DOI: 10.4149/BLL_2018_135

Bratisl Med J 2018; 119 (12)
743 – 746

ANATOMICAL STUDY

Subserosal localization of myenteric ganglia in normal human appendix: immunostaining with neuronal and glial markers

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ABSTRACT
OBJECTIVES: Visualization of unexpected distribution of myenteric ganglia in normal human appendiceal wall by immunofluorescence.
BACKGROUND: The myenteric plexus is located between the longitudinal and circular muscle layers of the GIT. However, recently the irregular distribution of myenteric ganglia was revealed in human appendix.
METHODS: The cryosections prepared from normal human appendices were examined by immunofluorescence methods using antibodies to neurofilaments (NF) and glial fibrillary acidic protein (GFAP).
RESULTS: Indirect immunofluorescence revealed the positive staining of myenteric ganglia with both neuronal and glial marker antibodies. Double labeling for NF/GFAP staining showed close assotiation between glia and neurons inside ganglia. GFAP-positive cells were often observed as the cells surrounding myenteric ganglia. The staining confirmed the irregular distribution of myenteric ganglia in human appendiceal wall and revealed the small ganglia in the subserosal area.
CONCLUSION: Our results showed that localization of myenteric ganglia in human appendix differs from other parts of GIT. GFAP immunostaining is available for visualization of smaller myenteric ganglia located mainly in the subserosal area. Our studies may find application in current HIV research focused on enteric neuropathogenesis and in diagnostic laparoscopy for chronic abdominal pain: to detect the irritation of subserosal ganglia *(Fig. 2, Ref. 26)*. Text in PDF *www.elis.sk.*
KEY WORDS: human appendix, myenteric ganglia, intermediate filaments, GFAP, NF.

Introduction

Gastrointestinal tissues are inervated by the enteric nervous system (ENS), composed of enteric neurons and enteric glia. The neurons of the ENS are organized into ganglia which together with nerve fibres form myenteric (Auerbach's) and submucosal (Meissner's) plexi. The submucosus plexus is located in the submucosa and controls epithelial and vascular functions of the mucosa. The myenteric plexus localization is described between the longitudinal and circular muscle layers in the gastrointestinal tract and together with intestinal cells of Cajal is responsible mainly for intestinal motility (1, 2)

The meyenteric plexus in human appendix is poorly examined. It could be because for a long time appendix was considered as a vestigial organ. The greatest attention was paid on well developped rabbit appendix that when compared with humans support the hypothesis of the appendix vestigiality (3). The most important question about the human appendix is its pathology and poorly understood etiology of appendicitis in children and young adults. In animals, appendicitis was described only in anthropoid apes (4). However, the morphological similarities of narrow worm-shaped appendix are restricted to humans and apes. On the other hand, easy empting of large open appendix present in many primates and mammals is probaly protective against appendicitis.

Previously, it was believed that appendicitis is caused by obstruction of the lumen (5). However, several studies revealed that the obstruction is not a causative factor of acute appendicitis (6, 7). Recently, we described the atypical myenteric ganglia distribution in relation to appendix pathology (8). Here, we examined the co-expression of neuronal and glial markers in normal human appendiceal wall.

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Acknowledgement: This study was supported by VEGA grant No. 1/3439/06 and ITMS: 26240120023, co-financed by the European Regional Development.

Material and methods

Bioptic samples

Fresh tissue samples of normal and inflamed human appendices were kindly provided by the Children´s Faculty Hospital Bratislava. Experiments with human bioptic samples were approved by the Ethical Committee of UNB Bratislava. For this study we chose appendices from five patients of similar age (12–14 years) which

were found to be normal after appendectomy. The appendices were cut along their longitudinal axes. Specimens for cryosections were embedded with OCT and cut into 10 um thick sections.

Immunofluorescence

Indirect immunofluorescence staining was performed using antibodies to neurofilament (1:100 dilution, clone NF-01, Exbio, Prague) and polyclonal sera to GFAP (1:100 dilution, DAKO). Cryosections were fixed in methanol-acetone (1:1) solution for 15 min at (–15 °C). temperature. They were incubated for 1 hour with primary and for 30 minutes with 1:50 diluted secondary antibodies (Sigma). Double labeling for NF/GFAP was performed with primary, and afterwards with appropriate mixtures of secondary antibodies for 1 hour and 30 minutes, respectively. Nu-

