Cardiovascular Pathology 24 (2015) 110–120

Contents lists available at ScienceDirect

Cardiovascular Pathology

Original Article

Interference of doxycycline pretreatment in a model of abdominal aortic aneurysms

Karina M. Mata [a], Cristiane Tefé-Silva [a], Elaine M. Floriano [a], Cleverson R. Fernandes [a], Elen Rizzi [b], Raquel F. Gerlach [c], Marc Q. Mazzuca [a], Simone G. Ramos [a,*]

[a] Department of Pathology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil
[b] Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil
[c] Department of Morphology and Physiology, Dental School of Ribeirão Preto, University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 28 October 2014
Accepted 29 October 2014

Keywords:
Abdominal aortic aneurysm
Inflammatory response
Metalloproteinases 2 and 9
Doxycycline pretreatment

ABSTRACT

Background: Abdominal aortic aneurysm (AAA) is characterized by chronic inflammation and degradation of the extracellular matrix, mediated by matrix metalloproteinases (MMPs). Doxycycline has been reported to control the progression of AAA by regulation of MMP. We hypothesized that doxycycline pretreatment in a rat model of AAA would cause reduction in gelatinolytic activity of MMP-2 and -9 and the inflammatory response in the wall of an aneurysm, consequently decreasing the formation and development of AAAs.
Methods: Male Wistar rats were divided into the following four groups: aneurysm (A); control (C); aneurysm +doxycycline (A+D) and control+doxycycline (C+D), with 24 animals per group subdivided into $n=6$ animals at different time points [1, 3, 7, and 15 days postsurgery (dps)]. The (A) and (A+D) groups simultaneously received the injury and extrinsic stenosis of the aortic wall. The (C) and (C+D) groups received sham operation. The treated animals received doxycycline via gavage (30 mg/kg/day) from 48 h before surgery until the end of experiment. At 1, 3, 7, and 15 dps, the animals were euthanized, and the aortas were collected for morphological analyses, immunohistochemistry, and zymography.
Results: The animals from the (A) group developed AAAs. However, the animals treated with doxycycline showed a 85% decrease in AAA development, which was associated with a large reduction in gelatinolytic activity of MMP-2 and -9, and decreased inflammatory response ($P<.05$).
Conclusions: These results suggest that pretreatment with doxycycline before surgery inhibited the activity of MMP-2 and -9, as well as the inflammatory response, and may play an important role in the prevention of the development of AAAs.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Abdominal aortic aneurysms (AAAs) are dynamic vascular lesions that are a major cause of cardiovascular-related morbidity and mortality [1]. Its underlying pathogenesis is complex. A potentially significant contributor to their development and progression is the protease-driven destruction of collagen and elastin fibers in the media and adventitia layers, resulting in a weakening of the aortic wall and a progressive expansion of its diameter [2–7]. This destructive process is likely due in part to a disruption in the balance between proteolytic enzymes and their inhibitors, leading to excessive degradation of the connective tissue that maintains the aortic wall structural integrity. Inflammation can be a major pathological component of aneurysm development, playing a fundamental role in the degradation of the extracellular matrix (ECM) of the aortic wall due to increases in the amount of matrix metalloproteinases (MMPs) secreted by inflammatory cells, particularly the secretion of gelatinases MMP-2 and -9 [7,8]. Therefore, blocking inflammation and inhibiting MMPs are attractive targets for pharmacologic agents aimed at reducing aneurismal expansion and rupture [9].

