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Tibolone and its delta-4, 7α-methyl norethisterone metabolite are reversible inhibitors of human aromatase☆

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Abstract

Tibolone is used for the treatment of climacteric symptoms and osteoporosis in menopausal women. After ingestion, it is rapidly converted to a number of metabolites including 3α- and 3β-hydroxy derivatives and the delta-4, 7α-methylnorethisterone (7α-MeNET) metabolite, which is rapidly cleared from circulation. Tibolone and some of its metabolites act in a tissue-selective manner to inhibit steroid sulphatase (STS) and 17β-hydroxysteroid dehydrogenase Type 1 (17β-HSD1) activities but also stimulate steroid sulphotransferase and 17β-HSD2 activities. In the present study we have examined whether the ability of tibolone and its 7α-MeNET metabolites to regulate the activities of enzymes involved in oestrogen formation or inactivation extends to another key enzyme involved in oestrogen synthesis, the aromatase, which converts androstenedione to oestrone. Using JEG-3 choriocarcinoma cells, which have a high level of aromatase activity, tibolone and 7α-MeNET, but not the 3α- or 3β-hydroxy metabolites, were found to inhibit aromatase activity in intact cells and also lysates prepared from these cells (up to 61% inhibition at 10 μM). An investigation into the nature of aromatase inhibition by these compounds revealed that they inhibit aromatase activity by a reversible mechanism. Tibolone and 7α-MeNET also inhibited aromatase activity in MCF-7 breast cancer cells, which have a much lower level of aromatase activity than JEG-3 cells. It is concluded that, in addition to inhibiting STS and 17β-HSD1, tibolone and 7α-MeNET may exert some of their tissue-selective effects in regulating oestrogen synthesis by also inhibiting aromatase activity.

Keywords: Tibolone; Hormone-replacement therapy; Aromatase; Steroid sulphatase; Oestrogens; Steroid metabolism

1. Introduction

Tibolone is a synthetic steroidal compound used for the treatment of climacteric symptoms and osteoporosis in menopausal women [1,2]. Tibolone (Fig. 1, **1**) is a 19-nortestosterone derivative, which exerts oestrogenic effects on the CNS and bone but not on the breast or endometrium [3]. Although lacking an aromatic A ring which is usually required for oestrogenic activity it is now known that tibolone is rapidly metabolised *in vivo* to the 3α- and 3β-hydroxymetabolites [4]. These metabolites have a low affinity for the oestrogen receptor (ER) and account for the oestrogenic effects associated with tibolone use [5]. Like oestrogens, the 3α- and 3β-hydroxymetabolites of tibolone circulate mainly as the inactive mono- or di-sulphate conjugates [6]. These conjugates may undergo reactivation to their unconjugated, active, forms in tissues such as bone via the action of steroid sulphatase (STS) [7]. Breast, and possibly endometrial tissue, is considered to be protected from the potential oestrogenic effects of tibolone sulphates as tibolone and some of its metabolites act as potent STS inhibitors [8,9]. Inhibition of the transformation of endogenous oestrone sulphate to oestrone may also contribute to the lack of oestrogenic effects on breast tissues that is associated with tibolone use.

Tibolone also undergoes isomerisation to the delta 4 metabolite, 7α-methylnorethisterone (Fig. 1, **2**, 7α-MeNET) mainly in liver and the intestines. In addition, 7α-MeNET can be formed from tibolone, or the 3α-/3β-hydroxy metabolites, which can be converted back to tibolone in endometrial tissues [10]. Furthermore, 7α-MeNET possesses progestagenic

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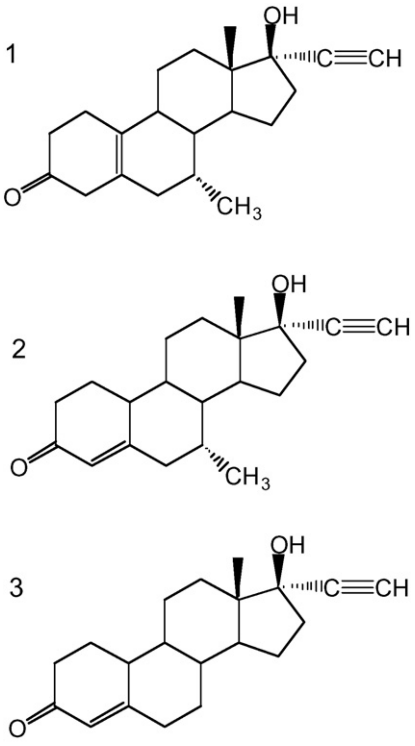

