Vitamin D receptor agonist VS-105 improves cardiac function in the presence of enalapril in 5/6 nephrectomized rats

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Wu-Wong JR, Chen Y, Wessale JL. Vitamin D receptor agonist VS-105 improves cardiac function in the presence of enalapril in 5/6 nephrectomized rats. Am J Physiol Renal Physiol 308: F309–F319, 2015. First published December 10, 2014; doi:10.1152/ajprenal.00129.2014.—Vitamin D receptor (VDR) agonists (VDRAs) are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). Patients with CKD experience extremely high risks of cardiovascular morbidity and mortality. Observations show that VDRA therapy may be associated with cardio-renal protective and survival benefits in patients with CKD. The 5/6 nephrectomized (NX) Sprague-Dawley rat with established uremia exhibits elevated serum parathyroid hormone (PTH), hypertension, and abnormal cardiac function. Treatment of 5/6 NX rats with VS-105, a novel VDRA (0.05 and 0.5 μg/kg po by gavage), once daily for 8 wk in the presence or absence of enalapril (30 mg/kg po via drinking water) effectively suppressed serum PTH without raising serum calcium. VS-105 alone reduced systolic blood pressure (from 174 ± 6 to 145 ± 9 mmHg, P < 0.05) as effectively as enalapril (from 174 ± 6 to 144 ± 7 mmHg, P < 0.05). VS-105 improved cardiac functional parameters such as E/A ratio, ejection fraction, and fractional shortening with or without enalapril. Enalapril or VS-105 alone significantly reduced left ventricular hypertrophy (LVH); VS-105 plus enalapril did not further reduce LVH. VS-105 significantly reduced both cardiac and renal fibrosis. The lack of hypercalcemic toxicity of VS-105 is due to its lack of effects on stimulating intestinal calcium transport and inducing the expression of intestinal calcium transporter genes such as CalB3 and TRPV6. These studies demonstrate that VS-105 is a novel VDRA that may provide cardiovascular benefits via VDR activation. Clinical studies are required to confirm the cardiovascular benefits of VS-105 in CKD.

PTH; vitamin D receptor; vitamin D analog; cardiac function; chronic kidney disease

SYNTHESIS OF VITAMIN D3 occurs naturally in the skin with adequate sunlight exposure. However, vitamin D3 is not active and needs to be converted to 1,25-dihydroxyvitamin D3 [1,25(OH)2D3, calcitriol]. Calcitriol is a secosteroid hormone that binds to the vitamin D receptor (VDR), a nuclear receptor, to activate multiple signaling pathways in various cells and tissues (60).

Chronic kidney disease (CKD) progresses through five stages; stage 5 CKD requires renal replacement therapy (dialysis or transplantation). Patients with CKD experience an extremely high rate of cardiovascular complications and mortality (3, 17, 18, 22, 42). Deficient calcitriol production is an early sign of CKD (32) and may be linked to complications in patients with CKD such as secondary hyperparathyroidism (SHPT), and bone and cardiovascular disorders (20). Clinical observations suggest that vitamin D receptor agonists (VDRAs) such as calcitriol, paricalcitol, and doxercalciferol may be associated with cardiovascular and survival benefits for patients with CKD (4, 6, 11, 25, 27, 28, 31, 33, 38, 44, 45, 48–50, 53, 57, 58). In the field of CKD, VDRA is currently indicated only for managing SHPT (20, 35). A narrow therapeutic window (efficacy vs. hypercalcemic side effect) and lack of cardio-renal benefits in the nonhypercalcemic dose range are some of the factors limiting the expanded use of on-market VDRA.

