World Journal of Gastroenterology

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DOI: 10.3748/wjg.v2.i4.200

World J Gastroenterol 1996 December 15; 2(4): 200-202
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Changes of neurotensin and endotoxin in rats with intestinal ischemia

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Author contributions: All authors contributed equally to the work.

Supported by Military Science Foundation of Chinese PLA, No. 02006-2.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

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Received: July 12, 1996
Revised: August 26, 1996
Accepted: October 25, 1996
Published online: December 15, 1996

Abstract

AIM: To investigate the changes of neurotensin (NT) and endotoxin in rats with segmental intestinal ischemia.

METHODS: The distal ileal mesenteric arteries in rats were ligated to make segmental intestinal ischemia models. At the 2nd, 6th and 12th hours after intestinal ischemia, endotoxin levels in portal blood were tested by limulus lysate test and NT levels in plasma from the heart and in intestine tissues (ischemia and peri-ischemia areas) were assayed by radioimmunoassay. Histological changes of the mucosa were examined under light and electron microscopes.

RESULTS: NT levels decreased significantly in intestinal ischemia and peri-ischemia areas (34.07 ± 5.93 *vs* 40.14 ± 5.38, $P < 0.05$; 7.47 ± 1.38 *vs* 40.14 ± 5.38, $P < 0.01$), especially lower in ischemia area (34.07 ± 5.93 *vs* 7.47 ± 1.38, $P < 0.05$. However, NT level increased obviously in plasma (0.76 ± 0.16 *vs* 0.47 ± 0.10, $P < 0.05$). Levels of endotoxin elevated obviously in portal blood (389.0 ± 105.0 *vs* 55.1 ± 6.7, $P < 0.01$), and the mucosa was injured both in ischemia and peri-ischemia areas.

CONCLUSION: Intestinal ischemia injures intestinal mucosa and leads to decrease of intestinal NT level, which is accelerated by endotoxemia and increase of blood NT level.

Key words: Neurotensin; Endotoxin; Intestinal ischemia

Ruan CP, Wang YH, Wang LG, Wang YX. Changes of neurotensin and endotoxin in rats with intestinal ischemia. *World J Gastroenterol* 1996; 2(4): 200-202 Available from: URL: http://www.wjgnet.com/1007-9327/full/v2/i4/200.htm DOI: http://dx.doi.org/10.3748/wjg.v2.i4.200

INTRODUCTION

Intestinal ischemia may cause impairment of gut barrier and leads to systemic dissemination of bacteria and endotoxin from the gastrointestinal (GI) tract, which is called bacterial translocation[1]. The GI mucosa is a complicated multifunctional tissue that not only serves as the major site of digestion and absorption of nutrients, but also has metabolic, endocrine and immune functions, all of which play important roles in gut barrier. In order to study the endocrine changes in bacterial translocation, we adopted the model of segmental intestinal ischemia to investigate changes of endotoxin in portal blood and neurotensin (NT) in plasma and intestinal tissues.

MATERIALS AND METHODS

Animals

Ninety SD rats were acclimated for 1 wk and fed standard rat chow ad libitum. After acclimation, the rats were weighed (300-350 g) and randomly assigned into six groups ($n = 15$, each): intestinal ischemia for 2 h, 6 h, and 12 h (experiment groups) and corresponding controls (2, 6, and 12 h). The distal ileal mesenteric arteries of rats in control groups were not ligated.

Animal models and sample processing

After being fasted overnight with free access to water, the rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight). The abdomen was opened and 2-3 distal ileal mesenteric arteries were ligated to make a 20 cm intestinal ischemia area, then the abdomen was closed. The rats were allowed to stay for 2, 6 and 12 h according to each group before undergoing the second stage of surgical procedure.

After anesthesia, the abdomen was reopened and the samples were taken in the following procedure: (1) Blood samples of 5-6 mL from the heart were collected in tubes containing trasylol (200 U each), and the plasma was immediately separated by centrifugation, then was frozen and stored at -20 ℃ until analysis; (2) Portal blood (2 mL) was collected in heparinized tubes; (3) The small intestine tissues (both in ischemia and peri-ischemia aresa) were removed, rinsed, weighed, and then were put into a tube with boiling water (W/V = 1/2). The tube was plunged into vigorously boiling water for 10 min, then it was cooled down and homogenized for 10 min. After centrifugation at 3000 r/min for 5 min, the supernatant was saved and stored at -20 ℃ until assay; and (4) Samples of the small intestine for histological study were taken immediately in the same parts stated above. The tissue from the ischemia area for light microscopy was fixed in 10% buffered formalin and embedded in

Table 1 Changes of NT in plasma (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	0.45 ± 0.08	0.49 ± 0.09	0.47 ± 0.10
Experiment	0.74 ± 0.08[a]	0.81 ± 0.18[a]	0.76 ± 0.16[a]

[a]$P < 0.05$, *vs* control.

