Lack of vitamin D receptor causes stress-induced premature senescence in vascular smooth muscle cells through enhanced local angiotensin-II signals

Petya Valcheva a, Anna Cardus a, Sara Panizo a, Eva Parisi a, Milica Bozic a, Jose M. Lopez Novoa c, Adriana Dusso a, Elvira Fernández b,1, Jose M. Valdivielso a,*,1

a Experimental Nephrology Laboratory, Department of Experimental Medicine, Biomedical Research Institute of Lleida (IRBLLEIDA), Lleida, Spain
b Nephrology Service and UDETMA, University Hospital Arnau de Vilanova, Lleida, Spain
c Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain

ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 9 April 2014
Accepted 1 May 2014
Available online 13 May 2014

Keywords:
VSMC
Vitamin D
Angiotensin-II
ROS
Senescence

ABSTRACT

Objectives: The inhibition of the renal renin-angiotensin system by the active form of vitamin D contributes to the cardiovascular health benefits of a normal vitamin D status. Local production of angiotensin-II in the vascular wall is a potent mediator of oxidative stress, prompting premature senescence. Herein, our objective was to examine the impact of defective vitamin D signalling on local angiotensin-II levels and arterial health.

Methods: Primary cultures of aortic vascular smooth muscle cells (VSMC) from wild-type and vitamin D receptor-knockout (VDRKO) mice were used for the assessment of cell growth, angiotensin-II and superoxide anion production and expression levels of cathepsin D, angiotensin-II type 1 receptor and p57Kip2. The in vitro findings were confirmed histologically in aortas from wild-type and VDRKO mice.

Results: VSMC from VDRKO mice produced more angiotensin-II in culture, and elicited higher levels of cathepsin D, an enzyme with renin-like activity, and angiotensin-II type 1 receptor than wild-type mice. Accordingly, VDRKO VSMC showed higher intracellular superoxide anion production, which could be suppressed by cathepsin D, angiotensin-II type 1 receptor or NADPH oxidase antagonists. VDRKO cells presented higher levels of p57Kip2, impaired proliferation and premature senescence, all of them blunted upon inhibition of angiotensin-II signalling. In vivo studies confirmed higher levels of cathepsin D, angiotensin-II type 1 receptor and p57Kip2 in aortas from VDRKO mice.

Conclusion: The beneficial effects of active vitamin D in vascular health could be a result of the attenuation of local production of angiotensin-II and downstream free radicals, thus preventing the premature senescence of VSMC.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Adverse cardiovascular events are the leading cause of death among chronic kidney disease (CKD) and end-stage-renal disease (ESRD) patients. The higher incidence of atherosclerosis in these populations is a well-recognized contributor to their disproportionally high morbi-mortality. In the course of CKD, several mechanisms increase the risk for cardiovascular disease. The decrease in renal parenchyma function causes a deregulated activation of the renin-angiotensin system (RAS). The main physiological role of angiotensin-II (Ang-II) is the regulation of blood pressure, electrolyte balance and extracellular volume, but it is also involved in some pathological conditions including fibrosis, hypertrophy, cell proliferation and senescence [1]. Ang-II production could be initiated locally through the catalytic activity of renin or the renin-like enzyme cathepsin D (CatD), depending on the cell type. Local Ang-II binding to its main receptor, Ang-II type 1 receptor (AT1), exerts pro-inflammatory effects in the vascular wall through the induction of reactive oxygen species (ROS) and inflammatory cytokines [2]. Free radicals impair vascular cell life-span through the onset of cellular senescence [3], a common feature of vascular smooth muscle.
cells (VSMC) that accumulate in the atherosclerotic plaque enhancing its susceptibility to rupture [4]. Thus, the pathophysiological impact of local Ang-II production on vascular wall health deserves further investigation.

An important feature of CKD is also vitamin D (VD) deficiency. The inverse relationship between VD deficiency and cardiovascular health is well documented. However, the underlying mechanisms have not been fully elucidated [5,6]. One of the potential benefits of the active form of VD (1,25(OH)2D3) is the down-regulation of renin levels. Li et al. demonstrated higher Ang-II plasma levels in VD receptor-knockout (VDRKO) mice, due to increased renin expression and activity [7]. In vitro experiments showed that 1,25(OH)2D3 directly suppresses renin gene expression by targeting the cyclic-AMP response element (CRE) in the renin gene promoter [8].

