PATHOLOGICAL STUDY

Biological aspects of appendix pathology: irregular distribution of myenteric ganglia in human appendiceal wall

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ABSTRACT
OBJECTIVES: Although appendicitis is a common disease, basic questions about risk factors and its etiology remain unexplained.
BACKGROUND: An obstruction of the appendix lumen is usually considered to be the main cause of acute appendicitis. However, more studies are currently dealing with neuroimmune appendicitis.
METHODS: We studied samples of human appendices with the histological diagnosis of chronic appendicitis. Fixed cryosections of appendiceal walls were examined by immunofluorescence methods using neuronal anti-neurofilament antibody markers and beta III tubulin.
RESULTS: The immunostaining revealed an irregular distribution of myenteric ganglia in inflamed appendiceal walls and unexpected groups of large ganglia unequally distributed in the subserosal area. The comparative analysis of normal and inflamed appendix samples showed differences in the occurrence of myenteric ganglia in the subserosal area. They appeared more frequently on cryosections prepared from the inflamed appendiceal wall.
CONCLUSION: We propose that the high variability and irregular location of myenteric ganglia in the appendiceal wall are due to an alteration in the motility which results in flaccid appendix emptying. In addition, superficially located myenteric ganglia are exposed to abdominal irritation and may explain the chronic abdominal pain which is often considered to be a sign of chronic appendicitis (Fig. 2, Ref. 23).

KEY WORDS: chronic appendicitis, chronic abdominal pain, myenteric ganglia, neurofilaments, beta III tubulin.

Introduction

Appendicitis may occur either as acute or chronic inflammation of the appendix vermiformis. Despite the diagnostic and therapeutic advancement in medicine, appendicitis remains a clinical emergency and is one of the common causes of acute abdominal pain. Still, the etiology of appendicitis remains unclear. Acute appendicitis is most common between 10 to 20 years of age but can occur at all ages (1, 2). Increased risks for appendicitis were described chiefly as low-fiber diet (3, 4), infections due to edema causing obstruction of appendix lumen, and hereditary factors (5). Familial history of appendicitis is considered to be due to a particular positioning of the appendix, which predisposes it to infection (6). In animals, appendicitis has been described only in anthropoid apes (7) with characteristically narrow worm-shaped appendix. On the other hand, in many primates and mammals, the appendix is much more open and sack-shaped (8).

Recently, more attention has been paid to the appendix innervation in relation to appendix emptying. Normal gastrointestinal motility depends on the enteric nervous system and networks of interstitial cells of Cajal which generate the pacemaker signals (9, 10). Previously, we examined myenteric ganglia of apparently normal appendices using neuronal marker antibodies. The aim of this study was to demonstrate the differences in myenteric ganglia localization in the normal and inflamed appendiceal wall by immunohistochemical methods.

Material and methods

Patients and samples

Patients with the clinical diagnosis of appendicitis underwent appendectomy at Children’s Faculty Hospital, Bratislava. Parts of 50 appendices were obtained for our studies. The histological diagnosis was performed by pathologists at Cytopathos, s.r.o. Bratislava. Experiments with human biopptic samples were approved by the Ethical Committee of UNB Bratislava. For this study, we chose appendices from five patients (15–18 years of age) with the histological diagnosis of chronic appendicitis. The appendix samples were cut along their longitudinal axes. Specimens for cryosections were embedded with OCT and cut into 10-μm thick sections.
**Immunoﬂuorescence staining**

Cryosections were ﬁxed in methanol-acetone (1:1) solution for 15 min at −15 °C or with 4 % p-formaldehyde for 15 min at room temperature. Myenteric ganglia were detected by indirect immunofluorescence staining using neuronal anti-neuroﬁlament antibody marker (1 : 100 dilution, clone NF-01, Exbio, Prague) and two antibodies against beta III tubulin, monoclonal (1 : 100, TUJ 1, Abcam), and polyclonal sera developed in rabbit (1 : 100, Sigma). Fixed cryosections were incubated for 1 hour with primary antibodies and for 30 minutes with 1 : 50 diluted secondary antibodies (Sigma). Double labeling with NF-01 and polyclonal sera against beta III tubulin was performed with primary antibodies, and afterwards with appropriate mixtures of secondary antibodies for 1 hour and 30 minutes, respectively. The staining of cryosections ﬁxed with p-formaldehyde was preceded by cell membrane permeabilization with 0.5 % Triton X-100 for 15 min. Nuclei were stained with Hoechst 33342 (5 μg/ml in PBS, Sigma) 1 min.

