European Journal of Pharmacology 765 (2015) 322–331

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Selective androgen receptor modulator activity of a steroidal antiandrogen TSAA-291 and its cofactor recruitment profile

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ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form
3 August 2015
Accepted 26 August 2015
Available online 1 September 2015

Keywords:
Selective androgen receptor modulator
TSAA-291
Androgen receptor
Castrated mouse models
Mammalian two-hybrid system
Cofactor

ABSTRACT

Selective androgen receptor modulators (SARMs) specifically bind to the androgen receptor and exert agonistic or antagonistic effects on target organs. In this study, we investigated the SARM activity of TSAA-291, previously known as a steroidal antiandrogen, in mice because TSAA-291 was found to possess partial androgen receptor agonist activity in reporter assays. In addition, to clarify the mechanism underlying its tissue selectivity, we performed comprehensive cofactor recruitment analysis of androgen receptor using TSAA-291 and dihydrotestosterone (DHT), an endogenous androgen. The androgen receptor agonistic activity of TSAA-291 was more obvious in reporter assays using skeletal muscle cells than in those using prostate cells. In castrated mice, TSAA-291 increased the weight of the levator ani muscle without increasing the weight of the prostate and seminal vesicle. Comprehensive cofactor recruitment analysis *via* mammalian two-hybrid methods revealed that among a total of 112 cofactors, 12 cofactors including the protein inhibitor of activated STAT 1 (PIAS1) were differently recruited to androgen receptor in the presence of TSAA-291 and DHT. Prostate displayed higher PIAS1 expression than skeletal muscle. Forced expression of the PIAS1 augmented the transcriptional activity of the androgen receptor, and silencing of PIAS1 by siRNAs suppressed the secretion of prostate-specific antigen, an androgen responsive marker. Our results demonstrate that TSAA-291 has SARM activity and suggest that TSAA-291 may induce different conformational changes of the androgen receptor and recruitment profiles of cofactors such as PIAS1, compared with DHT, to exert tissue-specific activity.

1. Introduction

Androgens, such as testosterone (T) and dihydrotestosterone (DHT), have diverse physiological actions in men such as the maintenance of muscle mass/strength, sperm production, fat distribution, and sex drive (Mooradian et al., 1987). Various symptoms such as loss of libido, decreases in muscle mass/strength, and mood disorder in aging men with low blood T levels are improved by T treatment; however, T and DHT are associated with various undesirable side effects (Gooren, 2003). To overcome the limitations of T treatment, several strategies have been investigated. One major approach has been the development of selective androgen receptor modulators (SARMs). SARM is defined as compounds that specifically bind to the androgen receptor and exert agonistic/antagonistic effects in a tissue-specific manner (Negro-Vilar, 1999). SARM displaying tissue-selective anabolic effects in muscle and the bone without exerting adverse effects on prostate or latent prostate cancer would be a useful therapeutic option for sarcopenia, osteoporosis, muscle wasting associated with cancer cachexia, and aging-associated functional limitations (Allan et al., 2007; Miner et al., 2007).

While the molecular mechanism underlying the tissue selectivity of SARM activity is unclear, SARM activity may be understood in the context of the well-established concept of selective estrogen receptor modulator (SERM) activity. Shang and Brown (2002) reported that cell type- and promoter-specific differences in steroid receptor coactivator-1 recruitment play a critical role in determining SERM function in the breast and uterus and offered a paradigm for understanding the action of SERM in other important targets. The transcriptional activity of androgen receptor is modulated *via* its interactions with cofactors, including coactivators that enhance androgen receptor activity and corepressors that inhibit androgen receptor activity (Brady et al., 1999; Dotzlaw et al., 2002; Fu et al., 2000; Yang et al., 2002). However, to the best of our knowledge, no comprehensive cofactor recruitment analysis

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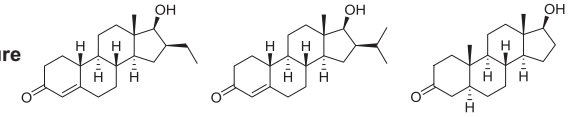
http://dx.doi.org/10.1016/j.ejphar.2015.08.052
0014-2999/