The role of VD signalling in VSMC proliferation is controversial. Thus, whereas some studies have shown decreases in proliferation [9–11], we and others have found the opposite effect [12–14]. Thus, previous studies from our laboratory have shown that 1,25(OH)2D3 increases proliferation and calcification of VSMC through a VD response element in the VEGF promoter [15,16] and via a RANK-BMP4 dependent pathway [17,18], respectively. These are two critical processes in the vasculature during atherosclerosis progression.

The present study examines the contribution of the lack of VD signalling in VSMC to enhance local Ang-II synthesis, increased free radical production and VSMC senescence, all of which may accelerate the onset of subclinical cardiovascular disease, independently of the degree of systemic RAS activation. Furthermore, it clarifies the role of vitamin D signalling in the regulation VSMC proliferation.

2. Methods

2.1. Experimental animals

VDRKO mice from the Tokyo strain [19], were kind gift from Dr. Shigeki Kato (University of Tokyo, Japan). For PCR genotyping four primers were used to amplify a 140 bp VDR band and a 455-bp Neo band from the inserted targeting vector, as described before [20]. In order to normalize mineral homeostasis and exclude hypocalcaemia as a confounding factor in our experiments, after weaning only VDRKO mice were fed a rescue diet [20% lactose, 2.0%Ca, 1.25%P and (Harlan Laboratories)]. Three- to six-months-old male and female VDRKO mice and their WT littermates, as a control group, were used for all experiments, following the National Institute of Health Guide for the Care and Use of Laboratory Animals. The protocols used in this study were approved by the Ethic Animal Experimentation Committee of University of Lleida.

2.2. Cells and culture conditions

Aortic VSMC from 3-months-old mice were obtained by explant culture as previously described [15]. Cells were grown in 10% heat-inactivated FBS-containing DMEM (GIBCO) and maintained at 37 °C in humidified incubator (5%CO2/95%air). Cells at passages 3–8 were serum-starved in 0.2%FBS DMEM during 24 h to synchronize the population in G0 phase of the cell cycle before treatments. Pepstatin A (PstA, Santa Cruz Biotechnology), inhibitor of aspartic proteases cathepsin D, pepsin and renin, was dissolved in water-free DMSO and added at 2.5 μg/ml. Losartan (Los, Sigma–Aldrich), an AT1 antagonist, was dissolved in water and used at 10 μmol/L. Diphenyleneiodonium (DPI, kind gift from Dr Jordi Boada (University of Lleida, Spain)), a NADPH oxidase inhibitor was dissolved in DMSO and used at 10 μmol/L. Sodium 4,5-dihydroxybenzene-1,3-disulfonate [Tiron, kind gift from Dr Xavier Dolcet (University of Lleida, Spain)], a scavenger of superoxide anions, was used at 10 μmol/L. 1,25-dihydroxyvitamin D3 (1,25D3, Sigma–Aldrich) was dissolved in absolute ethanol and used at 100 nmol/L.

2.3. RNA expression

Total cellular RNA was extracted using TRIzol® Reagent (Invitrogen) following the manufacturer’s instructions. The reverse transcription was performed using a First Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Diagnostics). For the Real-Time PCR TaqMan® probes for Cathepsin D (CatD, Mm00515587_m1), angiotensin-II receptor type 1a (AT1a, Mm01166161_m1), p57Kip2 (Mm00438170_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Mm99999915_g1), TaqMan® Universal PCR Master Mix, No AmpFlager® UNG and ABI PRISM® 7000 sequence detection PCR system from Applied Biosystems were used following the manufacturer’s instructions. The relative RNA amount was calculated by standard formulae (ΔΔCt method) using GAPDH as a housekeeping gene.

2.4. Western blot

Cells and arterial tissues were collected in lysis buffer [2% sodium dodecyl sulphate (SDS), 125 mmol/L Tris, pH 6.8, 1 × protease inhibitor cocktail (Sigma–Aldrich), 2 mmol/L phenylmethylene-sulfon fluoride (PMSF), Protein concentrations were determined with DC™ Protein Assay Kit (Bio-Rad). Western blot was performed as previously described [21]. Blots were incubated with primary antibody for CatD (1:5000, Abcam), AT1 (1:1000, Santa Cruz Biotechnology), p57Kip2 (1:500, Santa Cruz Biotechnology), and α-tubulin (1:10000, Sigma–Aldrich) overnight at 4 °C. After washing, appropriate horseradish peroxidase–conjugated secondary antibody at 1:10,000 dilution (anti-rabbit: Cell Signaling Technology® and anti-mouse: Jackson ImmunoResearch Laboratories, Inc.) was added for an extra hour. Blots were developed with the Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare Bio-Sciences AB) or EZ-ECL Chemiluminescence detection kit for HRP (Biological Industries) and the VersaDoc Imaging system Model 4000 (Bio-Rad).

