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MiniReview

Imbalanced Matrix Metalloproteinases in Cardiovascular Complications of End-Stage Kidney Disease: A Potential Pharmacological Target

Bernardo P. Marson[1], Carlos E. Poli de Figueiredo[2] and Jose E. Tanus-Santos[3]

[1]Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, São Paulo, Brazil, [2]Faculty of Medicine/IPB/HSL, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil and [3]Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil

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Abstract: End-stage kidney disease (ESKD) is a major health problem associated with very high morbidity and mortality secondary to cardiovascular complications, especially in ESKD patients on dialysis. Therefore, exploring key mechanisms underlying cardiovascular alterations associated with ESKD may offer reasonable pharmacological targets that may benefit these patients. Imbalanced matrix metalloproteinases (MMP) activities have been implicated in many cardiovascular diseases, and growing evidence now indicates that excessive MMP activities contribute to cardiovascular complications in ESKD patients. However, there is no study on the effects of MMP inhibitors (MMPIs) in such patients. MMPIs may prevent against the vascular and cardiac changes associated with ESKD. In this MiniReview, we aimed at reviewing current evidence supporting the idea that pharmacological inhibition of imbalanced MMP activities in ESKD may decrease the morbidity and mortality associated with cardiovascular complications in ESKD patients. However, MMPs have variable effects during different phases of kidney disease, and therefore optimal timing for MMP inhibition during a disease process may vary significantly and is largely undetermined. While current research shows that MMPs play a role in the pathogenesis of the cardiovascular alterations found in ESKD patients, clinical studies are required to validate the idea of using MMPIs in ESKD.

Chronic kidney disease (CKD) is a major health problem worldwide, and the increasing prevalence of diabetes and hypertension will further enhance the number of patients developing end-stage kidney disease (ESKD) [1]. Regardless of the initial insult, CKD comprises a complex pathophysiology with progressive interstitial fibrosis, glomerulosclerosis, renal and systemic vascular stiffening, and calcification [2]. Tubular epithelial cells become profibrotic, mesenchymal scarring cells, with fibroblast and cytokine activation leading to extracellular matrix (ECM) remodelling [2]. Importantly, a disequilibrium between increased synthesis of ECM components and decreased ECM degradation occurs as a result of imbalanced matrix metalloproteinases (MMP) activities [3], and this promotes vascular, glomerular and tubular alterations associated with CKD [4–7].

In this MiniReview, we aim at showing evidence that pharmacological inhibition of imbalanced MMPs in ESKD may decrease the morbidity and mortality associated with cardiovascular diseases, a major cause of death in ESKD patients [1].

Author for correspondence: Jose E. Tanus-Santos, Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirao Preto, SP, Brazil (fax +55 16 3602 0220, e-mails tanus@fmrp.usp.br; tanussantos@yahoo.com).

ESKD Patients Are at Increased Risk of Developing Cardiovascular Complications

Chronic kidney disease is an independent risk factor of cardiovascular diseases, and cardiovascular diseases promote CKD, thus resulting in a vicious cycle [8, 9]. As detailed in fig. 1, multiple risk factors interact, resulting in extremely high rates of cardiovascular events and mortality associated with a more aggressive natural history in ESKD patients [7, 10, 11]. Importantly, cardiovascular abnormalities increase gradually with progressive decreases in glomerular filtration rate [9, 10]. Risk factors typically related to mortality in general population, such as body mass index and cholesterol levels, are positively associated with improved survival in dialysis patients, highlighting particular mechanisms involved in the vascular disease in ESKD patients [10]. The main issue in these patients is not the number or volume of atherosclerotic plaques but its composition (reduced fibrous component and greater calcification), which makes them more unstable and prone to rupture [10–12].