Vitamin D receptor agonist VS-105 improves cardiac function in the presence of enalapril in 5/6 nephrectomized rats. Because VS-105, a novel VDRA, is intended for treating CKD, it is important to evaluate the efficacy of VS-105 in a CKD animal model. The CKD field has the advantage of the 5/6 nephrectomized (NX) uremic rat model, which, although being a difficult model to manage, is highly predictive of the human disease state. In addition, 5/6 NX rats, similar to human patients with CKD, develop cardiovascular complications, which makes them useful for assessing a compound’s cardiovascular protective effects. We have previously reported that VS-105 does not affect serum calcium (Ca) in the dose range that suppresses parathyroid hormone (PTH), improves endothelial function, and regresses left ventricular hypertrophy (LVH) in 5/6 NX uremic rats after 2 wk of either intraperitoneal or oral dosing (61). This report demonstrates that chronic (2 mo) daily oral dosing of VS-105 significantly improves cardiac function with or without enalapril in 5/6 NX rats.

METHODS

Materials. VS-105 (1(R),3R)-5-((E)-2-((3S,7aS)-1-((R)-1-((S)-3-hydroxy-2,3-dimethylbutyloxy)ethyl)-7α-methylidihydro-1H-inden-4(2H,5H,6H,7H,7αH)-ylidene)ethylidene)2-methylene cyclcophene-1,3-diol) and paricalcitol [19-nor-1α,25(OH)2D3] were synthesized by Vidasym (Chicago, IL). Two different lots of VS-105 with >95% purity were tested yielding identical results. The synthesis scheme of VS-105 was published previously (10). All other reagents were of analytical grade.

Subtotally NX rats. Nephrectomy was performed on male Sprague-Dawley rats weighing 200–220 g using a standard two-step surgical ablation procedure (47). Rats were maintained on a normal diet containing 1% calcium and 0.7% phosphorus. At 6 wk after the second surgery, when uremia was firmly established [as indicated by elevated serum creatinine and blood urea nitrogen (BUN)], rats were treated with vehicle (20% hydroxypropyl-β-cyclodextrin, 1.65 ml/kg, once daily by oral gavage), enalapril alone (30 mg/kg po via drinking water), VS-105 alone at 0.05 and 0.5 μg/kg in vehicle (once daily by oral gavage), or enalapril in drinking water plus VS-105 at 0.05 and 0.5 μg/kg (once daily by oral gavage) for 8 wk. Each treatment group consisted of 8 to 12 animals. Age-matched, vehicle-treated sham rats (ie, undergo surgery but without removal of kidneys) served as controls. Blood was drawn on day 0 (24 h before the first dose) and...
day 57 (24 h after the last dose), and assayed for creatinine, BUN, PTH, total Ca, and phosphorus (as phosphate, Pi). For some studies, rats at week 6 after surgery were treated with vehicle (ip: 5% ethanol + 95% propylene glycol, 0.4 ml/kg, three times per week; oral: 20% hydroxypropyl-β-cyclodextrin, 1.65 ml/kg, once daily by oral gavage) or test agent (in vehicle) for 12 days. At the end of a study, aorta, heart, small intestines, and remnant kidney were collected for further analyses.

The animal studies were conducted under the auspices 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 National Institutes of Health (NIH Publication No. 85-23, revised 1996; Bethesda, MD).

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 Immutopsics (San Clemente, CA). Serum Pi was determined using a phosphate colorimetric assay (K410–500; BioVision, Milpitas, CA). Serum creatinine and BUN concentrations were measured using a standard chemistry analyzer.

Echocardiographic evaluation and blood pressure determination. For echocardiographic evaluation, the animals were sedated with isoflurane (1% inhaled). The chest was shaved and animals were placed in the decubitus position on a warming pad to maintain normothermia. Transthoracic two-dimensional, M-mode and pulsed Doppler images were acquired using a high-resolution echocardiographic system (Sequoia C256 system with a 16-MHz transducer; Acuson, Mountain View, CA). The cardiac parameters were assessed using Millar conductance data acquisition and analysis software (PVAN 3.2). Systolic blood pressure was measured after treatment using the tail-cuff method in conscious rats.

