Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 204–207

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

A novel nonsecosteroidal VDR agonist (CH5036249) exhibits efficacy in a spontaneous benign prostatic hyperplasia beagle model☆

K. Taniguchi[a,*], K. Katagiri[b], H. Kashiwagi[c], S. Harada[c], Y. Sugimoto[b], Y. Shimizu[b], H. Arakawa[b], T. Ito[b], M. Yamazaki[c], T. Watanabe[c], A. Kato[c], E. Hoshino[b], T. Takahashi[c], T. Esaki[c], M. Suzuki[c], S. Takeda[c], F. Ichikawa[c], A. Harada[c], N. Sekiguchi[c], M. Ishigai[c], H. Kawata[a], T. Yoneya[a], E. Onuma[a], M. Sudoh[a], Y. Aoki[a]

[a] Research Division, Chugai Pharmaceutical Co., Ltd., Kamakura Research Labs., 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan
[b] Chugai Research Institute for Medical Science, Inc., Suwa Research Labs., 6598 Toyoda, Oo-aza, Suwa, Nagano 392-0016, Japan
[c] Research Division, Chugai Pharmaceutical Co., Ltd., Gotemba Research Labs., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan

ARTICLE INFO

Article history:
Received 26 October 2009
Received in revised form 9 March 2010
Accepted 11 March 2010

Keywords:
Nonsecosteroidal vitamin D receptor agonist
Spontaneous benign prostatic hyperplasia
Prostate stromal cells

ABSTRACT

To date, there have been no reports showing the efficacy of nonsecosteroidal vitamin D receptor (VDR) agonists in a benign prostatic hyperplasia (BPH) animal model. To examine the efficacy of CH5036249, a novel nonsecosteroidal VDR agonist, we orally administered the compound at 0.03 μg/kg to a beagle model with spontaneous BPH. Prostate volume was checked by rectal ultrasonic probe periodically during 11 months of administration and the prostate tissues histologically examined. CH5036249 inhibited prostate growth in two out of three dogs compared with vehicle-treated dogs. In the prostate specimens, substantial atrophy of the epithelium was observed in all dogs administered CH5036249. At the dose given, serum calcium levels slightly increased in the CH5036249-treated dogs but stayed within a normal range. We next examined the cell growth inhibition of CH5036249 using human prostate stromal cells and found the cell growth inhibitory activity of CH5036249 to be comparable to that of $1\alpha,25(OH)_2D_3$. The bioavailability from oral administration in rats was 95.1% with a $t_{1/2}$ of 17.6 h. Both micro-AMES and micronucleus tests were negative. Although the results are still preliminary, we consider the novel nonsecosteroidal VDR agonist CH5036249 to be a possible new drug candidate for the treatment of BPH in humans.

1. Introduction

Benign prostatic hyperplasia (BPH) is an age-related disease and histological evidence of BPH is present in almost all men by age 80 [1]. Alpha1 blockers are the gold standard for 1st line therapy in BPH treatment [2]. When clinical symptoms such as urination difficulties worsen, surgery of transurethral resection of the prostate (TURP) is conducted but the complication rate of the surgery is 16.1% [2]. Recently, in a placebo-controlled phase 2 trial, a secosteroidal VDR agonist (BXL-628) showed positive effects on prostate volume reduction, symptomatic relief, no adverse events, and, in particular, normal blood calcium (Ca) levels were maintained [3,4]. In addition, when administered to young adult dogs at 5 μg/kg for 9 months, BXL-628 inhibited prostate growth [5].

CH5036249 is a novel nonsecosteroidal VDR agonist and has favorable characteristics in ADME, toxicity and VDR agonist activity. To examine the possibility of CH5036249 in BPH treatment, we chronically administered the compound in a spontaneous BPH beagle model.

2. Materials and methods

2.1. VDR binding assay and cell growth inhibition assay

The binding affinity to human VDR was measured using the method described by Wecksler [6]. Human prostate stromal cells (PrSC, Ronza CC-2508) were seeded at 200 cells/96-well plate using SCGM medium (CC-3205). Six hours later, serially diluted CH5036249 or $1\alpha,25(OH)_2D_3$ was added to the cells. After 5 days of incubation, the proliferative activity was detected using a 5-bromo-2′-deoxyuridine (BrdU) ELISA kit (Roche, 1647229).

