Review

Evolution and modulation of age-related medial elastocalcinosis: Impact on large artery stiffness and isolated systolic hypertension

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Abstract

Arteriosclerosis, characterized by remodeling and stiffening of large elastic arteries is the most significant manifestation of vascular aging. The increased stiffening is believed to originate from a gradual mechanical senescence of the elastic network, alterations in cross-linking of extracellular matrix components, fibrosis and calcification of elastic fibers (medial elastocalcinosis). The stiffening of large arteries reduces their capacitance and accelerates pulse wave velocity, thus contributing to a widening of pulse pressure and to the increased prevalence of isolated systolic hypertension with age. Current antihypertensive drugs were mainly designed to reduce peripheral resistance and are not adequate to alter the pathological process of vascular stiffening or even to selectively reduce systolic blood pressure in isolated systolic hypertension. This review puts forward the concept that elastocalcinosis is a valuable therapeutic target and presents evidence that this process can be prevented and reversed pharmacologically.

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1. Introduction

In the field of hypertension, elevation of diastolic blood pressure (DBP), which is found mainly in middle age population, was the major concern less than 15 years ago. Today, the elevation of systolic (SBP) and pulse pressures (PP), which occurs “normally” later in life [1,2], is attracting considerable interest, especially since the publication of clinical trials showing the marked benefit of reducing these variables in the elderly population [3]. Unfortunately, current antihypertensive therapies have evolved from the concern of reducing systolo-diastolic hypertension and are not necessarily suited for isolated systolic hypertension (ISH), the most prevalent form of the disease in the elderly population. The diagnosis and treatment of high SBP is now a clear mandate of national and international advisory committees on hypertension management [1]. After an overview of the alterations in composition and structure of large arteries during aging, this review will focus on medial calcification (elastocalcinosis) and its pharmacological modulation as a potential new therapeutic approach for ISH.

2. Age-induced arteriosclerosis

At the levels of large elastic arteries, arteriosclerosis, a process defined by lumen enlargement with wall thickening (remodeling) and a reduction of elastic properties (stiffening), is the main manifestations of aging [1,4]. In fact, aging is the main factor leading to arterial stiffening [5]. However, this process is strikingly heterogeneous along the arterial tree, with distal muscular arteries not exhibiting the same age-dependent stiffening [6]. Development of intimal atheromatous plaques, referred to as atherosclerosis has a different pathophysiological evolu-
tion. Although the two clinical conditions can certainly coexist, they can also be observed separately both spatially and temporally [1,4,7]. Discussion of intimal calcification of atherosclerotic plaques is beyond the scope of this review.

The media of large arteries is mainly composed of an integrated assembly of vascular smooth muscle cells (VSMC), elastic lamellae and collagen fibrils, into functional musculo-elastic sheets. Cross-links between extracellular matrix (ECM) components and cell-matrix interactions confer adequate mechanical properties [8,10]. Smooth muscle cells, representing 30% to 50% of the volume, are probably the less rigid component in the vascular wall [8,10]. The very extensible elastic lamellae, representing 25% of the volume, largely determine the elasticity of the vessel wall in the normal physiological range of pressures [8,10]. Collagen fibers, which represent 35% of the volume, are at least two orders of magnitude stiffer than elastin and VSMC, thus maintaining vascular integrity (tensile strength) [8,10]. Collagen is recruited mainly at higher pressures, when the arteries are significantly distended [8,10,11]. Thus, the elasticity of large arteries decreases with pressure loading, since the stiffer components of the arterial wall are recruited sequentially. This efficient organization is modified during aging and seems to contribute to the progressive stiffening of large arteries.

2.1. Vascular smooth muscle cells

Most studies have found that the number of VSMC declines with age [12,13]. This has been attributed to a generalized reduction of cellular activity that cannot compensate for cellular apoptosis that occurs in the vessel wall [12]. Fibrotic scars seem to occupy cellular gaps left by deleted VSMC [12] and remaining cells become progressively larger and embedded in a richer collagen “capsule” with fewer cellular contacts [12,13]. The contribution of VSMC to large artery stiffness appears proportional to their level of active tone, as suggested by studies using vasoconstrictors [10,14]. Nonetheless, deactivation of VSMC does not abolish the age-related stiffness and downstream muscular arteries do not get stiffer during aging, suggesting that structural components are major contributors [14,15].