Two different patterns of vascular injury co-exist in uraemic patients: arteriolosclerosis and atherosclerosis [9–11]. The former is a premature ageing that involves loss of elastic fibres, reduced cushioning function and increased stiffness with

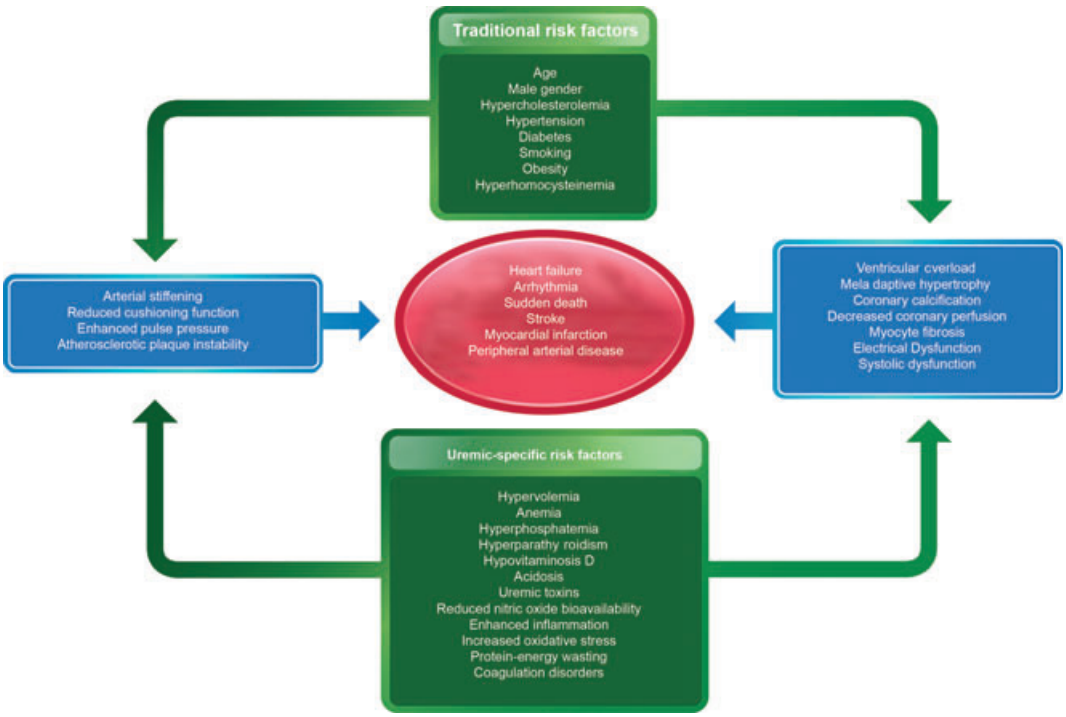

Fig. 1. Multiple risk factors, both traditional and uraemic-specific, interact to promote the vascular and cardiac alterations, which eventually cause clinical outcome in the centre of this figure.

arterial medial calcification and larger pulse pressure. The latter corresponds to intima-media thickening and altered conduit function because of obstructive plaques. Renal failure probably does not induce atherosclerosis, and rather aggravates pre-existent lesions [10], and maladaptive vascular remodelling is the final result of a complex conjunction of classic risk factors and specific ESKD features [13].

Uremic patients with alterations including increased volume overload, left ventricular hypertrophy and diastolic dysfunction [10] are more exposed to ischaemic damage resulting from reduced coronary reserve and ventricular dilation, which worsens microvascular oxygen diffusion [11, 14, 15].

MMPs and Cardiovascular Alterations in ESKD

Matrix metalloproteinases are enzymes involved in tissue remodelling and are usually secreted as pro-MMPs that are eventually activated [16]. They are regulated at multiple levels including gene transcription, interaction with their endogenous inhibitors [the tissue inhibitors of MMP (TIMPs)], and by other factors including oxidative stress [17].

