European Journal of Pharmacology 735 (2014) 77–85

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Pharmacologic profiles of investigational kisspeptin/metastin analogues, TAK-448 and TAK-683, in adult male rats in comparison to the GnRH analogue leuprolide

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ARTICLE INFO

Article history:
Received 13 October 2013
Received in revised form
26 March 2014
Accepted 27 March 2014
Available online 18 April 2014

Keywords:
Kisspeptin
KISS1R
Testosterone
Prostate cancer
Desensitization
Kisspeptin analogue

ABSTRACT

Kisspeptin/metastin, a hypothalamic peptide, plays a pivotal role in controlling gonadotropin-releasing hormone (GnRH) neurons, and we have shown that continuous subcutaneous administration of kisspeptin analogues suppresses plasma testosterone in male rats. This study examined pharmacologic profiles of investigational kisspeptin analogues, TAK-448 and TAK-683, in male rats. Both analogues showed high receptor-binding affinity and potent and full agonistic activity for rat KISS1R, which were comparable to natural peptide Kp-10. A daily subcutaneous injection of TAK-448 and TAK-683 (0.008–8 μmol/kg) for consecutive 7 days initially induced an increase in plasma luteinizing hormone and testosterone levels; however, after day 7, plasma hormone levels and genital organ weights were reduced. Continuous subcutaneous administrations of TAK-448 ($\geq$ 10 pmol/h, ca. 0.7 nmol/kg/day) and TAK-683 ($\geq$ 30 pmol/h, ca. 2.1 nmol/kg/day) induced a transient increase in plasma testosterone, followed by abrupt reduction of plasma testosterone to castrate levels within 3–7 days. This profound testosterone-lowering effect was sustained throughout 4-week dosing periods. At those dose levels, the weights of the prostate and seminal vesicles were reduced to castrate levels. These suppressive effects of kisspeptin analogues were more rapid and profound than those induced by the GnRH agonist analogue leuprolide treatment. In addition, TAK-683 reduced plasma prostate specific antigen (PSA) in the JDCaP androgen-dependent prostate cancer rat model. Thus, chronic administration of kisspeptin analogues may hold promise as a novel therapeutic approach for suppressing reproductive functions and hormone-related diseases such as prostate cancer. Further studies are warranted to elucidate clinical significance of TAK-448 and TAK-683.

1. Introduction

Prostate cancer is the second most common cancer in the world (Jemal et al., 2011). Since Huggins and Hodges first proposed hormonal therapy to treat prostate cancer in 1941, testosterone-lowering therapy has been the mainstay for prostate cancer treatment. Though the majority of prostate cancer is diagnosed as localized disease and curative treatments such as radical prostatectomy are standard, patients with metastatic prostate cancer are encouraged to receive androgen deprivation therapy such as bilateral orchiectomy (ORX) or gonadotropin-releasing hormone (GnRH) analogue-based therapy. Currently, continuous administration of GnRH analogues, such as leuprolide, is the mainstay of treatment for prostate cancer. Therapy with GnRH analogues is initially associated with substantial testosterone elevation especially during the first week of therapy (Bubley, 2001). This is followed by desensitization of the pituitary, which eventually leads to a decrease in testosterone levels.

Kisspeptin/metastin, a hypothalamic peptide hormone encoded by the *KiSS*-1 gene, is the cognate ligand for a G-protein coupled receptor KISS1R (also known as GPR54) (Ohtaki et al., 2001), and this kisspeptin/KISS1R system is now considered to be a key regulator of hypothalamic GnRH neurons. We and others have demonstrated that central or peripheral administration of kisspeptin results in marked GnRH and/or gonadotropin release in several

Abbreviations: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; ORX, bilateral orchiectomy; PSA, prostate specific antigen

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http://dx.doi.org/10.1016/j.ejphar.2014.03.058
0014-2999/

species including humans (Matsui et al., 2004; Messager et al., 2005; Gottsch et al., 2004; Shahab et al., 2005; Dhillo et al., 2005).

While acute administration of kisspeptin activates the reproductive system, continuous administration of kisspeptin has been shown to suppress reproductive functions in rats (Thompson et al., 2006), monkeys (Seminara et al., 2006; Ramaswamy et al., 2007), and humans (Jayasena et al., 2009). Because natural kisspeptin peptides are highly unstable, and large amounts are required to suppress reproductive functions, we have developed several kisspeptin analogues (Asami et al., 2012a, 2012b). The most promising clinical candidates are TAK-448 and TAK-683, which have enhanced in vivo activity and stability compared to the native peptides (Asami et al., 2013). We have shown that in male rats, continuous administration of TAK-448 suppresses plasma testosterone levels more rapidly and profoundly than leuprolide; this inhibitory effect can be explained by suppressed GnRH pulses (Matsui et al., 2012).

To explore the pharmacodynamic effects of kisspeptin analogues, we evaluated in vitro properties of TAK-448 and TAK-683 in receptor binding and agonistic assays. We then compared the effect of repetitive (once daily) or continuous administration of TAK-448 and TAK-683 with that of leuprolide on plasma drug and testosterone levels as well as the weights of the reproductive organs in adult male Sprague-Dawley rats. We also evaluated the therapeutic potential of TAK-683, as a representative of kisspeptin agonist analog, in a preclinical rat model of androgen-dependent human prostate cancer JDCaP (Kimura et al., 2009) by measuring serum prostate specific antigen (PSA) levels as a biomarker of antitumor activity.

