Research Paper

Journal of Vascular Research

J Vasc Res 2007;44:11–18
DOI: 10.1159/000097812

Received: July 15, 2006
Accepted after revision: September 28, 2006
Published online: December 7, 2006

Vitamin D Analogs Modulate the Expression of Plasminogen Activator Inhibitor-1, Thrombospondin-1 and Thrombomodulin in Human Aortic Smooth Muscle Cells

J. Ruth Wu-Wong   Masaki Nakane   Junli Ma

Abbott Laboratories, Abbott Park, Ill., USA

Key Words
Vitamin D receptor · Cardiovascular disease · Plasminogen activator inhibitor-1 · Thrombospondin-1 · Thrombomodulin

Abstract
Background/Aims: Plasminogen activator inhibitor-1 (PAI-1), thrombospondin-1 (THBS1) and thrombomodulin (TM) are involved in atherothrombosis. Vitamin D receptor agonists (VDRAs) provide survival/cardiovascular benefits for chronic kidney disease patients. ***Methods:*** The effects of VDRAs on regulating PAI-1, THBS1 and TM in human aortic smooth muscle cells (SMC) and endothelial cells (EC) were studied. ***Results:*** In SMC, paricalcitol and calcitriol downregulated the expression of PAI-1 mRNA and protein in a dose-dependent manner (EC_{50} = 0.7 and 4.4 nM, respectively). Both drugs also downregulated THBS1 mRNA and protein (EC_{50} = 1.6 and 3.9 nM, respectively). In contrast, paricalcitol and calcitriol upregulated TM mRNA and protein (EC_{50} = 28.9 and 25.5 nM, respectively). EC did not express VDR, and VDRAs failed to induce CYP24A1, a VDR target gene. The effect of paricalcitol on THBS1 in SMC was blocked by cycloheximide, while its effect on TM and CYP24A1 was not affected, suggesting that the regulation of THBS1 by VDR may be mediated through intermediate factors, but that TM is likely a direct target of VDR. ***Conclusion:*** VDR may play a role in atherothrombosis via regulation of PAI-1, THBS1 and TM.

Copyright © 2007 S. Karger AG, Basel

Introduction

The steroid hormone 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$, calcitriol] activates multiple signaling pathways in various cells and tissues. Although the synthesis of vitamin D_3 occurs naturally in the skin with adequate sunlight exposure, vitamin D_3 is not active and needs to be converted to 25(OH)D_3 by 25-hydroxylase in the liver. From the liver, 25(OH)D_3 is transported to the kidney and hydroxylated by 25-hydroxyvitamin D-1α-hydroxylase to form the active hormone, calcitriol [1]. Calcitriol is metabolized by 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) [2] to yield the biliary excretory product calcitroic acid. The binding of 1,25$(OH)_2D_3$ or its analogs to the vitamin D receptor (VDR), a nuclear receptor, activates VDR to recruit cofactors to form the VDR/cofactor complex, which binds to vitamin D response elements in the promoter region of target genes to regulate gene transcription [3].

Chronic kidney disease (CKD) patients experience a high mortality rate from cardiovascular disease [4, 5]. Vitamin D analogs (or VDR agonists, VDRAs) such as paricalcitol and calcitriol that activate VDR are commonly used to manage secondary hyperparathyroidism associated with CKD [6]. Recent retrospective clinical observations show that VDRAs provide survival benefit for stage 5 CKD patients in the effectiveness order of paricalcitol > calcitriol > no VDRA therapy, independent of the parathyroid hormone and calcium levels [7–9]; the survival

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2007 S. Karger AG, Basel
1018–1172/07/0441–0011$23.50/0

Accessible online at:
www.karger.com/jvr

Dr. J. Ruth Wu-Wong
Abbott Laboratories, R46R, AP9
100 Abbott Park Rd.
Abbott Park, IL 60064 (USA)
Tel. +1 847 938 8492, Fax +1 847 936 1550, E-Mail ruth.r.wuwong@abbott.com

benefit is associated with a decrease in cardiovascular-related incidents [10]. More recent studies using the time-dependent analyses suggest that the survival benefit is in the order of intravenous paricalcitol > intravenous calcitriol > oral vitamin D analogs = no vitamin D analogs [11, 12].

Although data from clinical studies demonstrate the positive impact of VDRAs on the cardiovascular system, the mechanism of action is largely unknown. CKD patients are known to have advanced atherosclerotic disease [13–15]. Atherosclerosis, the principal cause of myocardial infarction, stroke and peripheral vascular disease, is a process that involves a complex interplay among different factors such as inflammation, thrombosis and various cell types including smooth muscle cells (SMC) and endothelial cells (EC). Atherosclerosis is asymptomatic during a long period and dramatically changes its course when complicated by thrombosis. Atherothrombosis and the rupture of vulnerable plaques are responsible for coronary thrombosis, the main cause of unstable angina, acute myocardial infarction, and sudden cardiac death [16]. Under normal conditions, many factors involved in atherothrombosis are predominantly localized in EC, but vascular injury often results in increased expression of these factors in SMC [17–21].