2.5. Angiotensin-II determination

Ang-II concentration in the culture medium was determined by EIA [Angiotensin-II SPIE-IA kit (BertinPharma)], according to the manufacturer’s instructions. The concentration of Ang-II was normalized to cell number.

2.6. Superoxide anion detection

For detection of intracellular superoxide anions, after 24 h in 10%FBS DMEM, cells were detached and re-suspended in PBS alone, with PstA, LOS or DPI and kept at 37 °C for 30 min. During the last 20 min of incubation, 10 μmol/L dihydroethidium (DHE, Sigma–Aldrich) was added. Then, the cells were transferred into 5 ml polystyrene round bottom tubes (BD Biosciences) and analyzed in FACSCanto™II flow cytometer (BD Biosciences), using FACSdiva Version 6.1.1 software.

2.7. Senescence-associated-β-galactosidase (SA-β-Gal) activity

SA-β-Gal activity of the cells was assessed, as described previously [22], with minor modifications. Briefly, cells were fixed
during 4 min at RT in 0.5% glutaraldehyde and rinsed in PBS (pH 7.2, supplemented with 1 mmol/L MgCl₂). Finally, the cells were incubated overnight at 37 °C (without CO₂) with a freshly-prepared staining solution for SA-β-Gal: 1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactoside (Sigma–Aldrich), 5 mmol/L potassium ferrocyanide, 5 mmol/L potassium ferricyanide, 2 mmol/L MgCl₂ in PBS, pH 6.0. In order to count the total cell number, the cell nuclei were further stained with Hoechst 33258 dye (Sigma–Aldrich) during 10 min. The number of SA-β-Gal positive cells and the total cell number were counted and documented by a digital imaging system (Nikon). In order to assess the impact of RAS over the appearance of premature senescence, we treated the cells with PstA (Santa Cruz Biotechnology) or LOS (Sigma–Aldrich) during 14 days before the SA-β-Gal activity assessment.

2.8. Growth curves

The growth patterns of VSMC were studied in 10% FBS-supplemented DMEM. Cells (10 × 10³ cells/cm²) were left to attach overnight and counted in Neubauer chamber at 0, 3, 6, 18, 24, 32 and 48 h. For the experiments with the AT1 antagonist, cell number was counted 72 h after LOS (Sigma–Aldrich) addition.

2.9. Cell cycle fluorescence-activated cell sorter analysis

Cells (10 × 10³ cells/cm²) were cultured in 10% FBS DMEM and allowed to reach 50% of confluence, establishing this point as time 0. After 48 h the cells were trypsinized and fixed with 70% ethanol overnight at 4 °C. Then, the cells were incubated with 1 mg/ml
RNAase for 30 min at 37 °C, and afterwards cell nuclei were stained with 50 μg/ml propidium iodide during 30 min. Cell fluorescence distributions were obtained using Coulter Epics XL flow cytometer (Beckman Coulter).

2.10. Immunohistochemistry

Arteries were extracted from isoflurane-anesthetized 6-months-old mice and fixed overnight in 20% formalin solution. After paraffin inclusion, 5 μm thick slices were used for immunohistochemistry as previously described [23]. Sections were incubated with the following primary antibodies: anti-CatD (1:100, Abcam), anti-AT1 (1:400, Santa Cruz Biotechnology) and anti-p57kip2 (1:100, Santa Cruz Biotechnology). After washings, the sections were incubated with biotinylated secondary antibody (1:50) and stained with VECTASTAIN® ABC and peroxidase substrate (DAB) kits (Vector Laboratories) according to manufacturer’s instructions. Haematoxylin (EnVision™ FLEX, Dako) was used to counterstain cell nuclei.

2.11. Statistical analysis

Data are reported as mean ± standard deviation (SD). Statistical analysis was performed using Graph Pad Prism (release 5). Results were evaluated by t-test or by two-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc tests as appropriate. A value of p < 0.05 was considered to denote statistical significance.