Histological assessment. The tissue was fixed in 4% formaldehyde-phosphate-buffered saline (pH 7.4). The samples were embedded in wax and cut into 4-μm sections. The sections were stained with hematoxylin-eosin. For fibrosis, sections were stained with Masson trichrome reagent, and imaged and analyzed using a Vectra Intelligent Multispectral Slide Analysis System (Perkin-Elmer, Waltham, MA) in a blinded manner.

Urinary albumin determination. For urinary albumin determination, each animal was placed in a metabolic cage before the first dosing (predosing) and also after the last dosing on day 56, and urine was collected during a period of 24 h. Urinary albumin concentrations were determined using a rat albumin ELISA quantitation kit (Bethyl Laboratories, Montgomery, TX). Total urinary albumin levels during a 24-h period were determined.

Measurement of gastrointestinal transport. Duodenal calcium absorption was measured ex vivo as described previously (37). Briefly, segments of proximal small intestine were removed from each rat, everted, and filled with incubation buffer (125 mM NaCl, 10 mM fructose, 0.25 mM CaCl2, 30 mM Tris, pH 7.4 at 37°C). Gut sacs were incubated for 90 min in incubation buffer at 37°C with occasional shaking. At the end of the incubation period, the calcium concentration in the serosal and mucosal compartments was measured and the serosal/mucosal calcium ratio was calculated.

Cell culture. Rat cardiomyocytes were isolated from 4-day-old rat pups using the Worthington’s Neonatal Cardiomyocyte Isolation System. The myocytes were cultured in RPMI Medium 1640 with 10% horse serum, 5% fetal bovine serum, and 1% antibiotics at 37°C in 5% CO2 and used within 1 wk.

Real-time RT-PCR. Real-time RT-PCR was performed using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Each sample had a final volume of 25 μl containing 200 ng of mRNA, 100 nM (final concentration) each of the forward and reverse PCR primers, and 250 nM (final concentration) of the TaqMan probe (Applied Biosystems). Temperature conditions consisted of a step of 30 min at 48°C and a step of 10 min at 95°C, followed by 45 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 using a software package (Applied Biosystems). Threshold cycles were determined for each gene.

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.

Results

Effects of VS-105 and enalapril on physiological parameters in 5/6 NX rats. Effects of VS-105 on physiological parameters in 5/6 NX rats after chronic (2 mo) dosing were determined. As summarized in Table 1, serum creatinine and BUN were significantly elevated in 5/6 NX rats (vs. sham-vehicle rats), indicating a uniform uremic state. No significant changes in serum creatinine or BUN levels were observed after VS-105 and/or enalapril treatment. Both serum Ca and Pi were not significantly changed in the 5/6 NX-vehicle group (vs. the sham-vehicle group), and VS-105 and/or enalapril had no significant effect on serum Ca and Pi. Serum PTH was elevated in 5/6 NX rats, and was suppressed by VS-105 in a dose-dependent manner.

Effects of VS-105 and enalapril on cardiac parameters in 5/6 NX rats. The 5/6 NX rats are known to develop left ventricular hypertrophy and abnormal cardiac function (59).

Figure 1A shows that at 14 wk after the renal ablation surgery, the left ventricle weight (LVW) vs. body wt (BW) ratio as a percentage of control was significantly higher in 5/6 NX rats (vs. sham-vehicle rats). Previously, we reported that increased LVW/BW was present in 5/6 NX rats at 6 or 8 wk after the second surgery (61, 62). Treatment with enalapril produced a significant effect on reducing the LVW/BW ratio. VS-105 alone also reduced the LVW/BW ratio in a dose-dependent manner. No significant synergistic effect in reducing the LVW/BW ratio was observed in the VS-105 + enalapril treatment groups. Similar observations were made when the heart weight (HW) vs. BW ratio was examined (Fig. 1B).