☆ Special issue selected article from the 14th Vitamin D Workshop held at Brugge, Belgium on October 4–8, 2009.

* Corresponding author. Tel.: +81 467 47 2220; fax: +81 467 45 6782.
E-mail address: taniguchiknj@chugai-pharm.co.jp (K. Taniguchi).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.03.043

2.2. Spontaneous BPH beagle model

Male beagles, 7–10 years old, were used following the Institutional Animal Care and Use Committee policy promulgated by Chugai. The animals were kept under standard conditions (individually caged, 12-h/12-h light/dark cycle, 18–29 °C). Prostate weight was monitored by transrectal ultrasonography (TRUS) according to a method previously described [7]. The beagles ($n = 6$) were divided into two groups based on prostate weight: the vehicle-treated group showed weights of 20.02, 22.05 and 18.57 g (Animal Nos. 1–3, respectively) (mean: 20.21 g) and the CH5036249-treated group showed weights of 21.52, 13.65 and 26.62 g (Animal Nos. 4–6, respectively) (mean: 20.60 g). CH5036249 (initial dose: 0.01 μg/kg) or vehicle (medium chain triglyceride; Nisshin Oillio Group) was administered for 5 days per week for 48 weeks. Serum Ca levels were measured every week using a Fuji DRI-CHEM 3030. The serum Ca level and body weight measurements were performed weekly and gross observations were conducted daily. Escalations (+25% or +50%) and reductions (−20%) of the weekly-fixed dosage of CH5036249 were subsequently conducted. At the end of the study, serum Ca levels were measured at 24 h after the last administration. At termination of the experiment, the animals were euthanized by the intravenous administration of 30 mg/kg pentobarbital and the prostate tissues were dissected.

2.3. Histopathological study

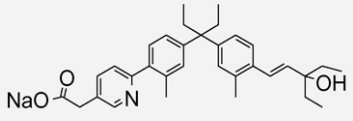
Whole-mount sections of the prostate glands were stained with hematoxylin–eosin (HE). Morphometric analysis was conducted according to a method described elsewhere [8,9].

3. Results

3.1. CH5036249 showed favorable characteristics in ADME, toxicity and VDR agonist activity

As shown in Table 1, CH5036249 bound to VDR at an affinity rate of 37% based on the rate of 1α,25(OH)$_2$D$_3$ (100%) in a competition assay using 1α,25-dihydroxy[26,27-methyl-^{3}H]cholecalciferol. CH5036249 showed high bioavailability (95.1%) and a long $t_{1/2}$ (17.6 h) in rats administered 100 μg/kg and also showed excellent metabolic stability after incubation in human and rat liver microsome (0.8 mL/min/mg and 0.3 mL/min/mg, respectively). Furthermore, CH5036249 showed favorable toxicity results and VDR agonist activity; the VDRE was 88.7% and the TRPV6 was 122% of the control.

Table 1
CH5036249 shows favorable characteristics for ADME, toxicity and VDR agonist activity.

CH5036249	
F (%)	95.1
$t_{1/2}$ (h)	17.6
Metabolic stability (mL/min/mg)	0.8 (human) 0.3 (rat)
Micro-AMES, MNT[a]	Negative
RGA[b] via VDRE[c] (1,25D = 100%)	88.7
TRPV6 (1,25D = 100%)	122
VDR binding (1,25D = 100%)	37
HSP binding affinity[d]	>10 μM

[a] MNT: micronucleus test.
[b] RGA: reporter gene assay.
[c] VDRE: vitamin D response element.
[d] HSP: human serum protein.

