Journal of Steroid Biochemistry & Molecular Biology 155 (2016) 18–25

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

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

Vitamin D-induced ectodomain shedding of TNF receptor 1 as a nongenomic action: D_3 vs D_2 derivatives

Won Seok Yang[a], Hoon Yu[a], Jin Ju Kim[b], Mee Jeong Lee[c], Su-Kil Park[a,*]

[a] Division of Nephrology, Department of Internal Medicine, Asan Medical Center, College of Medicine, University of Ulsan, 88 Olympic-Ro 43-Gil, SongPa-Gu, Seoul 138-736, Republic of Korea
[b] Asan Institute for Life Sciences, 88 Olympic-Ro 43-Gil, SongPa-Gu, Seoul 138-736, Republic of Korea
[c] Department of Pediatrics, College of Medicine, Dankook University, Anseo-dong San 16-5, Cheonan, Chungcheongnam-do 330-715, Republic of Korea

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form 10 September 2015
Accepted 12 September 2015
Available online 15 September 2015

Keywords:
ADAM10
1,25-Dihydroxyvitamin D_2
25-Hydroxyvitamin D_3
ERp57
L-type Ca^{2+} channel
TNF receptor 1

ABSTRACT

As a nongenomic action, 1,25-dihydroxyvitamin D_3 (1,25D_3) induces L-type Ca^{2+} channel-mediated extracellular Ca^{2+} influx in human aortic smooth muscle cells (HASMCs), which activates a disintegrin and metalloprotease 10 (ADAM10) to cleave and shed the ectodomain of tumor necrosis factor receptor 1 (TNFR1). In this study, we examined the potencies of other vitamin D_3 and D_2 analogs to stimulate the ectodomain shedding of TNFR1 in HASMCs.

25-Hydroxyvitamin D_3 (25D_3), a precursor of 1,25D_3, and elocalcitol, an analog of 1,25D_3, caused ectodomain shedding of TNFR1 within 30 min, whereas 1,25-dihydroxyvitamin D_2 (1,25D_2) and paricalcitol, a derivative of 1,25D_2, did not. Both 25D_3 and elocalcitol rapidly induced extracellular Ca^{2+} influx and markedly increased intracellular Ca^{2+}, while 1,25D_2 and paricalcitol caused only small increases in intracellular Ca^{2+}. 25D_3- and elocalcitol-induced TNFR1 ectodomain sheddings were abolished by verapamil and in Ca^{2+}-free media. Both 25D_3 and elocalcitol caused the translocation of ADAM10 to the cell surface, which was inhibited by verapamil, while 1,25D_2 and paricalcitol did not cause ADAM10 translocation. When ADAM10 was depleted by ADAM10-siRNA, 25D_3 and elocalcitol could not induce ectodomain shedding of TNFR1. The plasma membrane receptor, endoplasmic reticulum stress protein 57 (ERp57), but not the classic vitamin D receptor, mediated the nongenomic action of vitamin D to induce ectodomain shedding of TNFR1.

In summary, like 1,25D_3, 25D_3 and elocalcitol caused ADAM10-mediated ectodomain shedding of TNFR1, whereas 1,25D_2 and paricalcitol did not. The difference may depend on their affinities to ERp57 through which extracellular Ca^{2+} influx is induced.

1. Introduction

Besides calcium and phosphate homeostasis [1], 1,25-dihydroxyvitamin D_3 (1,25D_3) regulates cell functions unrelated to mineral metabolism including immune regulation, and has anti-inflammatory effects [2]. The diverse actions of 1,25D_3 are initiated by its binding to vitamin D receptor (VDR). Following binding of 1,25D_3, VDR localizes in the nucleus and regulates target gene transcription as a transcription factor [3]. In addition to the genomic actions, 1,25D_3 also elicits rapid nongenomic responses independently of gene transcription, such as opening of Ca^{2+} or Cl^- channels and rapid intestinal Ca^{2+} absorption [4]. Effects of vitamin D analogs in human spermatozoa proved the nongenomic nature of such actions because their DNA is packed with protamines instead of histones, which allows no transcription, and sperm cells have no endoplasmic reticulum and Golgi apparatus and are therefore unable to respond like somatic cells [5]. VDR and endoplasmic reticulum stress protein 57 (ERp57, also known as 1,25D_3-membrane associated rapid response steroid-binding receptor) that are located in the plasma membrane have been suggested to mediate these nongenomic actions of vitamin D analogs [6]. Plasma membrane VDR is different from nuclear VDR in the preferred shape of binding ligands [4].