The 5/6 NX rats are known to develop abnormal cardiac function. Figure 2 shows that the E/A ratio, fractional shortening, and ejection fraction as determined by echocardiography were significantly lower in NX 5/6 rats. For E/A ratio, enalapril alone exhibited a modest effect; VS-105 alone also had a modest effect. The optimal effect was observed in the group treated with VS-105 at 0.5 μg/kg plus enalapril. For fractional shortening and ejection fraction, enalapril exhibited a modest effect, whereas VS-105 improved the parameters to the levels observed in sham-vehicle rats in a dose-dependent manner. Consistent with the results in Fig. 1, the representative data in Fig. 2D show that enalapril or VS-105 alone reduced the septum relative wall thickness ratio.

Effects of VS-105 and enalapril on blood pressure in 5/6 NX rats. Systolic blood pressure was measured. Previously, we reported that systolic pressure was significantly elevated in 5/6 NX rats treated with vehicle, and although paricalcitol at 0.042 μg/kg was effective at suppressing serum PTH with elevated serum calcium, it had no significant effect on blood pressure (64). Figure 3 shows that, consistent with our previous report, systolic blood pressure was significantly higher in 5/6 NX rats. VS-105 alone at 0.5 μg/kg reduced blood pressure significantly, equivalent to that of enalapril alone. However, no
Physiological parameters in sham vs. 5/6 nephrectomized rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 57</th>
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<th>Day 57</th>
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<tbody>
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<td>Creatinine, mg/dl</td>
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<td>0.34±0.01*</td>
<td>1.0+4.2</td>
<td>1.0+4.2</td>
<td>0.80±0.05a</td>
<td>0.83±0.03a</td>
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<td>0.81±0.03a</td>
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<tr>
<td>Serum calcium, mg/dl</td>
<td>10.96±0.18</td>
<td>10.81±0.38</td>
<td>10.64±0.38</td>
<td>10.64±0.38</td>
<td>11.04±0.38</td>
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<tr>
<td>Phosphate, mmol/liter</td>
<td>3.33±0.16</td>
<td>2.83±0.24</td>
<td>3.33±0.16</td>
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Statistical test: One-way ANOVA Dunnett’s test with 95% confidence intervals of difference was performed for statistical comparisons. ### P<0.001 vs. sham. *P<0.05, **P<0.01, ***P<0.001 vs. sham.

Effects of VS-105 and enalapril on fibrosis in 5/6 NX rats

We have previously reported that compared with sham rats, a significant increase in collagen deposition was observed in the left ventricle (LV) of 5/6 NX rats treated with vehicle, and treatment with VS-105 at 0.01 and 0.5 μg/kg for 2 wk substantially reduced LV fibrosis staining (61). Similar results were observed in this study as shown in Fig. 4; namely, that VS-105 alone effectively reduced LV fibrosis, whereas enalapril alone exhibited a lesser effect. In addition to LV fibrosis, we examined the renal kidney for fibrosis. Representative images and summary data are shown in Fig. 5. Compared with sham rats, a significant increase in collagen deposition was observed in the remnant kidney in the NX-vehicle group. Although enalapril alone did not show a significant effect, treatment with VS-105 alone substantially reduced the fibrosis staining. The quantitative determination of tissue collagen abundance is also shown. These results suggest the potential cardiac and renal protective benefits of VS-105.


As predicted, there was a significant increase in urinary albumin in sham rats. However, when VS-105 and enalapril were administered together, a significant decrease in urinary albumin was observed (Fig. 2).
5/6 NX rats even at 6 wk after surgery (predosing), which further increased by 62% at the end of 2 mo in the vehicle group (Fig. 6). Enalapril alone exhibited an effect on reducing urinary albumin (by 24%). VS-105 at 0.05 µg/kg in the absence or presence of enalapril exhibited a reduction in urinary albumin by 24% vs. 5/6 NX rats. Enalapril alone exhibited an effect on reducing urinary albumin (by 24%). VS-105 at 0.05 µg/kg in the absence or presence of enalapril (30 mg/kg po via drinking water) for 8 wk as described in METHODS. Blood pressure was determined as described in METHODS. Group means ± SE are presented. A t-test was used to assess differences between two groups. *P < 0.05, ##P < 0.01 vs. sham rats. *P < 0.05 vs. 5/6 NX rats.

