetrite GS, Kloosterboer HJ, Philippe JC, Pasqualini JR (1999b) Effects of Org OD14 (Livial®) and its metabolites on 17β-hydroxysteroid dehydrogenase activity in hormone dependent MCF-7 and TD-47D breast cancer cells. *Anticancer Res* 19:261-268.

Clarkson TB, Anthony MS, Cline JM, Lees CJ, Ederveen AGH (2004) Multisystem evaluations of the long-term effects of tibolone on postmenopausal monkeys. *Maturitas* 48:S24-S29.

Clarkson TB, Anthony MS, Mikkola TS, StClair RW (2002) Comparison of tibolone and conjugated equine estrogens effects on carotid artery atherosclerosis of postmenopausal monkeys. *Stroke* 33:2700-2703.

Cline JM, Register TC, PhD, Clarkson TB (2002) Comparative effects of tibolone and conjugated equine estrogens with and without medroxyprogesterone acetate on the reproductive tract of female cynomolgus monkeys. *Menopause* 9:242-252.

Conner P, Christow A, Kersemaekers W, Soderqvist G, Skoog L, Carlstrom K, Tani E, Mol-Arts M, Von Schoultz B (2004) A comparative study of breast cell proliferation during

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DMD Fast Forward. Published on April 9, 2007 as DOI: 10.1124/dmd.106.014118
This article has not been copyedited and formatted. The final version may differ from this version.

hormone replacement therapy: Effects of tibolone and continuous combined estrogen-progestogen treatment. *Climacteric* 7:50-58.

Dimitrakakis C, Zhou J, Wang J, Belanger A, LaBrie F, Cheng C, Powell D, Bondy C (2003) A physiological role for testosterone in limiting estrogenic stimulation of the breast. *Menopause* 10:292-8.

Doeren M, Rubig A, Coelingh Bennink H, Holzgreve W (2001) Differential effects on androgen status in postmenopausal women treated with tibolone and continuous combined estradiol and norethindrone acetate replacement therapy. *Fertil Steril* 75:554-559.

Falany JL, Falany CN (1996) Regulation of estrogen sulfotransferase in human endometrial carcinoma cells by progesterone. *Endocrinology* 137:1395-1401.

Falany JL, Macrina N, Falany CN (2004) Sulfation of tibolone and tibolone metabolites by expressed human cytosolic sulfotransferases. *J Steroid Biochem Mol Biol* 88:383–391.

Gibbs RB, Nelson D, Anthony MS, Clarkson TB (2002) Effects of long-term hormone replacement and of tibolone on choline acetyltransferase and acetylcholinesterase activities in the brains of ovariectomized cynomolgus monkeys. *Neuroscience* 113:907-914.

Goldzieher JW, Mileikowsky G, Newburger J, Dorantes A, Stavchansky SA (1988) Human pharmacokinetics of ethynyl estradiol 3-sulfate and 17-sulfate. *Steroids* 51:63-79.

Gooyer ME de, Deckers GH, Schoonen WGEJ, Verheul HAM, Kloosterboer HJ (2003) Receptor profiling and endocrine interactions of tibolone. *Steroids* 68:21-30.

Gooyer ME de, Overklift Vaupel Kleyn GT, Smits KC, Ederveen AGH, Verheul HAM, Kloosterboer HJ (2001) Tibolone: a compound with tissue specific inhibitory effects on sulfatase. *Mol Cell Endocrinol* 183:55-62.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 9, 2024

DMD Fast Forward. Published on April 9, 2007 as DOI: 10.1124/dmd.106.014118
This article has not been copyedited and formatted. The final version may differ from this version.

Hofling M, Carlstrom K, Svane K, Azavedo E, Kloosterboer H, von Schoultz B (2005) Different effects of tibolone and continuous combined estrogen plus progestogen hormone therapy on sex hormone binding globulin and free testosterone levels – an association with mammographic density. *Gynecol Endocrinol* 20:110-115.

Jelinek J, Kappen A, Schönbaum E, Lomax P (1984) A primate model of human postmenopausal hot flushes. *J Clin Endocrinol Metab* 59:1224-1228.

