Journal of Steroid Biochemistry & Molecular Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Vitamin D receptor agonist VS-105 directly modulates parathyroid hormone expression in human parathyroid cells and in 5/6 nephrectomized rats

Kaichiro Sawada[a], J. Ruth Wu-Wong[b,*], Yung-wu Chen[b], Jerry L. Wessale[b], Genta Kanai[a], Takatoshi Kakuta[a], Masafumi Fukagawa[a]

[a] Tokai University School of Medicine, Kanagawa, Japan
[b] Vidasym, Chicago, IL, USA

ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form 19 October 2016
Accepted 24 October 2016
Available online xxx

Keywords:
PTH
Vitamin D receptor
Vitamin D receptor activator
Calcium-sensing receptor
Secondary hyperparathyroidism
Chronic kidney disease

ABSTRACT

Vitamin D receptor (VDR) agonists (VDRAs) are commonly used to treat secondary hyperparathyroidism (SHPT) associated with chronic kidney disease (CKD). Current VDRA therapy often causes hypercalcemia, which is a critical risk for vascular calcification. Previously we have shown that a novel VDRA, VS-105, effectively suppresses serum parathyroid hormone (PTH) without affecting serum calcium levels in 5/6 nephrectomized (NX) uremic rats. However, it is not known whether VS-105 directly regulates PTH gene expression. To study the direct effect of VS-105 on modulating PTH, we tested VS-105 and paricalcitol in the spheroid culture of parathyroid cells from human SHPT patients, and examined the time-dependent effect of the compounds on regulating serum PTH in 5/6 NX uremic rats (i.p. 3x/week for 14 days). In human parathyroid cells, VS-105 (100 nM) down-regulated PTH mRNA expression (to 3.6% of control) and reduced secreted PTH (to 43.9% of control); paricalcitol was less effective. VS-105 effectively up-regulated the expression of VDR (1.9-fold of control) and CaSR (1.8-fold of control) in spheroids; paricalcitol was also less effective. In 5/6 NX rats, one single dose of 0.05–0.2 μg/kg of VS-105 or 0.02–0.04 μg/kg of paricalcitol effectively reduced serum PTH by >40% on Day 2. Serum PTH remained suppressed during the dosing period, but tended to rebound in the paricalcitol groups. These data indicate that VS-105 exerts a rapid effect on suppressing serum PTH, directly down-regulates the PTH gene, and modulates PTH, VDR and CaSR gene expression more effectively than paricalcitol.

1. Introduction

The expression of parathyroid hormone (PTH) is modulated by calcium (Ca), phosphate and active vitamin D (1,25D, calcitriol) [1]. Secondary hyperparathyroidism (SHPT), a common complication of chronic kidney disease (CKD), develops as an adaptive response to impaired control of Ca, phosphate and 1,25D during the renal disease progression.

Vitamin D analog therapy has been used to treat SHPT in CKD patients for more than 25 years. VDRAs such as calcitriol, paricalcitol [2], and maxacalcitol (mainly in Japan) [3] are commonly used to treat SHPT. However, current VDRAs in therapy exhibit a narrow therapeutic index (TI) as determined by comparing doses required for PTH suppression (efficacy) vs. hypercalcemic toxicity. Even paricalcitol and maxacalcitol, supposed to be vitamin D analogs with a reduced hypercalcemic side effect profile, exhibit only a 3-4-fold improved TI (vs. 1-fold for calcitriol) [4,5]. Hypercalcemia is a serious clinical problem because normal Ca homeostasis is important to many body functions. Thus, current VDRA therapy requires frequent serum Ca monitoring and dose adjustment, and is primarily limited to treatment of Stage 5 CKD hemodialysis patients where clinical management of patients is more accommodating to management of hypercalcemic episodes.

Recently VS-105, a novel VDRA, has been shown to suppress serum PTH in a wide dose range without causing hypercalcemia in 5/6 NX uremic rats, demonstrating a TI at >50-fold (vs. 1-fold for calcitriol) [6,7]. In addition, VS-105 improves endothelial and cardiac function in the 5/6 NX uremic rat [7,8]. However, it has not

Abbreviations: CaSR, calcium-sensing receptor; CKD, chronic kidney disease; NX, nephrectomized; PTH, parathyroid hormone; SHPT, secondary hyperparathyroidism; VDR, vitamin D receptor; VDRA, vitamin D receptor activator or VDR agonist.

* Correspondence to: 2201W. Campbell Park Dr. Suite 13, Chicago, IL 60612, USA.
E-mail address: ruth.wuwong@prodigy.net (J. R. Wu-Wong).

http://dx.doi.org/10.1016/j.jsbmb.2016.10.008
0960-0760/

been shown whether or not VS-105 directly regulates PTH gene expression in human parathyroid cells. In this study, we used the spheroid culture of human parathyroid cells prepared from CKD patients with parathyroidectomy to investigate the effect of VS-105 on PTH expression in the absence of other factors such as calcium and phosphate; paricalcitol was used as a control. In preparation for clinical studies needing a suitable dosing schedule for VS-105, we also investigated the time-dependent effect of VS-105 on serum PTH in the 5/6 NX rat (vs. paricalcitol).

2. Methods

2.1. Materials

VS-105 ((1R,3R)-5-((E)-2-((3αS,7αS)-1-((R)-1-((S)-3-hydroxy-2,3-dimethylbutoxy)ethyl) -7α-methyldihydro-1H-inden-4 (2H,5H,6H,7H,7αH)-ylidene)ethylidene)-2-methylenecyclohexane-1,3-diol) and paricalcitol (19-nor-1α,25(OH)$_2$D$_2$) were synthesized by Vidasym (Chicago, IL, USA). The synthesis scheme of VS-105 was published previously [9]. All other reagents used were of analytical grade.

