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Role of mast cell chymase and tryptase in the progression of atherosclerosis: study in 44 autopsied cases

Luciana Santos Ramalho, MD [a], Lívia Ferreira Oliveira, MD [a], Camila Lourencini Cavellani, PhD [a], Mara Lúcia da Fonseca Ferraz, PhD [a], Flávia Aparecida de Oliveira, PhD [b], Rosana Rosa Miranda Corrêa, PhD [a,*], Vicente de Paula Antunes Teixeira, PhD [a], Sanívia Aparecida De Lima Pereira, PhD [c]

[a] General Pathology Division, Triângulo Mineiro Federal University (UFTM), Uberaba-MG 38015-050, Brazil
[b] General Pathology Division, Tropical Pathology and Public Health Institute (IPTSP), Goiás Federal University (UFG), Goiânia, GO 74605-050, Brazil
[c] Laboratory of Biopathology and Molecular Biology, University of Uberaba (UNIUBE), Uberaba 38055-500, Brazil

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ABSTRACT

The aim of this study was to describe the role of mast cell chymase and tryptase in the progression of atherosclerosis. Forty-four sections of aortas were obtained from autopsies. We assessed the macroscopic degree of atherosclerosis, microscopic intensity of lipid deposition in the tunica intima, percentage of collagen in the tunica intima, and density of immunostained mast cells. There was no significant difference between the density of mast cell tryptase and chymase concerning ethnicity, sex, cause of death, or degree of atherosclerosis. The density of mast cell chymase was significantly higher in the nonelderly group. The percentage of collagen was significantly higher in elderly patients. There was a positive and significant correlation between the degree of macroscopic atherosclerosis and lipidosis, the density of mast cell chymase and the percentage of collagen, the density of mast cell tryptase and the percentage of collagen, and lipidosis and the density of mast cell tryptase. The degree of macroscopic lesion of atherosclerosis increased proportionally with the increase in the density of mast cell chymase and tryptase and in the intensity of lipid deposition and with the percentage of collagen in the atherosclerotic plaques. Thus, mast cells may play a crucial role in aggravating atherosclerotic lesions.

1. Introduction

Atherosclerosis is a chronic inflammatory disease that is the main cause of cardiovascular morbidity and mortality all over the world [1]. It affects peripheral and central blood vessels with prolonged gradual evolutions of lesions that tend to worsen with time [2]. Atherosclerosis is characterized by the progressive accumulation of cholesterol in the intimal layer of arterial walls of large- and medium-sized arteries, leading to the formation of plaques that can obstruct vascular light [3–5]. Inflammatory cells such as lymphocytes, macrophages, neutrophils, and mast cells are involved in the pathogenesis of atherosclerotic plaque rupture, as they cause the fibrous plaque to weaken because of the enzyme activity of the leukocytes that degrade the extracellular matrix [4].

Proteases produced by mast cells have also been found at sites of plaque rupture [6–8]. Mast cells are derived from pluripotent hematopoietic stem cells, which are released in the blood flow, and then migrate to the tissue where they proliferate, differentiate, and become resistant [9,10]. Two types of mast cells, differing in neutral (cytoplasmic) proteases, are identified: mast cells that contain tryptase and mast cells that contain tryptase and chymase. Those that contain tryptase are found in the mucous membranes, mainly in the respiratory and gastrointestinal tracts. Mast cells that contain tryptase and chymase also have carboxypeptidase and cathepsin G and are found in the connective tissues of the skin, peritoneum, perivascular tissues, and synovial membranes. Another subtype has also been identified, mast cells that contain chymase and carboxypeptidase [10] or chymase and cathepsin G [9], and may be found in different sites. Mast cells tryptase and mast cells chymase and tryptase are found in the human aortic and coronary intima and adventitia [11].

The physiologic role of chymase and tryptase is still uncertain, and the activity of such enzymes is only observed in damaged tissues such as those of people with atherosclerosis [12]. It is known that the efflux of high-density lipoprotein (HDL) in macrophages prevents the accumulation of cholesterol; however, mast cell neutral proteases degrade HDL, hindering the removal of cholesterol from macrophage foam cells [13,14]. Thus, mast cells play a vital role in the physiopathogenesis of atherosclerosis by actively contributing to its evolution. They may even aggravate the degree of atherosclerosis depending on the number and type of mast cells in the tissue [15].

* Corresponding author. Disciplina de Patologia Geral. Universidade Federal do Triângulo Mineiro. Rua Frei Paulino, 30. CEP 38025-180. Uberaba-Minas Gerais, Brasil. Tel.: +55 34 33185428; fax: +55 34 33185846.
E-mail address: rosana@patge.uftm.edu.br (R.R. Miranda Corrêa).

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Few studies report the role of mast cells and its proteases, chymase and tryptase, in atherogenesis and in the formation of fibroatheromas [6,12,16]. Therefore, this study aimed to describe the influence of mast cell neutral (cytoplasmic) proteases, tryptase and chymase, in the morphological evolution of atherosclerosis in aortas of autopsied patients.

2. Materials and methods

This study was approved by the research ethics committee of Universidade Federal do Triângulo Mineiro under protocol 888. Forty-four sections of aortas were obtained from autopsies performed at Hospital de Clínicas (General Hospital) of Universidade Federal do Triângulo Mineiro from 1963 to 2008. Patients ranged from 18 to 85 years, regardless of the cause of death or underlying disease. Variables such as age, sex, ethnicity, and cause of death were obtained from the autopsy reports. The patients who are 60 years or older were considered to be elderly, whereas the other patients were classified as nonelderly. Autopsied patients were divided into white and nonwhite groups.