Effects of VS-105 on regulating brain natriuretic peptide. NPPB is the gene that encodes for brain natriuretic peptide (BNP), which is a biomarker for cardiovascular disease (16, 43). The effects of VS-105 and paricalcitol on the expression of NPPB in neonatal rat cardiomyocytes were compared. Figure 7A shows results from real-time PCR that the addition of angiotensin II (1 µM) to cardiomyocytes significantly elevated the expression of NPPB (to 149.6% of control), and both VS-105 and paricalcitol at 0.01 and 0.1 µM suppressed NPPB expression. To confirm that the effect of VS-105 on NPPB was mediated via VDR, the expression of 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) and VDR was also measured. Figure 7B shows that VS-105 stimulated the expression of CYP24A1, a known target gene of VDR, in a dose-dependent manner. Figure 7C shows that VS-105 also increased the expression of VDR. To further confirm the NPPB observation in cultured cardiomyocytes, 5/6 NX rats were treated with VS-105 (doses as indicated) for 2 wk. Figure 7D shows that expression of NPPB was significantly higher in the left ventricle of 5/6 NX rats, and VS-105 treatment significantly reduced NPPB. The results are consistent with a previous report that VDRAs suppress NPPB expression (51).

Effects of VS-105 on intestinal calcium transport parameters in 5/6 NX rats. Hypercalcemia induced by VDR agonists, an undesirable side effect, remains a serious concern, which may counteract and thus mask potential cardiovascular benefits derived from VDR activation. Intestine, bone, and kidney are major organs involved in regulating Ca metabolism. In CKD, the hypercalcemic toxicity of VDRAs is likely linked to upregulated intestinal Ca absorption. Thus it is important to examine the Ca transport parameters in the intestine. The epithelium-specific calcium channel, CaT1/ECaC2, and the cytosolic calcium binding protein, calbindin D9K, play important roles in intestinal Ca absorption (8). Using quantitative-PCR (Q-PCR), Fig. 8, A and B, show that VS-105 at 0.05 and 0.5 µg/kg after 2 mo of treatment did not significantly affect

Fig. 2. Cardiac function. Sham and 5/6 NX rats were given vehicle or VS-105 at indicated doses (once daily by oral gavage) with or without enalapril (30 mg/kg po via drinking water) for 8 wk as described in METHODS. Cardiac function was determined as described in METHODS. A: E/A ratio. B: fractional shortening. C: ejection fraction. D: septum relative wall thickness ratio [(IVSd + LVPWd)/LVIDd]. Group means ± SE are presented. A t-test was used to assess differences between two groups. *P < 0.05, ##P < 0.01 vs. sham rats. *P < 0.05 vs. 5/6 NX rats. IVSd: interventricular septum thickness in diastole; LVIDd: left ventricular internal diameter in diastole.
the expression of Calb3 (the gene encoding calbindin D9K) or TRPV6 (the gene encoding CaT1 and ECaC2) in the intestine. To confirm the Q-PCR results, Fig. 8C shows the results from a functional assay that VS-105 at the tested doses did not affect intestinal calcium transport.

Previously, we reported that paricalcitol is slightly less potent than calcitriol in upregulating Calb3 and TRPV6 in the intestine, but paricalcitol still induced intestinal Ca transport (vs. NX-vehicle) (37, 63). To confirm the observations made about VS-105, we compared the effect of paricalcitol vs. VS-105 on intestinal Ca transport parameters in 5/6 NX rats. Figure 9, A and B, show that paricalcitol but not VS-105 increased Calb3 and TRPV6 expression in the intestine. Consistently, paricalcitol at 0.1 µg/kg, but not VS-105, increased intestinal calcium transport (Fig. 9C).

DISCUSSION

We have demonstrated in this report that chronic dosing of VS-105, a novel VDRA, effectively suppresses PTH, reduces
fibrosis, modulates the expression of BNP, and improves blood pressure and cardiac function in the dose range that has no effects on serum Ca, intestinal Ca transport, or intestinal Calb3 and TRPV6 gene expression. The effect of VS-105 is mediated via VDR activation, as evidenced by the induction of CYP24A1, a known target gene of VDR and a key enzyme involved in the metabolism of endogenous vitamin D (40, 41).