Recently, we found a novel biologic effect of 1,25D_3 on vascular smooth muscle cells [7]. 1,25D_3 suppressed the effect of TNF-α on human aortic smooth muscle cells (HASMCs), and our data revealed that 1,25D_3 causes ectodomain shedding of TNF receptor 1 (TNFR1) within 30 min and thereby decreases the responsiveness of the cells to TNF-α. The ectodomain shedding of TNFR1 was mediated by a disintegrin and metalloprotease 10 (ADAM10). As a

* Corresponding author. Fax: +82 2 3010 6963.
E-mail address: skpark@amc.seoul.kr (S.-K. Park).

http://dx.doi.org/10.1016/j.jsbmb.2015.09.019
0960-0760/

nongenomic action, $1,25D_3$ rapidly stimulates extracellular Ca^{2+} influx via L-type Ca^{2+} channels. The extracellular Ca^{2+} influx triggered translocation of ADAM10 to the cell surface, which may allow it to cleave the ectodomain of TNFR1.

In humans, the main source of vitamin D is cholecalciferol (D_3) that is synthesized in the skin, while a small portion of it is derived from dietary sources as animal cholecalciferol (D_3) or plant derived ergocalciferol (D_2) [8]. In the liver, D_3 is transformed to 25-hydroxyvitamin D_3 ($25D_3$) after 25-hydroxylation. In the kidney, $25D_3$ further undergoes 1α-hydroxylation to become $1,25D_3$, the active metabolite [8]. Synthetic $1,25D_3$ has been used to control secondary hyperparathyroidism in chronic kidney disease [9], since it inhibits parathyroid cell proliferation [10] and PTH gene transcription [11]. However, it causes hypercalcemia and hyperphosphatemia as side effects [12]. To avoid hypercalemic side effect of $1,25D_3$, other vitamin D_3 analogs have been developed. Elocalcitol is one of the $1,25D_3$ analogs that has less hypercalcemic side effect [13].

Like D_3, D_2 is converted to 25-hydroxyvitamin D_2 ($25D_2$) and thereafter 1,25-dihydroxyvitamin D_2 ($1,25D_2$) [8]. $1,25D_2$ also binds to VDR [14] and regulates target gene transcriptions, and it thereby exhibits biological responses identical to those reported for $1,25D_3$ [15,16], including suppression of PTH secretion [17–19]. Therefore, chemically synthesized vitamin D_2 derivatives have been used to correct vitamin D deficiency. Paricalcitol is an analog of $1,25D_2$ that lacks the C19 methylene group in ring A [20]. It suppresses PTH secretion with less hypercalcemic effect and is currently used to treat secondary hyperparathyroidism of dialysis patients [20,21].

Though $1,25D_2$ has the genomic effects of vitamin D, it is not clear whether it also exerts nongenomic actions because the receptors mediating the genomic and nongenomic actions are not the same. In the blood, $25D_3$ is the predominant form of vitamin D_3. $25D_3$ is the precursor form of $1,25D_3$, but it was also shown to induce Ca^{2+} influx in osteoblasts and osteosarcoma cells [22,23]. In the present study, to know whether the vitamin D_2 and D_3 analogs other than $1,25D_3$ have the regulatory effect on TNFR1, we examined the potencies of $25D_3$, elocalcitol, $1,25D_2$ and paricalcitol to induce ectodomain shedding of TNFR1 in HASMCs, and also investigated the receptor mediating this action of vitamin D.

