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BLOOD VESSELS IN GANGLIA IN HUMAN ESOPHAGUS MIGHT EXPLAIN THE HIGHER FREQUENCY OF MEGAESOPHAGUS COMPARED WITH MEGACOLON

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SUMMARY

This study aimed to determine the existence of blood vessels within ganglia of the myenteric plexus of the human esophagus and colon. At necropsy, 15 stillborns, newborns and children up to two years of age, with no gastrointestinal disorders, were examined. Rings of the esophagus and colon were analyzed and then fixed in formalin and processed for paraffin. Histological sections were stained by hematoxylin-eosin, Giemsa and immunohistochemistry for the characterization of endothelial cells, using antibodies for anti-factor VIII and CD31. Blood vessels were identified within the ganglia of the myenteric plexus of the esophagus, and no blood vessels were found in any ganglia of the colon. It was concluded that the ganglia of the myenteric plexus of the esophagus are vascularized, while the ganglia of the colon are avascular. Vascularization within the esophageal ganglia could facilitate the entrance of infectious agents, as well as the development of inflammatory responses (ganglionitis) and denervation, as found in Chagas disease and idiopathic achalasia. This could explain the higher frequency of megaesophagus compared with megacolon.

KEYWORDS: Enteric nervous system; Vascularization of esophageal ganglia; Myenteric plexus; Megaesophagus; Megacolon; Immunohistochemistry.

INTRODUCTION

Most studies describing the ganglia of the enteric nervous system have been performed in animals, and to a lesser extent in humans. However, in general, it is not mentioned whether or not these ganglia were vascularized[5,10,12,15,16,18]. According to GERSHON &BURSZTAJN (1978), GABELLA (1979, 1990), TAFURI & RASO (1979) and KIERNAN (1996), ganglia of the enteric nervous system do not contain blood vessels. It is thought that a thin collagen-elastic capsule containing vessels surrounds each ganglion. From that capsule, unlike sympathetic and cardiac ganglia, no conjunctive septa penetrate the ganglion[22]. Nutrients and other substances contained in blood diffuse through the capillary endothelium of blood vessels of the capsule and reach the interior of ganglia[8]. According to GABELLA (1990), "a single basal lamina surrounds the entire ganglion and no collagen fibrils, connective cells or blood vessels are found inside ganglia". However, ADAD described the presence of blood vessels in ganglia of the myenteric plexus of the human esophagus[1], although such vessels were not identified in colonic ganglia[2]. Nevertheless, these studies were performed on histological sections stained by hematoxylin-eosin (H&E) and Giemsa only, and they were not conducted in a systematized manner to investigate the vascularity of the ganglia. No studies were found on ganglia of the enteric nervous system using immunohistochemical techniques for the labeling of endothelial cells, which could facilitate the identification of blood vessels.

The importance of determining whether ganglia are vascularized is related mainly to inflammation. Avascular structures become inflamed only indirectly, i.e. by contiguity with the surrounding tissues. However, vascular structures can become inflamed directly without previous inflammation of adjacent tissues, which could affect the genesis of esophageal and colonic diseases, which are associated with ganglionitis and denervation, such as Chagas disease and idiopathic achalasia.

Given the paucity of data and the controversy regarding vascularity of the ganglia of the human enteric nervous system, this study was conducted to evaluate in a systemized manner whether there is vascularization within the ganglia of the myenteric plexus of the esophagus and colon. Only fetuses, newborns and children up to two years were selected for the study, as ganglionitis in the esophagus is frequently observed in adult controls[3,7] and could be caused by inflammation triggering neovascularization.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro.

Fifteen autopsy cases with no gastrointestinal alteration, of people who died of disease in other organs, were studied. Nine cases were stillbirths with a gestational age ranging from 23 to 37 weeks, and there

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were three newborns and three cases aged between six months and two years of age. Nine cases were male (60%). All serological tests for Chagas disease, performed in pericardial fluid, were negative.

One ring of the lower esophagus and one ring of a distal part of the sigmoid colon were removed for each case and fixed in buffered formalin for 48 hours. The rings were processed for paraffin embedding to obtain 7-µm-thick histological sections. Four en-echelon histological sections were stained with H&E and Giemsa, and immunohistochemistry was performed to characterize endothelial cells.