We have previously shown that in primary culture of human coronary artery SMC, VDR is involved in modulating the expression of plasminogen activator inhibitor-1 (PAI-1) [22], one of the risk markers for coronary heart disease [23]. It is known that thrombomodulin (TM), a monomeric transmembrane protein that serves as a cell surface receptor for thrombin [24], and thrombospondin-1 (THBS1), a large glycoprotein that is released into the extracellular matrix by several cell types including SMC [25], also play roles in fibrinolysis and thrombogenicity. In this study, we investigated the effects of VDRAs on modulating PAI-1, THBS1 and TM expression in primary culture of human aortic SMC and EC.

Materials and Methods

Materials

1α,25-dihydroxyvitamin D_3 (calcitriol) and 19-nor-1α,25-dihydroxyvitamin D_2 (paricalcitol) were from Abbott Laboratories. Drugs were dissolved in ethanol to make a 10-mM stock solution. Other reagents were of analytical grade.

Cell Cultures

Primary culture of human aortic SMC (Cambrex, Walkersville, Md., USA) was grown in SmGM-2 containing 5.5 mM glucose, 5% FBS, 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, 5 μg/ml insulin, 2 ng/ml hFGF, and 0.5 ng/ml hEGF at 37°C in humidified 5% CO_2/95% air. Cells were grown to >80% confluence and used within five passages. Primary culture of human aortic EC (Cambrex) was grown in EGM-2 Bullet Kit medium containing 2% FBS (Cambrex) at 37°C and used within four passages.

Real-Time Reverse Transcription Polymerase Chain Reaction

Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed with an iCycler (BioRad, Hercules, Calif., USA). Each sample has a final volume of 25 μl containing 100 ng of cDNA, 0.4 μM each of the forward and reverse PCR primers and 0.1 μM of the TaqMan™ probe for the gene of interest (Applied Biosystems). Temperature conditions consisted of a step of 5 min at 95°C, followed by 40 cycles of 60°C for 1 min and 95°C for 15 s. Data were collected during each extension phase of the PCR reaction and analyzed with the software package (BioRad). Threshold cycles were determined for each gene.

SDS-PAGE and Western Blot Analysis

Cells (1×10^6 cells per sample) pretreated with or without test agents were solubilized in 50 μl of SDS-PAGE sample buffer (Invitrogen, Carlsbad, Calif., USA), and the protein content in each sample was determined by the Pierce (Rockford, Ill., USA) bicinchoninic acid protein assay. Samples were resolved by SDS-PAGE using a 4–12% NuPAGE gel (Invitrogen), and proteins were electrophoretically transferred to polyvinylidene fluoride membrane for Western blotting. The membrane was blocked for 1 h at 25°C with 5% nonfat dry milk in PBS Tween-20 (PBS-T) and then incubated with a mouse anti-PAI-1 monoclonal antibody (1,000-fold dilution; Santa Cruz Biotechnology, Santa Cruz, Calif., USA), a mouse anti-THBS1 monoclonal antibody (2,000-fold dilution; Calbiochem, La Jolla, Calif., USA), or a mouse anti-TM monoclonal antibody (2,000-fold dilution; Santa Cruz Biotechnology) in PBS-T overnight at 4°C. The membrane was washed with PBS-T and incubated with a horseradish peroxidase-labeled anti-mouse antibody for 1 h at 25°C. The membrane was then incubated with detection reagent (SuperSignal WestPico, Pierce). Specific bands were visualized by exposing the membrane to Kodak BioMax films. Band intensity was quantified by Quantity One (BioRad). For VDR and retinoid X receptor (RXR) detection, membranes were incubated with a rabbit anti-VDR (Active Motif, Carlsbad, Calif., USA) or RXR (Santa Cruz Biotechnology) polyclonal antibody (500-fold dilution), followed by a horseradish peroxidase-labeled anti-rabbit antibody.

Results

Results from real-time RT-PCR analysis show that the PAI-1 mRNA level was reduced by both paricalcitol and calcitriol in a dose-dependent manner in SMC (fig. 1a). The maximal suppression of PAI-1 mRNA expression (approximately 60%) was achieved at 0.1 μM of paricalcitol or calcitriol. The EC_{50} values (concentration that achieves 50% of maximum effect) of paricalcitol and calcitriol on inhibiting PAI-1 mRNA were 0.7 and 4.4 nM,