2.2. Collagen

Collagen isoforms found in the aorta are mainly (80–90%) of type I and III, with some type IV [9,11] and their concentration gradually increases after the age of 50 [11]. In young rats, collagen is organized in large linear bundles along the elastic lamellae, to become spread diffusely in the intimal space with age [12]. Intermolecular cross-linking, which is the molecular event that gives the tensile strength, could also contribute to the stiffening and some reports suggest that this process is increased with age (see Section 2.4).

2.3. Elastic fibers

One of the most striking features of arterial aging is the progressive thinning, splitting, fraying, and fragmentation of elastic lamellae [12,13]. Elastin is the most abundant protein of the vascular wall of large arteries and represents 90% of elastic fiber content, which are also composed of glycoproteins such as fibrillin-1 [8,9]. Elastic fibers are formed from soluble tropoelastin monomers assembled and cross-linked on several residues [16]. The metabolism of elastin appears to be age-dependent, being mainly synthesized during early development with a subsequent slow turnover [13]. Elastin is thus one of the most stable proteins in the body with the consequence of being most vulnerable to alterations with minimal coordinated repair [9,17]. Furthermore, several elastin cross-links are decreasing with age, thus contributing to reduce the rubber-like properties of the polymer [16]. Although most studies suggest a relative reduction of elastin density, the most important aspect is its structural distortion contributing to limit the function of providing capacitance during systole and recoil during diastole.

The prevailing hypothesis in the literature for the gradual disorganization of the elastic lamellae is a slow mechanical senescence of the network by repetitive influences of systolic stretching [1,18]. Accordingly, a study looking at different species reported that the combined effects of age and heart rate are positively associated with structural alterations of elastin [19]. Other factors that could also be involved were reviewed by Atkinson [18] and are depicted in Fig. 1. As an example, oxidative stress and cytokines, which are enhanced with age, could trigger elastase activation and disrupt the global architecture of the fibers [9,17].

2.4. Advance glycation end-products (AGEs)

Two distinct processes are involved in ECM cross-linking: the enzymatic lysyl oxidase and the non-enzymatic Maillard reaction [20]. This non-enzymatic reaction involving sugars results in the formation of glycosylated proteins (also called Amadori products), which undergo cycles of condensation with dehydration and oxidation to form irreversible advanced glycated end-products (AGEs) [21]. Since only protein catabolism removes AGEs, collagen and elastin are highly susceptible to AGE accumulation because of their slow turnover. Increased cross-linking confers a resistance to enzymatic degradation of collagen that promotes its accumulation in the arterial wall [20]. Other relevant effects of AGEs include their binding to RAGE (their receptors) to promote the release of fibrotic cytokines and inhibition of cell adhesion that could enhance apoptosis [22]. Advanced glycation end-products accumulation on
collagen and elastin, and age-related aortic stiffness were correlated [23]. Moreover, experiments using aminoguanidine and pyridoxamine, inhibitors of AGEs cross-linking formation, reported a prevention of arterial stiffening [24,25]. Furthermore, AGE cross-link breakers restored large artery properties in aging rats, providing scientific rationale to their development [21,26].

2.5. Medial calcification (elastocalcinosi)

2.5.1. Localization and impact on stiffness

The presence of calcium deposition in the media of large arteries is not a new observation, suggesting that recent lifestyle changes are not essential culprits [27]. In 582 postmortem aortic specimens, Blumenthal reported that only
4% of patients aged 20–30 years had significant calcification, while this value increased to 98% in individuals above 50 years [28]. In the latter group, there was marked intensity in 52% of cases. It is suggested that vascular calcium content may increase 30 to 40 times in a lifespan, including both intimal atherosclerotic calcification and medial elastocalcinosis [29,30].