Immunohistochemistry was performed using the polymer technique (Novocastra Novolink Polymer - USA) for characterization of endothelial cells, using the antibodies for anti-factor VIII (Von Willebrand Factor) and CD31 (endothelial cell). Sections were deparaffinized through xylene and dehydrated in graded alcohols. To unmask antigenic sites, sections were boiled in a Pascal pressure chamber (DakoCytomation, USA) for 30 min in citrate buffer (pH 6.0). Endogenous peroxide activity was inhibited by incubation with 3% hydrogen peroxide for 15 min. The sections were incubated in Protein Block for 10 min. The sections were then incubated with the primary antibody, anti-factor VIII (Von Willebrand Factor, Monclonal Mouse Anti-human - clone F8/86, DAKO, Carpinteria, CA, USA; code M061601; diluted 1: 50) and CD31 (endothelial cell, Monclonal Mouse Anti-human - Clone JC/70A, Abcam - Cambridge, MA - USA; code ab9498; diluted 1: 100), for 16 hours at 4 °C. The sections were then incubated with the post-primary reagent (Novolink Polymer) for 30 min. Each incubation period was followed by gentle washing in phosphate buffered saline. Activity was demonstrated by incubation with diaminobenzidine (Liquid DAB, DAKO, Carpinteria, CA - USA) for five min. Slides were counterstained with Harris hematoxylin (Sigma), dehydrated in graded alcohols, and mounted in synthetic mounting media.

RESULTS

Analysis of histological sections of the esophagus and colon revealed no ganglionitis or alterations in the mucosa, submucosa, muscularis propria, adventitia and/or serosa in any case. Blood vessels were observed in the capsule and within ganglia of the myenteric plexus of the esophagus in all histological sections stained with H&E and Giemsa for all 15 cases (Fig. 1A, B). With regard to the colon, blood vessels were observed only in the capsule involving ganglia, and blood vessels were not identified within the ganglia in any cases (Fig. 1E, F). An immunohistochemical study confirmed the findings from H&E and Giemsa-stained sections. For the esophagus, positive immunostaining for CD31 and factor VIII in cells that lined vessels, both within the ganglia and in the capsule (Fig. 1C, D), was observed. In the colon, however, positivity was observed for only endothelial cells in the capsule of the ganglia of the myenteric plexus (Fig. 1G, H).

DISCUSSION

The results demonstrated the presence of blood vessels within ganglia of the myenteric plexus of the esophagus. These findings are different from those by GERSHON & BURSZTAJN (1978), GABELLA (1979, 1990), TAFURI & RASO (1979) and KIERNAN (1996), who reported avascular ganglia of the enteric nervous system. Experimental studies have demonstrated differences in the ganglia of the enteric nervous system among species and regions of the digestive system[10]. It is likely that the

