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In vitro and *in vivo* evaluation of a 3β-androsterone derivative as inhibitor of 17β-hydroxysteroid dehydrogenase type 3

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ABSTRACT

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3 or HSD17B3) catalyzes the last step in the biosynthesis of the potent androgen testosterone (T), by stereoselectively reducing the C17 ketone of 4-androstene-3,17-dione (4-dione), with NADPH as cofactor. Since T plays an important role in androgen-sensitive diseases, this enzyme is thus an interesting therapeutic target. In an attempt to design compounds to lower the level of T, we synthesized androsterone derivatives substituted at position 3 as inhibitors of 17β-HSD3, and selected one of the most potent compounds for additional studies. In an enzymatic assay in homogenized and whole HEK-293 cells overexpressing 17β-HSD3, the inhibitor RM-532-105 efficiently inhibited the conversion of natural substrate 4-dione (50 nM) into T with an IC_{50} of 26 nM and 5 nM, respectively. Moreover, the inhibitor RM-532-105 (10 mg/kg) reached a plasma concentration of 250 ng/mL at 7 h (AUC 24 h: 3485 ng h/mL) after subcutaneous (s.c.) injection in the rat. In order to mimic the human situation in which 4-dione is converted to T in the testis, we used intact rats. Treatment for 7 days with 17β-HSD3 inhibitor RM-532-105 by s.c. injection or oral gavage exerted no effect on the testis, prostate and seminal vesicle weight and no modification in the levels of plasma steroids. However, after this treatment, the concentration of inhibitor in plasma increased depending on the dose. We thereafter determined the concentration of inhibitor in the testis and we discovered that the compound was slightly present. In fact, at 10 mg/kg, the inhibitor RM-532-105 seems to have difficulty penetrating inside the testis and was found to be concentrated in the testicular capsule, and therefore unable to inhibit the 17β-HSD3 located inside the testis. However, with a higher dose of 50 mg/kg injected s.c. in rats, RM-532-105 significantly decreased the level of T and dihydrotestosterone measured in plasma at 2 h.

1. Introduction

Prostate cancer accounts for about 29% of cancer cases in men and is the second cause of death in men after lung cancer [1]. This cancer is generally an androgen-sensitive disease and the androgens testosterone (T) and dihydrotestosterone (DHT) play an important role in the development, growth and progression of prostate cancer [2–4]. The optimal androgen blockade therapy presently used consists in the administration of an LHRH agonist (medical castration) in combination with a pure antiandrogen (flutamide or bicalutamide) [5,6]. However, studies have shown that the widely used chemical castration with depot LHRH agonist fails to achieve castrate levels of T in men in nearly 20% of cases [7,8]. Moreover, the relative weak affinity of the antiandrogens flutamide and bicalutamide for the androgen receptor (AR) [9] leaves the residual androgens free to interact with the AR and potentially activate the growth of prostate cancer cells. To find a better cure against hormone-dependent prostate cancer, some different enzymes that

Abbreviations: AR, androgen receptor; A-diol, 5α-androstane-3α,17β-diol; A-dione, 5α-androstane-3,17-dione; 5-diol, 5-androstene-3β,17β-diol; 4-dione, 4-androstene-3,17-dione; ADT, androsterone; AUC, area under curve; CTX, castrated; CTL, control; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DMSO, dimethyl sulfoxide; E2, estradiol; E1, estrone; EDTA, ethylenediaminetetraacetic acid; GC, gas chromatography; IC_{50}, half maximal inhibitory concentration; P450c17, 17β-hydroxylase/17,20-lyase; 17β-HSD3 or HSD17B3, 17β-hydroxysteroid dehydrogenase type 3; INT, intact; i.p., intraperitoneal; LH, luteinizing hormone; LHRH, luteinizing hormone-releasing hormone; MS, mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; p.o., per os or gavage; RIA, radioimmunoassay; 5α-Rs, 5α-reductases; s.c., subcutaneous; T, testosterone.

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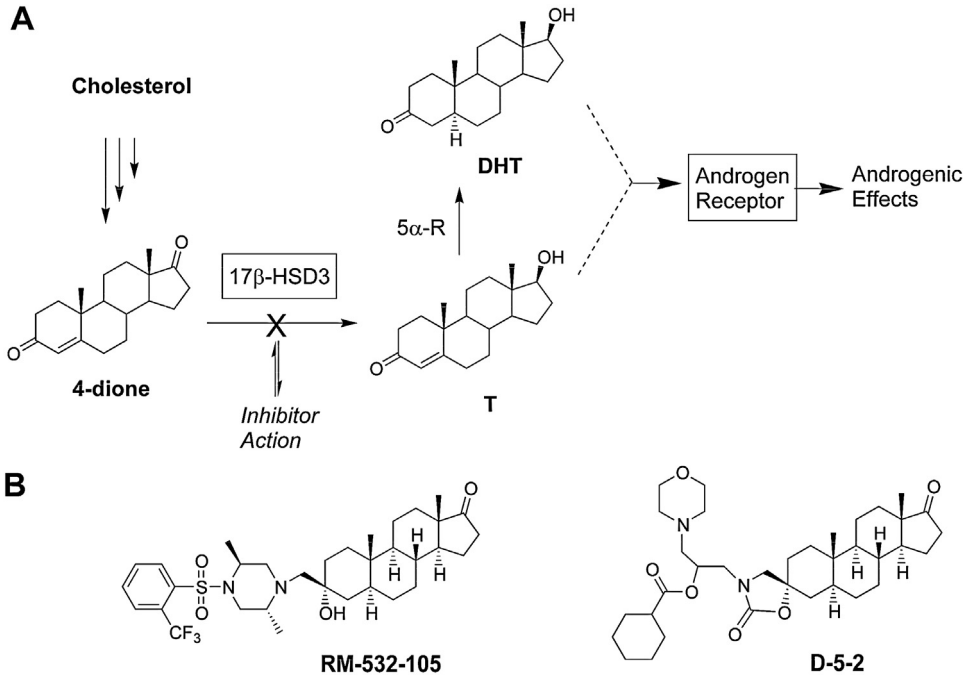