Kenemans P, Speroff L, for the international tibolone consensus group (2005) Tibolone: clinical recommendations and practical guidelines A report of the international tibolone consensus group. *Maturitas* 51:21-28.

Kloosterboer HJ (2004) Tissue selectivity: The mechanism of action of tibolone *Maturitas* 49:S30-S40.

Kullak-Ublick GA, Fisch T, Oswald M, Hagenbuch B, Meier PJ, Beuers U, Paumgartner G (1998) Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain. *FEBS Letters* 424:173-176.

Landgren MB, Helmond FA, Engelen S (2005) Tibolone relieves climacteric symptoms in highly symptomatic women with at least seven hot flushes and sweats per day. *Maturitas* 50:222-230.

Liyou NE, Buller KM, Tresillian MJ, Elvin CM, Scott HL, Dodd PR, Tannenberg AEG, McManus ME (2003) Localization of a brain sulfotransferase, SULT4A1, in the human and rat brain: an immunohistochemical study. *J Histochem Cytochem* 51:1655-1664.

Lundström E, Christow A, Kersemaekers W, Svane G, Azavedo E, Söderqvist G, Mol-Arts M, Barkfeldt J, von Schoultz B (2002) Effects of tibolone and continuous combined hormone replacement therapy on mammographic breast density. *Am J Obstet Gynecol* 186:717-722.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 9, 2024

DMD Fast Forward. Published on April 9, 2007 as DOI: 10.1124/dmd.106.014118
This article has not been copyedited and formatted. The final version may differ from this version.

Morris EP, Wison POG, Robinson J, Rymer JM (1999) Long term effects of tibolone on the genital tract in postmenopausal women. *Br J Obstet Gynaecol* 106:954-959.

Purohit A, Malini B, Hooymans C, Newman SP (2002) Inhibition of oestrone sulphatase activity by tibolone and its metabolites. *Horm Metab Res* 1:1-6.

Schatz F, Kuczynski E, Kloosterboer HJ, Buchwalder L, Tang C, Krikun G, Lockwood CJ (2005) Tibolone and its metabolites enhance tissue factor and PAI-1 expression in human endometrial stromal cells: evidence of progestagenic effects. *Steroids* 70:840-845.

Simoncini T, Mannella O, Fornari L, Caruso A, Varone G, Garibaldi S, Genazzani AR (2004) Tibolone activates nitric oxide synthesis in human endothelial cells. *J Clin Endocrinol Metab* 89:4594-4609.

Stearns V, Ullmer L, Lopez J, Smith Y, Isaacs C, Hayes DF (2002) Review: Hot flushes. *Lancet* 360:1851-1861.

Steckelbroeck S, Nassen A, Ugele B, Ludwig M, Watzka M, Reissinger A, Clusman H, Luetjohann D, Siekman L, Klingmueller D, Hans VH (2004) Steroid sulfatase (STS) expression in the human temporal lobe: enzyme activity, mRNA expression and immunohistochemistry study. *J Neurochem* 89:403-417.

Steckelbroeck S, Oyesanmi B, Jin Y, Lee S-H, Kloosterboer HJ, Penning TM (2006) Tibolone metabolism in human liver is catalyzed by the 3α/3β-hydroxysteroid dehydrogenase activities of the four human isoforms of the aldo-keto reductase (AKR)1C subfamily. *J Pharmacol Exp Ther* 316:1300-1309.

Stute P, Register TC, Blair RM, Cline JM (2006) Effects of tibolone on estrogen biosynthesis in the mammary tissue of postmenopausal monkeys. *Menopause* 13:232-240.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 9, 2024

DMD Fast Forward. Published on April 9, 2007 as DOI: 10.1124/dmd.106.014118
This article has not been copyedited and formatted. The final version may differ from this version.

Swanson SG, Drosman S, Helmond FA, Stathopoulos VM (2006) Tibolone for the treatment of moderate to severe vasomotor symptoms and genital atrophy in postmenopausal women: a multicenter, randomized, double-blind, placebo-controlled study. *Menopause* 13:917-925.

Takanashi K, Itoh Y, Watanabe K, Yoshizawa I (2003) Metabolism of [6,7-3H,35S] estradiol 17-sulfate in rats. *Steroids* 68:383-392.