3. Results

3.1. Lack of VDR increases local Ang-II signals

In order to provide conclusive evidence for the role of VD in vascular health, we examined the expression of several factors involved in Ang-II signalling in primary cultures of aortic VSMC, isolated from WT and VDRKO mice. There was no detectable renin expression in these cells. However, VDRKO cells showed an increase in mRNA levels of the acid protease CatD (Fig. 1a). Moreover, when we treated WT cells with 100 nM 1,25(OH)2D3 during 24 h, we found a significant decrease in the CatD mRNA levels. The fact that CatD could be regulated negatively upon VDR activation, led us focus on CatD-downstream signalling pathways. The protein levels of CatD were higher in VSMC lacking the VDR (Fig. 1b). Ang-II levels in the cell culture media, measured by EIA, were significantly increased in VDRKO cells (Fig. 1c). Furthermore, AT1 expression was also up-regulated in VDRKO cells at both mRNA (Fig. 1d) and protein levels (Fig. 1e). These results suggest that the absence of VDR signals activates local RAS in VSMC.

Fig. 2. VDRKO cells show higher intracellular superoxide anion levels and premature senescence. (a) Representative flow cytometry histograms showing the intracellular superoxide anions levels measured by dehydroethidium (DHE) in VSMC lacking vitamin D receptor (VDRKO) and WT controls from passage 5. (b) Quantification of the DHE oxidation products, expressed as mean fluorescence units (MF). ROS production was assessed after the addition of PstA (2.5 μg/mL), LOS (10 μmol/L) and DPI (10 μmol/L). (c) Representative photographs of WT and VDRKO cells, stained for senescence-associated-β-galactosidase (SA-β-Gal) activity at pH6.0. Quantification is shown on the right (n = 16, quadruplicates from four independent experiments). Bars represent mean ± SD of at least three independent experiments. Statistical significance, ns: non significant,***: p < 0.001 vs. WT cells under identical experimental conditions.
3.2 VDRKO VSMC cells present higher superoxide anion levels and eliciting features of premature senescence

Systemic RAS activation is known to increase ROS production in VSMC [24]. To determine local ROS production, the content of superoxide anions was measured by flow cytometry in WT and VDRKO cells. Dihydroethidium-derived oxidation products were higher in VDRKO cells, indicating a higher production of superoxide anions (Fig. 2a). To examine whether the enhancement in free radicals content in VDRKO cells resulted from an increase in Ang-II-induced NADPH oxidase activity, WT and VDRKO cells were incubated with Pepstatin A (PstA, an aspartyl protease inhibitor), Losartan (LOS, an AT1 antagonist), or diphenyleneiodonium (DPI, a NADPH oxidase inhibitor). As shown in Fig. 2b, all inhibitors significantly decreased the superoxide anion content in VDRKO cells to levels similar to those observed in WT controls. The finding that VSMC lacking VDR grow under a higher oxidative stress could also explain their distinct morphology in culture. Specifically, VDRKO cells have a bigger size, a flattened star-like shape and lots of vacuoles in their cytosol (data not shown), all features of cell senescence. Staining for Senescence-Associated-β-Galactosidase (SA-β-Gal) activity showed that 22.5% of the VSMC obtained from VDRKO animals presented a blue perinuclear staining. Instead, WT cells were all negative (Fig. 2c), confirming the appearance of premature senescence in VDRKO cells.

3.3 Decreased proliferation rates in VDR lacking VSMC

WT VSMC grew faster than VDRKO, as assessed by the comparison of the growth curves slopes (p < 0.0001). WT cells followed a typical exponential curve, while VDRKO cells showed a slower, almost linear growth (Fig. 3a). Moreover, FACS analysis also revealed a reduction in the percentage of cells in the S-phase of the cell cycle. VDRKO cells presented 73.0 ± 4.3% in G1-phase and 3.9 ± 0.3% in S-phase after 48 h in culture compared to 64.2 ± 2.8% and 15.4 ± 0.8%, respectively for WT cells (Fig. 3b). Importantly, the percentage of dead cells was similar for both cell types and below 5%. To further elucidate the downstream signalling mechanism by which VDR depletion induced cell cycle arrest and senescence, the expression levels of cell cycle checkpoint proteins involved in senescent phenotype were examined. An interesting finding was the very high expression of the cyclin-dependent kinase inhibitor (CDKi) p57Kip2 in VDRKO cells (Fig. 3c).