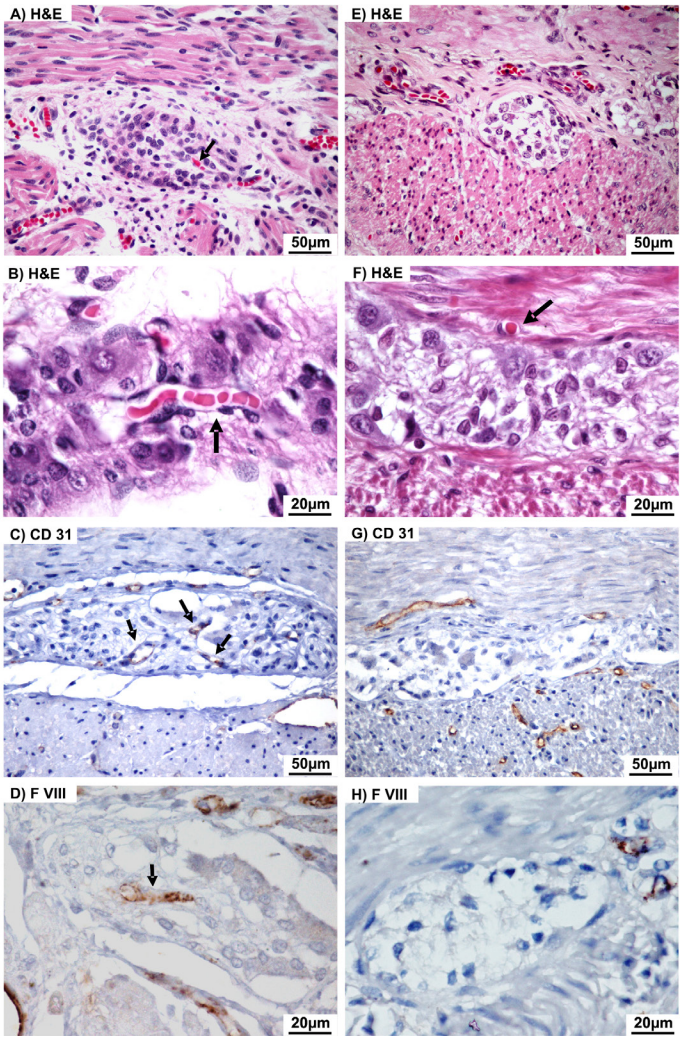

Fig. 1 - Photomicrographs of histological sections stained with hematoxylin-eosin (A, B, E, F) and immunohistochemistry (C, D, G, H). All histological sections were photographed with the following orientation: circular muscle layer on the upper portion, longitudinal muscle layer on the lower portion (not visualized in B) and the myenteric plexus on the center. Note the presence of blood vessels (arrows) within ganglia of the myenteric plexus of the esophagus (A, B, C, D), which is in contrast to the colon (E, F, G, H) where the vessels do not exceed the limits of the capsule.

ganglia of the esophageal myenteric plexus in humans are different from those in animals.

Frequent ganglionitis in the esophagus of adult controls was described by ECKARDT *et al.* (1978) and ADAD *et al.* (1991). Vascularization within the esophageal ganglia could facilitate the entrance of infectious agents, as well as the development of inflammatory responses.

The presence of blood vessels within the ganglia of the myenteric plexus of the esophagus and their absence within the ganglia of the colon is important in the pathogenesis of esophageal and colonic diseases associated with ganglionitis and denervation. This could explain the higher frequency of idiopathic megaesophagus (achalasia) compared with idiopathic megacolon. It would also allow more severe denervation

of the esophagus compared with the colon, favoring earlier onset of megaesophagus compared with megacolon[6,19,20,21], despite the need for a higher degree of denervation in megaesophagus than in chagasic megacolon[1,2,3,4,14].

Reductions in the number of neurons in the myenteric plexus of the esophagus occur in the elderly[7,17,23], possibly due to the aging process itself. However, vascularization of ganglia could contribute to this slow and progressive loss of neurons throughout life, by facilitating the entry of infectious agents with consecutive ganglionitis, favoring the appearance of denervation and presbyesophagus. The absence of ganglionitis in fetuses and newborns, in the present study, the frequent finding of discrete ganglionitis in younger adult controls[3,7] and more severe ganglionitis in older controls[7], support this hypothesis, as well as the absence of ganglionitis in the colon of the same individuals.[2]

It was concluded that the ganglia of the esophagus are vascularized and the ganglia of the colon are avascular. This has important implications in the pathogenesis of esophageal and colonic diseases, which are associated with ganglionitis and denervation, such as Chagas disease and achalasia.

RESUMO

Vasos sanguíneos em gânglios no esôfago humano poderiam explicar a maior freqüência de megaesôfago comparado com megacolon

Este estudo teve como objetivo avaliar se existem ou não vasos sanguíneos no interior de gânglios do plexo mientérico do esôfago e cólon humano. Foram examinados 15 casos de necrópsias de natimortos, recém-nascidos e crianças de até dois anos de idade, sem alterações gastrintestinais, que faleceram por doenças em outros órgãos. Foram analisados anéis do esôfago e cólon, fixados em formol e processados para inclusão em parafina. Cortes histológicos escalonados foram corados pelas técnicas de hematoxilina-eosina, Giemsa e imuno-histoquímica para caracterização das células endoteliais, utilizando-se os anticorpos anti-fator VIII e CD 31. Foram identificados vasos sanguíneos no interior de gânglios do plexo mientérico do esôfago em todos os casos e não foram vistos vasos sanguíneos em nenhum gânglio do cólon. Concluímos que os gânglios do plexo mientérico do esôfago são vascularizados e, os do cólon, avasculares. A vascularização no interior dos gânglios do esôfago pode facilitar a entrada de agentes infecciosos, bem como o desenvolvimento de respostas inflamatórias (ganglionite) e denervação, como encontrados na doença de Chagas e na acalásia idiopática. Isso pode explicar a frequência maior de megaesôfago comparado com megacólon.

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