Tang B, Markiewicz L, Kloosterboer HJ, Gurpide E (1993) Human endometrial 3β-hydroxysteroid dehydrogenase/isomerase can locally reduce intrinsic estrogenic/progestagenic activity ratios of steroidal drugs. *J Steroid Biochem Mol Biol* 45:345-351.

Timmer CJ, Houwing NS (2002a) Dose proportionality of three different doses of tibolone. *Pharmacotherapy* 22:6-13.

Timmer CJ, Verheul HAM, Doorstam DP (2002b) Pharmacokinetics of tibolone in early and late postmenopausal women. *Br J Clin Pharmacol* 54:101-106.

Traish AM, Kim N, Min K, Munarriz R, Goldstein I (2002) Role of androgens in female genital sexual arousal: receptor expression, structure and function. *Fertil Steril* 77:S11-S18.

Utian WH, Shoupe D, Bachmann G, Pinkerton JV, Pickar JH (2001) Relief of vasomotor symptoms and vaginal atrophy with lower doses of conjugated equine estrogens and medroxyprogesterone acetate. *Fert Steril* 75:1065-1079.

Verheul HAM, Timmer CJ, Iersel MLPS van, Delbressine LPC, Kloosterboer HJ (2007) Pharmacokinetic parameters of tibolone and metabolites in plasma, urine, feces and bile from ovariectomized cynomolgus monkeys after a single or multiple doses of tibolone. *Drug Metab Disp* 2007: in press

Vos RME, Krebbers SFM, Verhoeven CHJ, Delbressine LPC (2002) The in vivo human metabolism of tibolone. *Drug Metab Dispos* 30:106–112.

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DMD Fast Forward. Published on April 9, 2007 as DOI: 10.1124/dmd.106.014118
This article has not been copyedited and formatted. The final version may differ from this version.

Wang M, Ebmeier CC, Olin JR, Anderson RJ (2006) Sulfation of tibolone metabolites by human postmenopausal liver and small intestine sulfotransferases (SULTs). *Steroids* 71:343-351.

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Figure legends

Figure 1

Structures of tibolone metabolites

Figure 2

Time-concentration curves of tibolone metabolites in necropsy plasma. Panel A [all non-sulfated metabolites] and B [sum (∑) of (3α + 3β mono-S) and sum (∑) of (3α + 3β di-S metabolites)] following multiple doses of tibolone given to cynomolgus monkeys.

Figure 3

Time-concentration curves of non-sulfated tibolone metabolites in hypothalamus (panel A) and endometrium (panel B) following multiple doses of tibolone given to cynomolgus monkeys. The levels of the sulfated metabolites are not included.

Figure 4

AUC of tibolone metabolites in hypothalamus, proximal vagina, endometrium, mammary glands, and plasma. The combined AUCs are presented of the estrogenic metabolites [sum (∑) of (3αOH-tib + 3βOH-tib)], the progestagenic/androgenic [sum (∑) of (Δ^4-tib+tib)] metabolites, the mono-sulphated [sum (∑) of (3αS,17βOH-tib + 3βS,17βOH-tib)] metabolites and the di-sulphated [sum (∑) of (3αS,17βS-tib + 3βS,17βS-tib)] metabolites

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Table 1. Concentration and pharmacokinetic parameters of tibolone metabolites in plasma at necropsy

A. Concentration (ng/mL)

Sampling time (hours)	**3αOH-tib**	**3βOH-tib**	**Δ^4-tib**	**3αS, 17βOH-tib**	**3βS, 17βOH-tib**	**3αS, 17βS-tib**	**3βS, 17βS-tib**
1	30	2	2	32	11	342	105
1.25	6	1	1	23	19	885	464
2.25	10	0	1	18	5	499	223
4	1	0	0	5	4	438	318
6	1	0	0	7	4	95	79
24	1	0	0	2	0	36	4

B. Pharmacokinetic parameters

Parameter	**3αOH-tib**	**3βOH-tib**	**Δ^4-tib**	**3αS, 17βOH-tib**	**3βS, 17βOH-tib**	**3αS, 17βS-tib**	**3βS, 17βS-tib**
AUC_{0-24} (ng/mL*h)	58	3	7	151	74	3541	2079
C_{max} (ng/mL)	30	2	2	32	19	885	464
t_{max} (hours)	1	1	1	1	1.25	1.25	1.25

Values have been rounded to the nearest integer; tibolone was not detectable.

