Review

Matrix metalloproteinases: Targets for doxycycline to prevent the vascular alterations of hypertension

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\textbf{A R T I C L E   I N F O}

Keywords:
Hypertension
Doxycycline
Matrix metalloproteinases
Vascular remodeling

\textbf{A B S T R A C T}

Hypertension is associated with well known structural and functional alterations in both resistance and conduit arteries, which may be the result from long-lasting high blood pressure and may also be the cause of maintained hypertension and its complications. Therefore, in addition to lowering blood pressure, therapeutic strategies targeting the structural and functional modifications found in hypertensive patients may prevent the cardiovascular events and decrease the death rates associated with hypertension. Mounting evidence indicates that many vascular alterations associated with sustained hypertension are due to imbalanced matrix metalloproteinases (MMPs), a family of zinc-endopeptidases that degrade not only proteins of extracellular matrix (ECM) but several other substrates. Recent observations showed that abnormal MMP activity is a feature of the pathogenesis of hypertension and other diseases, thus justifying the development of drugs aiming at MMP downregulation. This review focuses on the extracellular actions of MMPs in hypertension-induced chronic vascular alterations. We then discuss the effects of MMP inhibitors, especially doxycycline, on the vascular changes associated with hypertension. There is now strong evidence that MMP inhibition with doxycycline (and maybe other MMP inhibitors) may attenuate the functional and structural alterations associated with hypertension, including increases in arterial stiffness. These beneficial effects may be, at least in part, independent of their antihypertensive effects.

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1. Introduction

Hypertension is one of the main public health problems in the world and is associated with increased morbidity and mortality. According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, more than 1 billion of people have hypertension and approximately 7.1 million of deaths per year are a consequence of it. This disease is considered a strong risk factor for the development of other cardiovascular diseases with many deaths resulting from heart failure and stroke [1].

Hypertension is associated with well known structural and functional alterations in both resistance and conduit arteries, which may result from long-lasting high blood pressure and may also be the cause of maintained hypertension and its complications [2–6]. Therefore, in addition to lowering blood pressure, therapeutic
strategies targeting the structural and functional modifications found in hypertensive patients may prevent the cardiovascular events and decrease the death rates associated with hypertension.

Mounting evidence indicates that many vascular alterations associated with sustained hypertension are due to imbalanced matrix metalloproteinases (MMPs) [5,7,8], a family of zinc-endopeptidases, first discovered for their ability to degrade several proteins of extracellular matrix (ECM) [9,10]. These recent observations showing that abnormal MMP activity is a key feature of the pathogenesis of hypertension (and other diseases) [11–13] have justified the development of drugs aiming at MMPs down-regulation. Although other important mechanisms are clearly involved in the maladaptive vascular alterations of hypertension, such as activated renin–angiotensin–aldosterone pathway and increased oxidative stress, it is now becoming evident that MMPs act as important downstream mediators, thus promoting vascular remodeling and functional alterations [14–18].

In this review we will focus on the extracellular actions of MMPs in hypertension-induced chronic vascular alterations. We then discuss the effects of MMP inhibitors, especially doxycycline, on the vascular changes associated with hypertension.

2. MMPs and their regulation

MMPs are a group of zinc-dependent endopeptidases that degrade a variety of components of extra-cellular matrix (ECM). Their activities in the vascular tissues contribute to vascular remodeling associated with cardiovascular diseases. While other MMPs may be relevant, we will focus on MMP-2 and MMP-9 (gelatinases A and B, respectively) because these two enzymes play key roles in the pathogenic mechanisms underlying the vascular alterations associated with hypertension [5,7,8]. Structurally, the catalytic domains of gelatinases differ from those found in other MMPs because they contain three fibronectin type II-like domains, which confer specificity to cleave denatured collagen (gelatin) and collagen type IV. They also have a signaling peptide at the N-terminus that is responsible for their transport out of the cells, a hinge region, and a haemopexin-like C terminal domain. Several excellent articles provide detailed information on the structure and relevant biochemistry of MMPs [10,19–22].