2.2. Spheroid culture of parathyroid cell

Cell culture spheroid formation was performed as previously described with minor modifications [10]. It was previously shown that these cells maintain responsiveness to calcium for up to one month [10]. Briefly, the parathyroid glands were obtained by parathyroidectomy from 4 CKD patients suffering from advanced secondary hyperparathyroidism under informed consent. The glands were minced, and incubated in DMEM/F12 + GlutaMax-1 medium (Thermofisher, Waltham, MA, USA) containing 10% fetal calf serum and 0.2% collagenase type II (Worthington, Lakewood, NJ, USA) at 37 °C for 30 min. Parathyroid cells were dispersed by gentle pipetting and filtered through a 70 micro-meter mesh. After washing out the collagenase with medium, parathyroid cells were suspended in the same medium containing 10% fetal calf serum, 50 U/ml penicillin and 50 micro-gram/ml streptomycin. Approximately 5×10^5 cells/well were inoculated into wells of non-adherent round-bottom 96-well plates (Sumitomo Bakelite, Tokyo, Japan) and incubated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C for 5–7 days with fresh medium added every other day until spheroid formation was observed. Then, spheroids were transferred into wells of non-adherent flat-bottom 24-well plates (Corning, Corning, NY, USA) and incubated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C with/without addition of vitamin D receptor agonists at 100 nM. Due to the limited supply of the human parathyroid spheroids, only one concentration of each compound was tested; the concentration at 100 nM was chosen based on our previous publication using pig parathyroid cells [11]. Based on our previous experience using the spheroid culture of human parathyroid cells [10], the concentration of intact PTH secreted into the medium was measured with a human intact PTH ELISA kit obtained from Immutopics (San Clemente, CA) for serum PTH on day 5. This study protocol was approved by the Institutional Review Board of Tokai University School of Medicine. (No. 14R-167)

2.3. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR)

RNA was recovered from individual spheroid lysed with Isogen II (Nippon Gene, Tokyo, Japan) and converted to cDNA using SuperScript III First-Strand Synthesis System (Thermofisher). DNA was simultaneously extracted, purified and the amount determined by an absorbance of UV at 260 nm. Real-time PCR was performed by the method of TaqMan Gene Expression Assays (Applied Biosystems, Foster, CA, USA) with primers and probes for human PTH (Assay ID: Hs0017488_m1), human CaSR (Assay ID: Hs01047795_m1), and human VDR (Assay ID: Hs00172113_m1). PCR was carried out on the StepOne Plus Realtime PCR System (Applied Biosystems). Relative amounts of expressed mRNAs were determined using beta-actin as an internal control.

2.4. Sub-totally nephrectomized (NX) rats

5/6 nephrectomy (5/6 NX) was performed on male, Sprague Dawley rats weighing 200–220 g using a standard two-step surgical ablation procedure [6,8]. Rats were maintained on a standard rodent diet used in the animal facility at University of Illinois at Chicago (Teklad LM-485, 7912, Harlan Laboratories, Madison, WI; containing 1% calcium and 0.7% phosphorus). At 6 weeks after the second surgery, when uremia was firmly established (as indicated by elevated serum creatinine and BUN), rats were treated with vehicle (5% ethanol + 95% propylene glycol, 0.4 ml/kg, i.p., 3x/week), VS-105, or paricalcitol (doses as indicated) for 2 weeks. Age-matched sham rats treated with vehicle served as controls. Blood was drawn on Days 0 (24 h before the first dose), 2, 5, 9, 12, and 14 (24 h after the last dose), and assayed for PTH. Blood collected from Days 0 and 14 was also assayed for creatinine, BUN, total calcium, and phosphorus. Because of the difficulty in handling the 5/6 NX rats, the studies described in the report were staged such that each time ~30 rats were handled. The animal assignment was conducted such that each independent study would include sham, vehicle-treated rats, and then groups treated with the test agent of interest. At the end of the studies, the data were compiled. The number of rats in each group is shown in the figure legends.

The animal studies were conducted under the auspice of the Office of Animal Care and Institutional Biosafety, University of Illinois at Chicago. The studies conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Anesthesia was accomplished by i.p. injection of a mixture of ketamine/xylazine (1 ml medical grade ketamine at 100 mg/ml mixed with 0.5 ml medical grade xylazine at 20 mg/ml) at 1.5 ml per kg of body weight. At the end of the study (termination of experiments), blood was collected by cardiac puncture, followed by cardiectomy. At other time points, blood was collected via the tail vein.

2.5. Measurements of physiological parameters

Serum Ca was measured using the Stanbio LiquiColor calcium assay kit (Boerne, TX). Serum PTH was measured using a rat intact PTH ELISA kit obtained from Immutopics (San Clemente, CA). The serum phosphate was determined using a phosphate colorimetric assay (Catalog #K410-500, BioVision, Milpitas, CA). Serum creatinine and BUN concentrations were measured using a standard chemistry analyzer.

2.6. Data analysis

Differences across different treatment groups were assessed using a one-way ANOVA followed by a Dunnett's post-hoc test. A *t*-test was used to assess differences between two treatment groups.

3. Results

3.1. Effects of VS-105 and paricalcitol on regulating PTH in cultured parathyroid spheroids

In order to determine the direct effect of VDRAs on PTH expression, the culture system of human parathyroid cells was

Please cite this article in press as: K. Sawada, et al., Vitamin D receptor agonist VS-105 directly modulates parathyroid hormone expression in human parathyroid cells and in 5/6 nephrectomized rats, J. Steroid Biochem. Mol. Biol. (2016), http://dx.doi.org/10.1016/j.jsbmb.2016.10.008