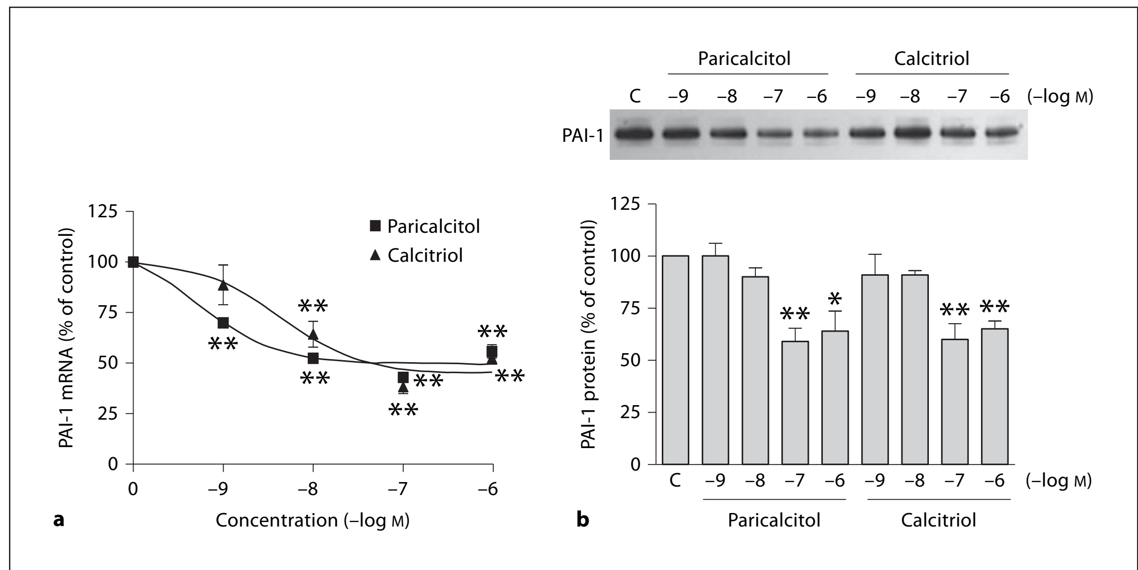

Fig. 1. Effect of paricalcitol and calcitriol on PAI-1 in SMC. **a** SMC in serum-free medium were treated with paricalcitol or calcitriol at indicated concentrations for 24 h. RNA was isolated using Trizol (Invitrogen) followed by a Qiagen RNeasy mini kit. Real-time RT-PCR was performed as described in Materials and Methods. The expression level of PAI-1 mRNA was first normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA level and then calculated as percent of control (untreated, 100%). Values shown are means ± standard deviations (n = 4). **b** Cells were treated with paricalcitol and calcitriol at indicated concentrations for 48 h. Cells were then solubilized and the protein content in each sample was determined by the Pierce bicinchoninic acid protein assay. Western blotting was performed as described in Materials and Methods. The specific bands were visualized and the density of each band was measured. The band was first normalized to the protein content in each sample and then calculated as percent of control (untreated, 100%). Values shown in the bar graph are means ± standard deviations (n = 3). The band picture shown is representative of 3 independent experiments. C = Control, no drug treatment. Statistical comparisons were performed by ANOVA. * $p < 0.05$, ** $p < 0.01$.

respectively. Figure 1b shows that paricalcitol and calcitriol, analyzed by Western blotting, downregulated the expression of PAI-1 protein in a dose-dependent manner. These results are consistent with our previous observations in human coronary artery SMC that vitamin D analogs downregulated PAI-1 expression [22].

Similarly, the THBS1 mRNA level was reduced by both paricalcitol and calcitriol in a dose-dependent manner (fig. 2a). The maximal suppression of THBS1 mRNA expression (approximately 50%) was achieved at 0.1 μM of paricalcitol or calcitriol. The EC_{50} values of paricalcitol and calcitriol on suppressing THBS1 mRNA were 1.6 and 3.9 nM, respectively. Figure 2b shows that paricalcitol and calcitriol downregulated the expression of THBS1 protein in a dose-dependent manner.

In comparison, results from real-time RT-PCR analysis showed that the TM mRNA level was upregulated by both paricalcitol and calcitriol in a dose-dependent manner in SMC (fig. 3a). At 0.1 μM, paricalcitol and calcitriol stimulated an increase in the TM mRNA level by 4.9 and 6.0 fold, respectively. The EC_{50} values of paricalcitol and calcitriol on upregulating TM mRNA were 28.9 and 25.5 nM, respectively. Consistent with the real-time RT-PCR results, figure 3b shows that paricalcitol and calcitriol increased the expression of TM protein in a dose-dependent manner.

Interestingly, studies comparing SMC and EC indicated paricalcitol at 1–100 nM did not exhibit a significant effect on the expression of PAI-1 or TM in EC (data not shown). The results were consistent with our previous report that VDRAs, in human coronary artery EC, do not affect the expression of PAI-1 due to the absence of VDR [22].

