

THE ROLE OF GUT INNERVATION IN THE ETIOPATHOGENESIS OF CROHN'S DISEASE

I. Stoyanova

*Department of Anatomy, Faculty of Medicine, Thracian University,
P.O.Box 1025, BG-6010 Stara Zagora, Bulgaria;
stoyanovai@yahoo.co.uk*

ABSTRACT. Crohn's disease is a chronic form of intestinal inflammation, which is segmental, transmural, and granulomatous, and affects any part of the intestine, but most commonly it occurs in the distal ileum and proximal part of the colon. Over the past decade, attention has been paid to the role of neuronal structures and mast cells in regulating the inflammatory and immune responses in inflammatory bowel diseases. This study was aimed to investigate the chemical coding of neurons and neuronal fibres in the small intestine of patients with Crohn's disease.

Specimens from five patients with histologically confirmed Crohn's disease were investigated with immunocytochemical techniques for detection of substance P (SP) and vasoactive intestinal polypeptide (VIP). Normal intestinal tissue, obtained from three patients operated on for rectal carcinoma was used as a control. Significant alterations in the neuronal structures innervating the gut were observed in all layers of the intestine with Crohn's disease. The number of SP- and VIP-immunoreactive neurons was higher than in the controls. In addition, there was a chaotic display of nerve fibres containing the neuroactive substances VIP and SP with high frequency of enlarge varicosities in the circular muscle layer and in the submucosal and the serosa layer.

These results show quantitative and qualitative changes in the neurochemical composition of enteric nerve fibres and nerve cell bodies in Crohn's ileum. These findings suggest that nerve-immune interactions may have a significant role in the process of the inflammatory changes in Crohn's ileitis.

KEYWORDS. Crohn's disease, peptidergic gut innervation, immunohistochemistry

INTRODUCTION

Crohn's disease is a chronic form of intestinal inflammation, which is segmental, transmural, and granulomatous, and affects any part of the intestine, but most commonly it occurs in the distal ileum and proximal part of the colon. Over the past decade, attention has been paid to the role of neuronal structures in regulating the inflammatory and immune responses in inflammatory bowel diseases (IBD).

The peristalsis of the intestine comprises two separate reflexes of the circular muscle layer; an ascending orally directed excitatory reflex, SP mediated, and a descending anally directed inhibitory reflex, VIP mediated (Karila et al., 1998; Pederzoli et al., 2004). The action of SP on smooth muscle activity is also consistent with regulation of the local blood flow as SP-utilizing innervation of small blood vessels was observed all along the intestine of different species (Domeneghini et al., 2004). Substantial evidence implicates the neuropeptide SP in mucosal immunoinflammatory response. The strategic localization and upregulation of neurokinin-1 receptor in inflamed intestine also suggests the involvement of SP in the pathophysiology of IBD (Goode et al., 2000).

Therefore, this study aimed to investigate the effect of Crohn's disease on the chemical coding of neurons and enteric nerve fibers in the small intestine of patients with Crohn's disease.

MATERIAL AND METHODS

Specimens from five patients with histologically confirmed Crohn's disease were investigated with immunocytochemical techniques for detection of substance P (SP) and vasoactive intestinal polypeptide (VIP). Normal intestinal tissue, obtained from three patients operated on for rectal carcinoma was used as a control. The staining was performed using the avidin-biotin method on free-floating sections with primary antibodies, rabbit anti-SP and anti-VIP for 24 h at room temperature. After rinsing in PBS, sections were incubated with the secondary antibody, biotinylated goat anti-rabbit IgG. After washing the sections, the ABC complex was applied. Following rinsing, peroxidase activity was visualized using 0.05% diaminobenzidine. Finally, sections were dehydrated in a graded series of alcohol and xylene, and embedded in Entellan.

RESULTS

Pathomorphology:

Microscopically specimens exhibited an active stage of the disease with shallow ulcers extending into the superficial submucosa, edema, loss of the normal mucosal texture, and noncaseating granulomas in the terminal ileum. The granulomas contained epithelioid cells, Langhans-type giant cells and lymphocytes, and were localized in both submucosa and subserosa. There were also aggregates of inflammatory cells in the serosa layer of the affected segments of Crohn's ileum.

Immunohistochemistry:

The number of SP- and VIP-immunoreactive neurons was higher than in the controls. In addition, there was a chaotic occurrence of nerve fibers containing the neuroactive

substances VIP and SP with a high frequency of enlarged varicosities in the circular muscle layer and in the submucosal and the serosa layers (Fig. 1). SP-immunoreactivity also appeared in the gut epithelial cells. In some regions, all the cells were SP-positive (Fig. 2).

DISCUSSION

The study results show quantitative as well as qualitative changes in the neurochemical composition of enteric nerve fibers and neurons in Crohn's ileum. Such a dramatic increase in neuronal fibers and cell bodies expressing SP and VIP indicates that these neuronal structures contribute to the impaired gut motility as observed in patients with CD, an entity characterized with persistent relaxation of the affected gut segments.