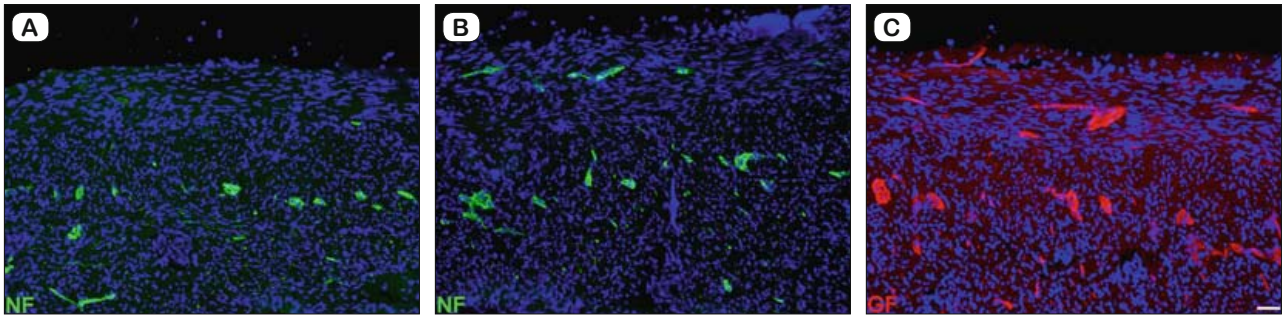

Fig. 1. Indirect immunofluorescence of human appendix, longitudinal cryosections. The irregular distribution of myenteric ganglia positively stained for NF (A, B) and GFAP (C). Nuclei stained with Hoechst. Scale bar: 50 µm.

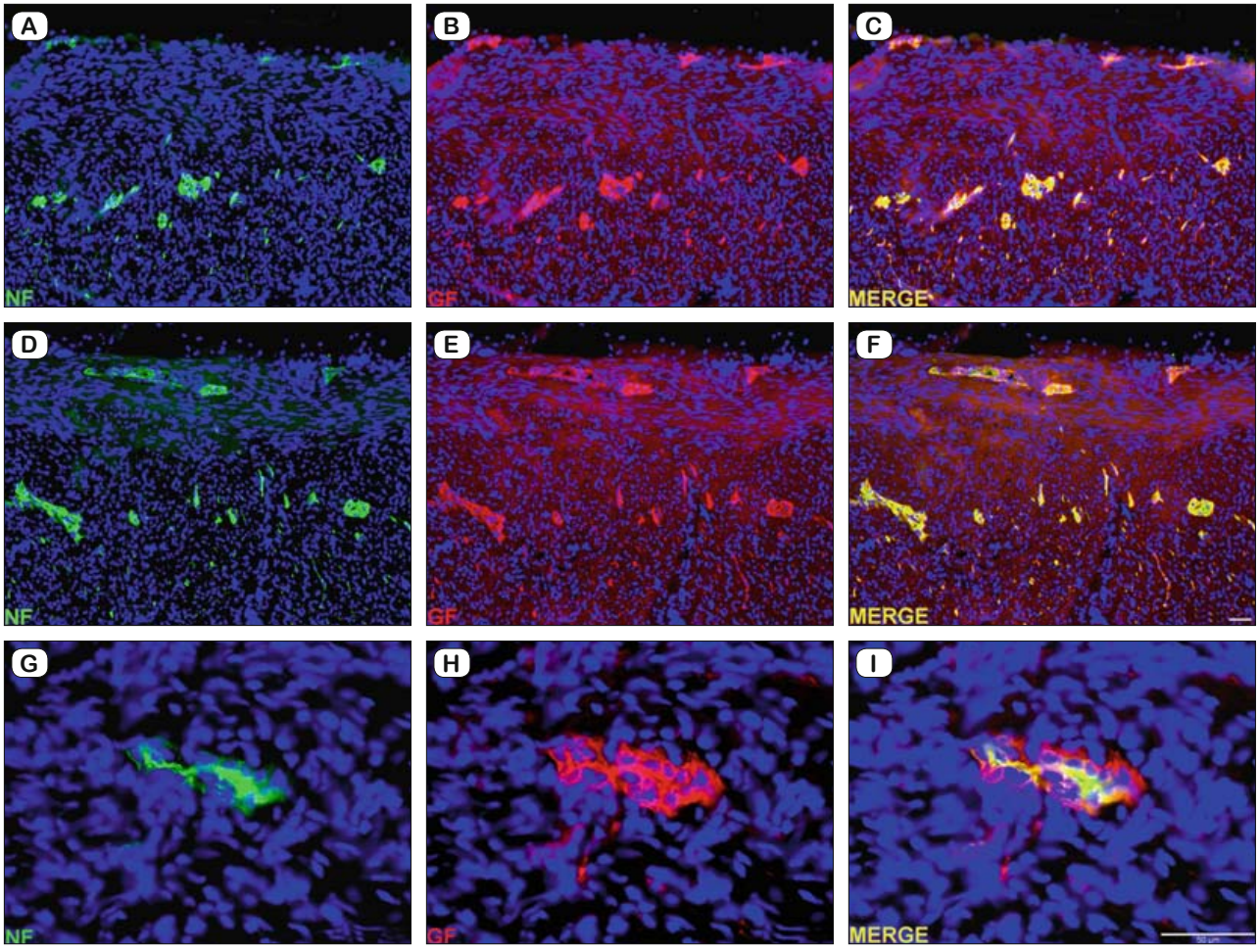

Fig. 2. Double labeling for NF and GFAP of human appendix, longitudinal cryosections. The staining showed close association between glia and neurons within myenteric ganglia (A–F). GFAP-positive cells were also observed as myenteric ganglia surrounding cells (G–I). Nuclei stained with Hoechst. Scale bars: 50 µm.

clei were stained with Hoechst 33342 (5 μg/ml in PBS, Sigma) for 1 minute.

