STEROIDS 71 (2006) 639–646

available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/steroids

Lack of aromatisation of the 3-keto-4-ene metabolite of tibolone to an estrogenic derivative

Bindumalini Raobaikady, Michael F.C. Parsons, Michael J. Reed, Atul Purohit*

Endocrinology and Metabolic Medicine, Faculty of Medicine, Imperial College, St. Mary's Hospital, London W2 1NY, UK

ARTICLE INFO

Article history:
Received 21 November 2005
Received in revised form 20 March 2006
Accepted 30 March 2006

Keywords:
Tibolone
Aromatase
7α-Methyl norethisterone
7α-Methyl ethinylestradiol
Hormone-replacement therapy

ABSTRACT

Tibolone is used for the treatment of climacteric symptoms in postmenopausal women. It is metabolised in a tissue-specific manner so that while some metabolites exert estrogenic effects on bone and the CNS, others are thought to protect the breast and endometrium from estrogenic stimulation. Tibolone is a 7α-methyl derivative of 19-norethynodrel. Since the introduction of synthetic progestagens for therapeutic use there has been considerable controversy as to whether they can undergo aromatisation to give rise to the potent estrogen, ethinylestradiol. In this study, we examined whether the delta-4-ene (7α-methyl norethisterone) metabolite of tibolone, which has a similar delta-4-ene A-ring structure to that of the estrone precursor, androstenedione, could undergo aromatisation to the potent estrogen, 7α-methyl ethinylestradiol. For these studies, JEG-3 choriocarcinoma cells were employed as they have a very high level of aromatase activity. TLC and HPLC procedures were developed to separate phenolic from non-phenolic compounds and were initially used to confirm that JEG-3 cells readily aromatised androstenedione to estrogens (up to 74%). The aromatisation of androstenedione to estrogens by these cells could be completely blocked with the potent aromatase inhibitor letrozole. When [^{3}H] 7α-methyl norethisterone was incubated with JEG-3 cells no evidence for its conversion to [^{3}H] 7α-ethinylestradiol was obtained. Radioactivity detected on the TLC plate or HPLC fractions where standard 7α-methyl ethinylestradiol was located, revealed that similar levels were present when 7α-methyl norethisterone was incubated with culture medium alone or with JEG-3 cells in the absence or presence of letrozole. From these investigations, it is concluded that 7α-methyl norethisterone does not undergo aromatisation to an estrogenic derivative.

1. Introduction

Tibolone (Fig. 1, 1) is a 7α-methyl derivative of the synthetic progestagen, norethynodrel and is used as a hormone replacement therapy for postmenopausal women [1,2]. In postmenopausal women after its oral administration 75% of the total dose is present in the circulation as sulfated metabolites [3]. Tibolone exerts estrogenic effects on bone and the CNS as a result of being metabolised to the 3α- and 3β-hydroxy tibolone metabolites which have a low affinity for the estrogen receptors (ER) α and β [4,5]. In contrast, it is not considered to exert any estrogenic effects on the breast or endometrium [6]. These differential effects of tibolone are thought to result from the ability of tibolone, and some of its unconjugated and sulfated metabolites, to inhibit steroid sulfatase (STS) activity in breast tissues and the formation of the 3-keto-4-ene isomer (7α-methyl norethisterone, 7αMeNET, Fig. 1, 2) in the endometrium [7–9]. Since the introduction of synthetic progestagens, such as norethynodrel and norethisterone (NET Fig. 1, 3) in the 1960s, there has been considerable controversy

* *Corresponding author*. Tel.: +44 207 886 1738; fax: +44 207 886 1790.
E-mail address: m.reed@imperial.ac.uk (M.J. Reed).
0039-128X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2006.03.006