2. Materials and methods

2.1. Materials

Vitamin D analogs and the materials used in this study were obtained as follows; $1,25D_3$, $25D_3$, $1,25D_2$ and elocalcitol (BXL-628, 1-α-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol) from Tocris Bioscience (Bristol, UK); paricalcitol (19-nor-1α,$25(OH)_2D_2$) from Abbott Korea Co., Ltd. (Seoul, Korea); probenecid, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and calcium chloride from Sigma–Aldrich (St. Louis, MO, USA); control-siRNA, ADAM10-siRNA (Ambion®), Alexa Fluor 488-conjugated anti-goat IgG secondary antibody, Fluo-4 AM (Molecular probes®) from Life Technologies (Seoul, Korea); VDR-siRNA, ERp57-siRNA and antibodies against human TNFR1 (H-5; epitope within the extracellular domain), ADAM10, VDR, ERp57 and actin from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell culture

HASMCs, obtained from Lonza Walkersville, Inc. (Walkersville, MD, USA), were cultured in 10% fetal calf serum (FCS, Biological Industries Ltd., Cumbernauld, UK)-supplemented RPMI 1640 media (Life Technologies). For the starvation before the experiments, the cells were incubated in 1% FCS-supplemented RPMI 1640 media for 24 h. Thereafter, the media was replaced with calcium-supplemented RPMI 1640 media (Ca^{2+} 1.2 mM) or DMEM media (Life Technologies; Ca^{2+} 0 or 1.2 mM) without FCS prior to the addition of vitamin D analogs.

2.3. Transfection of siRNA

Using lipofectamine® reagent (Life Technologies), siRNA was transfected to the cells cultured for 24 h after seeding in a 6-well plate. In brief, siRNA-lipofectamine complexes were made by mixing siRNAs (100 pmol) with lipofectamine reagent (10 μl). The cells, placed in culture medium without FCS, were incubated with the siRNA-lipofectamine complexes for 6 h, followed by further incubation with FCS containing culture medium for 18 h. Thereafter, the cells were subjected to the experiments after serum starvation as mentioned above.

2.4. Western blot analysis

Whole cell lysates were obtained after cell lysis on ice for 10 min in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.25% sodium deoxycholate, 1% NP-40, inhibitors of protease and phosphatase). In case of cell culture supernatants, the secreted proteins were collected by precipitation with 20% trichloroacetic acid, washing with aceteone and air dry. The proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred to an Immobilon-P membrane (Millipore, Bedford, MA, USA). After incubation with the primary antibody, the membrane was washed and further incubated with horseradish peroxidase conjugated secondary antibody. Using an enhanced chemiluminescence agent (Amersham International), the protein bands were visualized.

2.5. Measurement of intracellular Ca^{2+}

Fluo-4 AM is a cell membrane permeable Ca^{2+} indicator that becomes fluorescent when bound with Ca^{2+}. To measure the intracellular Ca^{2+}, HASMCs were loaded with Fluo-4 AM (2 μM) and probenecid (1.5 mM), the latter to increase the loading efficiency. After washing, the cells were placed in Hank's balanced salt solution (HBSS; Ca^{2+} 1.2 mM or 0 mM) and fluorescence images (excitation 494 nm, emission 506 nm) were obtained using Zeiss LSM710 laser-scanning confocal microscope (Carl Zeiss, Germany) while the cells were treated with vitamin D analogs. The fluorescence intensity in each cell was measured by ZEN 2011 imaging Software (Carl Zeiss, Germany).

2.6. Immunofluorescence Staining

To localize ADAM10 in the subcellular compartments, the cells were prepared in the following orders; fixation for 10 min with 4% paraformaldehyde, permeabilization for 5 min with 0.3% Triton X-100 and blocking of nonspecific binding for 60 min with 1% bovine serum albumin in phosphate buffered saline. Afterwards, the cells were incubated with goat anti-ADAM10 antibody, followed by incubation with Alexa Fluor 488-conjugated anti-goat IgG secondary antibody. Using a confocal microscope, the fluorescence images were obtained.

2.7. Statistical analysis

In each experiment, the data are expressed as mean ± SE (standard error) with the number of independent experiments. The differences between the groups were analyzed by an analysis of variance with Dunnett multiple-comparisons test, and it was considered significant when the p value was less than 0.05.