One could then wonder if calcification is linked to other age-related alterations. Calcification is primarily associated with the elastic elements rather than with muscle cells [18,28]. Interestingly, syphilitic patients, with a characteristic disappearance of the elastic lamellae, had very low calcification as compared to age-matched subjects [28]. In addition, the intensity of calcification during aging follows the elastin gradient, with large arteries having proportionally higher levels of elastin and calcification [18]. It is tempting to postulate that the gradual disruption of elastic fibers could facilitate the calcification process. Accordingly, an increased polarity of elastin with age is associated with increased mineralization [31]. Indeed, elastin has been shown to have a very strong affinity for calcium [17]. Other components of the elastic fibers could also be mineralized and these alternative hypotheses were reviewed elsewhere [30]. Thus, the age-related elastic calcification will be referred to as medial elastocalcinosis (MEC) throughout the text.

The next important question is whether calcification contributes to arterial stiffness. Using a rat model of MEC without changes of global structure, the group of Atkinson elegantly demonstrated a strong relationship between the extent of aortic calcification and stiffness measured by several approaches [32]. We recently confirmed these data by measuring PWV in another model of drug-induced MEC [33]. Fig. 2 presents a correlation between PWV and aortic calcification in this model, showing that at least one third of the stiffness was related to elastocalcinosis.

In man, the associations between age and vascular stiffness, and between age and MEC coincide, with a threshold at around 50 years of age [28]. Moreover, a direct relationship between large artery calcification and stiffness has been demonstrated in patients with end-stage renal disease (ESRD) [34]. Thus, it appears that calcification can contribute to arterial stiffening in man as well.

2.5.2. Is elastocalcinosis a regulated process?

The gradient of calcium within the aging media reveals that its mid-portion appears mostly calcified, with less calcification in the inner and outer areas. This distribution suggests local modulation rather than simple passive deposition [29]. Interestingly, medial calcification is associated with local expression of mineralization-regulating proteins normally expressed in osteogenesis [35]. Since only VSMC are found in MEC, these cells appear as the source of such unusual proteins in the vascular wall. These data clearly indicate that the vessel wall is not a passive bystander. Indeed, knockout or pharmacological inhibition of some of these mineralization-regulating proteins can lead to MEC [36].

2.5.3. Pathological modulation of elastocalcinosis

Several pathological conditions have been shown to accelerate or amplify MEC. Aortic specimens from hypertensive individuals aged from 30 to 60 showed accelerated calcification [28]. There seems to be a general agreement that hypertension contributes to or accelerates arterial stiffness [37] and amplifies intimal and medial calcification in man [38]. In fact, hypertension is considered to produce premature vascular aging, at least in a subset of patients with the disease [39].

Type 2 diabetes is also associated with amplification of MEC [40] and with the accelerated formation of AGEs. Interestingly, AGE-modified elastin has been shown to enhance MEC [41], potentially explaining the acceleration of calcification, and the different distribution of calcification, involving also arteries of the lower limbs [40].

Another condition associated with MEC is ESRD, in which patients have stiffer arteries than age-matched controls [42]. Guérrin reported that the density of arterial calcification increased with age, duration of haemodialysis, and the dose of calcium-based phosphate binders [34]. Calcification of the aorta and common carotid arteries was associated with increased stiffness in this condition.

2.6. Remodeling

Large artery remodeling, the second hallmark of arteriosclerosis, is characterized by an enlarged lumen and wall thickening [12]. Although the number of cells decreases with time, they are replaced by fibrosis and the residual cells undergo hypertrophy. These changes seem to explain the increase in wall thickness and the elevation of the collagen/
elastin ratio, demonstrated in rats by Fornieri [13]. The intima and adventitia could also contribute to the thickening of large arteries [43]. It is more likely that alterations in matrix composition and organization affect the structure, rather than the reverse. As proposed by Atkinson, the decay of the elastic network could gradually transfer the wall tension to the collagen fibers, which are normally recruited at higher pressure and thus at greater diameter [18]. Since the collagen fibers are stiffer than the elastic network, the vessel would also offer greater resistance to further dilatation (Fig. 1).

Additional cross-linking by AGEs and elastocalcinosis would further increase the stiffness of the arteries. Mineralization could also promote the destruction of elastic fibers to exacerbate the aging process [30,44]. It is noteworthy, however, that calcification that is induced upon normal elastic lamellae does not trigger vascular remodeling [32,33], suggesting that elastocalcinosis is not a requirement for structural changes. Thus, in atherosclerosis, MEC and remodeling seem to be two parallel consequences of the degradation of the elastic network (Fig. 1).