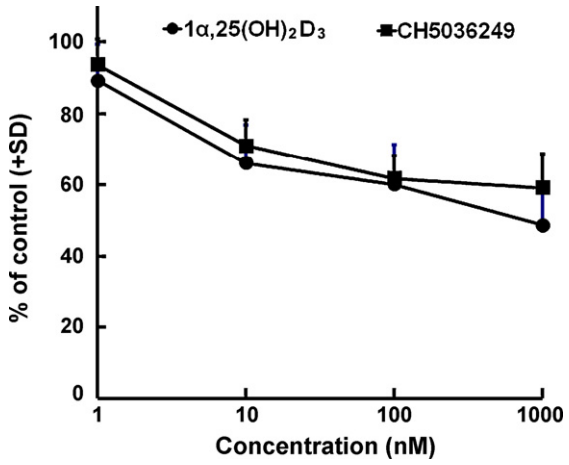


Fig. 1. Dose-dependent inhibition of human prostate stromal cells by CH5036249. Human prostate stromal cells were seeded at 200 cells/96-well plate. After 6 h, serially diluted CH5036249 or 1α,25(OH)$_2$D$_3$ was added to the cells (1–1000 nmol/L). After 5 days of incubation, proliferative activity was detected using a BrdU ELISA kit. Cell growth is expressed as the percentage (+SD) of the control. The inhibitory effect of CH5036249 was comparable to 1α,25(OH)$_2$D$_3$.

3.2. Human prostate stromal cell inhibition of CH5036249 comparable to 1α,25(OH)$_2$D$_3$

Because the prostate in the spontaneous BPH model mainly consisted of glandular epithelium and was not rich in stroma, we examined the inhibitory activity of CH5036249 in human prostate stromal cells using a BrdU ELISA kit. Inhibition of the proliferation of human PrSC by CH5036249 was comparable to that of 1α,25(OH)$_2$D$_3$ (Fig. 1).

3.3. Prostate weight slope index slightly reduced by CH5036249

CH5036249 was administered to the spontaneous BPH beagle model for 48 weeks. The dosage of CH5036249 was escalated from an initial dose of 0.01 to 0.03 μg/kg and fixed at 16 weeks based on serum Ca levels, body weight and gross observation. Serum Ca levels in the CH5036249-treated dogs were slightly increased but stayed within a normal range of 9–11.5 mg/dL during the period of administration [10]. The serum Ca levels at the end of the study in dogs (Nos. 1–6) were 10.2, 9.4, 9.7, 11.0, 9.4 and 10.2 mg/dL, respectively. Body weight measurements for the CH5036249-treated group were similar to the vehicle-treated group and varied only slightly from the initial levels of the CH5036249-treated group (data not shown). Prostate weight was periodically monitored during the administration and linear regression of individual prostate weight data determined the time-generated slope index. The mean slope index showed that CH5036249 slightly reduced prostate weight compared with the vehicle-treated group (Fig. 2A). Changes from baseline prostate weight data at day 336 indicated that CH5036249 inhibited prostate growth in two out of three dogs (Nos. 4 and 6) compared with vehicle-treated dogs (Fig. 2B).

3.4. CH5036249 inhibited BPH epithelium

Histological examination of the maximal area of the whole-mount sections of prostate gland revealed that spontaneous BPH epithelium was reduced by CH5036249 (Fig. 3A and B). As canine