**Results**

We have studied myenteric ganglia by indirect and double-immunofluorescence methods using neuronal anti-neuroﬁlament (expressed only in neuronal cells) and anti-III tubulin antibody markers. Monoclonal antibodies NF-01 recognize an epitope on heavy neuroﬁlament protein (210 kDa) of various species (11). Beta III tubulin was found in the central and peripheral nervous systems, and appears to be speciﬁc for neurons (12, 13). However, beta III tubulin was also found in human fetal astrocytes (14). By double labeling, we previously demonstrated a similar staining with both neuronal marker antibodies on non-inﬂamed appendix cryosections (15). In this study we found a similar co-expression of neuroﬁlaments and beta III tubulin on inﬂamed appendiceal wall sections. In addition, the staining with neuroﬁlament was brighter than that with beta III tubulin. This is because the majority of cryosections were examined by indirect immunofluorescence with neuroﬁlament antibodies.

The indirect immunofluorescence showed strong staining of myenteric ganglia with neuroﬁlaments (Fig. 1A, B) and beta III tubulin antibodies (Fig. 1C, D). They were of different sizes and shapes irregularly distributed mainly in the circular layer (Fig. 1B, D) or within both muscle layers (Fig. 1A, C). We observed single or cumulated large ganglia in the subserosal area (Fig. 1A, C). Subserosal myenteric ganglia were very unequally distributed in all inﬂamed appendiceal samples.

**Discussion**

Previously, we demonstrated an irregular distribution and atypical subserosal localization of myenteric ganglia in the normal human appendiceal wall. In this study, we examined biotic samples from patients with the histological diagnosis of chronic appendicitis. To perform the comparative analysis of myenteric plexuses in normal and inﬂamed appendiceal walls we used the same methods and neuronal marker antibodies (15). Cryosections were stained with neuroﬁlaments and beta III tubulin antibodies. Similarly to the normal appendiceal wall, the immunostaining revealed an irregular distribution of myenteric ganglia in inﬂamed appendices. However, some differences were found in the occurrence of myenteric ganglia in the subserosal area. In the normal appendix, small ganglia rarely appeared in the subserosa. On the other hand, in inﬂamed appendices, they occurred more frequently. It is remarkable that several myenteric ganglia of varying size and shape were cumulated in the subserosa. Single or cumulated subserosal ganglia appeared to be very unequally distributed in

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**Fig. 1.** Localization of myenteric ganglia in human appendiceal wall with the diagnosis of chronic appendicitis. Indirect immunofluorescence for neuroﬁlaments (A, B) and beta III tubulin (C, D), stained longitudinal cryosections. Myenteric ganglia were distributed mainly within the circular layer (A, C) or within longitudinal and circular muscle layers (B, D). Nuclei stained with Hoechst. Scale 200 μm.

**Fig. 2.** Concentrated myenteric ganglia in the subserosal area of human appendix with diagnosed chronic appendicitis. Indirect immunofluorescence staining on longitudinal cryosections using antibodies against neuroﬁlaments (A, B). Stained for neuroﬁlaments and nuclei stained with Hoechst (B, D). Scale 200 μm.
all inflamed appendixes. The irregular distribution of myenteric ganglia in human appendix was previously described in some studies (16, 17). On the other hand, Hanani (18) revealed that in most cases, the innervation of the external muscles of the appendix consisted of three concentric networks of ganglia located between the circular and longitudinal muscle layers as well as within them.

The most important question about the human appendix is the poorly understood etiology of appendicitis in children and young adults. Obstruction of the appendix lumen is usually considered to be the main cause of acute appendicitis (19). However, recent studies measuring the pressure in the appendix perioperatively suggest that the obstruction is not an important causative agent of acute appendicitis, but might develop as a result of the inflammatory process (20). Other authors described no significant correlation between gangrenous/perforated appendicitis and the presence of a fecalith (21). Currently more studies are dealing with neuroimmune appendicitis or neuronal hypertrophy in acute appendicitis (22, 23).

These results confirm our lately advanced hypothesis that the irregular distribution of myenteric ganglia occurs due to an alteration in motility which results in flaccid appendix emptying and subsequent pathogenesis of human appendicitis (15). In this study, we revealed areas of subserosal large myenteric ganglia in an inflamed appendiceal wall. Thus, superficially located myenteric ganglia are exposed to abdominal irritation and may help explain the chronic abdominal pain which is often considered to be a sign of chronic appendicitis. We infer that the most important role in appendicitis etiology is the variability and irregular distribution of myenteric ganglia in the appendiceal wall, while the mentioned risks for appendicitis may only develop this disease.

References


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