3. Impact of arterial stiffening

3.1. Impact on pulse pressure

The two major direct consequences of arterial stiffening are a reduced capacitance and a faster PWV (Fig. 1, pulse wave velocity). Pulse wave velocity represents the time taken by the pulse to travel between two points of know distance, normally the carotid and the femoral arteries, and is proportional to the rigidity of the artery under investigation [1,45]). In turn, these two events will impact on the cardiovascular system by modifying central hemodynamics. Indeed, SBP increases by a reduction of the ejected fraction accommodation, generating a larger pressure wave for a similar flow wave [1,46]. SBP is amplified even further by the reflected wave that now returns during systole because of the rapid PWV. Indeed, when PWV is sufficiently slow, the reflected waves normally return during diastole [47]. Since SBP contributes prominently to cardiac afterload, arterial stiffness has a major impact on the heart. As far as DBP is concerned, it is reduced by the smaller diastolic recoil and by the premature reflected wave return during systole [1]. Reduction of DBP limits coronary filling pressure and blood flow and may impact on other organs as well [1]. These two opposite pressure effects lead to an elevation of PP, which can be used as a marker of large artery stiffness, although it also depends on other factors, such as peripheral resistance and cardiac output. Nonetheless, PP elevation is one of the most important clinical manifestations of central arterial stiffness, and is emerging as a potent and independent predictor of cardiovascular events (see below).

3.2. Impact on small arteries

In hypertension, remodeling of resistance arteries is believed to contribute to target organ damage [48] by limiting flow reserve [49], and drug-induced reverse remodeling has been shown to improve cardiac perfusion [50]. These arteries adapt their structure to local hemodynamic conditions to normalize their wall stress, thus leading to increased media thickness to lumen diameter ratio (M/L) [51]. The structure of small arteries in aging has been seldom reported in man, but appears to involve wall thickening [7]. In hypertensive patients older than 60 years, eutrophic remodeling (no increase in cross-sectional area, CSA) was found, but patients had systolo-diastolic hypertension and not ISH [52]. Thus results from animal models provide the only insight on large artery stiffening-induced small artery remodeling. Two studies using different strain of rats reported an increase of small mesenteric arteries CSA (hypertrophy) with advanced aging [53,54]. The remodeling appeared as an outward hypertrophic remodeling characterized by an increase of media thickness and lumen diameter, but unchanged M/L [53]. We have recently reported similar findings in middle cerebral and small mesenteric arteries after 4 weeks of a drug-induced MEC model [55], suggesting that the impact of vascular aging on small arteries can be reproduced in an accelerated fashion (Fig. 3). Moreover, the mechanical properties, showing a preserved distensibility, were also similar in the two experimental conditions. To confirm the role of PP on remodeling, Baumbach reported that a selective elevation of PP, by a surgical arteriovenous fistula, leads to hypertrophy of cerebral arterioles [56]. Thus, although the actual structural and mechanical changes of small arteries during aging and ISH in man are unknown, the animal studies predict hypertrophic remodeling that may contribute to the deleterious consequences of large artery stiffening on target organs.

3.3. Impact on target organs

Epidemiological findings suggest that PP and SBP are powerful and robust predictors of cardiovascular risks and target organ damage [2,57]. Moreover, meta-analysis of eight outcome trials involving elderly subjects (≥60 years) with ISH showed that in untreated patients, SBP was a more precise predictor of morbidity and mortality than DBP [58]. In fact, it now appears that for a similar value of SBP, elderly patients with higher DBP fare better than those with lower DBP, due to the reduction of PP [2]. More specifically, it was reported that the development of left ventricular hypertrophy (LVH), myocardial infarction, LV dysfunction and congestive heart failure can be predicted by SBP and PP [59,60]. At the kidney level, PP elevation and arterial stiffness have been linked to plasma creatinine concentration and the presence of microalbuminuria in subjects with mild and moderate renal insufficiency [61].
Local PP also appears to be a key element of large artery remodeling, as suggested by work from the group of Laurent [62]. Elevation of PWV is emerging as a serious risk factor in hypertension [61,63,64] and in patients with ESRD [65]. Furthermore, vascular calcification has been reported as a strong and independent predictor of cardiovascular disease risk in type 2 diabetic patients [66], as well as of coronary heart disease in asymptomatic adults [38]. Thus, in addition to hemodynamic parameters such as SBP and PP, more direct indices of vascular stiffness, including PWV and vascular calcification, are associated with increased risks of developing cardiovascular diseases.