2.1. Degree of atherosclerosis

The selected aortas were 15 cm long or more. The extension of the atheromatous plaques as well as the intensity of fibrosis and of calcification was the basis upon which we assessed how affected the aortas were. Taking this criterion into account, 3 researchers described the degree of atherosclerosis separately. Then, the aortas were analyzed using a standardized scale of 0.0 to 12.0 cm, whereby each researcher recorded a point corresponding to the diagnosed degree of atherosclerosis. After that, the distance of 0.0 cm to the point in the scale was measured with a ruler. This analysis was performed by 5 researchers, separately, and the overall mean found was used to determine the degree of atherosclerosis of each aortic section analyzed. Then, the degree of atherosclerosis was classified as mild when measurement ranged from 0.1 to 4.0 cm; moderate, 4.1 to 7.0 cm; and severe, 7.1 to 12.0 cm.

2.2. Fragment collection and processing

A transverse aortic section measuring about 2 × 2 cm was collected from each patient, regardless of its location (thoracic or abdominal). The cuts were made mainly in sites that are more affected by atherosclerosis, based on its degree, and they were then fixed in 3.7% formaldehyde and processed for histologic analysis. For histologic staining, 4-μm cuts were made, and for immunohistochemistry, 2-μm cuts were made.

2.3. Microscopic intensity of lipid deposition in the tunica intima

The aortic sections, which had been previously fixed in formaldehyde, were washed with a buffer (buffered saline solution) and were frozen at liquid nitrogen temperature (−180°C), and the sections were cut using a cryostat at −23°C. We performed 8-μm-thick serial sections in cryostat at 23°C. The sections were stained with Sudan red, and the positive areas were established at a final magnification of ×800 using an image analyzer KS-300 (Kontron-Zeiss, Thornwood, NY). Then, the percentage of deposition per field area analyzed was found.

2.4. Quantification of collagen in the tunica intima

The sections were stained by Picrosirius. After hydrating the 5-μm sections, the slides were stained for 1 hour in a 0.1% Sirius Red solution (Direct Red 80, Sigma Aldrich, São Paulo, Brazil, diluted in saturated picric acid solution), followed by rapid washing in running tap water and counterstained with Harris hematoxylin for 15 minutes, dehydrated and mounted in synthetic resin. Picrosirius-stained sections were evaluated by ordinary polychromatic and polarized light microscopies, and collagen fibers were quantified. The quantification of collagen was performed in the tunica intima of the aorta, with picrosirius-stained sections examined under polarized light using image analyzer KS-300 software. The values were expressed in percentage of collagen per field area analyzed.

2.5. Evaluation of the density of immunostained mast cells

Immunostaining of mast cell cytoplasmic proteases was done as a single batch by using the following primary antibodies: antichymase (DBS/Diagnostic BioSystems, 1:2000) and antitryptase (DBS/Diagnostic BioSystems, 1:1000). Quantification of immunostained mast cells was performed using a video camera connected to a conventional light microscope and to a computer with KS-300 image analysis software. Mast cells were quantified along the length of the cut, in the intima, media, and adventitia layers of the aorta using final magnification of ×500. After that, the slides were photographed by a Sanyo VCC-5974 camera (San Diego, CA) coupled to the microscope. Then, the area of the sections was measured by Image J software (Bethesda, MD), with the cuts having been outlined with the aid of a cursor. Hence, it was possible to calculate the density of mast cells, which was expressed in number of mast cells per square centimeter.

2.6. Statistical analysis

A Microsoft Excel spreadsheet (Redmond, WA) and SigmaStat 2.03 software (SPSS Inc., Chicago, IL) were used for data analysis. Variable distribution type was verified using Kolmogorov-Smirnov statistical test. Mann-Whitney *U* test was used to compare 2 groups, and Kruskal-Wallis test was used to compare 3 or more groups. Then, Spearman correlation coefficient was used. Differences in which the probability (P) was less than 5% ($P < .05$) were considered statistically significant.

3. Results

The average age of the patients was 52 ± 9.8 years, ranging from 36 to 69 years. Twelve patients were elderly, whereas 32 were nonelderly; 24 patients (66.7%) were male, and 28 (64.5%) were white. Regarding the cause of death, 16 patients (36.4%) were affected by cardiovascular cause of death; 19 (42.6%), by infectious disease; 7 (16%), by neoplasic cause; and 2 (5%), by other causes. Depending on the degree of atherosclerosis, patients were divided in 3 groups, in which 24 patients (41.9%) had mild atherosclerosis, 13 (26.2%) had moderate atherosclerosis, and 7 (31.9%) had severe atherosclerosis (Table).

There was no significant difference between the density of mast cell tryptase and chymase concerning ethnicity, sex, cause of death, or degree of atherosclerosis. The density of mast cell chymase was significantly higher in the nonelderly group (107.775 vs 27.230 per cm^2, $P = .047$). On the other hand, such significant difference was not observed concerning the density of mast cell tryptase (135.113 vs 82.333 per cm^2, $P = .148$). The percentage of collagen was

Table
Demographic characteristics of the 44 autopsied patients

Demographic characteristics, n = 44		
Age (y)		52 ± 9.8
Sex, n (%)	Male	24 (66.7)
	Female	20 (33.3)
Skin color, n (%)	White	28 (64.5)
	Nonwhite	16 (35.5)
Cause of death, n (%)	Cardiovascular	16 (36.4)
	Noncardiovascular	28 (63.6)
Degree of atherosclerosis	Discrete	24 (41.9)
	Moderate	13 (26.2)
	Severe	07 (31.9)