Indeed, when we examined the expression of VDR, no VDR was detected in human aortic EC (fig. 4a). As a control, RXR was detected in these cells. Figure 4a also shows that SMC expressed VDR (molecual weight 55 kDa), which was increased by paricalcitol in a dose-dependent manner (a 3.5-fold increase observed at 0.1 μM paricalcitol). RXR was also detected in SMC, but its expression level was not affected by paricalcitol treatment. As expected, figure 4b shows that paricalcitol profoundly stim-

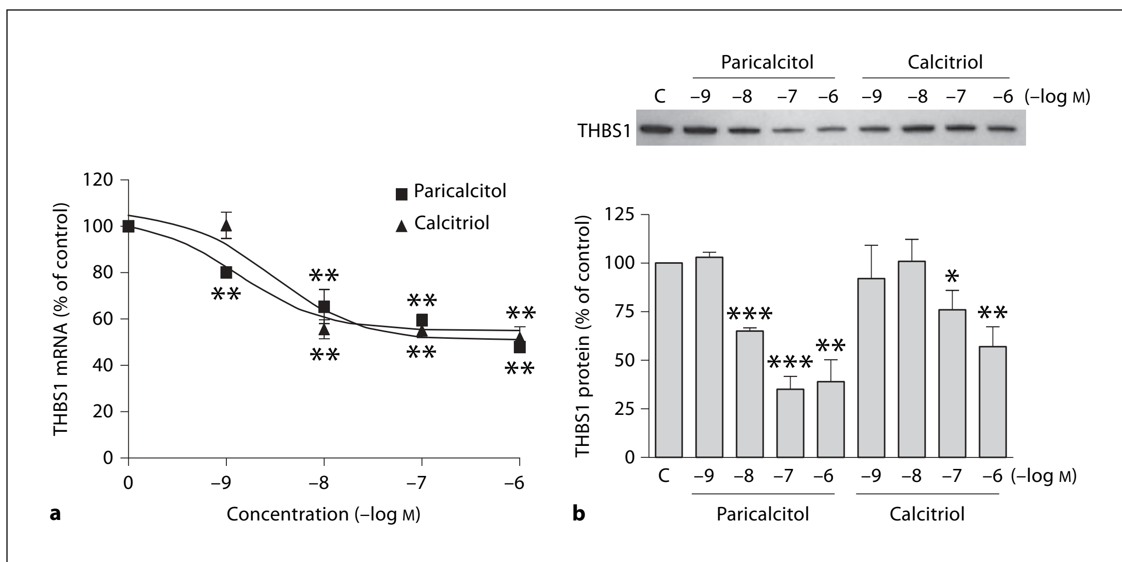

Fig. 2. Effect of paricalcitol and calcitriol on THBS1 in SMC. **a** SMC in serum-free medium were treated with paricalcitol or calcitriol at indicated concentrations for 24 h. Samples were processed for real-time RT-PCR and data analysis done as in figure 1a. Values shown are means ± standard deviations (n = 4). **b** Cells were treated with paricalcitol and calcitriol at indicated concentrations for 48 h. Samples were processed for Western blotting and data analysis was done as in figure 1b. Values shown are means ± standard deviations (n = 3). C = Control, no drug treatment. Statistical comparisons were performed by ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

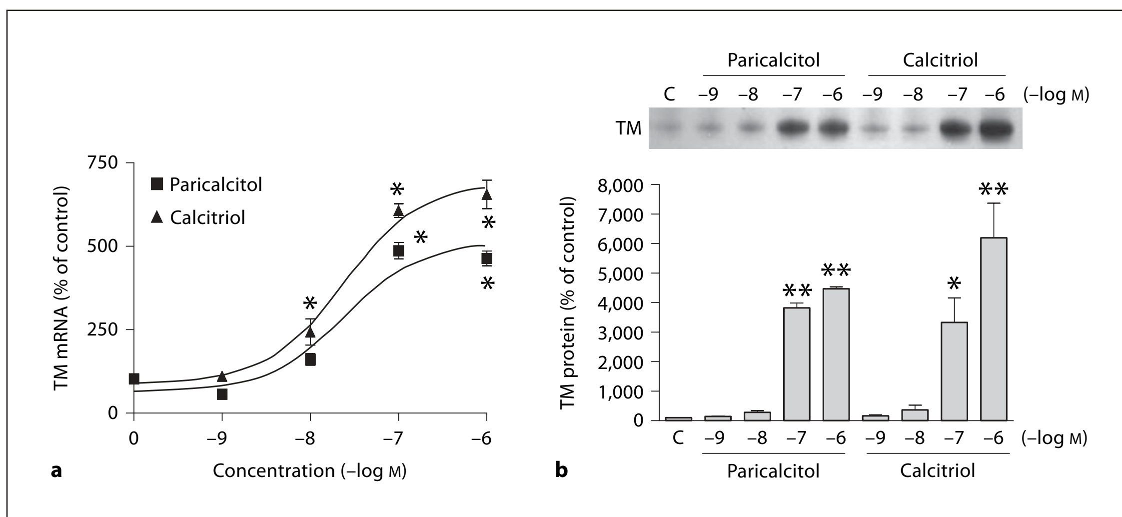

Fig. 3. Effect of paricalcitol and calcitriol on TM in SMC. **a** SMC in serum-free medium were treated with paricalcitol or calcitriol at indicated concentrations for 24 h. Samples were processed for real-time RT-PCR and data analysis was done as in figure 1a. Values shown are means ± standard deviations (n = 4). **b** Cells were treated with paricalcitol and calcitriol at indicated concentrations for 48 h. Samples were processed for Western blotting and data analysis was done as in figure 1b. Values shown are means ± standard deviations (n = 3). C = Control, no drug treatment. Statistical comparisons were performed by ANOVA. * $p < 0.01$, ** $p < 0.001$.