4. Experimental models of drug-induced medial arterial elastocalcinosis

From the previous discussion, it seems quite obvious that large artery stiffening is not a trivial phenomenon and drugs that can selectively affect this process are needed [67]. To that end, MEC represents a potential therapeutic target. Medial elastocalcinosis can be studied in aging rats, but the phenotype is less impressive than in man [68]. A defect in the expression of the klotho gene in the mouse results in a syndrome that mimics human aging, including extensive aortic elastocalcinosis [69]. This model could therefore be used for pharmacological studies, but in order to better isolate MEC and to improve flexibility for pharmacological interventions, drug-induced models of MEC have been developed. Other models, including transgenic mouse models, have been reviewed more extensively elsewhere [36].

4.1. Vitamin D3 and nicotine (VDN) rat model

Vitamin D3 plus nicotine (VDN) administration produces calcium overload which was initially used to demonstrate the therapeutic benefit of calcium channel blockers (CCB) [70,71]. Following the administration of vitamin D3, there is a temporary hypercalcemia, presumably the first step in ectopic calcium accumulation [68]. Nicotine alone produces minor increases in calcium levels, but its contribution may involve release of catecholamines [72]. The model is characterized by elastocalcinosis in large elastic arteries, but also in some downstream arteries irrigating the heart and the kidneys [68,73]. Using this model, the group of Atkinson showed that calcification was associated with elastin fragmentation, stiffening of large elastic arteries, increase of SBP (ISH) and left ventricular hypertrophy [30].

4.2. Warfarin/vitamin K1 (WVK) rat model

Matrix Gla protein (MGP) is a 10 kD protein synthesized by many cell types, including VSMC [74], and present in cartilage, the heart, arteries, kidneys, and lungs [75]. On MGP, five glutamic acid residues are modified by the action of the vitamin K-dependent enzyme γ-glutamate carboxylase to form carboxyglutamic acid (Gla) [76]. These Gla residues bind minerals and are believed to limit calcium phosphate salt precipitation in the ECM [77]. Using high doses of warfarin, which inhibits the regeneration of the biological active form of vitamin K, the group of Price has been able to induce MEC [78]. Indeed, MGP is found at areas of calcification, but in its inactive state [79]. Concomitant vitamin K1 administration is necessary to prevent bleeding (hepatic synthesis of coagulation factors), but does not interfere with the effect of warfarin on arteries, due to the presence of different enzymes [80]. Interestingly, the MGP null mice develop to term but die within 2 months due to a fracture of the heavily calcified thoracic or abdominal aorta [74]. This knockout mouse model, with its remarkable phenotype, established the critical role of MGP in the inhibition of soft tissue calcification.

Thus, an impairment of an anti-mineralization defense mechanism seems sufficient for elastocalcinosis to occur. One must keep in mind that blood is a metastable solution supersaturated in calcium and phosphate ion concentration, and various physicochemical forces and certain inhibitory

![Fig. 3. Comparison of lumen size and media cross-sectional area, an index of vascular hypertrophy, of small mesenteric arteries in rats of 2 (white bars) and 26 months (black bars) of age (Aging) or of medial cerebral arteries in 3-month-old controls (white bars) or rats treated for 4 weeks with warfarin and vitamin K (black bars) to induce MEC (MEC). The results obtained in the two studies [53,55] are qualitatively similar. *P<0.05 vs. respective controls.](image-url)
proteins prevent its crystallization [81]. In line with this, vitamin D administration amplifies calcification in the WVK model [79]. Others have also suggested that calcification could evolve from the stimulation of mineralization-related proteins such as BMP-2 [36]. Indeed, since MGP inhibits the mineralization properties of BMP-2, the WVK treatment could favor the mineralization process [82]. However, we were unable to detect presence of BMP-2 histologically in areas of calcification in the WVK model (Essalihi and Moreau, 2004, unpublished observation).