Results

IF are usually considered as cell type specific proteins. NF are the major component of the neuronal cytoskelet. The three component proteins (NF-L, NF-M and NF-H) are reffered as the neurofilament triplet. We used monoclonal antibodies which recognize an epitope on NF-H (9). GFAP is specific for cells of astroglial origin and also is marker protein for enteric glia (10). To avoid the cross-reactivity of IF antibodies we used separately the staining for NF and GFAP. Myenteric ganglia were detected by indirect immunofluorescence using antibodies to NF and GFAP. Immunofluorescence showed a strong staining of ganglia with both marker antibodies. They were of different size and shape (Fig. 1 A–C). Irregular distribution of myenteric ganglia was present in all examined samples. The layer of myenteric ganglia never appeared only between longitudinal and circular muscles layers as is described in the large and small intestines. They were distributed mainly in the circular layer (Fig. 1A) or within both muscle layers (Fig. 1 B, C).

Double labeling was performed with monoclonal antibodies to NF and polyclonal sera against GFAP. The staining showed close assotiation between glia and neurons inside myenteric ganglia (Fig. 2A–F). GFAP-positive cells were also present outside ganglia as myenteric ganglia surrounding cells (Fig. 2C–I). Single GFAP-positive cells were not identified within both muscular layers. The small subserosal ganglia were found unequally distributed under a thin layer of serosa. They were revealed mainly with GFAP immunostaining. It is because ganglia surrounding GFAP-positive cells increased the size of small ganglia created by several neurons (Fig. 2 A–C).

Discussion

In this report we demonstrate the immunostaining of myenteric ganglia with neuronal (NF) and glial (GFAP) marker antibodies. Indirect imunofluorescence on cryosection prepared from normal human appendices revealed the positive staining of myenteric ganglia with both marker antibodies. Double labeling NF/GFAP showed close assotiation between glia and neurons inside ganglia. In addition, GFAP-positive cells were observed as myenteric ganglia surrounding cells. Immunofluorescence staining showed the irregular distribution of myenteric ganglia in human appendiceal wall which were often concentrated in the circular muscle layer. Small ganglia appeared in the subserosal area. For the visualization of smaller myenteric ganglia antibodies to GFAP are more suitable, because GFAP-positive cells surround the neurons and enlarge these ganglia. Enteric glia are peripheral glial cells which share morphological similarities with astrocytes. They are present in the myenteric and submucosal plexuses, within the circular muscle and in the lamina propria of the mucosa (11). However, we did not observe single enteric glial cells with astrocyte-like morphology in longitudinal or circular muscle layers. However, similarly as in appendix enteric glial cells surrounding myenteric ganglia were described in colon (10, 12).

Numerous studies revealed that myenteric ganglia in gastrointestinal tract are located between longitudinal and circular muscle layer. On the other hand, only several reports with conflicting results are dealing with myenteric ganglia in human appendix. Recently we described the irregular distribution of myenteric ganglia in human appendix (13) and the presence of myenteric ganglia in the subserosal area which occured more frequently in appendices with diagnosis of chronic appendicitis (8). Irregular distribution of myenteric ganglia was described in some studies (14, 15). However, Hanani (16) revealed that in most cases, the inervation of human appendix consisted of three concentric networks of ganglia located between the circular and longitudinal muscle layers as well as within them.

New findings and new questions.

We suppose that different degree of irregular distribution, mainly ganglia concentrated in the circular muscle layer, may cause altered appendix motility leading to inflamatory appendix pathology. On the other hand, subserosal localization of myenteric ganglia may be a reason of chronic abdominal pain in the right lower quadrant, caused by direct irritation of surrounding abdominal organs. The appendix can be a rare cause for chronic abdominal pain in this quadrant, however, a significant reduction of pain was achieved after an appendectomy (17). Increase interest of human appendix function, revealed the role of appendix in Crohn's disease or colitis ulcerosa (18, 19), appendix as a source of mesenchymal stem cells (20) or appendix as reservoir of good bacteria (21). Based on these new findings, appendectomy for chronic abdominal pain, similarly prophylactic appendectomy of a normal-appearing appendix during laparoscopic surgery remains disputable (22, 23, 24). New question arises: what is the benefit for patients? It could be useful to consider the new methods, how to save apparently normal appendix in patients with diagnosis of chronic right lower quadrant abdominal pain, how to protect and cover the irritated subserosal myenteric ganglia in human appendiceal wall.

Abdominal pain and gastrointestinal disorders are major clinical features in patients with AIDS. However, it is still not clear why acute appendicitis occurs at a 4-fold higher prevalence among HIV-infected patients compared with the general population (25). In addition, acute appendicitis was described as the initial clinical presentation of primary HIV-1 infection (26). The study of myenteric ganglia in human appendix may contribute to an understanding of neuroinflamatory processes reported in HIV patients.

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Received July 11, 2018.
Accepted September 7, 2018.