Tetracyclines, including doxycycline, are a group of broad-spectrum antibiotics that interfere with protein synthesis at the ribosomal level [10] and down-regulate MMP-9 gene transcription [11]. Initially, doxycycline has been used to treat periodontal disease by inhibiting MMPs, and these drugs are now being investigated for their wide range effects [12]. Their properties include the inhibition of inflammation, proteolysis, and angiogenesis [13–15], induction of macrophage apoptosis [16], and the reduction of the levels of the interleukin-1 [17] and reactive oxygen species [18]. Doxycycline may also affect the degree of aortic inflammation, diameter enlargement, and wall fragmentation in AAA disease [19]. Doxycycline has been used to prevent AAA formation in

Abbreviations: AAA, abdominal aortic aneurysm; AA, aortic aneurysm; A, Aneurysm group; A+D, Aneurysm+Doxycycline group; C, Control group; C+D, Control +Doxycycline group; dps, days postsurgery; ECM, extracellular matrix; SMC, smooth muscle cell; TIMPs, tissue inhibitors of metalloproteinases.

Funding: This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (2007/04212-1) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

* Corresponding author at: Department of Pathology, Ribeirão Preto Medical School, University of São Paulo, Av. Bandeirantes, 3900, Ribeirão Preto, SP, Brazil.
E-mail address: sgramos@fmrp.usp.br (S.G. Ramos).

http://dx.doi.org/10.1016/j.carpath.2014.10.004
1054-8807/© 2014 Elsevier Inc. All rights reserved.

animals through the inhibition of MMP expression [20–22], and currently, doxycycline is considered the lead candidate for pharmaceutical stabilization of AAAs [23]. However, whether doxycycline pretreatment inhibits MMP-2, 9 activity, and the inflammatory response and consequently decrease the development and progression of AAA, is still unclear.

We have previously created a novel experimental model of AAA in our laboratory showing that MMP secretion and activation due to acute inflammation and turbulent blood flow causes the development of AAA [8]. When these causative conditions were combined, enormous aneurysms developed due to inflammatory processes, abundant MMP-2 and MMP-9 secretion and activation, and the promotion of elastin degradation. Thus, the aim of this study was to investigate whether pretreatment with doxycycline 48 h before surgical induction of AAA was able to inhibit formation and development of AAA. We also evaluated the MMP-2 and -9 gelatinolytic activity, inflammatory response, and the amount of aortic wall remodeling in this AAA model. We postulated that early administration of doxycycline may reduce the gelatinolytic activity of MMPs and prevent the infiltration of inflammatory cells and may be an important therapeutic target for the treatment of AAA.

2. Materials and methods

2.1. Animals and treatment

The animal protocols were approved by Committee on Animal Research of Ribeirão Preto Medical School of University of São Paulo (Protocol number: 134/2007), and the animals were handled according to the guiding principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Wistar rats (150 g) obtained from the colony at the University of São Paulo were maintained in a 12-h light/dark cycle at 25°C, with free access to rat chow and water. The animals were divided into the following four groups: aneurysm (A); control (C); aneurysm+doxycycline (A+D) and control+doxycycline (C+D). The treated groups received doxycycline via gavage (30 mg/kg/day) starting 48 h before the surgical procedure, until the end of the experiment, whereas the nontreated groups received an appropriate volume of distilled water via gavage (vehicle) [23–25] [26]. Each group contained 24 animals that were divided into four subgroups (6 animal per group), according to the following time intervals: 1, 3, 7, and 15 days postsurgery (dps). All efforts were made to minimize animal suffering.

2.2. Experimental design

The surgical procedure was previously described [8]. Briefly, with the animals under anesthesia, the aorta was constricted using a 0.94-diameter diamond dental bur to place a cotton thread ligature around the aorta. When the diamond dental bur was removed, the vessel lumen was reduced, leading to approximately 80% stenosis. During its withdrawal, the diamond dental bur induced traumatic injury to the outside layer of the aorta in the (A) and (A+D) groups. In the (C) and (C+D) groups, the aorta was only manipulated. Sodium dipirone solution (10-mg/100-g body weight, ip) was administered at the start of surgery, then 6 and 14 h postsurgery for pain relief. Rats were awakened from anesthesia, monitored to ensure adequate recovery, and returned to the animal facility.