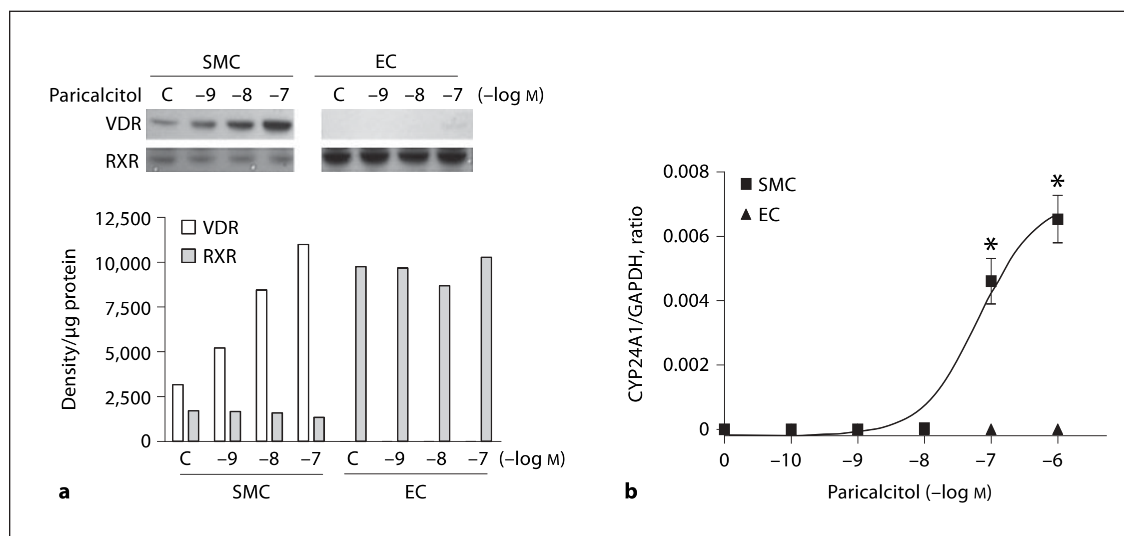

Fig. 4. VDR/RXR expression and effect of paricalcitol on CYP24A1 mRNA in SMC and EC. **a** Cells in serum-free medium were treated with paricalcitol at indicated concentrations for 48 h. Western blotting was performed as in figure 1. Results shown are representative of two independent experiments. C = Control, no drug treatment. **b** Cells were treated with paricalcitol at indicated concentrations for 24 h, and RNA was isolated for real-time RT-PCR. The expression level of CYP24A1 mRNA was normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA level. Values shown are means ± standard deviations (n = 4). Statistical comparisons were performed by ANOVA. * $p < 0.01$.

Table 1. Effect of paricalcitol on THBS1 and TM expression differentially blocked by cycloheximide

Paricalcitol nM	THBS1 mRNA, % of control		TM mRNA, % of control		CYP24A1 mRNA, % of control	
	no CHX	+ CHX	no CHX	+ CHX	no CHX	+ CHX
Control	116.3 ± 31.5	99.9 ± 10.6	100.0 ± 26.67	99.9 ± 26.1	100.0 ± 10.7	100.0 ± 11.4
1	82.39 ± 14.7	99.3 ± 5.4	156.5 ± 27.9	106.7 ± 4.8	3,389.1 ± 319.7**	778.1 ± 17.2**
10	69.2 ± 7.2	98.3 ± 13.5	711.2 ± 145.76**	841.3 ± 35.7**	12,581.8 ± 385.0**	10,960.2 ± 561.9**
100	43.1 ± 12.5*	113.2 ± 12.3	1,346.4 ± 24.8**	1,452.1 ± 34.8**	14,015.4 ± 489.5**	13,899.9 ± 2,079.5**
1,000	40.0 ± 0.6**	98.7 ± 15.2	1,295.1 ± 233.2**	1,611.6 ± 147.1**	12,812.9 ± 792.6**	14,135.4 ± 2,115.6**

SMC in growth medium containing 5% FBS were treated with 3 µg/ml cycloheximide (CHX) for 4 h, followed by addition of paricalcitol at indicated concentrations for 24 h. RNA was isolated and real-time RT-PCR was performed as described. The expression level of THBS1, TM or CYP24A1 mRNA was first normalized to the glyceraldehyde-3-phosphate dehydrogenase mRNA level and then calculated as percent of control (untreated, 100%). Values shown are means ± standard deviations (n = 4). Statistical comparisons were performed by ANOVA.