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Table 2. AUCs of tibolone metabolites and ratios and percentages of the metabolites in plasma and efficacy tissues

	Area under the curve (AUC, ng/g*h)							percentage of 3OH metabolites		Tissue ratio
Plasma/Tissue	**3αOH -tib**	**3βOH -tib**	**Δ^4 – tib**	**3αS, 17βOH**	**3βS, 17βOH**	**3αS, 17βS**	**3βS, 17βS**	**total S**	**mono-S**	**Δ^4-tib / (3αOH+3βOH)**
Plasma	58	3	7	151	74	3541	2079	99	4	0.1
Cortex	172	13	26	117	103	34	11	59	83	0.1
Brain stem	387	16	37	7	3	21	4	8	28	0.1
Cerebellum	166	5	26	9	14	31	11	28	36	0.2
Hippocampus	323	10	32	169	10	18	7	13	50	0.1
Midbrain	281	13	9	5	1	142	32	38	3	<0.1
Corpus callosum	480	21	49	73	49	6	1	20	95	0.1
Corpus striatum	223	11	34	21	18	21	7	22	59	0.1
Hypothalamus	235	11	4	3	1	47	24	23	5	<0.1

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	Area under the curve (AUC, ng/g*h)							percentage of 3OH metabolites		Tissue ratio
Plasma/Tissue	**3αOH -tib**	**3βOH -tib**	**Δ^4 – tib**	**3αS, 17βOH**	**3βS, 17βOH**	**3αS, 17βS**	**3βS, 17βS**	**total S**	**mono-S**	**Δ^4-tib / (3αOH+3βOH)**
Vagina (proximal)	24	14	36	194	140	272	244	96	39	0.9
Vagina (distal)	19	8	12	31	22	455	183	96	8	0.4

Ratios and percentages calculated using AUCs and rounded to one decimal place and integers, respectively. <0.1: ratio ranges from 0.01-0.04

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Table 3. AUCs of tibolone metabolites and ratios and percentages of the metabolites in plasma and safety tissues

	Area under the curve (AUC, ng/g*h)							percentage of 3OH metabolites		Tissue ratio
Plasma/Tissue	**3αOH -tib**	**3βOH -tib**	**Δ^4 – tib**	**3αS, 17βOH**	**3βS, 17βOH**	**3αS, 17βS**	**3βS, 17βS**	**total S**	**mono-S**	**Δ^4-tib / (3αOH+3βOH)**
Plasma	58	3	7	151	74	3541	2079	99	4	0.1
Endometrium	22	3	66	18	9	822	441	98	2	2.7
Myometrium	38	5	57	18	6	566	698	97	2	1.4
Mammary glands	31	2	37	13	7	896	515	98	1	1.1
Mammary fat	88	4	101	10	4	520	326	90	2	1.1

Ratios and percentages calculated using AUCs and rounded to one decimal place and integers, respectively.