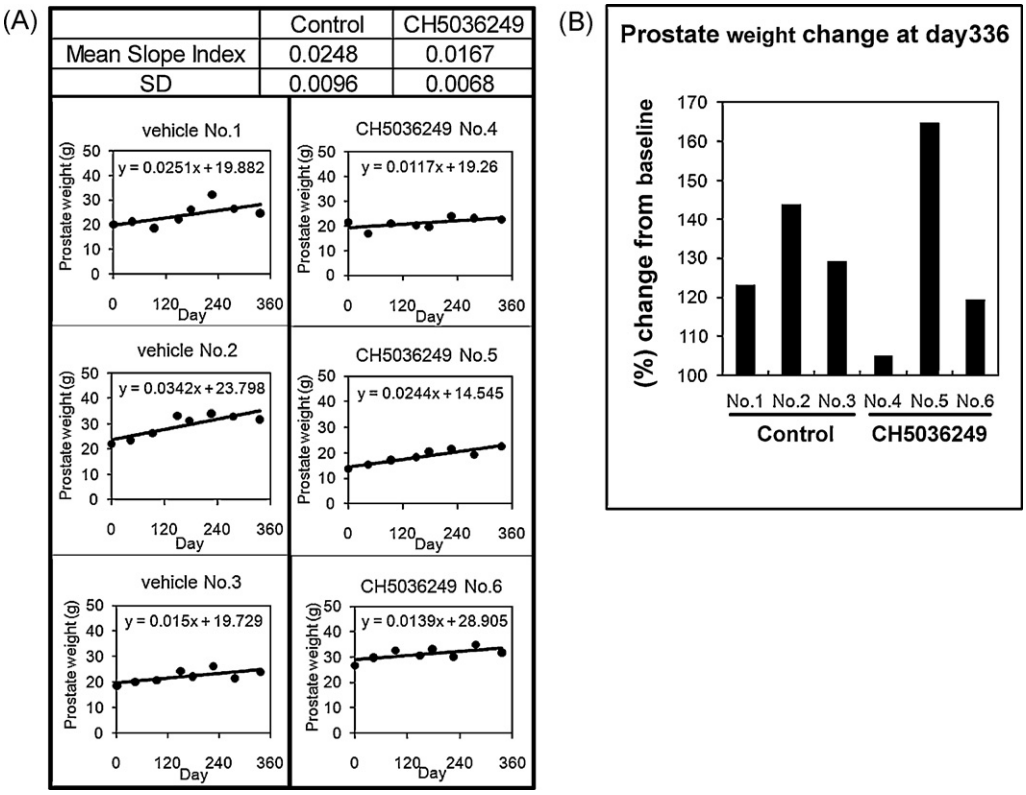


Fig. 2. Prostate weight changes from baseline. Prostate weight was monitored by TRUS. (A) Time-dependent changes in individual prostate weights. Slope index was calculated by the linear regression of time-dependent changes of individual prostate weights. The mean slope index was slightly reduced by CH5036249. (B) Prostate weight change at day 336. Two out of three dogs (Nos. 4 and 6) in the CH5036249-treated group inhibited prostate growth.