2.3. Macroscopy and harvesting and preparing the aortas for histopathological, immunohistochemical, and morphometric analysis

After 1, 3, 7, and 15 dps, the animals were euthanized under anesthesia. The protocol of harvesting and measuring the morphometric analysis of aorta were performed as previously described [8]. Immunohistochemical staining was utilized to identify macrophages (CD68, clone ED-1, 1/100, Serotec, London, UK), B lymphocytes (CD20, M-20, 1/100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), T lymphocytes (CD3, MCA772, clone 1F4, 1/100, AbD Serotec Ltd, London, UK), and mesenchymal proliferation (smooth muscle, clone 1A4, 1/100, Research Diagnostics,, San Francisco, CA, USA), whereas neutrophils and evidence of neovascularization were directly detected in hematoxilin and eosin (H&E) stained sections by visual analysis by an experienced pathologist in noncoincident fields at a magnification of 400×. Quantification was performed from each slide ($n=6$) using ImageJ software (National Institutes of Health, Bethesda, MD, USA) in 10 randomly chosen noncoincident fields at a magnification of 400×. The mean values were calculated, and the values were expressed as cells or vessels/area (mm^2). Quantification of mesenchymal proliferation (α-actin) was based on the optical densities determined using image analysis (Leica QWin software; Leica Microsystems Image Solutions, Cambridge, UK). Thresholds were established for each slide after enhancing the contrast to the point at which the cells were easily identified as brown bands. Eight randomly chosen noncoincident fields were analyzed for each group at a magnification of 400×. The mean values were calculated, and the values are expressed as a percentage (%).

2.4. Measurement of levels of pro and active MMP-2 (72 and 64 kDa) and MMP-9 (92 kDa) in the aortic wall using gelatin zymography

Gelatin zymography was performed as previously described [27,28]. Tissue extracts were subjected to electrophoresis on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) gels copolymerized with gelatin (1%). Gelatinolytic activities were detected as unstained bands against the background of Coomassie-blue-stained gelatin, assayed by densitometry using ImageJ (NIH).

2.5. Fluorimetric assay to determine the gelatinolytic activity in the rat aortas

To determine the total amount of gelatinolytic activity in the aortic homogenates, a method based on the “dequenching” of the substrate by the gelatinases present in the extract was used [29]. The total protein in the aortic homogenates was determined using the Bradford method (Sigma, St Louis, MO, USA). Sixty micrograms of freshly prepared aortic extract was used in each well, and all of the determinations were conducted in duplicate. The gelatinolytic activity was measured using DQ Gelatin as the substrate (E12055, Molecular Probes, OR, USA) at a concentration of 5 μg/ml in a Tris–$CaCl_2$ buffer (50-mM Tris, 10-mM $CaCl_2$). This activity was evaluated using a microplate fluorimeter (at $\lambda_{excitation}$ of 495nm, $\lambda_{emission}$ of 515nm; Gemini EM, Molecular Devices, Sunnyvale, CA, USA) after 120 min of incubation at 37°C because the maximal dequenching of the substrate occurred at this time. A standard curve of gelatinolytic activity was prepared as recommended by the manufacturer of the Gelatinolytic Activity Kit (E12055, Molecular Probes).

2.6. Statistical analysis

Experiments were conducted in aorta from $n=24$ animals per group subdivided into $n=6$ animals at different time points (1, 3, 7, and 15 dps), with “n” value representing the number of rats per group. These data were expressed as the means±S.D. The data were analyzed using GraphPad Prism software (V. 5.01; GraphPad Software, San Diego, CA, USA). The data were first tested for normality and equal variance before performing parametric post-hoc analysis. Comparisons of the values were made using a one-way analysis of variance (ANOVA). When a statistical difference was observed, data were further analyzed using Newman–Keuls' post-hoc corrections for multiple comparisons. A value of $P<.05$ was considered statistically significant.

Download English Version:

https://daneshyari.com/en/article/2898645

Download Persian Version:

https://daneshyari.com/article/2898645

Daneshyari.com