In our protocol of WVK administration, MEC increases after the second week, to stabilize at 4 weeks of treatment (Fig. 4). Pulse wave velocity increased significantly, suggesting that calcification of normal elastic lamellae in young rats contributes to the arterial stiffness [33]. The hemodynamic consequences of the WVK treatment are in line with the observed aortic stiffening: increased PP due to a selective elevation of SBP, with normal mean blood pressure and slightly reduced diastolic pressure [33,55]. Although this model bypasses the degradation of the elastic network found during aging as a potential substrate for calcification (Fig. 1), it reproduces many features found in aging arteries and was used to study the pharmacological modulation of elastocalcification.

5. Pharmacological modulation of medial elastocalcification

The number of clinical trials looking at pharmacological interventions to reduce large artery stiffness is increasing, although candidate drugs are scarce [46,67,83]. Indeed, nearly all antihypertensive drugs have been developed as vasodilators and act mainly on the reflected wave to decrease PP rather than on stiffness itself [84]. However, some recent clinical studies are reporting improvement of PWV and other indices of large artery stiffness with omapatrilat or a combination of diuretic/ACE inhibitor [46,83,85,86]. Nitric oxide donors also appear promising and a review of their role in vascular stiffness is presented elsewhere [87]. The mechanism of action of these drugs remains to be elucidated and current hypotheses have been reviewed [46,88,89]. This section will focus on the modulation of MEC.

5.1. Prevention

5.1.1. A connection between bones and arteries?

Aging is associated with MEC, but also with bone resorption, and evidence linking cardiovascular diseases and osteoporosis is mounting [90]. Moreover, PWV has been associated with reduced bone mineral density [91]. Many factors such as estrogen deficiency, dyslipidemia, oxidative stress, decreased nitric oxide, inflammation, and sedentary lifestyle have been linked to both conditions [90]. Thus, the concept that bone mineral loss could contribute to elastocalcification has been proposed. Considering that vascular calcification also appears to be a regulated process, such a relationship would suggest that bones and large arteries have opposite mineralization regulation. The group of Price has elegantly shown that interventions aiming at reducing bone resorption (bisphosphonates, osteoprotegerin, and SB 242784, a V-H⁺-ATPase inhibitor) also prevented the development of MEC in the WVK model [92]. In addition, in the VDN model there is a shift of calcium from the skeleton to the arteries, evidenced by a decreased calcium content in the femur and an increased plasma calcium concentration [68]. In man, the relationship is less conclusive, although there is also some evidence [90]. Thus, treating osteoporosis, or preventing bone mineral loss, may prove beneficial for the arterial aging process. In that respect, bisphosphonates and even statins, which have cholesterol-independent effects, may deserve some attention [90].

5.1.2. Antihypertensives

In the WVK model, we have shown that the diuretic hydrochlorothiazide (HCTZ), the AT₁ receptor antagonist irbesartan and the ETA-receptor antagonist darusentan prevented MEC [55]. Hydrochlorothiazide is known to inhibit carbonic anhydrase, the major source of protons used by osteoclasts to perform bone resorption [93]. In line with this hypothesis, we have administered acetazolamide, a diuretic working mainly through inhibition of carbonic anhydrase, and found that it inhibits MEC (Fig. 4). Thus, the prevention could be explained by a reduction of bone resorption, as discussed in the previous section. In that context, endothelin has been shown to have complex interactions with bones and its antagonism could also prevent calcification through a related mechanism [94,95].

Using the VDN model, Wu et al. also observed a reduction of calcification with an ET receptor antagonist,
thus confirming the involvement of this peptide in elastocalcinosi s [96]. In the same model, Henrion et al. have tested the effect of different antihypertensive agents on the development of elastocalcinosi s. Isradipine, a CCB, and perindopril, an ACE inhibitor, did not affect the development of elastocalcinosi s in large arteries [97,98]. Their results are at variance with that of Fleckenstein, which showed a prevention of vascular calcification with a CCB [70]. We also have recent evidence that the CCB amlodipine prevents elastocalcinosi s in the WVK model [99]. The different response in the VDN and the WVK models could be explained by the mechanism responsible for the induction of calcification. Since VDN induces a massive calcium overload, it may be more difficult to modulate than the WVK model, which reduces an anti-mineralization mechanism. Despite these encouraging results, current antihypertensive drugs do not seem to prevent the elevation of PP with age [100], but additional studies are clearly needed, as this could be very important clinically.