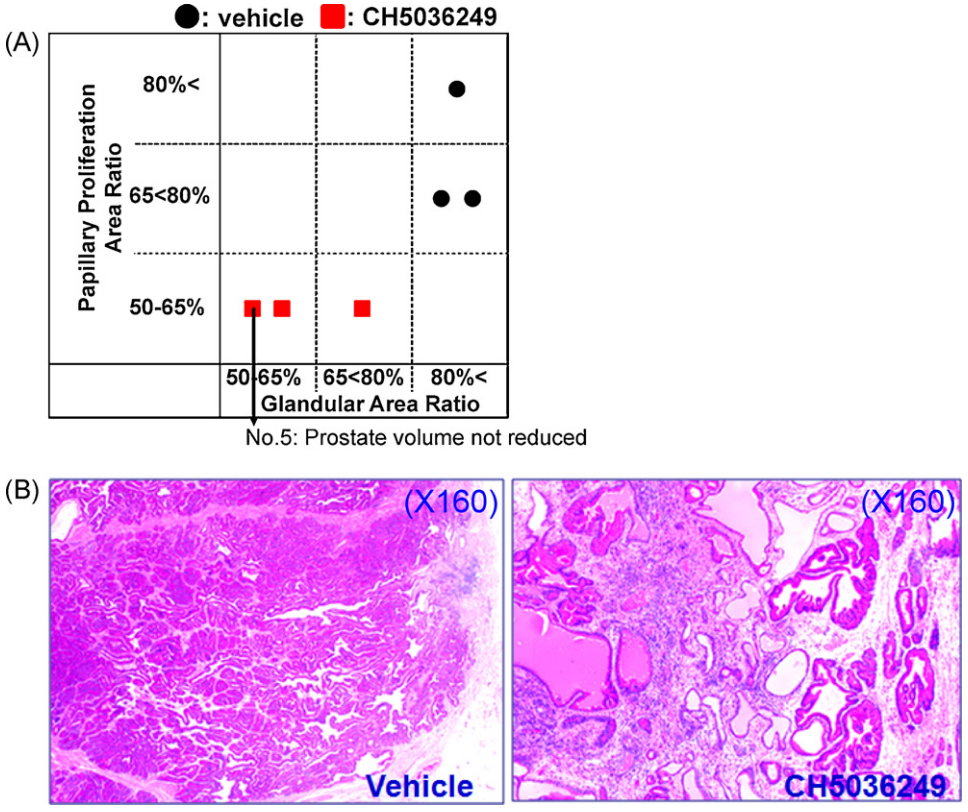


Fig. 3. Histological evaluation of prostate epithelial atrophy by CH5036249. Histological examination was conducted using the maximal area of whole-mount sections of the prostate. (A) CH5036249 decreased the two indexes indicating BPH epithelial proliferation (the ratio of the papillary proliferation area to the entire prostate epithelium area and the ratio of glandular area to the entire prostate area). (B) CH5036249 strongly inhibited BPH epithelium (figure magnification: 160×).

BPH progresses, areas of papillary proliferation gradually emerge within the glandular tissue [11] and the prostate in the spontaneous BPH model mainly consisted of glandular epithelium (Fig. 3B). Therefore, we defined two indexes indicating BPH epithelial proliferation: the ratio of the area of papillary proliferation to the entire prostate epithelium area and the ratio of the glandular area to the entire prostate area. Both indexes were reduced by CH5036249: the papillary proliferation area ratio for the CH5036249-treated group was 50–65% compared with a ratio of >65% for the vehicle-treated dogs and the ratio for the glandular area of the CH5036249-treated group was 50–80% compared with a ratio of >80% for the vehicle-treated dogs (Fig. 3A). Even in the case of No. 5, whose prostate weight was not reduced by CH5036249, both indexes decreased (Fig. 3A). Histological examination using HE staining showed that CH5036249 strongly inhibited BPH epithelium (Fig. 3B).

4. Discussion

CH5036249, a novel nonsecosteroidal VDR agonist, exhibits favorable characteristics with potential as a new drug candidate for the treatment of BPH. At the very low dosage of 0.03 μg/kg, the compound showed efficacy in a spontaneous BPH beagle model (Figs. 2 and 3). In addition, the compound has no toxic killing factor and showed high bioavailability and metabolic stability (Table 1). The dosage of 0.03 μg/kg was almost maximal in terms of the following observations: serum Ca levels for the CH5036249-treated group slightly increased (see Section 3.3) but were within a normal range for canines [10]. The body weight changes in the CH5036249-treated group were similar to those in the vehicle-treated group and there were no toxic signs which suggest hypercalcemic side effects (data not shown). However, the dosage may have been an overdose based on the following findings after the administration of CH5036249: histological examination of the prostate revealed that epithelial atrophy was accompanied by the infiltration of inflammatory cells in stroma and with edema (3/3 dogs, data not shown) and squamous cell metaplasia occurred (3/3 dogs) but less frequently in the vehicle-treated group (1/3 dogs, data not shown), which may be a physiological remodeling process in the spontaneous BPH model.

VDR agonists are apparently more efficacious for symptom relief than for the reduction of prostate volume in human BPH. In a placebo-controlled phase 2 trial, BXL-628 slightly reduced prostate volume (mean change from baseline: +0.41% for elocalcitol 150 μg and +2.55% for placebo at 24 weeks) and the extent of the prostate volume reduction was much weaker than that of 5 mg of finasteride, a steroid 5α-reductase inhibitor (−19.2% median change from baseline at 12 months) [4,12]. However, a symptomatic parameter such as urinary urgency was clearly improved in subgroups of patients with three or more urgency episodes per day at baseline (episodes/day: −3.12 for elocalcitol 150 μg and −1.82 for placebo) [4]. The improvement of maximum urinary flow rate (Q_{max}) was similar to or better than that of finasteride (2.04 mL/s for elocalcitol 150 μg at 24 weeks and 1.6 mL/s for finasteride 5 mg at 12 months) [4,12]. Abundant stromal tissue in human nodular BPH is a possible cause of urination difficulties [2,11] and may be the reason why a VDR agonist is more efficacious for symptom relief than prostate volume reduction. As shown in Fig. 1, CH5036249 inhibited the proliferation of human PrSC comparable to 1α,25$(OH)_2D_3$ and may improve symptomatic parameters in human BPH, even though the effect on prostate volume reduction was not very potent in the epithelial-dominant BPH model (Figs. 2 and 3). In conclusion, a novel nonsecosteroidal VDR agonist, CH5036249, is a possible new drug candidate for the treatment of BPH and whether or not CH5036249 improves symptomatic parameters in the clinical setting of human BPH is extremely intriguing.