* $p < 0.05$, ** $p < 0.01$ versus control.

ulated the expression of CYP24A1 mRNA in a dose-dependent manner in SMC, but failed to have an inducing effect on CYP24A1 mRNA in EC. These results confirm that primary culture of human aortic EC, similar to coronary artery EC, do not express a functional VDR, and paricalcitol fails to evoke a functional response.

Previously, we have shown that the effect of paricalcitol on suppressing PAI-1 in human coronary artery SMC was blocked by cycloheximide, suggesting that protein synthesis was involved. Here, we investigated the mechanism for VDR-mediated THBS1 suppression and TM up-regulation in SMC. Aortic SMC were treated with 3 µg/ml cycloheximide for 4 h before addition of paricalcitol.

Table 1 shows that in cells treated with cycloheximide, the suppression of THBS1 by paricalcitol was no longer statistically significant, while paricalcitol induced TM mRNA in a dose-dependent manner and the effects were not significantly affected by cycloheximide (EC_{50} = 10.6 and 11.7 nM in the presence or absence of cycloheximide, respectively). As a control, paricalcitol induced CYP24A1 mRNA in a dose-dependent manner, and the induction was not significantly affected by cycloheximide (EC_{50} = 2.3 and 4.9 nM in the presence or absence of cycloheximide, respectively).

Discussion

PAI-1, THBS1 and TM are known to be involved in fibrinolysis and thrombogenicity. Upregulation of PAI-1 and THBS1 is associated with the development of thrombosis, atherosclerosis and vascular injury [26, 27], while an early loss of TM expression resulted in an enhanced thrombin generation [24, 28], and a local overexpression of TM prevented atherothrombosis [29]. Previously, it has been shown that TM mRNA expression is decreased in aorta, liver and kidney prepared from the VDR knockout mice [30], and calcitriol upregulates TM expression in human leukemic cells and monocytes [31, 32]. Recently, THBS1 has also been shown as an anti-angiogenic agent [27], but the role of VDR in the regulation of THBS1 in human SMC has not been reported before.

We have previously reported that human coronary artery EC do not express a functional VDR at 55 kDa, and both paricalcitol and calcitriol downregulated the expression of PAI-1 mRNA and protein in human coronary artery SMC [22]. A review of the literature shows that there are inconsistent results regarding whether EC express VDR, and also about the effect of vitamin D analogs on PAI-1 expression. For example, it has been reported that paricalcitol suppresses the expression of PAI-1 protein, while calcitriol stimulates the expression of PAI-1 in human umbilical vein EC [33]. It is possible that cells of different origins may differ in VDR expression and in their responses to vitamin D analogs. Therefore, it is important to study SMC and EC prepared from different types of tissues in order to develop a better understanding of the role of VDR in the vasculature. Our current study demonstrates that human aortic EC do not express VDR either. Furthermore, we found that VDR plays a role in downregulating the expression of PAI-1 not only in coronary artery SMC, but also in aortic SMC. In addition, we have extended the studies to include TM and THBS1. Our results unequivocally show that VDRAs upregulate TM and downregulate THBS1 in human aortic SMC.

Our study finds that aortic EC do not express VDR, and VDRAs fail to induce CYP24A1 mRNA expression in these cells, which is consistent with our previous report that human coronary artery EC do not express VDR [22]. Although under normal conditions many of these factors involved in atherothrombosis are predominantly localized in EC, vascular injury often results in altered expression patterns of these factors. For example, in both human and mouse, the expression of PAI-1 is prevalent in activated EC at the early stages of atherosclerosis, but its expression in advanced atherosclerotic lesions is predominant in SMC [17]. In atherosclerotic vessels, TM has been shown to be markedly downregulated in EC, and SMC may become a relevant source of TM under pathological conditions such as advanced atherosclerosis [18–20]. THBS1 expression in the vascular wall is significantly increased in injured vessels and in stent-induced neointima [34]. Furthermore, increased expression of THBS1 has been detected in the adventitia of blood vessels from diabetic rats and in cultured vascular SMC in response to glucose stimulation [21].

The effect of paricalcitol on suppressing THBS1 in aortic SMC is blocked by cycloheximide, while the upregulation of TM by paricalcitol is not. In the control study, the effect of paricalcitol on inducing the CYP24A1 gene, which is known to be a direct target gene of VDR, is not affected by cycloheximide treatment either. This result suggests that the regulation of THBS1 by VDR may be mediated through intermediate factors involving protein synthesis, but TM is likely a direct target gene of VDR. Our previous studies in coronary artery SMC show that VDR-mediated suppression of PAI-1 can also be blocked by cycloheximide. These results suggest that at least in the case of CYP24A1 and TM, VDR may directly bind to VDR response elements in the promoter region of target genes to regulate gene expression. However, when VDR is involved as a negative regulator such as in the case of PAI-1 and THBS1, an indirect mechanism may be involved. More studies are required to elucidate the molecular mechanism of how VDR mediates the suppression of PAI-1 and THBS1.