5.2. Regression

With the growing evidence that vascular calcification is regulated by mineralization-related proteins, and since bone resorption is a physiological process coordinated by some of these proteins, it is possible that mineral loss could also occur in arteries. The first direct evidence that ectopic calcification can regress comes from Giachelli’s group. They implanted gluteraldehyde-fixed aortic valve leaflets (GFAV) and observed amplification of calcification in osteopontin (OPN)-null and heterozygote mice as compared to wild-types [101]. This is in line with the accelerated calcification in MGP-OPN double knockout mice [102]. Although these results suggest that OPN, a secreted phosphoprotein associated with ectopic calcification, prevents mineral deposition, they also observed regression in heterozygotes. Indeed, there was a time-dependent mineral dissolution on GFAV by the induction of carbonic anhydrase II (CA II) expression. In the GFAV model, binding of OPN to bioapatite provided a recognition site for macrophages and giant cells leading to localize accumulation as well as up-regulation of CA II [101].

Using the WVK model, we made the unexpected finding that an endothelin receptor antagonist (ETRA), darusentan, not only prevented but also regressed MEC (Fig. 4) [55]. Indeed, aortic calcium content was reduced towards control values when the drug was administered after 4 weeks of WVK treatment. The treatment also reduced PP and normalized the collagen/elastin ratio. We recently found that amlodipine also induces mineral loss and improves hemodynamic parameters [99]. Interestingly, CCB are known to inhibit the production [103] and some of the effects of ET [104,105]. Endothelin production is suppressed by NO and we recently observed that sinitrotil, a NO donor with large artery selectivity [83], also induced mineral loss (Gilbert and Moreau 2004, unpublished observation). Thus, ET may be involved in the maintenance of MEC and a reduction of its production or effect could promote mineral loss. In contrast, HCTZ and irbesaran t, although they prevented calcification, did not regress elastocalcinosi s [55]. Moreover, stopping WVK after an initial 4 weeks of treatment does not allow spontaneous regression (Essalihi and Moreau 2004, unpublished observation), suggesting that MGP is mainly an initiation inhibitor.

The mechanisms by which ETRA and other drugs regress aortic elastocalcinosi s are currently under investigation. In the WVK model, OPN and CA II are expressed exclusively at sites of calcification [106,107]. However, their expression was not modulated by an ETRA, suggesting that they may not contribute to the regression process. Macrophages and osteoclasts were not present locally, suggesting that in contrast to the introduction of a foreign body (GFAV), vascular mineral loss is most probably mediated by phenotypically modified VSMC. Indeed, these cells do not express smooth muscle α-actin, but express OPN [106]. We recently found that CA IV, a membrane-bound isoform present in VSMC, was overexpressed and hyperactive only in animals treated with darusentan, suggesting that this isoenzyme may contribute to the pharmacological induction of mineral loss [107].

6. Conclusion and perspectives

The majority of old age mortality seems related to cardiovascular failure [4]. Arteriosclerosis, characterized by vascular remodeling and stiffening, appears to contribute to this process by producing a “natural” elevation of SBP and PP. Isolated systolic hypertension has dramatic cardiovascular consequences and appropriate management is now strongly advised. In the near future, we may see a refinement of the predictive value of other arteriosclerosis-related consequences, such as PP and PWV elevation. Unfortunately, current therapies were designed to reduce DBP and the control of SBP is inadequate [108]. More appropriate therapy will come from a better understanding of the pathological process of vascular stiffening induced by aging, hypertension or diabetes [46,67]. Thus, drugs that can prevent large artery stiffening or improve vascular elasticity could certainly be of therapeutic value, especially if they are designed to target pathological processes without affecting healthy tissue integrity. We propose that pharmacological modulation of MEC represents a worthy therapeutic goal that deserves further investigation with current drugs and with the development of more specific agents.

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