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Suppression of the hypothalamic–pituitary–gonadal axis by TAK-385 (relugolix), a novel, investigational, orally active, small molecule gonadotropin-releasing hormone (GnRH) antagonist: Studies in human GnRH receptor knock-in mice

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ABSTRACT

TAK-385 (relugolix) is a novel, non-peptide, orally active gonadotropin-releasing hormone (GnRH) antagonist, which builds on previous work with non-peptide GnRH antagonist TAK-013. TAK-385 possesses higher affinity and more potent antagonistic activity for human and monkey GnRH receptors compared with TAK-013. Both TAK-385 and TAK-013 have low affinity for the rat GnRH receptor, making them difficult to evaluate in rodent models. Here we report the human GnRH receptor knock-in mouse as a humanized model to investigate pharmacological properties of these compounds on gonadal function. Twice-daily oral administration of TAK-013 (10 mg/kg) for 4 weeks decreased the weights of testes and ventral prostate in male knock-in mice but not in male wild-type mice, demonstrating the validity of this model to evaluate antagonists for the human GnRH receptor. The same dose of TAK-385 also reduced the prostate weight to castrate levels in male knock-in mice. In female knock-in mice, twice-daily oral administration of TAK-385 (100 mg/kg) induced constant diestrous phases within the first week, decreased the uterus weight to ovariectomized levels and downregulated GnRH receptor mRNA in the pituitary after 4 weeks. Gonadal function of TAK-385-treated knock-in mice began to recover after 5 days and almost completely recovered within 14 days after drug withdrawal in both sexes. Our findings demonstrate that TAK-385 acts as an antagonist for human GnRH receptor *in vivo* and daily oral administration potently, continuously and reversibly suppresses the hypothalamic–pituitary–gonadal axis. TAK-385 may provide useful therapeutic interventions in hormone-dependent diseases including endometriosis, uterine fibroids and prostate cancer.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide, which is synthesized in the hypothalamus. It plays a pivotal role in the secretion of gonadotropins, such as luteinizing hormone and follicle-stimulating hormone (Counis et al., 2005). Since sustained administration of GnRH agonists decreases circulating gonadal hormones as a result of desensitization of the gonadotrophs and a decline in both the synthesis and secretion of gonadotropins, GnRH agonists are generally used for the treatment of hormone-dependent diseases such as prostate cancer, premenopausal breast cancer, endometriosis and uterine fibroids (Broekmans, 1996; Labrie et al., 2005; Olive, 2004; Robertson and Blamey, 2003). However, GnRH agonists can cause transient exacerbation of clinical symptoms associated with an initial surge of gonadotropins and gonadal hormones. In contrast to GnRH agonists, GnRH antagonists achieve immediate decreases of these hormones and thus fast onset of therapeutic effects without adverse effects induced by the flare-up. To date, several peptide GnRH antagonists have been developed, including: cetrorelix and ganirelix, which are used for *in vitro* fertilization; and degarelix for advanced prostate cancer (Huirne and Lambalk, 2001; Doehn et al., 2009). These peptide antagonists, however, must be administered by frequent subcutaneous injection or sustained release formulation due to lack of oral bioavailability. Therefore, an unmet clinical need still exists for orally available GnRH antagonists to circumvent

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problems associated with existing peptide antagonists including injection site reactions and inability to discontinue treatment during the release period. Various non-peptide GnRH antagonists have also been developed by several research groups; some have been evaluated in clinical trials including TAK-013 (sufugolix), previously discovered by our laboratories (Mezo and Manea, 2009; Sasaki et al., 2003). Recently, elagolix, a uracil derivative, also demonstrated suppression of gonadotropins and estradiol in premenopausal women by oral administration and is now in clinical trials for the treatment of endometriosis (Struthers et al., 2009) and uterine fibroids (ClinicalTrials.gov).

TAK-385 (relugolix), which builds on previous work on TAK-013 (Sasaki et al., 2003), is an investigational, novel thienopyrimidine derivative and possesses higher affinity and more potent antagonistic activity for human (binding IC_{50}=0.33 nM in the presence of serum) and monkey (IC_{50}=0.32 nM) GnRH receptors compared with TAK-013 (Miwa et al., 2011). Both compounds, however, have low affinity for the rat GnRH receptor (TAK-385: IC_{50}=9800 nM) (Miwa et al., 2011), which makes it difficult to evaluate their antagonistic effects in rodent models. Therefore, we generated human GnRH receptor (hGNRHR) knock-in mice, in which mouse *Gnrhr* was substituted with human *GNRHR*, to evaluate the effect of TAK-385 on the hypothalamic–pituitary–gonadal axis and to characterize the pharmacological properties *in vivo*.

2. Materials and methods

2.1. Chemicals

TAK-385 (relugolix; 1-{4-[1-(2,6-difluorobenzyl)-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea) and TAK-013 (sufugolix; 1-{4-[5-{[benzyl-(methyl)amino]methyl}-1-(2,6-difluorobenzyl)-2,4-dioxo-3-phenyl-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidin-6-yl]phenyl}-3-methoxyurea) were synthesized in house at Takeda Pharmaceutical Company Limited. Methylcellulose was purchased from Shin-Etsu Chemical (Tokyo, Japan). Citric acid monohydrate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Giemsa's Azure Eosin Methylene Blue Solution was purchased from Merck (Billerica, MA, USA).