Moreover, VS-105 alone is as effective as enalapril in reducing LVH and systemic blood pressure, and in improving left ventricular ejection fraction and fractional shortening. It is also worth noting that VS-105 is more effective than enalapril in reducing cardiac/renal fibrosis and urinary albumin in this uremic rat model.

The results from the present study provide preclinical evidence of a cardio-protective effect of VS-105 in CKD; however, our observations on the cardiovascular benefits of VS-105 will need to be validated in human trials. For this reason, it is worth discussing our current preclinical results in light of some recent clinical developments in the VDR field. In the PRIMO (Paricalcitol Capsule Benefits in Renal Failure-Induced Cardiac Morbidity) study (51), a multinational, double-blind, randomized placebo-controlled trial with 227 patients with stage 3/4 CKD receiving the standard of care including renin-angiotensin-aldosterone system (RAAS) inhibitors, 48 wk of treatment with paricalcitol did not further reduce left ventricular mass index (LVMI, the primary endpoint as determined by cardiac magnetic resonance imaging) in the presence of RAAS inhibitors. A more recent paper reported similar results from the OPERA trial (55). RAAS inhibitors are known to reduce cardiac fibrosis and left ventricular mass (34, 54, 56). Our current study shows that VS-105 in the presence of enalapril does not further reduce left ventricular mass, which is consistent with the observation in the PRIMO and OPERA
paricalcitol potentially reduces blood pressure in patients with CKD (14). However, our current observations differ from our previous report that paricalcitol at 0.042 μg/kg had no effect on blood pressure in 5/6 NX rats (64), and are also different from the PRIMO and OPERA studies showing that paricalcitol had no significant effect on blood pressure. Once again, it cannot be ruled out that the difference may be related to hypercalcemia. In the VITAL study and in this current report, no significant effect on serum Ca was observed after VDRA treatment, although hypercalcemia was present in our previous study testing paricalcitol in 5/6 NX rats, and hypercalcemic incidents were significantly increased in both the PRIMO and OPERA studies. Results from the current study also demonstrate that the lack of hypercalcemic toxicity of VS-105 is likely due to its lack of effects on stimulating intestinal Ca transport because VS-105 has minimal effects on inducing the expression of intestinal Ca transporter genes. However, whether hypercalcemia impedes or counteracts the effect of VDRAs on reducing blood pressure will require further investigation.

In the VITAL study a reduction in blood pressure was observed in patients with CKD receiving paricalcitol and stable doses of angiotensin 1-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) for at least 3 mo (14). Chronic dosing of current RAAS inhibitors, including ACE inhibitors, ARBs, and renin inhibitors (e.g., aliskiren) is known to

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)

Fig. 7. Effects of VS-105 or paricalcitol on expression of NPPB, CYP24A1, or vitamin D receptor (VDR). A: neonatal rat cardiomyocytes were treated with or without VS-105 or paricalcitol (concentrations as indicated) in the presence or absence of angiotensin II (1 μM) for 24 h. Cells were harvested, RNA was isolated, and NPPB mRNA (A), CYP24A1 mRNA (B), or VDR mRNA (C) levels were analyzed by real-time RT-PCR. The NPPB level was first normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and then expressed as % of control (no treatment). The CYP24A1 level was expressed as a ratio of GAPDH because there was no detectable CYP24A1 expression in the untreated samples (control) that could be used for normalization. The VDR level was also expressed as a ratio of GAPDH. Statistical analysis was performed by unpaired t-test. #P < 0.05, ##P < 0.001 compared with control (no treatment); *P < 0.05 compared with angiotensin II alone; n = 3 per condition. Results shown are representative of two independent experiments. B: 5/6 NX rats at week 6 after surgery were treated with vehicle (20% hydroxypropyl-β-cyclodextrin, 1.65 ml/kg once daily by oral gavage) or VS-105 (0.01 or 0.1 μg/kg in vehicle) for 12 days. The left ventricle from each rat was harvested, RNA was isolated, and NPPB mRNA level was analyzed by real-time RT-PCR. The NPPB level was first normalized with GAPDH and then expressed as % of sham (kidneys intact). Statistical analysis was performed by unpaired t-test. #P < 0.05 compared with sham rats; *P < 0.05 compared with 5/6 NX rats; n = 6 per condition.