In healthy individuals, the average level of calcitriol is approximately 45 pg/ml (equivalent to about 0.1 nM), while the precursor, $25(OH)D_3$, is at 29.6 ng/ml (about 74 nM) [35]. Calcitriol suppresses the expression of parathyroid hormone with 50% inhibitory concentration in the sub-nM range [36], which is consistent with its endocrine function in mineral homeostatsis and bone metab-

olism. However, we have observed in this study that calcitriol modulates the expression of PAI-1, THBS1 and TM in SMC with EC_{50} in the 1- to 30-nM doses which are 10- to 300-fold higher than the serum level. Although it is beyond the scope of this study, these data seem to suggest that calcitriol may act as a paracrine/autocrine hormone in the vasculature. Previously, Schwartz et al. [37] showed that human prostate cells synthesize calcitriol from 25(OH)D_3. More studies are required to investigate whether the vascular cells also express 25-hydroxyvitamin D-1α-hydroxylase to produce a high concentration of calcitriol locally.

In summary, in this report, we show that VDRAs modulate the expression of PAI-1, THBS1 and TM in human aortic SMC. Our data also suggest that TM is a direct target gene of VDR, and the regulation of THBS1 by VDR is likely mediated through intermediate factors. Although more studies are required to clarify whether VDR is involved in modulating vascular function, our data suggest that the involvement of the VDR in regulating PAI-1, THBS1 and TM in SMC may account for some of the factors contributing to the benefits of VDRA therapy on reducing mortality and morbidity risk in stage 5 CKD patients.

References

1 Andress DL: Vitamin D treatment in chronic kidney disease. Semin Dial 2005;18:315–321.
2 Brown AJ, Dusso AS, Slatopolsky E: Vitamin D analogues for secondary hyperparathyroidism. Nephrol Dial Transplant 2002;17:10–19.
3 Carlberg C, Quack M, Herdick M, Bury Y, Polly P, Toell A: Central role of VDR conformations for understanding selective actions of vitamin D(3) analogues. Steroids 2001;66:213–221.
4 Zebrack JS, Anderson JL, Beddhu S, Horne BD, Bair TL, Cheung A, Muhlestein JB: Do associations with C-reactive protein and extent of coronary artery disease account for the increased cardiovascular risk of renal insufficiency? J Am Coll Cardiol 2003;42:57–63.
5 Weiner DE, Tighiouart H, Amin MG, Stark PC, MacLeod B, Griffith JL, Salem DN, Levey AS, Sarnak MJ: Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol 2004;15:1307–1315.
6 Martin KJ, Gonzalez EA: Vitamin D analogs: actions and role in the treatment of secondary hyperparathyroidism. Semin Nephrol 2004;24:456–459.
7 Nakai S, Shinzato T, Nagura Y, Masakane I, Kitaoka T, Shinoda T, Yamazaki C, Sakai R, Ohmori H, Morita O, Iseki K, Kikuchi K, Kubo K, Suzuki K, Tabei K, Fushimi K, Miwa N, Wada A, Yanai M, Akiba T: An overview of regular dialysis treatment in Japan (as of 31 December 2001). Ther Apher Dial 2004;8:3–32.
8 Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R: Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. N Engl J Med 2003;349:446–456.
9 Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernan MA, Camargo CA Jr, Thadhani R: Activated injectable vitamin D and hemodialysis survival: a historical cohort study. J Am Soc Nephrol 2005;16:1115–1125.
10 Shoji T, Shinohara K, Kimoto E, Emoto M, Tahara H, Koyama H, Inaba M, Fukumoto S, Ishimura E, Miki T, Tabata T, Nishizawa Y: Lower risk for cardiovascular mortality in oral 1α-hydroxy vitamin D_3 users in a haemodialysis population. Nephrol Dial Transplant 2004;19:179–184.
11 Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS, McAllister CJ, Budoff MJ, Salusky IB, Kopple JD: Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. Kidney Int 2006;70:771–780.
12 Young EW, Albert JM, Akiba T, Greenwood R, Kimata N, W. LN, Piera L, Saran R: Vitamin D therapy and mortality in the Dialysis Outcomes and Practice Patterns Study (DOPPS). J Am Soc Nephrol 2005;16:TH-PO0735.
13 Raggi P: Cardiovascular calcification in end stage renal disease. Contrib Nephrol 2005;149:272–278.
14 Amann K, Ritz C, Adamczak M, Ritz E: Why is coronary heart disease of uraemic patients so frequent and so devastating? Nephrol Dial Transplant 2003;18:631–640.
15 Schwarz U, Buzello M, Ritz E, Stein G, Raabe G, Wiest G, Mall G, Amann K: Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. Nephrol Dial Transplant 2000;15:218–223.
16 Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ: Atherothrombosis and high-risk plaque. 1. Evolving concepts. J Am Coll Cardiol 2005;46:937–954.
17 Laukkanen MO, Kivela A, Rissanen T, Rutanen J, Karkkainen MK, Leppanen O, Brasen JH, Yla-Herttuala S: Adenovirus-mediated extracellular superoxide dismutase gene therapy reduces neointima formation in balloon-denuded rabbit aorta. Circulation 2002;106:1999–2003.
18 Laszik ZG, Zhou XJ, Ferrell GL, Silva FG, Esmon CT: Down-regulation of endothelial expression of endothelial cell protein C receptor and thrombomodulin in coronary atherosclerosis. Am J Pathol 2001;159:797–802.
19 Yoshii Y, Okada Y, Sasaki S, Mori H, Oida K, Ishii H: Expression of thrombomodulin in human aortic smooth muscle cells with special reference to atherosclerotic lesion types and age differences. Med Electron Microsc 2003;36:165–172.
20 Tohda G, Oida K, Okada Y, Kosaka S, Okada E, Takahashi S, Ishii H, Miyamori I: Expression of thrombomodulin in atherosclerotic lesions and mitogenic activity of recombinant thrombomodulin in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1998;18:1861–1869.
21 Stenina OI, Krukovets I, Wang K, Zhou Z, Forudi F, Penn MS, Topol EJ, Plow EF: Increased expression of thrombospondin-1 in vessel wall of diabetic Zucker rat. Circulation 2003;107:3209–3215.
22 Wu-Wong JR, Nakane M, Ma J: Effects of vitamin D analogs on the expression of plasminogen activator inhibitor-1 in human vascular cells. Thromb Res, in press.
23 Nordt TK, Peter K, Ruef J, Kubler W, Bode C: Plasminogen activator inhibitor type-1 (PAI-1) and its role in cardiovascular disease. Thromb Haemost 1999;82:14–18.
24 Kim AY, Walinsky PL, Kolodgie FD, Bian C, Sperry JL, Deming CB, Peck EA, Shake JG, Ang GB, Sohn RH, Esmon CT, Virmani R, Stuart RS, Rade JJ: Early loss of thrombomodulin expression impairs vein graft thromboresistance: implications for vein graft failure. Circ Res 2002;90:205–212.
25 Raugi GJ, Mullen JS, Bark DH, Okada T, Mayberg MR: Thrombospondin deposition in rat carotid artery injury. Am J Pathol 1990;137:179–185.
26 Vaughan DE: PAI-1 and atherothrombosis. J Thromb Haemost 2005;3:1879–1883.