Table 2 Changes of NT in intestinal tissues (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	39.38 ± 5.80	38.94 ± 4.76	40.14 ± 5.38
Ischemia	11.71 ± 1.33[bd]	6.23 ± 0.79[bd]	7.47 ± 1.38[bd]
Peri-ischemia	33.25 ± 3.70[a]	32.42 ± 4.10[a]	34.0 ± 5.93[a]

[b]$P < 0.01$, *vs* control; [a]$P < 0.05$, *vs* control; [d]$P < 0.01$, *vs* peri-ischemia.

Table 3 Changes of endotoxin in portal blood (10^{-3} mg/L, mean ± SD)

Group	2 h	6 h	12 h
Control	50.4 ± 8.2	54.3 ± 9.8	55.1 ± 6.7
Experiment	297.0 ± 47.0[a]	336.0 ± 97.0[a]	389.0 ± 105.0[a]

[a]$P < 0.01$, *vs* control.

paraffin, then sections (4 μm) were stained with hematoxylin and eosin. Samples from the peri-ischemia area for electron microscopy was fixed in phosphate-buffered glutaradehyde (2.5%) and osmium tetroxide (1%), dehydration of the mucosa was accomplished with acetone solutions of increasing concentrations. The tissue was embedded in epoxy resin. Semithin (1 μm) sections through the mucosa were then cut and stained with toluidine blue. Then 600 angstrom-thin sections were made from a selected area of tissue defined by the semithin section, and these sections were stained with lead citrate and uranyl acetate.

Morphologic observation and tissue assay

Histological changes were observed with a light microscope in ischemia area and with an electron microscope in peri-ischemia area. NT levels in plasma and intestine tissue were determined by radioimmuno assay[2]. Endotoxin level in portal blood was assayed by limulus lysate test.

Statistical analysis

Data are expressed as mean ± SD and analyzed by Student's *t*-test. *P*-values < 0.05 were considered significant.

RESULTS

Histological features

Histological features were observed with a light microscope in ischemia area. Two hours after intestinal ischemia, the mucosal villi denuded, part of which degenerated and necrosed. Six hours later, the whole layer of the mucosa necrosed. Twelve hours later, the mucosa was digested and its construction disappeared. Microvilli observed had edema and part of them lifted down, and the degree of microvillus damage was positively correlated with the time of intestinal ischemia. No similar changes of the mucosa were found in the control groups.

Changes of NT in plasma

The NT levels in plasma are shown in Table 1. In the intestinal ischemia groups, statistical differences were found when compared with control groups ($P < 0.05$). The plasma NT level elevated obviously during intestinal ischemia.

Changes of NT in ischemia and peri-ischemia areas

The NT values in ischemia and peri-ischemia areas are summarized in Table 2. In peri-ischemia area, the NT values were significantly lower than those in the control groups ($P < 0.05$). In ischemia area, significant differences were found when compared with values of the control groups ($P < 0.01$) and peri-ischemia area ($P < 0.01$). NT values in ischemia area decreased and were even lower than those in peri-ischemia area.

Changes of endotoxin in portal blood

As shown in Table 3, endotoxin in portal blood increased obviously ($P < 0.01$) during intestinal ischemia.

DISCUSSION

This study demonstrates that NT levels increased obviously in plasma and decreased in intestinal tissues (ischemia and peri-ischemia areas) with particularly low level in ischemia area, levels of endotoxin elevated significantly in portal blood, and mucosa was injured in ischemia and peri-ischemia areas.

NT is a tridecapeptide originally isolated from bovine hypothalamus and is subsequently found to be distributed widely in the GI. NT-like immunoreactivity has been localized to a specific mucosal endocrine cell type (N cells), which is found in the highest density in the ileum, and in lower densities in the duodenum, jejunum and colon. From the duodenum to ileum, the level of NT elevates gradually. Because N cells are impaired by intestinal ischemia, NT level in ischemia area will decrease; but decrease of NT level in peri-ischemia area is related to endotoxemia, because a large amount of endotoxin from the GI entered blood due to impairment of gut barrier. Endotoxin can cause constriction of visceral blood vessel, and impair intestinal epithelial cells, including N cells.

Elevation of NT in plasma may be related to: stress caused hypersecretion of adrenal medulla; and entering of NT into bloodstream from the ruptured N cells.

In conclusion, intestinal ischemia injures the intestinal mucosa and leads to a decrease of intestinal NT accelerated by endotoxemia. NT has several protective functions on the gut barrier. It can stimulate small bowel and colonic mucosal growth[3,4], and affect different aspects of immune function by regulating neuroendocrine immune axis. For example, NT stimulates the chemotactic properties of leukocytes[5], augments phagocytosis by macrophages and neutrophils[6], and maintains the secretion of IgA in the GI[7]. Increase of blood NT during intestinal ischemia may benefit the gut barrier. Further studies are required to evaluate the significance of NT level changes in the intestine and plasma.

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S- Editor: Cao LB **L- Editor**: Wang TQ **E- Editor**: Zhang FF

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ISSN 1007-9327
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