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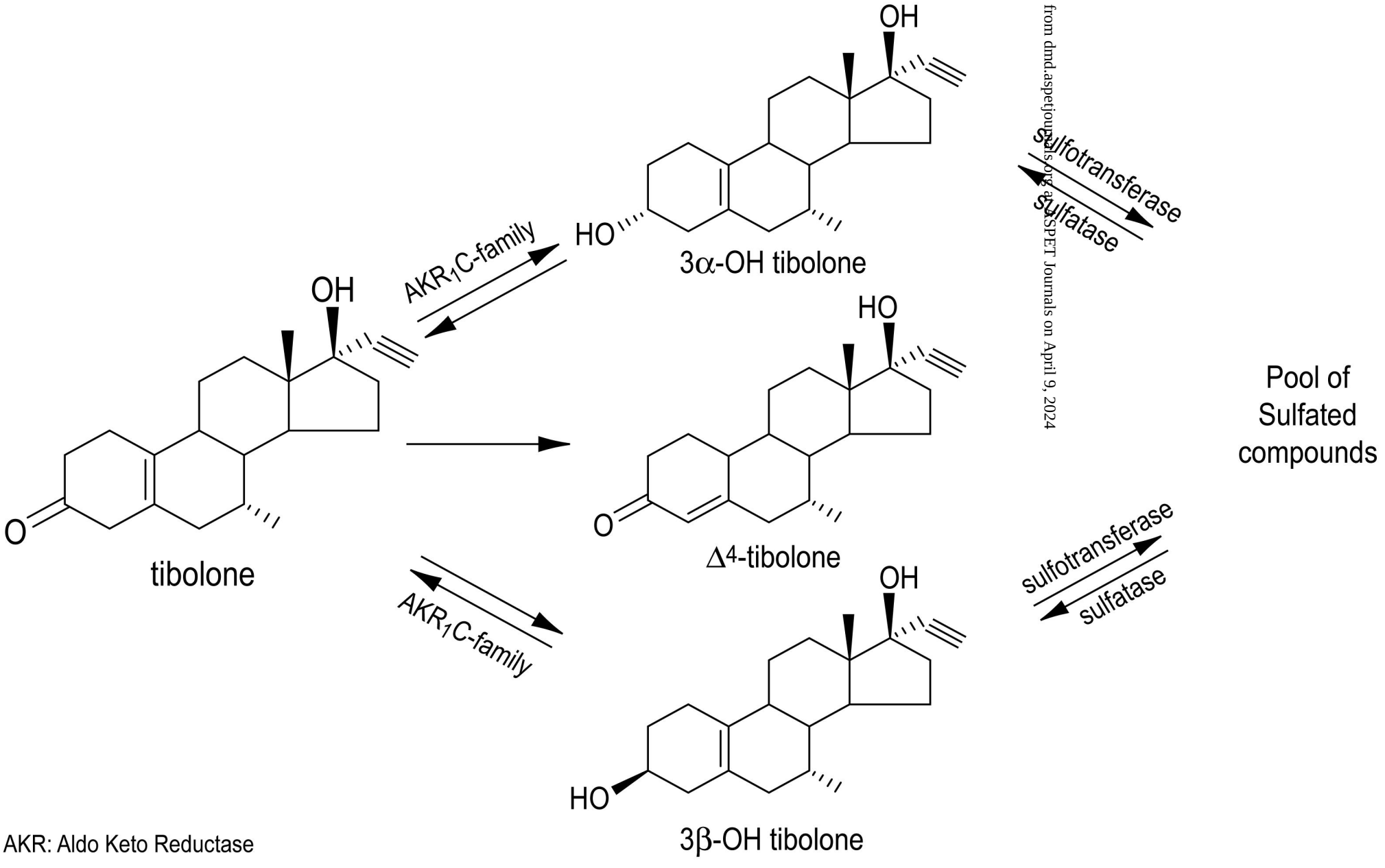
Figure 1