Acknowledgements

We are very grateful to Dr. Yoshio Hosaka for his valuable expertise and support of histological evaluations.

References

[1] S.J. Berry, D.S. Coffey, P.C. Walsh, L.L. Ewing, The development of human benign prostatic hyperplasia with age, J. Urol. 132 (3) (1984) 474–479.
[2] R.S. Kirby, T.J. Christmas, Clinical presentation and evaluation, in: Benign Prostatic Hyperplasia, 1st Japanese ed., Igaku Tosho Shuppan, Tokyo, 2001, pp. 25–42.
[3] E. Colli, P. Rigatti, F. Montorsi, W. Artibani, S. Petta, N. Mondaini, R. Scarpa, P. Usai, L. Olivieri, M. Maggi, BXL628, a novel vitamin D3 analog arrests prostate growth in patients with benign prostatic hyperplasia: a randomized clinical trial, Eur. Urol. 49 (1) (2006) 82–86.
[4] BioXell announces positive Phase IIb results for Elocalcitol in Benign Prostatic Hyperplasia. <http://www.bioxell.com/media/press-releases/2007/index.lbl/>, 2009 (accessed 23.10.09).
[5] L. Adorini, G. Penna, S. Amuchastegui, C. Cossetti, F. Aquilano, R. Mariani, B. Fibbi, A. Morelli, M. Uskokovic, E. Colli, M. Maggi, Inhibition of prostate growth and inflammation by the vitamin D receptor agonist BXL-628 (elocalcitol), J. Steroid Biochem. Mol. Biol. 103 (3–5) (2007) 689–693.
[6] W.R. Wecksler, A.W. Norman, An hydroxylapatite batch assay for the quantitation of 1alpha, 25-dihydroxyvitamin D3-receptor complexes, Anal. Biochem. 92 (2) (1979) 314–323.
[7] K. Suzuki, K. Ito, Y. Tamura, T. Suzuki, S. Honma, H. Yamanaka, Effect of aromatase inhibitor, TZA-2209, on the prostate of androstenedione-treated castrated dogs: changes in prostate volume and histopathological findings, Prostate 28 (5) (1996) 328–337.
[8] M Harada, Y. Kinoshita, M. Moriyama, I. Kondo, M. Nakahashi, H. Kumagai, K. Sasaki, M. Hosaka, Histological evaluation of benign prostatic hyperplasia treated by long-term administration of chlormadinone acetate (CMA), Prostate 25 (3) (1994) 147–155.
[9] Y. Yoshinaka, H. Kobayasi, J. Kirihara, F. Sato, S. Shakutou, H. Yamanaka, Effects of mepartricin (S-160) on spontaneous canine benign prostatic hyperplasia, Eur. Urol. 37 (4) (2000) 428–435.
[10] R. Nakamura, Chemical properties of the blood, in: Kachiku-Naika-Shindangaku, Yokendo, Tokyo, 1990, pp. 159–169.
[11] I. Leav, S.H. Schelling, F.B. Merk, Age-related and sex hormone-induced changes in the canine prostate, in: U. Mohr, W.W. Carlton, D.L. Dungworth, S.A. Benjamin, C.C. Capen, F.F. Hahn (Eds.), Pathobiology of the Aging Dog, 1st ed., Iowa State University Press, Ames, IA, 2001, pp. 310–329.
[12] E. Stoner, Three-year safety and efficacy data on the use of finasteride in the treatment of benign prostatic hyperplasia, Urology 43 (3) (1994) 284–294.