All MMPs are initially synthesized as inactive ‘pro-MMPs’ that eventually become fully active. MMPs are regulated at multiple levels including gene transcription, post-translational activation, and by interaction with their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs) [22]. Although four known members of the TIMP family (TIMP-1, –2, –3, and –4) have already been described in the vascular wall, TIMP-1 and –2 are the best characterized in hypertensive animals and humans [23,24]. Activation of pro-MMPs involves the disruption of a coordination between a thiol group in their propeptide and a Zn2+ present in the active site of the catalytic domain [25]. Interestingly, MMPs may activate other MMPs and act synergistically. For example, proteolysis of the propeptide domain of MMP-2 by membrane type-MMP-1 (MT-MMP-1; MMP-14) occurs extracellularly and leads to complete removal of the propeptide region, thus resulting in a smaller, 64 kDa, active MMP-2 form [21,22]. Moreover, recent studies showed that the 72 kDazymogen form of MMP-2 is also activated by peroxynitrite and glutathione, thus leading to conformational changes in the enzyme by disrupting the coordination between the cysteine thiol group and Zn2+ [26,27]. This activation results in the active MMP-2, as reported in conditions associated with increased oxidative stress [28]. Such a pathogenic mechanism involving oxidative stress-induced MMP-2 activation has been implicated in two kidney-one clip (2K-1C) hypertension [17]. Consistent with these findings, antioxidant drugs attenuated vascular MMP-2 activity and protected against the vascular dysfunction and remodeling found in 2K-1C hypertension [17].

3. Involvement of MMP-2 and -9 in the vascular remodeling associated with hypertension

Although vascular remodeling in hypertension may initially be adaptive so as to normalize vascular wall stress in response to increased intravascular pressure, it may become maladaptive and lead to vascular dysfunction [29]. Significant differences exist when the vascular remodeling of the resistance, small arteries is compared with that found in large, conduit arteries. While hypertension leads to increased media thickness, reduced internal and external diameters, and increased media thickness/lumen (M/L) ratio (inward eutrophic remodeling) in the small vessels, large vessel remodeling consists of wall hypertrophy characterized by increased media thickness without changes in arterial lumen [29,30]. Both types of vascular remodeling involve degradation and reorganization of the ECM scaffold, and hypertrophy or hyperplasia of vascular smooth muscle cells (VSMCs), thus contributing to thickened vessel wall and vascular stiffness [2–5]. These vascular structural changes maintain high blood pressure and contribute to the development of complications associated with hypertension.

Increased circulating and tissue MMP-2 and MMP-9 levels have been reported in clinical hypertension and in animal models [23,31–37]. Because MMPs can degrade a variety of components of ECM, it is now becoming clear that imbalanced MMPs activities play an important role in hypertensive vascular remodeling. However, a recent study suggested that MMP-9 exerts a beneficial role early on in angiotensin-II-induced hypertension because increased vascular stiffness and increased pulse pressure were found in hypertensive MMP-9 knock-out mice [31]. Conversely, sustained hypertension upregulates MMPs and leads to excessive ECM degradation, VSMCs proliferation and migration, and vascular hypertrophy [2,38]. Therefore, it is possible that temporal changes may exist in MMPs levels during the development of hypertension, which may be involved in the initial beneficial adaptive vascular remodeling that is followed by hypertrophic/eutrophic maladaptive remodeling.

MMPs activities may disrupt the interactions of ECM components with VSMCs, thus promoting new cells-matrix interactions, and VSMCs change from an inactive phenotype to cells that migrate and proliferate [39–43] (Fig. 1). These cellular changes lead to increased synthesis of ECM components and promote vessel wall thickening and increased vascular stiffness [40]. Integrin-mediated intracellular mechanisms are involved in this maladaptive structural vascular alterations [24,44,45], with upregulated MMP-2 and MMP-9 being consistently implicated, both in early and in late hypertrophic vascular remodeling associated with hypertension in patients and in animal models [23,31,32,34–36]. In addition, we have recently found evidence suggesting that increased vascular MMP-2 levels may cause vascular functional alterations [36]. Indeed, we found increased MMP-2 levels associated with impaired endothelial-dependent vasorelaxation, arterial wall hypertrophy, and excessive collagen and elastin deposition in the aortas from 2K-1C hypertensive rats [36]. Since these recent findings implicate MMPs in hypertension-induced vascular remodeling and dysfunction, MMPs inhibition became an attractive therapeutic aim in the treatment and/or prevention of such disease and its complications. While there are several effective antihypertensive drugs that have been widely used in the therapy of hypertension and its cardiovascular complications, controlling MMPs activities with MMPs inhibitors could help to prevent the clinical consequences of hypertension.
3.1. Doxycycline protects against the structural vascular alterations associated with hypertension

Doxycycline acts at low doses to inhibit MMPs, both in clinical and in experimental conditions. In fact, this drug is a surprisingly potent MMP inhibitor, and it has been shown to inhibit MMP activities at plasma concentrations well below those required to produce antimicrobial effects [46], as demonstrated in several disease models [47–51]. While this tetracycline inhibits MMP activities by binding directly to MMP’s zinc ion domains, thus altering their interactions with substrates [52,53], it can also downregulate the transcription of MMPs mRNA [54].