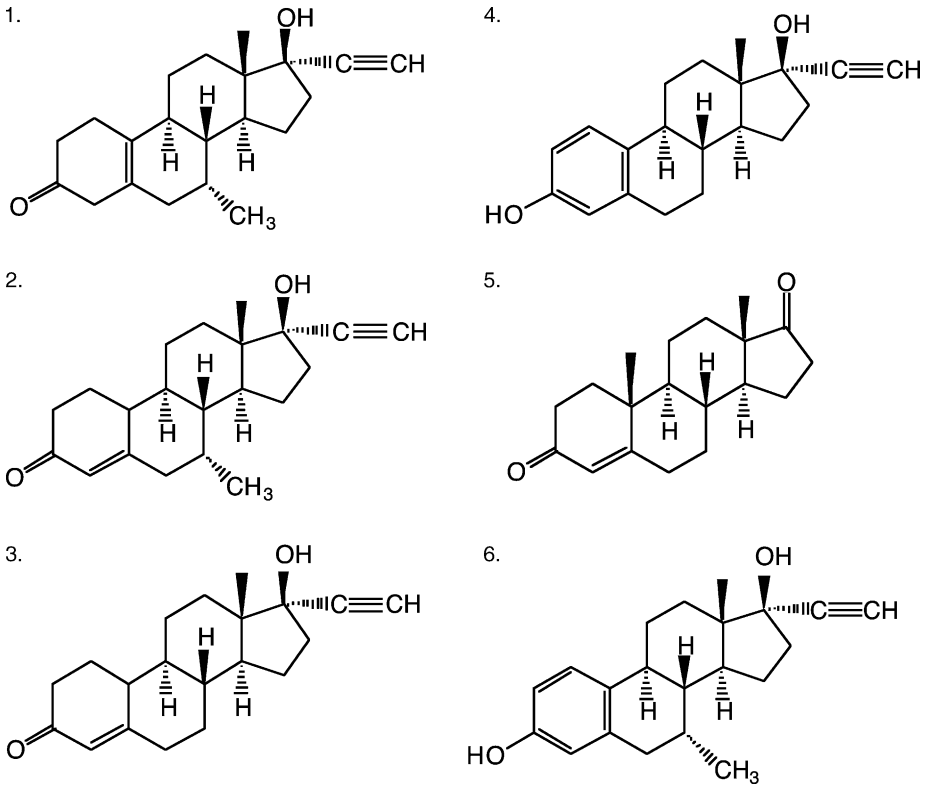

Fig. 1 – Structures. (1) Tibolone, 7α-methyl norethynodrel, (2) 7α-methyl norethisterone (7αMeNET), (3) norethisterone (NET), (4) ethinylestradiol (EE), (5) androstenedione, (6) 7α-methyl ethinyl estradiol (7αMeEE).

as to the extent, if any, to which these steroids are converted to the synthetic estrogen, ethinylestradiol (EE, Fig. 1, 4) [10]. Initial metabolic studies in humans provided evidence for the formation of EE from synthetic progestagens [11]. The possibility was raised, however, that the presence of EE found in urine in these studies might result from its artefactual formation due to the use of acids and bases in the hydrolysis and isolation procedures [12,13]. The extent of the artefactural formation of phenolic products after ingestion of norgestrel by such isolation procedures was investigated by Sisenwine et al. [14]. By adding sodium borohydride to urine and reducing the 4-ene-3-one grouping in the A-ring to a 4-en-3-ol the possibility of aromatisation is prevented. Using this procedure the percentage of the administered dose of norgestrel recovered in the phenolic fraction decreased from 0.63–0.88% to 0.17–0.22%. In vitro studies using placental microsomes or liver tissue appear to show that NET can be aromatised to EE [15,16]. Clinical studies using isotopically labeled 4-^{14}C NET, or unlabeled NET or NETacetate, have shown that a small, but significant, proportion (about 2%) of NET was converted to EE [17,18]. The main substrate for aromatase in postmenopausal women is androstenedione (Fig. 1, 5), which is aromatised to estrone (E1) mainly in adipose tissue [19]. Tibolone undergoes metabolism in the liver, and other tissues dependent on the enzymes present, to the delta-4-isomer of tibolone, 7αMeNET (Fig. 1, 2) which has the same 3-keto-4-ene A-ring structure as androstenedione, although lacking its angular methyl group at C19 [9]. It was recently reported that after the administration of tibolone it was possible to find significant concentrations of 7α-methyl EE (7αMeEE, Fig. 1, 6), in the blood of pre-menopausal women [20]. It was postulated that this was formed by the aromatisation of 7αMeNET. 7αMeEE is a potent estrogen being more active than EE or estradiol (E2) [21]. However, it remains a possibility that the finding of 7αMeEE in blood after tibolone ingestion results from its artefactual formation during the isolation and derivatisation procedures that were employed to measure it using a gas chromatographic mass spectrometric (GC-MS) technique [22].

Whether tibolone and its metabolites could be aromatised has been previously investigated using purified human recombinant aromatase with phenolic metabolites being detected by in vitro receptor bioassays [23]. This study failed to find any evidence in support of tibolone and its metabolites being aromatised. Furthermore, tibolone is known to exert estrogenic effects on bone in the aromatase knockout (ArKO) mouse suggesting that the formation of the 3α/β-hydroxy metabolites are sufficient to account for the estrogenic effects associated with the use of tibolone [24]. Obviously, any estrogenic contamination of tibolone (e.g. from an intermediate generated during its synthesis) would be estrogenic in the ArKO mouse model but there is no evidence for any contamination of this nature. In the present study, isotopically labeled 7αMeNET has been used to examine whether or not it can be aromatised to 7αMeEE. The choriocarcinoma cell line, JEG-3, was used for these investigations as it has a very high level of aromatase activity [25].