Fig. 1. The role of 17β-HSD3 inhibitor in reducing the production of androgens. (A) Last steps in the biosynthesis of androgens testosterone (T) and dihydrotestosterone (DHT) from cholesterol. (B) The chemical structure of 17β-HSD3 inhibitors RM-532-105 and D-5-2.

catalyze the biosynthesis of androgens have been targeted [10]. The last step in the formation of androgens and estrogens is controlled by the key steroidogenic enzymes 17β-hydroxysteroid dehydrogenases (17β-HSDs or HSD17Bs) and a variety of 17β-HSD isoforms are responsible for the interconversion of 17-ketosteroids (e.g., dehydroepiandrosterone (DHEA), 4-androstene-3,17-dione (4-dione) and estrone (E1)) as well as their corresponding more active 17β-hydroxysteroids (e.g., androst-5-ene-3β,17β-diol (5-diol), testosterone (T) and 17β-estradiol (E2)) [11–15]. Fundamental roles played by the 17β-HSD family in steroid biology probably explain the existence of a large series of isoforms having individual cell-specific expression, substrate specificity, and regulatory mechanisms. Among all 17β-HSD isoforms, we are particularly interested in type 3 (17β-HSD3 or HSD17B3), which is expressed almost exclusively in the testis, principally in the microsomal fraction of Leydig cells [16]. These cells produce T, the precursor of the most active androgen DHT, by a process that requires the reduction of C17 carbonyl of non-androgenic 4-dione [17] catalyzed by 17β-HSD3 and its cofactor NADPH (Fig. 1A) [18–21].

While 17β-HSD3 is expressed almost exclusively in the testis, it is sometimes expressed in other tissues. As examples, 17β-HSD3 mRNA increased over 30-fold in cancerous prostate biopsies [22] and the enzyme is overexpressed 8-fold in LuCaP-23 and LuCAP-35 cell lines, both obtained from metastatic tissues of a patient who was resistant to castration therapy [23–25]. The level of T within these metastatic tumors was thus sufficiently high to stimulate cancer cell proliferation despite the castrated level of T in the bloodstream. 17β-HSD3 was also up-regulated in AR-positive prostate cell line LNCaP after it was treated for 48 h with dutasteride, an inhibitor of 5α-reductases (5α-Rs) types 1 and 2 [26]. Produced from T by the action of 5α-Rs, DHT is the main intracellular androgen in the prostate and stimulates the growth of hormone-dependent prostate cancer via its interaction with the AR. Furthermore, it was reported that 17β-HSD3 catalyzes the biosynthesis of approximately 50% of the total amount of androgen in men [16]. The remaining 50% would result from the same enzymatic reaction catalyzed by 17β-HSD5 [27,28] or 17β-HSD15 [29] in peripheral tissues. In fact, the recently identified 17β-HSD15 is expressed in the prostate and is able to catalyze the transformation of 5α-androstane-3,17-dione (5α-dione) into DHT [29,30]. An alternative pathway, independent of DHEA, 4-dione and T as intermediates, was also recently reported for the synthesis of DHT from progesterone and androsterone (ADT) precursors [31,32]. In addition to 17α-hydroxylase/17,20-lyase (P450c17) and 5α-Rs, this backdoor pathway involves 17β-HSD3, for the conversion of ADT to 5α-androstane-3α,17β-diol (A-diol), and 17β-HSD6, for the conversion of A-diol to DHT. Inhibitors of these enzymes can be consequently used as a therapeutic approach for prostate cancer [10]. However, 17β-HSD3 is a very important enzyme in the production of T since a deficiency of this enzyme has been associated with pseudohermaphroditism [20]. These observations show the important role of 17β-HSD3 and suggest that this enzyme is an interesting therapeutic target. Thus, 17β-HSD3 inhibitors are attractive tools to lower the level of T (Fig. 1A) and to become a potential agent for androgen-dependent prostate cancer.

Several groups have so far developed inhibitors of 17β-HSD3 as reported in recently published review articles [19,33,34]. However, there are few reports of efficacy of these inhibitors in *in vivo* models and this weakness prompted us to study the *in vivo* efficacy of a new family of 17β-HSD3 inhibitors developed by our group. These new inhibitors were generated from a preliminary study on the inhibition of 17β-HSD3 that identified ADT as a lead compound with an IC_{50} value of 330 nM in microsomes of transfected HEK-293 cells [35]. Several libraries of ADT derivatives were next synthesized by adding a hydrophobic group at position 3β of ADT nucleus and measuring their inhibitory potencies and androgenicity [36,37]. Among all these compounds, some ADT derivatives were found to be potent inhibitors of 17β-HSD3 in homogenized and whole HEK-293 cells overexpressing 17β-HSD3 activity while devoid of androgenic activity [38]. Based on these results, we further investigated the potential of one of our best 17β-HSD3 inhibitors (RM-532-105; Fig. 1B) by carrying out *in vitro* and *in vivo* studies on rat models and measuring different biological parameters.