From the distribution of SP-positive epithelial cells we could conclude that besides the role of neurotransmitter, SP might have paracrine and/or autocrine activity in the gut physiology, which further may contribute to the damaged gut function in CD.

Recently, it was demonstrated that SP, VIP and other neuropeptides, such as calcitonin gene-related peptide, neurokinin A and B, and serotonin, act as a secretagogues in human intestinal mast cells when they are pre-activated by IgE during intestinal inflammation (Bischoff et al., 2004). On the other hand, mast cells activation is one of the mechanisms leading to hyperemia in the mucosa of the small intestine in IBD (Ruh et al., 1998). Thus, SP and VIP may contribute to the gut inflammation and fibrosis via mast cells-neuronal interaction.

In summary, our findings showed extensive changes in the enteric nerve system, which suggest that the gut innervation may play a significant role in the process of the inflammatory changes in Crohn's disease. However, the exact mechanisms and degree of importance in initiating and perpetuating the inflammatory process merit further investigation.

REFERENCES

- BISCHOFF, S.C., SCHWENGBERG, S., LORENTZ, A., MANNS, M.P., BEKTAS, H., SANN, H., LEVI-SCHAFFER, F., SHANAHAN, F., SCHEMANN, M. 2004. Substance P and other neuropeptides do not induce mediator release in isolated human intestinal mast cells. *Neurogastroenterol Motil* 16: 185-193.
- DOMENEGHINI, C., RADAELLI, G., ARRIGHI, S., BOSI, G., DOLERA, M. 2004. Cholinergic, nitrenergic and peptidergic (Substance P- and CGRP-utilizing) innervation of the horse intestine. A histochemical and immunohistochemical study. *Histol Histopathol* 19: 357-370.
- GOODE, T., O'CONNELL, J., ANTON, P., WONG, H., REEVE, J., O'SULLIVAN, G.C., COLLINS, J.K., SHANAHAN, F. 2000. Neurokinin-1 receptor expression in inflammatory bowel disease: molecular quantitation and localization. *Gut* 47: 387-396.
- KARILA, P., SHAHBAZI, F., JENSEN, J., HOLMGREN, S. 1998. Projections and actions of tachykininergic, cholinergic, and serotonergic neurons in the intestine of the atlantic cod. *Cell Tissue Res* 291: 403-413.
- PEDERZOLI, A., BERTACCHI, I., GAMBARELLI, A., MOLA, L. 2004. Immunolocalisation of vasoactive intestinal polypeptide and substance p in developing gut of *Dicentrarchus labrax* (L.). *Eur J Histochem* 48: 179-184.
- RUH, J., SCHMIDT, E., GEBHARD, M.M., KLAR, E., GLASER, F., HERFARTH, C. 1998. Effect of mast cell activation on microcirculation of intestinal mucosa in inflamed small intestine of the rat. *Langenbecks Arch chir Suppl Kongressbd* 115 (Suppl I); 109-112.

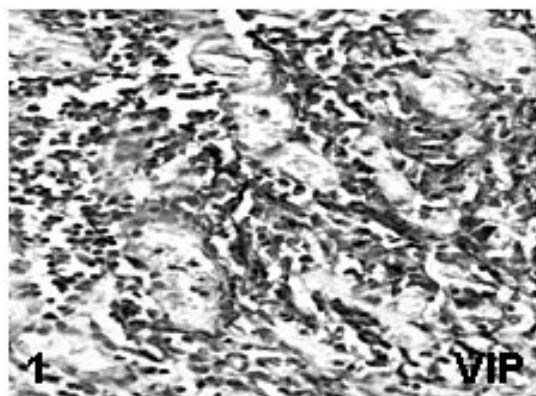


Fig.1. *Micrographs showing VIP-immunoreactive nerve fibers and nerve cell bodies in the myenteric plexus of the small intestine from patient with Crohn's disease. 100x*

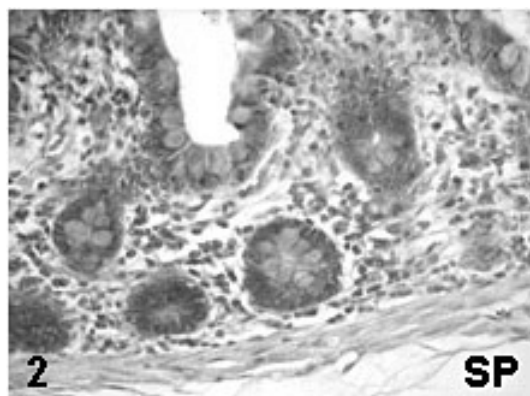


Fig. 2. *Transverse section of the gut showing SP- immunoreactivity in the gut epithelial cells. Note that in some regions all the cells are SP-positive. 400x*