The catalytic domain of MMPs has a zinc-binding site, and the prodomain contains a cysteine in coordination with the zinc in the catalytic domain, which keeps the enzyme in its inactive form. MMPs are activated by propeptide cleavage by proteases, or by detachment of the prodomain after the interaction between the prodomain and the catalytic site is disrupted, thus exposing the catalytic domain [18]. Many compounds may exert such effect, including reactive oxygen species, peroxynitrite, alkylating agents, heavy metals and disulphides [19].

Increased MMP activities enhance the degradation of ECM components, as well as the processing of non-matrix substrates including cytokines, cell adhesion molecules and growth factors [20, 21]. Although other MMPs may be relevant, this review is mainly focused on gelatinases (MMP-2, 72 kDa; MMP-9, 92 kDa) because these enzymes play important roles in the cardiovascular alterations associated with different cardiovascular diseases in different populations [22–27]. They cleave denatured collagen (gelatin), elastin and laminin [21, 24, 28], and abnormal MMP activity is a key feature in cardiovascular remodelling [26, 28, 29].

Atherosclerotic conditions such as metabolic syndrome, obesity and hypertension have been associated with increased MMP activities [30–32]. Both MMP-2 and MMP-9 were shown at increased levels in hypertension and may be involved in both vascular and cardiac remodelling of hypertension [33–35]. Moreover, imbalanced MMP activities may increase aortic stiffness, an independent marker of mortality in ESKD [36], and vascular MMP up-regulation is probably a result of activation of nuclear factor kappa-B pro-inflammatory pathways [21, 37]. Importantly, MMP-9 levels strongly correlated with carotid atherosclerosis burden independently of other factors in early, moderate and advanced CKD [38]. Similarly, the circulating levels of MMP-2 have been strongly linked to intima-media thickness in ESKD patients on haemodialysis [39]. These findings emphasize the role of imbalanced activities of pro-inflammatory proteases in the development of vascular alterations in CKD [21, 39]. In fact, inflammatory markers predict clinical events in ESKD patients and trigger sudden cardiac death by inducing plaque instability

or by directly affecting the myocardium electrical conduction system [14, 40].

The vascular alterations in ESKD include vascular thickening, smaller elastic fibres, calcification, vasomotor dysfunction and hypertrophy and apoptosis of smooth muscle cells, which may be related to MMP-2 activation because of mineral imbalance typically found in ESKD [7, 26, 41–43] (fig. 2). Supporting this idea, MMP-2 and MMP-9, two potent elastases, were up-regulated in arteries from diabetic ESKD patients, and these alterations correlated with vascular stiffness [25]. Interestingly, progressive CKD increased the circulating MMP-2 levels in association with increasing MMP-2, MMP-9 and TIMP-1 expression in the aorta [26]. Fragmented elastic lamellae may predispose to calcification, especially in the presence of uraemic factors such as hyperphosphataemia. Indeed, calcified deposits that develop in the media are associated with regions of elastin disorganization [25, 43]. In line with these results, uraemic rats showed increased blood pressure, arterial medial calcification, elastin degradation and increased vascular MMP-2 expression [42]. Moreover, MMP-2 over-expression and vascular smooth muscle cell phenotype change found in elastocalcinosis were shown as early events in CKD and preceded cell loss and arterial medial calcification [43].

MMP Levels in ESKD Patients and the Effect of Dialysis

A number of studies have been carried out to examine MMP/TIMP levels in ESKD patients on dialysis, and table 1 summarizes their findings. However, conflicting results have been reported, and MMP-9 levels were not different in three studies [39, 44, 45], higher in two studies [46, 47] and lower in one study [48], compared with healthy controls. These differences among studies may be explained by differences in ethnicity, age, type of dialysis membrane, causal diseases or other clinical conditions [49].

Matrix metalloproteinase-9 was positively correlated with severe hypertension in blacks, and significantly elevated when compared with healthy controls, although MMP-9 levels were similar to those found in hypertensive patients with normal renal function [47].

