ABSTRACT

Abdominal aortic aneurysm (AAA) represents a complex pathophysiological process of weakening and dilatation of the aortic wall associated with atherosclerosis, chronic inflammatory response and hemodynamic alterations. Degradation of the extracellular matrix by the matrix metalloproteinases (MMPs) and an imbalance between MMPs and their tissue inhibitors (TIMPs), have fundamental roles in the development of AAA. However, the exact pathogenetic mechanisms remain incompletely elucidated. In addition to the previous results already published “Interference of doxycycline pretreatment in a model of abdominal aortic aneurysms”, in this commentary we have complementary results. Here we have included new findings of the TIMPs 1 and 2 expressions in animals submitted to AAA surgical induction associated with doxycycline pretreatment. In this study, we used a new experimental model, developed in our laboratory, to induce AAA by combining two potential causes of MMP secretion: inflammation and turbulent blood flow. Male Wistar rats were divided into Control (C), Control+Doxycycline (C+D), Aneurysms (A) and Aneurysms+Doxycycline (A+D) groups. The rats were euthanized at 3, 7 or 15 days post-surgery (dps). The administrated doxycycline started 48 hours before the surgical induction of AAA until the end of the experiment. After 1, 3, 7, and 15 dps, the animals were euthanized under anesthesia and the vessels were collected to measurement of TIMPs 1 and 2 by western blot. Our results demonstrate an increased expression of TIMPs 1 and 2 in aneurysm group (A) probably in an attempt to counteract the increased activity of MMPs 2 and 9. In aneurysms groups submitted to doxycycline pretreatment (A+D) showed the regulation of expression of TIMP 1 and 2, remaining close to baseline levels from the third day, similar to expression found the control groups (C and C+D). This study suggests that the pretreatment with doxycycline balances the TIMPs 1 and 2 expressions with a protective effect on the progression of abdominal aortic aneurysms in experimental model.

Abdominal aortic aneurysm (AAA) characterized by dilation of the abdominal aorta is one of the top 10 causes of death among older men. Although the cause of AAA remains unknown in the majority of cases, several key regulators of AAA pathogenesis are known. AAA is known to develop mainly by the increased diameter of aorta through metalloproteinases (MMPs). MMPs have been shown to play a major role in progressive extracellular matrix (ECM) degradation in AAA. The activation of MMPs is tightly regulated under physiologic conditions by the tissue inhibitors of metalloproteinases (TIMPs) which comprise a family of 4 protease inhibitors: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. An imbalance between MMPs and TIMPs may serve to fragility of arterial wall and may contribute to the degenerative process of AAA formation, however, little is known about TIMPs/MMP behavior in aneurysmal formation.

Some studies have demonstrated that the regulated TIMPs suppress medial degradation and aneurysm formation. The
Inhibitors of MMPs that can be used in medical therapies\textsuperscript{12,13}. For this reason, there is great interest in developing synthetic inhibitors of MMPs that can be used in medical therapies\textsuperscript{12,13}. MMP inhibitors (natural and synthetic) have been tested in experimental models of vascular human and rodent studies\textsuperscript{23,24}, one study showed any effects of doxycycline in treatment of AAA patients\textsuperscript{25}. An additional trial is underway in the United States examining the efficacy of a higher dose of doxycycline in a sample of 248 patients\textsuperscript{26}. Based on the present state of knowledge, the authors agree with the hypothesis that inflammation results in AAA growth and elevates MMP-9 and high sensitivity C reactive protein (hs-CRP) levels as markers of this inflammation. Their hypotheses, doxycycline will produce a series of events: 1) therapeutic levels of doxycycline are achieved in the aneurysm; 2) reduction of MMP-9 levels; 3) reduction in hs-CRP; 4) reduction in aneurysm expansion. According to the authors this sequence of events takes time to change the course of aneurysm growth. No effect may be seen at early time points while doxycycline may prove effective in the longer term. Consequently, they designed the primary end point of this trial to be measured after 24 months\textsuperscript{26}.