studies. At the same time, however, both the PRIMO and OPERA studies reported some intriguing findings that cardiovascular-related hospitalizations were significantly reduced in the paricalcitol group, but no significant differences were observed in diastolic or systolic function. The effects of paricalcitol on cardiac function have previously been demonstrated in animal studies (2, 7). Our observations in this study, which agree with those reported from previous animal studies (2, 7), show that VDR activation by VS-105, after 2 mo of daily dosing administered in the presence of enalapril, improves the echocardiographic E/A ratio (an index of diastolic function), ejection fraction, and fractional shortening. Although it remains unclear why the incidence of cardiovascular-related hospitalizations was lower in patients with CKD treated with paricalcitol without significant differences being observed in cardiac function, it cannot be ruled out that hypercalcemia may negatively affect the benefits of VDR activation because paricalcitol at the doses tested (2 μg in the PRIMO study; 1 μg in the OPERA study) significantly increased hypercalcemic incidents.

In the present study, VS-105 alone at 0.5 μg/kg significantly reduced blood pressure. The results are consistent with observations from the VITAL clinical study that VDR activation by paricalcitol potentially reduces blood pressure in patients with CKD (14). However, our current observations differ from our previous report that paricalcitol at 0.042 μg/kg had no effect on blood pressure in 5/6 NX rats (64), and are also different from the PRIMO and OPERA studies showing that paricalcitol had no significant effect on blood pressure. Once again, it cannot be ruled out that the difference may be related to hypercalcemia. In the VITAL study and in this current report, no significant effect on serum Ca was observed after VDRA treatment, although hypercalcemia was present in our previous study testing paricalcitol in 5/6 NX rats, and hypercalcemic incidents were significantly increased in both the PRIMO and OPERA studies. Results from the current study also demonstrate that the lack of hypercalcemic toxicity of VS-105 is likely due to its lack of effects on stimulating intestinal Ca transport because VS-105 has minimal effects on inducing the expression of intestinal Ca transporter genes. However, whether hypercalcemia impedes or counteracts the effect of VDRAs on reducing blood pressure will require further investigation.

In the VITAL study a reduction in blood pressure was observed in patients with CKD receiving paricalcitol and stable doses of angiotensin 1-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) for at least 3 mo (14). Chronic dosing of current RAAS inhibitors, including ACE inhibitors, ARBs, and renin inhibitors (e.g., aliskiren) is known
to lead to a compensatory renin buildup due to disruption of the feedback inhibition of renin production (15), and a buildup of renin may increase the risk of angiotensin II-dependent and -independent organ damage (15). VDR is directly involved in regulating renin gene expression (66) and VDRAs may suppress renin production, leading to blood pressure modulation independent of RAAS inhibition. We did not observe any additional reduction of systolic blood pressure in the VS-105 plus enalapril group in this animal study. One possible explanation for our blood pressure observation is that because enalapril was dosed together with VS-105, there might not be a high level of renin buildup for us to observe the combined

Fig. 8. Calb3 and TRPV6 gene expression, and intestinal calcium transport. Sham and 5/6 NX rats were given vehicle or VS-105 at indicated doses (once daily by oral gavage) for 8 wk as described in METHODS. RNA samples were prepared from small intestines using a standard RNA isolation procedure. Real-time RT-PCR was performed according to the description in METHODS. Calb3 (A) or TRPV6 (B) values were first normalized with GAPDH, and then expressed as % of control (sham). C: segments of proximal small intestine were processed and calcium transport was determined in the mucosal-to-serosal direction according to the description in METHODS. Calcium levels were determined in both serosal and mucosal compartments and the serosal/mucosal ratio was calculated. The data were expressed as % of control (sham rats). A t-test was used to assess differences between two groups.