3.4 Increased local production of angiotensin-II induces free radicals generation and p57Kip2 upregulation thus contributing to decreased proliferation of VSMC from VDRKO mice

In order to check whether AT1 activation contributed to differences in proliferation rates, LOS exposure was prolonged to 72 h. LOS treatment increased cell proliferation in VDRKO cells. In contrast, in WT cells, AT1 blockade led to decreased proliferation rates (Fig. 4a). Next, to examine the role of Ang-II signals in the onset of premature senescence, VDRKO cells were cultured with PstA or LOS for 14 days. As shown in Fig. 4b, the prolonged exposure to these inhibitors prevented further increase in the senescence induced by the lack of VD signalling. None of the WT cells were stained for SA-β-Gal even upon the treatments. In view of the marked increases in p57Kip2 observed in VDRKO cells, we examined its role in the recovery of the proliferation rates of VDRKO cells after CatD or AT1 inhibition. Fig. 4c shows a significant reduction in the mRNA levels of the CDKi p57Kip2 upon CatD and AT1 blockage. Also the superoxide anion scavenger Tiron caused a similar decrease in p57Kip2 mRNA levels in VDRKO cells (Fig. 4c) as CatD and AT1 inhibitors.

3.5 VDRKO mice present increased aortic expression of key components of the angiotensin-II signalling cascade

To validate in vivo the link between the lack of VDR and enhanced Ang-II signalling, western blot and

![Fig. 3. VDRKO vascular smooth muscle cells show decreased proliferation rates in vitro. (a) Growth curves of WT (solid line) or VDRKO cells (dotted line) after stimulation with 10% FBS (n = 3, experiments performed in triplicate); (b) FACS analysis to quantify WT and VDRKO cell distribution throughout the cell cycle. (c) Representative western blot of p57Kip2 expression in WT and VDRKO VSMC. The graph represents optical density (OD) of p57Kip2 to OD of α-tubulin, as a loading control. Bars represent mean ± SD. Statistical significance: *: p < 0.05, **: p < 0.001 and ns = non significant versus WT.](image)
4. Discussion

The present study shows for the first time that the lack of VD signalling in VSMC induces an increase in local production of Ang-II, which leads to two processes associated with vascular ageing: oxidative stress and premature senescence. Accelerated vascular ageing is a characteristic of CKD patients. Even in children with end-stage renal disease, the artery wall presents structural abnormalities comparable with those found in adults [25]. The VD deficiency that characterizes CKD is known to compromise cardiovascular health in these patients. Indeed, the active form of VD, 1,25(OH)2D3, exerts pro-survival and anti-ageing actions. The activated VDR up-regulates Klotho (a gene with numerous anti-ageing properties) through multiple VD response elements in the Klotho promoter [26]. Moreover, Eeien et al. [27] have recently demonstrated that VD induces FOXO3a and SESN1 expression in osteoblasts. These are highly conserved proteins that protect against age-related pathologies, altering ROS levels. Furthermore, Kim et al. [28] have shown that activation of peroxisome proliferator-activated receptor β (PPARβ), a protein up-regulated by VD [29], controls Ang-II-induced premature senescence in endothelial cells by increasing the expression of the novel anti-ageing protein SIRT1, thereby modulating cellular ROS generation. Likewise, the fact that activated VDR decreases renin expression [7] may also play a role in the potential beneficial effects of VD on cardiovascular health.

Thus, vascular protection afforded by VD in VSMC could be mediated through several mechanisms, among which the local Ang-II pathway has been poorly investigated. It has been described that VSMC locally produce Ang-II. The rate-limiting enzyme in renal production of Ang-II is renin. We did not detect renin expression in mouse VSMC in vitro or in vascular tissue in vivo. Fukuda et al. also were not able to detect renin in rat VSMC, but they showed that in VSMC obtained from spontaneously hypertensive rats, the higher production of Ang-II in vitro was due to increased expression of CatD [30]. CatD is a lysosomal aspartyl protease with high sequence homology to renin, which displays renin-like enzymatic activities [31]. In the present study we show for the first time that in the absence of VDR, VSMC express higher levels of CatD that could explain the higher amount of Ang-II. Yuan et al. have demonstrated that VD blocks the activity of cAMP-response element (CRE) in the renin gene promoter [8]. Interestingly, the promoter of CatD elicits a putative CRE [32]. Indeed, in our experiments, the incubation with 1,25(OH)2D3 decreased significantly CatD gene expression in WT VSMC. Therefore, CatD expression could be subjected to the same negative regulation by 1,25(OH)2D3 as the renin gene.