AKR: Aldo Keto Reductase

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Figure 2

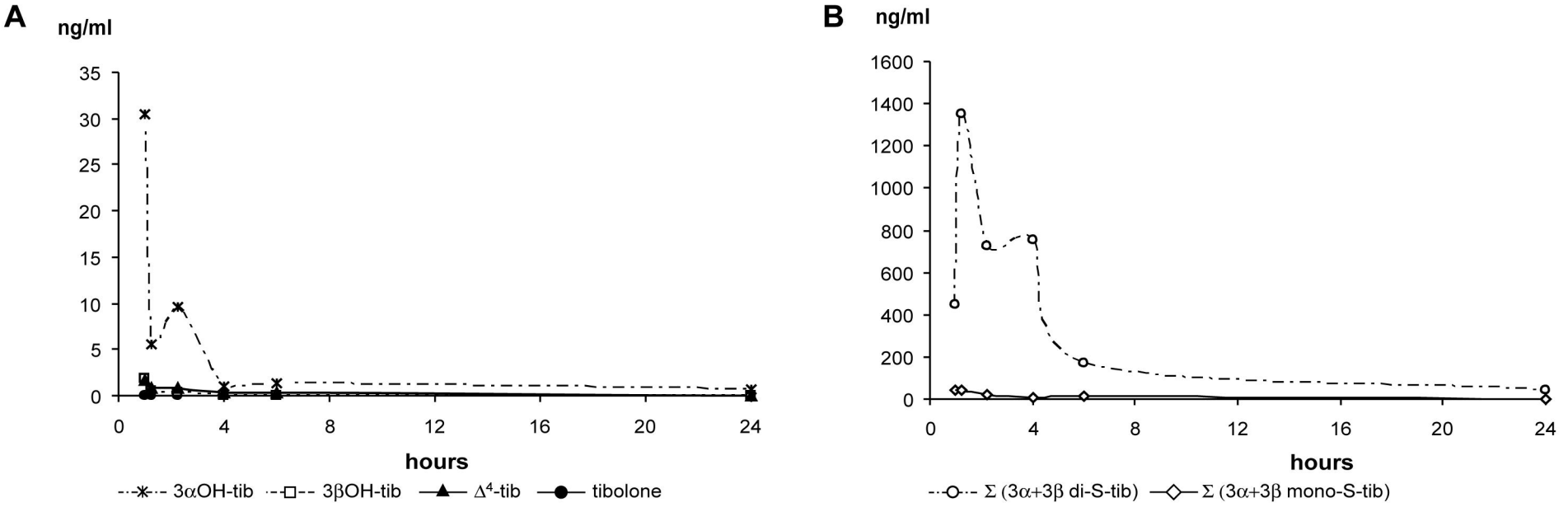

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Figure 3