2. Materials and methods

2.1. Materials

TAK-448 (Ac-[D-Tyr^{46},Hyp^{47},Thr^{49},azaGly^{51},Arg(Me)^{53}, Trp^{54}] metastin(46-54), MW: 1225.4), TAK-683 (Ac-[D-Tyr^{46}, D-Trp^{47}, Thr^{49},azaGly^{51},Arg(Me)^{53}, Trp^{54}]metastin(46-54), MW: 1298.5) (Asami et al., 2013), and leuprolide acetate (MW: 1209.40 as acetate-free form) were synthesized by Takeda Pharmaceutical Company, Limited. (Osaka, Japan). These peptide compounds were synthesized as the acetate form, and all doses and plasma drug concentrations in this study were expressed as the acetate-free form. Osmotic ALZET® minipumps (Model 2001 or 2004) were purchased from DURECT Corporation (Cupertino, CA). Other general reagents were aprotinin (Bayer HealthCare Pharmaceuticals, Leverkusen, Germany), EDTA-2Na (Dojindo Laboratories, Kumamoto, Japan), dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO).

2.2. Animals

Adult male Crl:CD (SD) rats and F344/N rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and Clea Japan (Tokyo, Japan), respectively. Animals were housed in a temperature – (23 ± 2 °C) and humidity – (55 ± 10%) controlled room with a 12 h:12 h light:dark cycle. All animals had free access to food and water. All animal experiments were approved by the Takeda Experimental Animal Care and Use Committee.

2.3. Study 1: in vitro Ca^{++} mobilization assay

Agonistic activities of TAK-448 and TAK-683 were evaluated as described previously (Ohtaki et al., 2001; Asami et al., 2013; Terao et al., 2004). Briefly, rat KISS1R-expressing Chinese hamster ovary (CHO) cells were stimulated with various concentrations of the peptides, and intracellular Ca^{++} mobilization was monitored by means of Ca^{++} indicator Fluo-3AM in FLIPR system (Molecular Devices, Sunnyvale, CA). The EC50 values and 95% confidence intervals (CI) ($n=3$) were calculated by logistic regression analysis using the SAS system version 8.2 (SAS institute Inc, Cary, NC). In this assay system we also evaluated Kp-10 as internal control; the EC50 and 95% CI were 310 (274–351) pM in a representative experiment.

2.4. Study 2: in vitro receptor binding assay

Receptor binding affinity of TAK-448 and TAK-683 were evaluated by competitive receptor binding assay using the membrane fraction of rat KISS1R-expressing CHO cells and ^{125}I-labeled Kp-15 (corresponding to N-terminal 15-aa residues of human Kp-54, originally called as metastin (40–54)) (Ohtaki et al., 2001). The IC50 values and 95% CI ($n=6$) were calculated by logistic regression analysis using the SAS system. Kp-10 was also evaluated as internal control; the IC50 and 95% CI values were 130 (130–140) pM in a representative experiment.

2.5. Study 3: 7-day repeat dosing of TAK-448 in male rats

Male SD rats (10 weeks of age, body weight range 372–460 g) were randomly assigned and subcutaneously injected once a day with either vehicle (5% glucose solution; Otsuka Pharmaceutical Laboratory, Tokushima, Japan) or TAK-448 (0.008, 0.08, 0.8, or 8 μmol/ml/kg) for consecutive 7 days ($n=4$). Blood samples (0.4 ml) were obtained from tail vein at 0 h (just before dosing) and 4 h (4 h after dosing) on days 1, 4 and 7. Blood samples were immediately mixed with aprotinin solution containing 10% w/v of EDTA-2Na, and centrifuged to obtain plasma samples, and then stored below −30 °C (for plasma hormone levels) or −80 °C (for plasma drug concentrations) until measurement. On day 8 the prostate, the seminal vesicles, and the testes were removed and weighed.

2.6. Study 4: 7-day repeat dosing of TAK-683 in male rats

Male SD rats (8 weeks of age, body weight range 284–308 g) were randomly assigned and subcutaneously injected once a day with either vehicle (5% glucose solution) or TAK-683 (0.008, 0.08, 0.8, or 8 μmol/ml/kg) for consecutive 7 days ($n=4$). The remaining procedure was identical as described above as Study 3.

2.7. Study 5: 4-week continuous administration of TAK-448 or TAK-683 in male rats

Two separate experiments were conducted. In the first experiment, male SD rats (9 weeks of age, body weight range=318–368 g) were assigned to vehicle control, bilateral orchiectomy (ORX), TAK-448 or TAK-683 (10, 30, or 100 pmol/h), or leuprolide (30, 100, or 300 pmol/h) based on the body weight ($n=10$). In the second experiment, male rats (9 weeks of age, body weight range=302–350 g) were assigned to vehicle, ORX, or TAK-448 (0.85, 3, or 10 pmol/h) based on the body weight ($n=9-10$) to evaluate the effect of TAK-448 at lower doses. In both experiments, an osmotic ALZET minipump containing vehicle (50% DMSO; mixture of 1 volume of DMSO and 1 volume of distilled water) or compounds was implanted subcutaneously, and blood samples were collected before dosing (experimental day 0), 1, 2, 3 days after the initiation of dosing, and every 7 days after day 7. For ORX animals, we performed bilateral orchiectomy under anesthesia by isoflurane after blood collection on day 0. Plasma samples were prepared and stored as described above. On day 28, weights of the prostate, seminal vesicles, and testes were recorded and expressed