Upon activation of its receptor AT1, Ang-II upregulates NADPH oxidase activity and consequently the production of ROS [33]. In our study, VDRKO VSMC showed a significant up-regulation of AT1 mRNA and protein levels, which could be mediated by increased Ang-II levels, as previously reported [34]. Furthermore, these cells elicited higher ROS levels, which were decreased to levels undistinguishable from those in WT after the inhibition of CatD activity, AT1 or NADPH oxidase downstream signalling. Thus, these results suggest that the lack of negative control by VD over CatD expression could cause higher Ang-II production with subsequent AT1-mediated NADPH oxidase activation and ROS production.

Ang-II induces AT1-mediated premature senescence in VSMC in vitro through an increased superoxide anion production by NADPH oxidase activity [35]. VSMC lacking VDR showed reduced proliferation and features of cell senescence. Ang-II usually contributes to enhanced VSMC growth which could be inhibited by AT1 receptor antagonists [36], a feature that we observed in WT cells.

However, it is possible that in VDRKO cells, LOS increased proliferation rates as a result of the blockade of Ang-II/immunohistochemical (IHC) analysis compared aortic samples from 6-months-old WT and VDRKO mice. The expression of CatD, AT1 and p57Kip2 were markedly higher in the VDRKO arteries (Fig. 5a). Similar findings were observed in IHC analysis (Fig. 5b).
NADPH oxidase-mediated ROS generation. Oxidative stress produces DNA damage, inducing cell cycle inhibitors that make cells exit the cell cycle. The lower proliferation capacity and the appearance of premature senescence in VDRKO cells could result from the higher expression of the cell cycle inhibitor p57Kip2, implicated in the senescent phenotype [37]. The fact that PstA, LOS and Tiron successfully decreased p57 Kip2 levels provides additional evidence for the contribution of CatD-mediated local RAS activation to the lower proliferation capacity and premature senescence of VSMC lacking VDR. Moreover, the inhibition of CatD activity and AT1-downstream signalling prevented new VDRKO cells to become senescent. Hence, the link between the absence of functional VDR and cellular ageing could be the excessive activation of local RAS.

The obvious premature ageing phenotype of VDRKO mice could be associated with the increased CatD, AT1 and p57Kip2 expression, found in their arteries. Undoubtedly, these increases could be partly accounted for the increased systemic RAS activation, demonstrated by Li et al. [7]. Furthermore, increased local production of Ang-II could partly account for the high blood pressure levels described in these animals. In vivo, VSMC are in quiescent state under normal conditions [38] and their re-entry into the cell cycle occurs in various pathological situations. For instance, in case of damage, VSMC are stimulated to proliferate in order to repair the vessel. However, the uncontrolled proliferation of VSMC has been linked to diseases such as hypertension and atherosclerosis [39].

The results presented here suggest that the cardiovascular protection showed by active vitamin D treatment in human CKD...
patients could be related, at least in part, to the effect of VDR activation on vascular senescence. Senescent VSMC cells have been found in atherosclerotic plaques [3,40], and recent results suggest that VSMC senescence could even promote atherosclerosis [41]. Thus, the accelerated atherosclerotic process that CKD patients suffer [42,43] could be partially enhanced by the chronic hypovitaminosis D associated with CKD. Indeed, recent results have shown that deletion of VDR gene in an animal model of atherosclerosis enhances plaque formation [44]. This effect could be mediated, at least in part, by the increase in VSMC senescence and also by the increase in blood pressure induced both by systemic and locally produced Ang-II. Although tempting, it is difficult to extrapolate animal data into humans, and experimental results should be taken cautiously until confirmed in clinical settings.

5. Conclusion

The findings of our work suggest that in absence of VD signalling an increase in the local production of Ang-II accounts for the appearance of premature vascular senescence (Fig 6), confirming that VD is an essential hormone in the regulation of VSMC proliferation. Thus, vitamin D could play a role in both, the normal repair of the vascular wall and in the prevention not only of systemic but also of local RAS-mediated VSMC hyperproliferation.

Disclosures

No conflicts of interest are declared by the authors.

Acknowledgements

This work was partially supported by grants from FIS PI12/01770, CEDAR and REDINREN (RD12/0021/0026). We thank Dr. Marti Aldea for lively discussions. Drs. Valcheva’s and Cardus’ work to complete this manuscript was partly supported by their scholarships from ERA-EDTA awarded on November 2011 and July 2012, respectively.

References