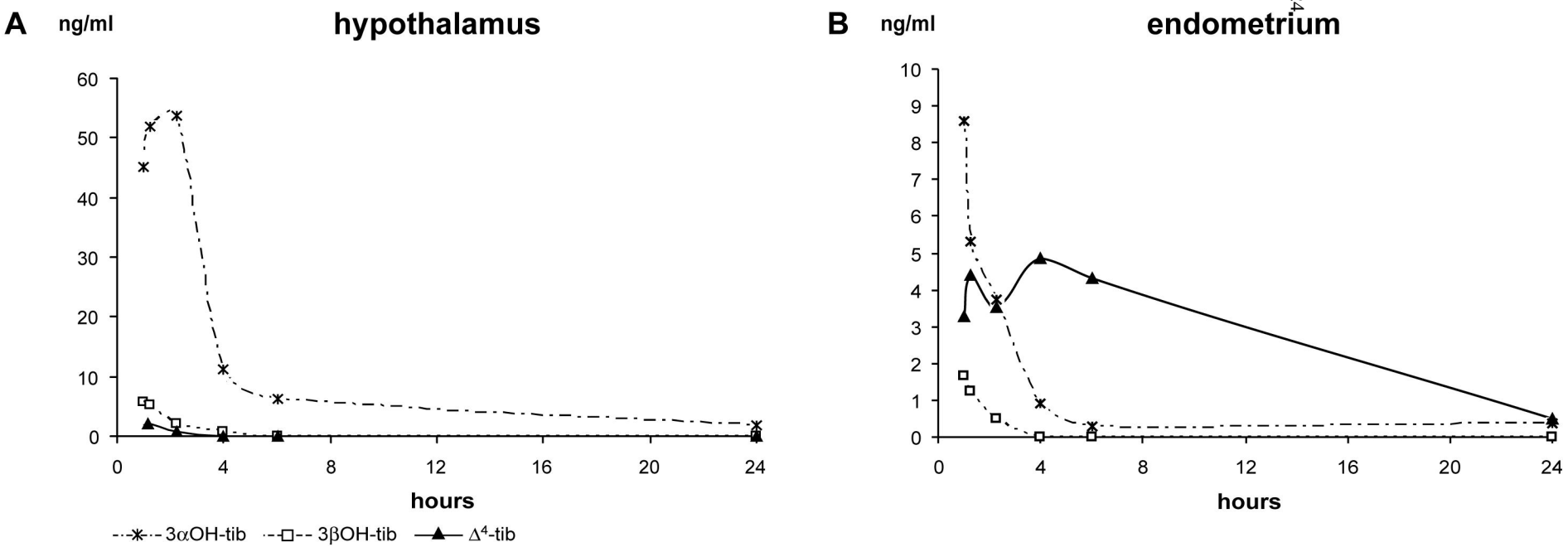

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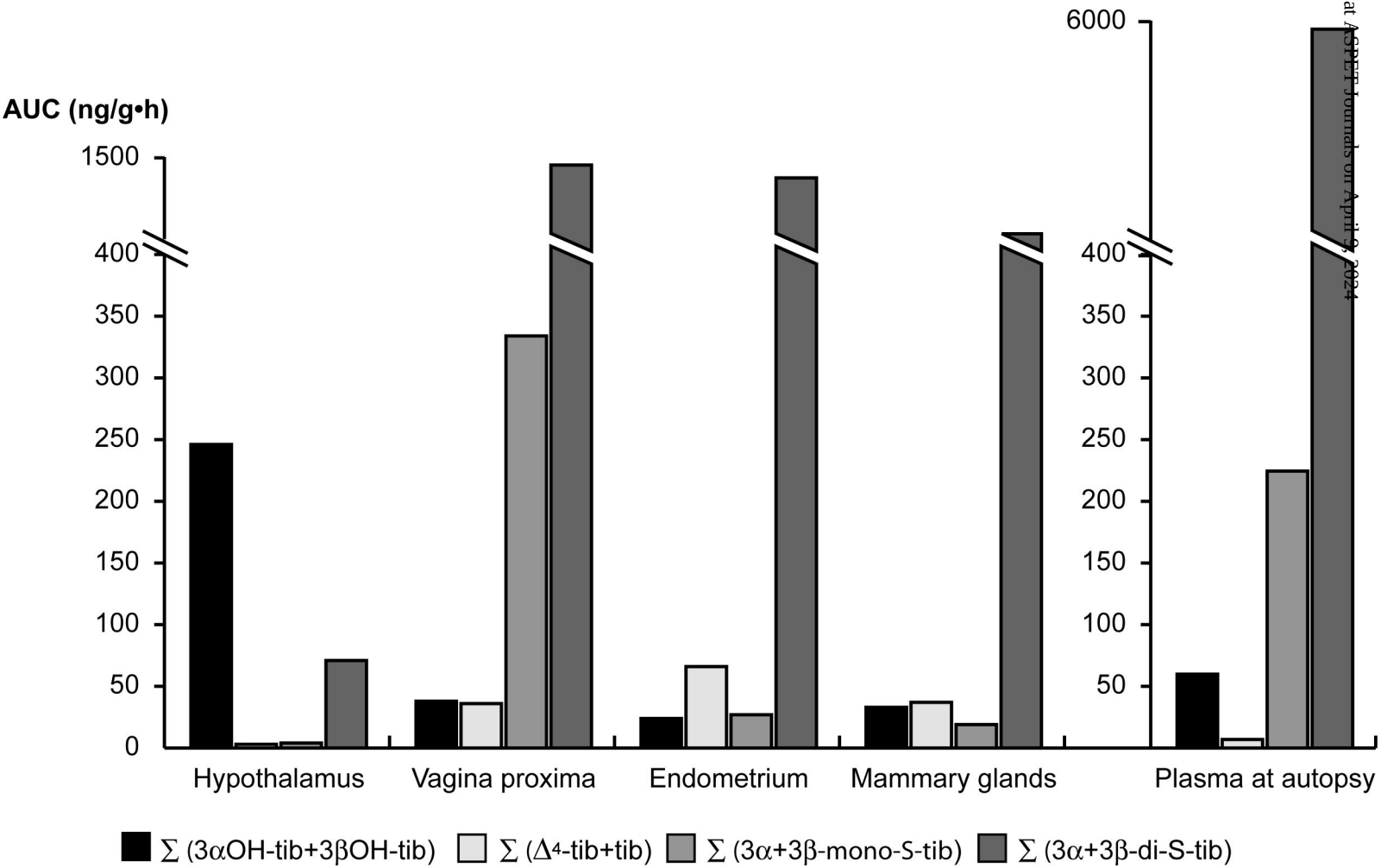
Figure 4



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