Fig. 9. VS-105 vs. paricalcitol on intestinal calcium transport parameters. 5/6 NX rats at week 6 after surgery were treated with vehicle (5% ethanol + 95% propylene glycol, 0.4 ml/kg), VS-105, or paricalcitol (0.1 µg/kg in vehicle) ip 3×/wk for 12 days. Calb3 (A) or TRPV6 (B) gene expression was determined as described in Fig. 8. C: intestinal calcium transport was determined as described in METHODS and Fig. 8. A t-test was used to assess differences between two groups. *P < 0.05, **P < 0.01 vs. sham rats.
drug effect such as that observed in the VITAL study. Evidently, more studies are needed to fully understand the conditions required for the effects of VDRAs on blood pressure.

ACE inhibitors are a preferred pharmacological option in the treatment of chronic heart failure; the effect is likely mediated via modulation of blood pressure, cardiac remodeling (e.g., fibrosis), endothelial function, and inflammation (1, 5, 56). The benefits of RAAS inhibitors in CKD are well documented, and many patients with CKD are treated with ACE inhibitors or ARBs (30). However, it is also well known that despite the extensive use of RAAS blockers, patients with CKD continue to experience an extremely high risk for cardiovascular complications and mortality (19, 21, 22, 29, 36). Large clinical trials have shown that RAAS blockade with multiple drugs does not improve the long-term renal or cardiovascular outcome in CKD (30, 35, 39, 46). It has been shown previously that in rats with CKD, ACE inhibition, although showing benefits of reducing blood pressure and cardiac hypertrophy, did not significantly affect renal fibrosis (9). However, an earlier study by Cruz et al. (12) reported that renal fibrosis in older rats could be improved by ACE inhibitors and taurine. Regarding the mechanisms of action for the cardiovascular effect of VS-105, it is known that VDR agonists modulate blood pressure, endothelial function, fibrosis (61, 62), and inflammation (24, 65), which is mediated by VDR independent of RAAS inhibition. Furthermore, observations from the current study show that VS-105 reduces cardiac/renal fibrosis and urinary albumin more effectively than enalapril. Taken together, the data suggest that VDRAs can provide additional cardiovascular effects in the presence of RAAS inhibitors.

Previously, we reported that VS-105 after 2 wk of dosing in 5/6 NX rats suppressed serum PTH in a dose-dependent manner starting at 0.004 µg/kg, and reduced serum PTH to the level observed in sham rats at higher doses (0.64 µg/kg ip or 0.5 µg/kg by oral gavage) (61). In this study, chronic dosing of VS-105 at 0.05 µg/kg suppressed serum PTH by 39% and at 0.5 µg/kg reduced it further to almost zero. Oversuppression of PTH can lead to adynamic bone disease (low-turnover bone disease), which is known to be associated with vascular calcification in CKD (23). Higher PTH concentrations have been reported to be associated with increased risk of CVD events, and PTH has been shown to be involved in cardiac remodeling (13, 52). Enalapril has no effect on PTH, and its effect on blood pressure, endothelial function, fibrosis (61, 62), and inflammation (24, 65), which is mediated by VDR independent of RAAS inhibition. The effects of vitamin D therapy on blood pressure, endothelial function, fibrosis (61, 62), and inflammation (24, 65), which is mediated by VDR independent of RAAS inhibition. The effects of vitamin D therapy on blood pressure, endothelial function, fibrosis (61, 62), and inflammation (24, 65), which is mediated by VDR independent of RAAS inhibition. The effects of vitamin D therapy on blood pressure, endothelial function, fibrosis (61, 62), and inflammation (24, 65), which is mediated by VDR independent of RAAS inhibition.