The doxycycline as a pharmacological strategy for the management of Thoracic aortic aneurysm (TAA) is considered to be of great potential. TAA are rare but potentially devastating condition with etiopathology multifactorial and still not well-defined but can be caused by genetic factors and can be involved in Marfan Syndrome\textsuperscript{27}. Several studies suggest that TAA the chronic inflammatory process and remodeling may be related to changes in balance between MMP/TIMP and this mechanism is similar to AAA\textsuperscript{28}. Doxycycline through its inhibitory effects on MMP-2 and MMP-9, acts on vessels and preserves aortic mechanical properties, integrity of elastic fibers, endothelium-dependent relaxation and SMC (smooth muscle cells) contractile function\textsuperscript{29,30}.

In previous studies performed in our laboratory, was created a new model of AAA induction by combining two potential causes of MMP secretion: acute inflammation and turbulent blood flow\textsuperscript{31}. In isolation, these causes were insufficient to provoke arterial dilatation and aneurysms but when combined, the abundant MMP-2 and MMP-9 secretion and activation promoted elastin degradation, wall remodeling and aneurysm formation in approximately 65\% of the animals. The technique of the new model was efficient, causing the development of extraordinarily dilated aneurysms with the morphology similar to that of human abdominal aneurysms only in 3 days. Data already published shows that initial inflammation is clearly acute and potent and seems to be autoregulated. After the seventh days post-surgery, the aneurysms maintained practically the same diameter, but the wall aneurysm changed completely to show a predominantly progressive remodeling process, characterized by mononuclear inflammatory cells intermixed in a remarkable intercellular matrix showing myofibroblastic proliferation, vascular neoformation, and collagen deposition. In contrast to the MMP-9 behavior, which shows a tendency to diminish after the initial peak, MMP-2 expression shows a progressive augmentation suitable for synergistic action in the wall remodeling process. Therefore, our hypothesis is that potent initial inflammatory stimuli lead to arterial wall weakness and dilation, which is maintained in the second step by the altered regulation of the endothelial cells submitted to turbulent flow in the post-stenotic area.\textsuperscript{31-33} However, the detailed mechanism of chronic inflammation in this experimental model needed to be more elucidated, but is similar to the literature. Moreover, in our previous study already published, was administrated doxycycline 48 hours before surgical induction of AAA. In this study was observed the inhibition of formation and development of AAA, reduction of the inflammatory response and gelatinolytic activity of MMP-2 and MMP-9 in the aortic wall.
wall and, consequently, limited AAA formation in 85% of rats in this AAA model.

In addition to the previous results already published, in this commentary we have complementary results regarding our paper “Interference of doxycycline pretreatment in a model of abdominal aortic aneurysms”32. Here we have included new findings of the TIMPs 1 and 2 expressions in animals submitted to AAA surgical induction associated with doxycycline pretreatment. The surgical procedure was previously described32. The doxycycline administration was started 48 hours before the surgical induction of AAA, following until the end of the experiment [1, 3, 7, and 15 days post-surgery (dps)]. Through the western blot analyses was measurement the expression of TIMPs 1 and 2. The entire protocol of harvesting, measuring the morphometric analysis of aorta and the western blot were performed as previously described31-33.

Here, the results show the overexpression of TIMPs 1 and 2 in aneurysms groups (A), followed the increase of MMP-9 and MMP-2 levels in animals with aneurysm formation32, and demonstrated that the pretreatment with doxycycline [Aneurysms+Doxycycline (A+D)] provide a great down-regulation of TIMPs 1 and 2 expressions (figures 1 and 2).