Recent studies by our group showed that doxycycline completely prevented the maladaptive vascular alterations associated with increased MMP-2 levels in experimental 2K-1C hypertension [36,55]. Hypertensive rats were treated with doxycycline (30 mg/kg/day by gavage) for 8 weeks after hypertension had been established. Lower increases in systolic blood pressure (SBP) were seen in doxycycline-treated 2K-1C rats when compared with 2K-1C controls treated with vehicle, thus indicating that treatment with doxycycline attenuated the increases in SBP, at least in part. Moreover, doxycycline blunted the increases in aortic MMP-2 activity found in hypertensive rats, and this effect was associated with prevention of vascular hypertrophic remodeling (Fig. 1). Interestingly, while doxycycline attenuated 2K-1C-induced increases in SBP by approximately 40%, this tetracycline completely prevented hypertension-induced increases in aortic media thickness, media/lumen ratio and cross sectional area, as well as the increases in vascular collagen and elastin contents [36]. The fact that doxycycline only partially attenuated 2K-1C hypertension and completely prevented the structural alterations usually found in this model of hypertension suggests that inhibiting MMPs activities blunts the development of structural vascular changes associated with hypertension, independently of the antihypertensive effects produced by this drug. However, it remains to be tested whether MMP inhibition may prevent the cardiovascular alterations found in clinical hypertension.

While previous studies are consistent with the idea that enhanced MMP-2 activity is critically involved in hypertensive maladaptive vascular remodeling of large arteries [36,55], another study suggested that significant differences may exist between large and small arteries [34]. Interestingly, while progressive increases in MMP-2 levels and activity were associated with hypertrophic remodeling of large arteries from rats with N-nitro-l-arginine methyl ester (L-NAME)-induced hypertension, only transient increases in MMP-2 activity were found in parallel with vascular remodeling in small (mesenteric) arteries [34]. Notwithstanding the fact that doxycycline significantly inhibited MMP-2 upregulation and MMP-2 activity in both small and large arteries, this drug prevented aortic hypertrophic remodeling without exerting significant effects on mesenteric eutrophic remodeling [34], thus suggesting that doxycycline may not prevent small vessels hypertensive remodeling.

In addition to MMP-2 inhibition, the protective effects exerted by doxycycline against the vascular changes associated with hypertension may be a result of inhibition of other MMPs. In fact, treatment with doxycycline was associated with attenuation of 2K-1C hypertension-induced increases in MMP-2, MMP-9, and MMP-14 levels, whereas no significant alterations in aortic TIMP-1, -2, -3, and -4 levels were found in this study [55], although increasing TIMP/MMP ratio could downregulate MMP activity. While MMP-14 is important for MMP-2 activation, MMP-14 activity has also been implicated in cell migration and invasion [56], thus possibly contributing to hypertrophic remodeling. In that study, we found enhanced MMP-9 levels mainly in the aortic intima layer from 2K-1C rats, whereas MMP-2 and -14 were more clearly
found in the aortic middle layer, in close interaction with VSMCs (Fig. 2) [55]. These observations suggest that these MMPs may contribute to both intimal thickening and medial VSMCs hypertrophy. Treatment with doxycycline completely prevented these structural vascular alterations in hypertensive rats (Fig. 2) [55]. In line with these findings, comparatively lower vascular MMP-9 than MMP-2 expression levels were also reported in DOCA-salt hypertensive rats [25], and MMP-9 appears to have a much more restricted tissue distribution, being mainly localized in the intima layer [56].

Inhibition of MMP-mediated vascular changes in 2K-1C hypertension by doxycycline is dose-dependent [57]. Given the importance of using subantimicrobial doses of doxycycline, the effects of three different doses of doxycycline (3, 10, and 30 mg/kg) on the vascular changes associated with 2K-1C hypertension were compared. Treatment with doxycycline at 30 mg/kg/day, but not with 3 or 10 mg/kg/day, decreased MMPs levels and gelatinolytic activity, and blunted the vascular alterations found in 2K-1C hypertensive rats [57]. These findings may be especially important when considering the possible side effects associated with doxycycline. While there is concern about the emergence of doxycycline-resistant bacteria, these findings suggest that beneficial effects may be lost if this drug is used at low doses [57]. However, the definition of this narrow dose range requires additional studies, and significant differences may exist when animal models are compared with clinical diseases. For example, doxycycline at doses escalating from 20 up to 100 mg per day presumably inhibited smooth muscle cell proliferation in association with a reduction in urinary MMP-2 and MMP-9 in a fatal lung disease, lymphangioleiomyomatosis, and eliminated the need for lung transplant [58]. Therefore, further studies are required to determine the doses that should be used in different conditions.