Fig. 1. Chemical structures. **1**, tibolone; **2**, 7α-methylnorethisterone (7α-MeNET); **3**, norethisterone (NET).

and androgenic properties, which are thought to protect the endometrium from oestrogenic stimulation [7]. In addition 7α-MeNET can also inhibit STS activity in breast cancer cells [8,9]. Tibolone and its metabolites can also act to inhibit oestradiol 17β-hydroxysteroid dehydrogenase Type 1(17β-HSD1) activity in breast cancer cells, the reaction that converts oestrone to the biologically active oestrogen, oestradiol while 17β-HSD2, the enzyme responsible for the oxidation of oestradiol to oestrone, is stimulated by tibolone and its metabolites [11]. In addition, the formation of inactive oestrone sulphate from oestrone is stimulated by tibolone at low concentrations [12]. There is, therefore, convincing evidence that tibolone acts as a selective tissue oestrogenic activity regulator (STEAR) by modulating the activities of a number of steroidogenic enzymes in a tissue-selective manner [13,14]. These enzymes all have pivotal roles in regulating the pre-receptor activation of oestrogen precursors.

Several studies have emphasised the important roles for STS, 17β-HSDs and sulphotransferase enzymes in modulating the effects of tibolone but relatively little attention has been paid to the possibility that tibolone might regulate aromatase activity, the key enzyme involved in oestrogen synthesis. In postmenopausal women oestrogens are derived almost exclusively from the peripheral conversion of androstenedione to oestrone [15]. While previous studies have found that some androgens and progestagens can inhibit aromatase activity, an investigation using recombinant human aromatase failed to find evidence to support an inhibitory effect of tibolone and its metabolites on the activity of this enzyme [16]. To further explore this possibility JEG-3 choriocarcinoma cells, which have a high level of aromatase activity, have been employed to examine if tibolone and its 7α-MeNET metabolite can inhibit aromatase activity in intact cells or lysates prepared from these cells. In addition, the ability of these compounds to inhibit aromatase activity in MCF-7 breast cancer cells was also examined.

2. Materials and methods

2.1. Steroids and other chemicals

Tibolone, 7α-MeNET and the steroid sulphatase inhibitor, oestrone-3-*O*-sulphamate (EMATE) used in this study were a kind gift from Dr. H.J. Kloosterboer (NV Organon, Oss, The Netherlands). All other chemicals, unless otherwise indicated, were from Sigma–Aldrich (Poole, Dorset, U.K.)

2.2. Cell culture

JEG-3 choriocarcinoma cells and MCF-7 breast cancer cells were obtained from the ECACC, Salisbury, U.K. Cells were maintained in minimum essential medium (MEM) with 10% foetal bovine serum (FBS), 2 mM glutamine and other essential nutrients. Cells were cultured at 37 °C in a humidified environment with 5% CO_2 and passaged at weekly intervals. For experimental procedures, cells were grown in 24-well plates and allowed to reach 80% confluency.

2.3. Measurement of aromatase activity

2.3.1. Intact cell assay

Aromatase activity was measured in intact cells by a tritrated water method using [1β ^{3}H] androstenedione (15–30 Ci/mmol, PerkinElmer LifeSciences, MA, USA) as the substrate. For this, intact JEG-3 cells or MCF-7 cells were incubated with substrate (2–3 nM) ± test compounds (10 μM or as stated in figure legends) for 3 h (JEG-3 cells) or 20 h (MCF-7 cells) and tritrated water formed was measured after diethyl ether and dextran-coated charcoal extraction [17]. Cell numbers were determined in each well using a Coulter cell counter. Representative results from at least two separate experiments are shown.

2.3.2. Cell lysate assay

JEG-3 cells were lysed using 0.2% Triton-X 100. Aromatase activity was measured by incubating cell lysates (250 μg protein) with substrate ± test compounds for 3 h. Tritrated water formed was measured as described previously.

2.4. Reversibility study

To investigate the nature of inhibition of aromatase activity by tibolone and 7α-MeNET (i.e. reversible or irreversible)