According to some authors, the overexpression of TIMP prevent the depletion of elastin and the aneurysm rupture in an experimental model of AAA induction, probably by inhibiting destruction of the wall, blocking the MMP-2 and MMP-934. In our results was detected an increase of TIMPs 1 and 2 expression in the aneurysms (A group), probably in an attempt to counteract the increased activity of MMPs 2 and 9. However, their increased weren't enough to inhibit the action of MMPs 2 and 9, because there was an increased of inflammatory cells, elastic fibers destruction and consequently development of AAA32. In the groups treated with doxycycline (A+D) the expressions of TIMP 1 and 2 remained close to baseline levels from the third day, similar to expression found in the control groups (C and C+D). These data indicate an imbalance between MMP-2 and MMP-9 and their inhibitors and additionally the doxycycline was able to regulate the expression of TIMP-1 and TIMP-2 in abdominal aortic aneurysm progression.

Similar to our findings, the study conducted by Kim et al., demonstrated the upregulated expression of TIMPs and MMP-9 in the arterial wall of patients harboring cerebral aneurysms and the aneurysm wall were markedly increased beyond levels in both extracranial arteries35. However, the role of TIMPs in the initiation and progression of aneurysms still unclear. Although there is no evidence that...
TIMPs are involved in transcriptional regulation of MMPs, TIMPs may enhance the gene expression of MMPs by some positive feedback mechanisms. TIMP-1 is also known to be an important modulator in the pathogenesis of AAA and is highly expressed in arterial walls of AAs compared to the control arteries. On the other hand, TIMP-2 gene expression did not correlate with AAA, suggesting that its role is not important in the initiation and progression of AAs. Nevertheless, it is highly possible that the altered balance between MMPs and TIMPs is also one of the major causes leading to the progression and rupture of aneurysms. Generally, when the expression of MMPs is increased, the TIMPs expression is decreased. However, it is still unclear whether the increase in MMPs activity, decreased TIMPs activity or loss of balance between MMPs and TIMPs that are the main biochemical disorders that lead to degeneration of the middle layer; and subsequently the dilation of the aortic wall. Furthermore, some studies demonstrate that doxycycline causes increased expression of TIMPs, but these results were found when the TIMPs expressions were decreased in aneurysmal tissue.

In conclusion we found the overexpression of TIMPs 1 and 2 following the increase of expression of MMPs 2 and 9, probably in an attempt to balance the MMP levels. Therefore, our data demonstrated that doxycycline pretreatment provide a regulation of TIMPs 1 and 2 expressions in this aneurysm model, and suggest that the balance of TIMPs have a protective effect on the progression of abdominal aortic aneurysms. Several studies demonstrated in both rat and mouse models that doxycycline suppresses formation of AAs, by inhibition of selective blockade of elastolytic MMP expression in infiltrating inflammatory cells, although in a few studies, doxycycline administration did not influence AAA progression and aortic rupture in angiotensin II-infused mice. Reasons for these divergences can be because doxycycline was administered to mice with established AAs. Although of science is gradually unraveling the TIMPs role in the development/ suppression of aneurysms, there are a discrepancy in results as well as little knowledge about the role of TIMPs in the development / suppression of AAs, and therefore further studies are needed.

Thus, the inhibitory mechanisms of doxycycline in the pathogenesis of AAA were not only decrease of the gelatinolytic activity but also regulation of the expression of TIMP-1 and TIMP-2 and ameliorating the inflammation. Doxycycline could effectively inhibit the onset and progression of AAA induced by mechanic surgical model, inhibiting of expression and activity of TIMP-1, TIMP-2, MMP-2 and MMP-9 and inflammatory factors. Therefore, doxycycline may have great potential to be used in clinic for treatment of the small aneurysms. Some studies including clinical trials in which patients with small aneurysms allocated to medical treatment with the intention of retarding aneurysm expansion were treated with doxycycline and other antibiotics. These studies showed that there is potentially promising evidence for antibiotics reducing the growth rates of small aneurysms. However, more research is needed to develop strategies to regulate specific MMPs and TIMPs, in a particular disease without affecting other metalloproteinases that are vital for maintaining normal physiological functions.

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References