Most studies showed increased MMP-2 levels in ESKD compared with controls [39, 44, 46, 47] and maybe associated

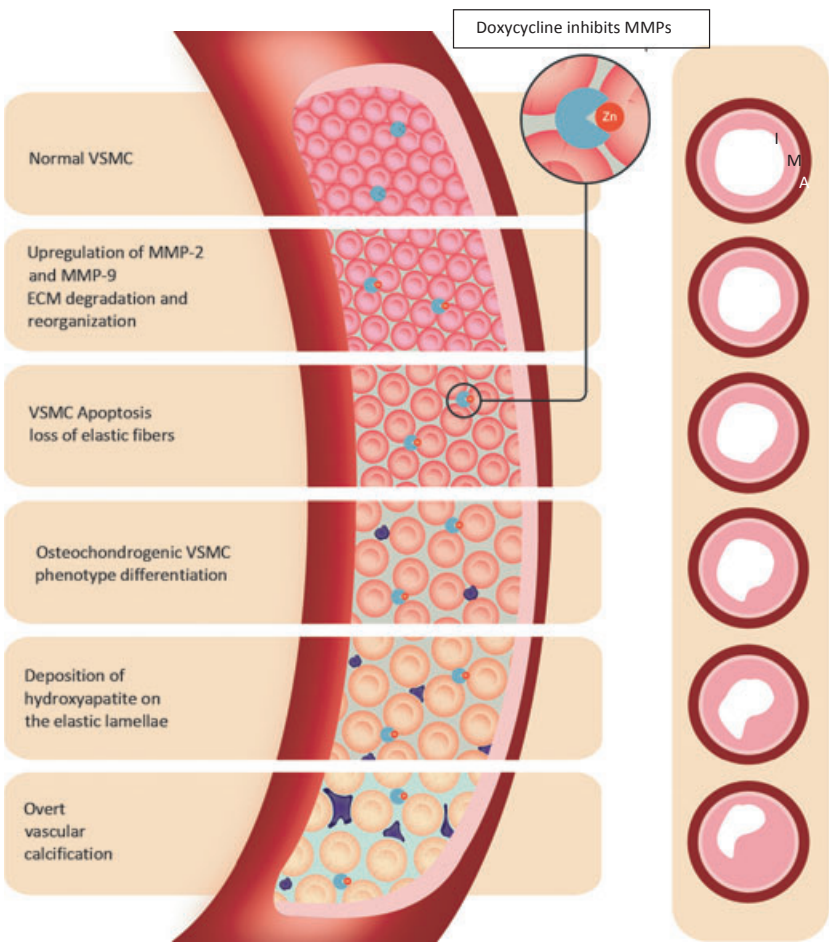

Fig. 2 Vascular alterations begin very early in chronic kidney disease (CKD) in a continuum in which MMPs are up-regulated, thus affecting the relation between vascular smooth muscle cells (VSMC) and the extracellular matrix (ECM). With time, arterial medial calcification becomes clinically detectable. Note the dark calcium deposits scattered in the later stages. Doxycycline may prevent these alterations and protect against the vascular structural modifications associated with CKD. Intima (I), media (M) and adventitia (A).

Table 1.
Studies to compare matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitors of metalloproteinase (TIMP)-1 and TIMP-2 levels in end-stage kidney disease (ESKD) patients on haemodialysis (HD) with those found in healthy controls (upper signals), or to compare the levels of these markers after HD with those before a HD session (lower signals).

References	Pat/Con	Post-HD measurements	MMP-2	MMP-9	TIMP-1	TIMP-2
[48]	18/15	Yes	=	↓	↓	↓
			↓↓	↔	↓↓	↔
[46]	19/30	Yes	↑	↑	↑	↑
			↓↓	↓↓	↓↓	↓↓
[44]	23/18	Yes	↑	=	NA	NA
			↔	↔		
[47]	30/18	No	↑	↑	NA	NA
[45]	21/20	Yes	NA	=	NA	NA
				↔		
[39]	40/20	No	↑	=	↑	↑
[50]	17/10	No	=	NA	NA	NA
[49]	40/–	Yes	NA	NA	NA	NA
			↓↓	↓↓	↔	↑↑