27 Adams JC, Lawler J: The thrombospondins. Int J Biochem Cell Biol 2004;36:961–968.
28 Shah PK: Thrombogenic risk factors for atherothrombosis. Rev Cardiovasc Med 2006;7:10–16.
29 Waugh JM, Yuksel E, Li J, Kuo MD, Kattash M, Saxena R, Geske R, Thung SN, Shenaq SM, Woo SL: Local overexpression of thrombomodulin for in vivo prevention of arterial thrombosis in a rabbit model. Circ Res 1999;84:84–92.
30 Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T, Hayashi H, Yamada Y, Endoh F, Fujimura M, Yoshida T, Yamaguchi H, Hashizume S, Kato M, Yoshimura K, Yamamoto Y, Kato S, Matsumoto T: Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. J Biol Chem 2004;279:35798–35802.
31 Koyama T, Shibakura M, Ohsawa M, Kamiyama R, Hirosawa S: Anticoagulant effects of 1α,25-dihydroxyvitamin D_3 on human myelogenous leukemia cells and monocytes. Blood 1998;92:160–167.
32 Ohsawa M, Koyama T, Yamamoto K, Hirosawa S, Kamei S, Kamiyama R: 1α,25-dihydroxyvitamin D(3) and its potent synthetic analogs downregulate tissue factor and upregulate thrombomodulin expression in monocytic cells, counteracting the effects of tumor necrosis factor and oxidized LDL. Circulation 2000;102:2867–2872.
33 Petrie MS, Harrell TE, Schwartz GG, Sane DC: Production of plasminogen activator inhibitor-1 (PAI-1) by endothelial cells: differential responses to calcitriol and paricalcitol. J Thromb Haemost 2004;2:2266–2267.
34 Sajid M, Hu Z, Guo H, Li H, Stouffer GA: Vascular expression of integrin-associated protein and thrombospondin increase after mechanical injury. J Investig Med 2001;49:398–406.
35 Bailie GR, Johnson CA: Comparative review of the pharmacokinetics of vitamin D analogues. Semin Dial 2002;15:352–357.
36 Ritter CS, Armbrecht HJ, Slatopolsky E, Brown AJ: 25-Hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. Kidney Int 2006;70:654–659.
37 Schwartz GG, Whitlatch LW, Chen TC, Lokeshwar BL, Holick MF: Human prostate cells synthesize 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3. Cancer Epidemiol Biomarkers Prev 1998;7:391–395.