3.2. Doxycycline protects against the functional vascular alterations associated with hypertension

In addition to the protection against the structural modifications associated with hypertension, as discussed above, there is now growing evidence that MMP inhibition with doxycycline also protects against functional vascular alterations associated with hypertension. Supporting this suggestion, there is mounting evidence that enhanced MMP-2 activity impairs vasodilation and promotes vasoconstriction, thereby contributing to increased vascular resistance in hypertension. Indeed, MMP-2 may promote the vasoconstrictor effects of big endothelin-1 (ET-1) by cleaving this peptide to a potent vasoconstrictor, ET-1-32 [59]. MMP-2 also cleaves the vasodilatory CGRP (calcitonin gene-related peptide) [60] and adrenomedullin [61], thereby promoting vasoconstriction. Therefore, it is possible that MMP inhibition with doxycycline restores vasodilation by inhibiting MMPs. In line with this suggestion, we found that doxycycline reversed the impaired, Ach-induced, endothelium-dependent responses found in 2K-1C rats [36]. Moreover, doxycycline produced similar improvements of endocardial endothelial response to acetylcholine [62]. While the improved responses to acetylcholine may be due to MMP-2 downregulation, MMP-9 inhibition by doxycycline may also contribute to the improved endothelium-dependent responses. This is because increased vasodilatory responses to acetylcholine were reported in MMP-9 knockout mice [63]. These observations highlight the notion that MMPs degrade a variety of non-ECM substrates [64], including β2-adrenergic receptors [65], which cause vasodilation. Curiously, lead exposure has been associated with MMPs upregulation [66] and hypertension, and doxycycline prevented lead-induced vascular MMPs activation and hypertension [67].

4. MMP inhibition may prevent arterial stiffness

An important consequence of long-term increases in MMP activity and hypertension-induced structural changes in large arteries is increased arterial stiffness. This alteration reduces vessel compliance and distensibility, thus resulting in amplification of the arterial pulse pressure and pulse wave velocity (PWV), a marker of arterial stiffness [68]. MMP-mediated excessive degradation of arterial elastin [69,70] and re-synthesis of collagen (the stiff
protein) [40] leads to increased arterial stiffness and fibrosis. Moreover, part of elastin that is re-synthesized after its degradation is less efficient and this change underlies the loss of arterial wall resilience [70]. Interestingly, products of elastic fiber degradation affect VSMC migration and proliferation [70]. MMPs also mediate more deposition of proteoglycans and fibronectin in the vasculature during hypertension, thus contributing to the increased vessel stiffness [71,72]. Indeed, clinical studies with hypertensive patients showed that increased plasma MMP-9 activity correlates positively with PWV in patients with isolated systolic hypertension, thus suggesting an MMP-9 participation in the development of this disease [32].

In line with these ideas, some studies suggest that aortic medial calcification may contribute to the development of aortic stiffness in hypertension [7], and MMP-9 may be involved in this process [69]. Using an animal model of arterial elastocinosis, it was shown that early arterial MMP-9 activation could induce both gradual elastin degradation and activation of transforming growth factor β (TGF-β) [69]. These alterations were associated with progressive increases in aortic medial calcium deposition and also long-lasting elastin fragmentation, thus contributing to increased aortic PWV and to the development of hypertension [69]. Importantly, treatment with doxycycline (30 mg/kg/day) prevented aortic calcification and increases in aortic PWV, thus suggesting MMP-9 is a key player in aortic medial calcification and increased stiffness of hypertension [69].

Taken together, these recent studies strongly suggest that MMPs inhibition with doxycycline may attenuate functional alterations associated with hypertension, including the increases in arterial stiffness.

5. Concluding remarks

It is now becoming clear that MMPs play relevant roles in the pathogenic mechanisms involved in the vascular alterations of hypertension, and the recent findings discussed in this review are consistent with the idea that inhibiting MMPs with doxycycline may offer improved cardiovascular protection to hypertensive patients. It is also possible that doxycycline may prevent the deleterious effects associated with other conditions that upregulate MMPs and are linked to cardiovascular diseases, such as periodontal disease [73,74]. In line with this conclusion, it is possible that some anti-hypertensive drugs that reduce arterial blood pressure and downregulate MMPs [15,17,75,76] may provide additional cardiovascular protection to that associated with their direct anti-hypertensive effects. However, the use of subantimicrobial doses of doxycycline combined with anti-hypertensive drugs may offer greater advantages in the pharmacological therapy of hypertensive patients. These suggestions warrant clinical studies to validate them. It should be noted that outstanding clinical trials have clearly shown that treatment with subantimicrobial doses of doxycycline may exert anti-inflammatory effects and protect against relevant cardiovascular events [77,78].

Acknowledgements

This study was funded by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP-Brazil) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil).

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