Table 1
Chemical structure and binding affinity to the androgen receptor

	TSAA-291	TSAA-272	DHT
Structure			
Ki (nM)	1.4	1.3	0.28

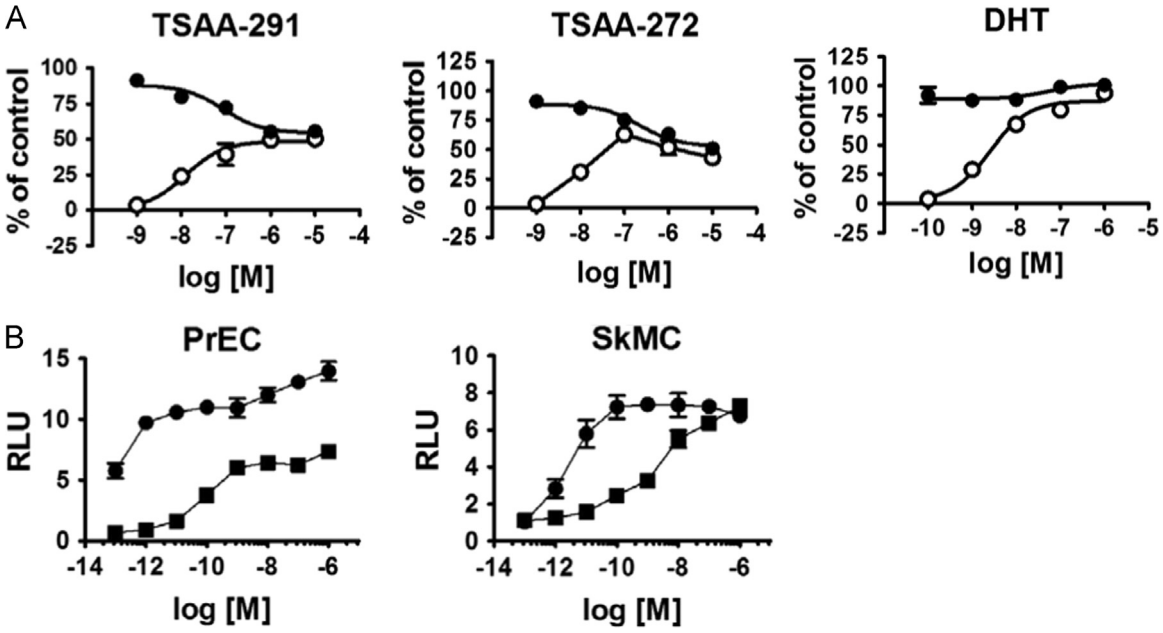


Fig. 1. *In vitro* androgen receptor transcriptional activity of SARM compounds. (A) The reporter assay using monkey kidney fibroblast cells (COS-7 cells). Agonist activity (○) and antagonist activity (●). The agonistic activities are shown relative to those of the 1 μM DHT treatment as 100%. The antagonistic activities by test compound are shown relative to those of the 0.1 μM DHT treatment as 100%. Data are shown as the mean ± standard error of mean (S.E.M.; $n=3$). (B) The reporter assay using human prostate epithelial and human skeletal muscle cells. The concentration of DHT (●) or TSAA-291 (■) was from 0.1 pM to 1 μM. The ordinate values are expressed as RLU, setting the value of the DMSO control as 1. Data are shown as the mean ± S.E.M. ($n=3$).

of androgen receptor has been performed with a mammalian two-hybrid (M2H) system using endogenous ligands and SARM.

TSAA-291 is a steroidal antiandrogen previously used for benign prostatic hyperplasia in Japan. In the present study, we investigated the SARM activity of TSAA-291 in mice because TSAA-291 was found to possess partial androgen receptor agonist activity in reporter assays. In addition, to clarify the mechanism underlying its tissue selectivity, we performed comprehensive cofactor recruitment analysis of androgen receptor using TSAA-291 and DHT.

2. Materials and methods

2.1. Reagents

TSAA-291 (16β-ethyl-17β-hydroxyestr-4-en-3-one) and TSAA-272 (17β-hydroxy-16β-isopropyl-estr-4-en-3-one) were synthesized in our company (Goto et al., 1978).

2.2. Animals

Male Crj:ICR mice (5 weeks old) were purchased from Charles River Laboratories, Japan (Yokohama, Japan). The animals were housed in a temperature-controlled room (23 ± 2 °C) with a 12-h/12-h light/dark cycle and given *ad libitum* access to food (CE-2; CLEA Japan, Inc. Tokyo, Japan) and water. All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee of Pharmaceutical Research Division, Takeda Pharmaceutical Company Limited.

2.3. In vivo Pharmacokinetic study

Male Crj:ICR mice were randomly assigned to treatment groups and subsequently treated with TSAA-291. Plasma was collected *via* heart puncture under anesthesia at the indicated time points (each time point, $n=3$), and the concentration of TSAA-291 was determined by high-performance liquid chromatography.

2.4. In vivo effects of selective androgen receptor modulator compounds on androgen-dependent organ weight

Male Crj:ICR mice were castrated at the beginning of the study. A group of sham-operated male mice was also included as an intact control. The castrated animals were allocated into groups of