Upper signals: ↑, higher levels than healthy controls; =, similar levels compared with healthy controls; ↓, lower levels than healthy controls. Lower signals: ↑↑, haemodialysis increases the levels; ↔, haemodialysis has no effects on the levels; ↓↓, haemodialysis decreases the levels. Pat/Con, number of ESKD patients/number of healthy controls; HD, haemodialysis; NA, not available.

with organ damage but not with hypertension [47]. However, two studies showed similar MMP-2 levels [48, 50] in patients compared with healthy controls (table 1). Moreover, TIMP-1 and -2 comparisons between ESKD and controls have also shown conflicting results [46, 48]. As detailed in table 1, a single session of haemodialysis apparently does not affect or may decrease both MMP-2 and MMP-9 levels, as well as TIMP-1 and TIMP-2 levels [44–46, 48, 49]. Collectively, these studies suggest increased MMP levels in ESKD patients, and dialysis may decrease, particularly MMP-2 levels. The potential reason for this decrease is not known, because MMP-2 is a large molecule (>60–70 kDa for the different forms), and this feature should preclude its dialysability. Measuring systemic MMP levels may not reflect precisely MMP activities at tissue level. Furthermore, care must be taken to avoid artificial results caused by lack of pre-analytical care, because there are remarkable differences between serum and plasma samples [51, 52], and the use of appropriate samples is of major relevance [33].

It is possible that genetic factors involving MMP-2 and MMP-9 may interact with ESKD to modulate MMP levels. Indeed, it has been suggested that genetic factors may interact with environmental and disease factors and affect MMP levels in some groups of patients [23, 53–55]. Further research is required to understand how MMP polymorphisms may modify the effects of long-term dialysis on plasma MMPs. The study of genetic MMP polymorphisms may help to identify patients with worse prognosis and that could respond to pharmacological interventions [56], possibly including MMP inhibitors (MMPIs).

Uncertain Effects of MMP Inhibitors in Non-dialytic CKD

Matrix metalloproteinase inhibitors have been studied in diabetic and hypertensive nephropathy, retardation of CKD, transplantation and attenuation of cystic diseases [2, 4, 26, 57–62]. Doxycycline, a non-selective MMPI that is easily available, improved elastic fibre integrity and reduced arterial stiffness in Marfan syndrome [63]. While earlier clinical trials of MMPIs in chronic cardiovascular disorders failed to show clear effects [64], doxycycline reduced proteinuria both in short open trials with patients and in animal models of diabetic nephropathy [60–62]. In line with these findings, chronic administration of MMPIs delayed CKD progression in hypertensive and diabetic nephropathy [59]. However, these findings were not reproduced in other forms of kidney diseases [65]. Moreover, the test of candesartan in the adriamycin kidney injury model resulted in regression of established glomerulosclerosis associated with increased glomerular MMP-2 activity, and such effect was attenuated by pre-treatment with doxycycline or by targeted deletion of MMP-2 gene [65], thus suggesting a protective effect of MMP-2 activity. These findings suggest that MMPs may not be deleterious in all kidney diseases, although most of the studies suggest that this may be true.

A selective MMPI for gelatinases was tested in a kidney transplantation model of chronic allograft nephropathy [58]. Adding complexity to the understanding of how MMPs affect kidney diseases, early MMP inhibition resulted in significantly lower-grade chronic allograft nephropathy in that study, whereas late inhibition induced higher proteinuria and more severe chronic allograft nephropathy [58], thus suggesting a time-dependent effect for the MMPI. Another important factor that should be taken into consideration to explain discrepancies between studies is that differences in the MMPI dosage scheme or species may affect drug responses [22], and further studies are required to better define useful doses of MMPIs, especially in human beings.