2.2. Generation of hGNRHR-knock-in mice

A targeting vector for homologous recombination was constructed by replacement of the first exon, which contains the initiation codon on the mouse *Gnrhr* locus, with a human *GNRHR* cDNA and neomycin resistant unit (Fig. 1A). The resulting vector was electroporated into AB2.2 embryonic stem cells (Lexicon Genetics, The Woodlands, TX, USA) and recombinant cells were selected in G418. The embryonic stem cells showing correct homologous recombination were screened by PCR genotyping and Southern blot analysis. PCR primer sets for the 5′ flanking region (P1: 5′-TACCTGCCTTATACCTGGTGTCCAG-3′ and P2: 5′-GTTCTTGGACTGATTCAGTTGTAG-3′; PCR product size= 3.3 kbp) and the 3′ flanking region (P4: 5′-GACGTGCTACTTCCATTTGTCACG-3′ and P6: 5′-CCATGACTCACAGTCTTATCAGTG-3′; PCR product size=5.5 kbp) were used for genotyping. The recombinant embryonic stem cells were injected into C57BL/6J blastocysts. Chimeric offspring were identified by coat color. Chimeric male mice with high embryonic stem cell contribution were backcrossed to C57BL/6J females and germ line transmission was predicted by coat color and confirmed by PCR genotyping.

2.3. Animal experiments

The protocols of animal experiments were approved by the Takeda Experimental Animal Care and Use Committee of Takeda

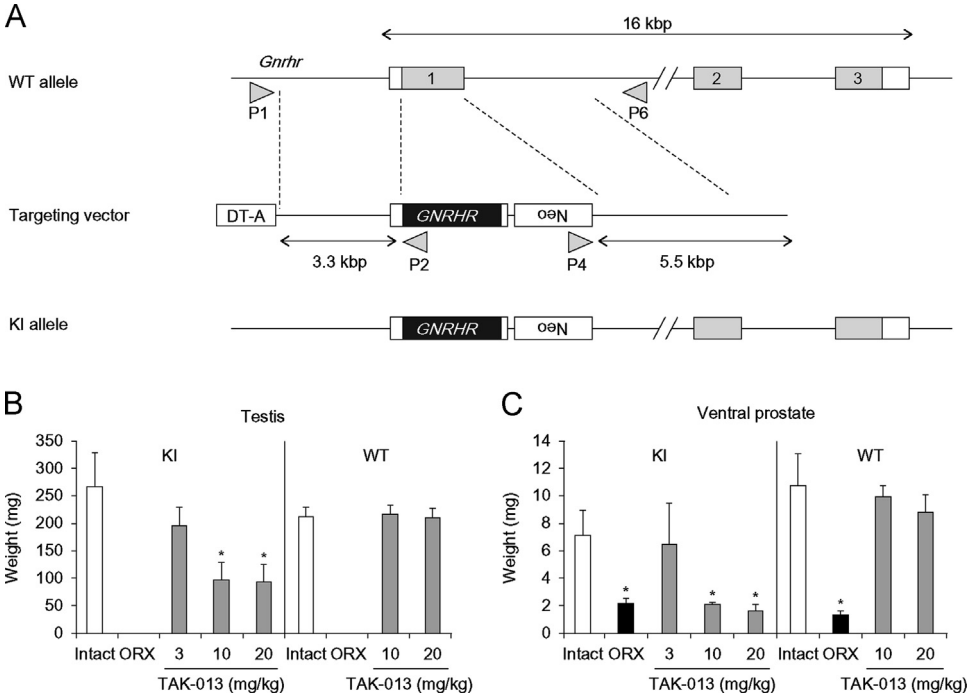

Fig. 1. Validation of hGNRHR-knock-in mice for evaluation of TAK-013. (A) Schematic diagrams of the WT *Gnrhr* allele, the targeting vector, and the resulting KI allele. The coding region in exon 1 was substituted for human *GNRHR* cDNA and neomycin resistant unit (pgk promoter, neomycin resistant gene and bgh polyA signal) by homologous recombination. The primers for PCR genotyping are shown by arrowhead (P1, P2, P4 and P6). (B and C) Effect of TAK-013 on the weights of testes (B) and ventral prostate (C) in male hGNRHR KI mice (left half of the graphs) and male WT mice (right half). TAK-013 was orally administered twice daily (3 and 10 mg/kg) or once daily (20 mg/kg) for 4 weeks. Data are expressed as mean ± S.D. *$P \leq 0.05$ vs. intact control by Dunnett's test. bgh: Bovine growth hormone, DT-A: diphtheria toxin fragment A, hGNRHR: human GnRH receptor, KI: knock-in, Neo: neomycin resistant unit, ORX: orchiectomized, pgk: mouse phosphoglycerate kinase, and WT: wild-type.