Increased MMP-2 and diminished MMP-9 activities were shown in patients with different stages of CKD [66]. There is now evidence for imbalanced MMP/TIMP activity and increased deposition of ECM in CKD at intermediate stages of disease [2, 5]. However, MMPs may have different and maybe

apparently contrasting effects during different disease phases [2]. Indeed, MMPs may play dual roles in a number of primary nephropathies, with an acute, harmful effect contributing to damage in the early phases and a protective, compensatory effect against deleterious ECM deposition in later-phases [4, 58]. Therefore, optimal timing for MMPs inhibition during a disease process may vary significantly and is largely undetermined [4]. Moreover, MMP expression varies according to localization, and therefore MMPI may have different effects along the entire nephron [5, 58]. A major challenge for future therapeutic interventions using MMPIs will certainly be how to achieve therapeutic effects without causing any harm. More detailed studies on the involvement of MMPs and TIMPs in CKD will help to improve our understanding of how MMPIs may be helpful.

It should be noted that a number of drugs affecting the cardiovascular system may down-regulate MMP activities. These drugs include diuretics [31], calcium channel blockers [67–70], angiotensin converting enzyme inhibitors [71] and statins [72], among others. However, it remains to be determined how these drugs may affect MMP activities in ESKD.

MMP Inhibition May Protect ESKD Patients against Cardiovascular Complications

Typical features of ageing and cardiovascular diseases in the general population are utterly accelerated in ESKD patients, leading to far amplified occurrence of CV events in dialysis patients [8, 73]. Despite receiving the best available therapy, a considerable proportion of ESKD patients die from cardiovascular issues [10]. Therefore, exploring key mechanisms underlying specific renal atherosclerotic events may offer reasonable pharmacological targets that may benefit these patients [74]. While there is evidence that imbalanced MMP activities contribute to cardiovascular diseases in ESKD patients, there is no study on the effects of MMPIs in such patients.

Imbalanced MMP activities in CKD may promote cardiovascular disease, and therefore it is possible that MMPIs may exert beneficial effects by postponing cardiac remodelling and vascular events more clearly in patients than in individuals with normal renal function. In line with this suggestion, doxycycline attenuated aortic calcification in a CKD animal model [26] and prevented elastin degradation caused by early MMP-9 activation [75], thus suggesting that inhibiting MMPs is a potential therapeutic strategy to protect against the vascular alterations commonly found in patients with ESKD. However, the use of MMPIs in the non-dialytic CKD may be risky [4]. The risk of accelerated CKD progression after MMP inhibition in non-dialytic patients may preclude them from using MMPIs to improve cardiovascular health. This is because MMPs apparently preserve residual kidney function in such patients [65]. Moreover, patients with moderate CKD have less severe cardiovascular diseases, and the possible benefits of MMPIs would probably be less evident in these patients [10].

Hypervolaemia is an important issue in patients with anuria, and it is aggravated by the presence of arteriovenous fistula because the fistula increases cardiac output by approximately 20%. Increased MMP activities preceded left ventricular remodelling induced by experimental chronic volume overload [76], and MMP inhibition attenuated this effect and prevented left ventricular dilation and hypertrophy, thus preserving ventricular function [76]. Whether this protective effect associated with MMP inhibition is found in ESKD patients on haemodialysis is not known.

In conclusion, mounting evidence indicates that cardiovascular complications deserve pharmacological intervention in ESKD patients. Taking into consideration that abnormal cholesterol levels are not the major issue in the pathogenesis of cardiovascular diseases in these patients, the development of new tangible targets has been encouraged [74]. Current data suggest that excessive degradation of the ECM is a critical step in the pathogenesis of the vascular alterations found in ESKD patients, and imbalanced MMP activities contribute to these alterations. While there is now reasonable evidence supporting the use of MMPIs to prevent these alterations, clinical studies are required to validate this suggestion. Genetic research may add important evidence because genetic polymorphisms may help to identify patients with worse prognosis that may have better responses to MMPIs.

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