Experimental study on the expression and diagnostic significance of I-FABP in acute intestinal ischemia

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ABSTRACT

Objective: To detect the expression and distribution of I-FABP in intestinal tissue and the changes of serum concentrations at different time of acute intestinal ischemia, and explore the significance and mechanism of I-FABP in early diagnosis of acute ischemic bowel disease. Methods: The selected 96 healthy adult SD rats were randomly divided into the experimental group and control group, 48 in each group. Each group was randomly subdivided into 6 groups with 8 rats in each group. The superior mesenteric artery was ligated in the experimental group and the peritoneal switch operation was performed in the control group. The venous blood samples were extracted from each group rats’ right ventricle at 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h after the operation and the concentration of I-FABP was tested respectively. Then the rats were killed, and the diseased intestinal tubes were cut out for paraffin sections. The I-FABP in intestinal tissue was stained by routine HE staining and direct immunofluorescence staining. Results: The I-FABP was mainly expressed in the epithelial villi of intestinal mucosa, and there was a small amount of expression in the intestinal submucosa and even the muscularis. Within 1 hour of intestinal ischemia, the number of I-FABP positive granules in the intestine and intestinal cavity increased gradually, and then gradually decreased after 1 hour. The difference has statistically significant between the experimental group and the control group (P < 0.05). The serum I-FABP: In the experimental group, the serum I-FABP concentration began to increase at 0.5 h, and reached a peak at 1 h (290.24 ± 156.69) μg·L⁻¹, then gradually decreased. Compared with the control group, the difference was statistically significant (P < 0.05). Conclusion: I-FABP usually mainly exists in the epithelial cells of intestinal mucosa. When acute intestinal ischemia occurs, the epithelial cells of intestinal mucosa permeability changes; I-FABP expression rapidly releases to intestinal tissue and intestinal cavity, and is absorbed into the blood. Therefore, I-FABP has certain clinical significance in early diagnosis and treatment of acute intestinal ischemia.

Keywords: Intestinal Fatty Acid Binding Protein; Acute Intestinal Ischemia; Immunofluorescence; Diagnosis

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1. Introduction

Acute intestinal ischemia (AII) is a kind of acute, critical and severe diseases of the digestive system. The early symptoms and signs are nonspecific, so it is difficult to judge the ischemic state of the intestinal tract in time, and it is very easy to develop into irreversible intestinal necrosis in a short time.¹ ² Therefore, the early diagnosis of AII is particularly important. It has been found that the early intestinal fatty
acid binding protein (I-FABP) in AII patients is higher than that in normal people\cite{3,4}. Blood I-FABP is significantly increased in patients with narrow intestinal obstruction compared with simple intestinal obstruction, so the detection of I-FABP content in blood can be used as an auxiliary means for the diagnosis of AII. However, some studies believe that the concentration of I-FABP in blood is positively correlated with the degree of intestinal ischemia in the early stage of AII\cite{5}. With the extension of ischemia time, the concentration of I-FABP in blood gradually decreases. In this study, the concentration of serum I-FABP in different periods of intestinal ischemia in rat acute mesenteric ischemia models was detected and combined with the I-FABP immunofluorescence labeling of intestinal tissue in the process of intestinal ischemia to understand the serological changes of I-FABP in the development of AII and its significance. The expression of intestinal ischemia further proves the significance of I-FABP as a laboratory serum biochemical index in the early diagnosis of AII, providing effective help for clinical diagnosis and treatment of acute small intestinal obstruction.

2. Materials and methods

2.1 Materials and instruments

Materials and instruments include experimental rats that were purchased from the animal room of Gannan Medical University, PE labeled rabbit anti-intestinal fatty acid binding protein antibody (Shanghai Anyan), centrifuge (LC-4012), microplate reader (SK202, Shenzhen, Sinotherink Company), biochemical analyzer (BECKMAN COULTER, AU680), electron microscope (OLYMPUS, CX31), and fluorescence microscopy (OLYMPUS, CX31).

2.2 Grouping of experimental animals and establishment of intestinal ischemia models

96 healthy SD rats (half male and half female) were randomly divided into two groups (the experimental group and control group), with 48 rats in each group. The two groups were randomly subdivided into 6 groups (I, II, III, IV, V, VI), with 8 rats in each group. The intestinal ischemia model was established by ligating the superior mesenteric artery in the experimental group, and the peritoneal switch operation was performed in the control group.

2.3 Blood samples being collected and assayed

3 mL of venous blood samples were extracted from each group rats’ right ventricle with sterile syringes at 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h after the operation. The supernatant was taken after centrifugation at 3000 r·min\(^{-1}\) for 10 min at 4 ℃, and serum I-FABP concentration was measured by ELISA.

2.4 Staining intestinal tissue by HE staining and staining I-FABP by immunofluorescence staining

Experimental animals in each group were killed immediately after blood collection, the diseased intestinal tubes were cut, then fixed, embedded, sectioned, HE stained, and intestinal pathological changes were observed by 400-fold microscopy. Sections were prepared in the same method, dewaxed, and antigen repaired; and I-FABP in intestinal tissue was stained by direct immunofluorescence staining of PE-labeled rabbit anti-I-FABP antibody. At the same time, a blank control group was also set and I-FABP expression in intestinal tissue at different periods of time of intestinal ischemia under a 100 x fluorescence microscope. The acquired images were analyzed by the Image-pro plus 6.0 software and the number of positive particles was calculated.

2.5 Statistical method

Statistics data were processed and analyzed by using the SPSS 22.0 statistical software. The measuring data of each group were shown as $\bar{x} \pm s$. One-way ANOVA was used for comparisons between groups, and SNK-q test for pairwise comparisons between groups, $P < 0.05$ was statistically significant.

3. Results

3.1 Measurement of serum I-FABP concentration in each group

The changes of serum I-FABP concentration in
each group are shown in Table 1. It began to rise at 0.5 h and reached the peak at 1 h ($P < 0.05$). Then, with the lasting of ischemia, the I-FABP concentration gradually decreased.

**Table 1.** Changes of serum I-FABP concentration in each group /μg·L$^{-1}$. $x \pm s$

| Time                        | Experimen-
|-----------------------------|--------------|
| Superior mesenteric artery | 182.63 ± 52.54 | 64.56 ± 29.36 |
| ligation for 0.5 h (I)      | 290.24 ± 50.10 | 156.69 ± 37.48 |
| Superior mesenteric artery | 251.91 ± 41.83 | 27.6 < 0.05   |
| ligation for 1 h (II)       | 109.62 ± 20.77 | 83 0.001      |
| Superior mesenteric artery | 228.09 ± 33.53 |             |
| ligation for 4 h (IV)       | 99.29 ± 7.06  |             |
| Superior mesenteric artery | 191.29 ± 35.74 |             |
| ligation for 8 h (V)        | 85.64 ± 12.14 |             |
| Superior mesenteric artery | 176.19 ± 51.48 |             |
| ligation for 12 h (VI)      | 86.02 ± 30.63 |             |

Note: * Comparison of each experimental group and control group, $P < 0.05$; @ comparison of II and I, $P < 0.05$; # comparison of III and II, $P < 0.05$; ※ comparison of IV and III, $P < 0.05$; ※※ comparison of V and VI, $P < 0.05$.

3.2 Analysis of immunofluorescence staining images and statistical analysis

The number of I-FABP positive particles in each experimental group and control group is shown in Table 2. Compared with the control group, the number of I-FABP positive particles in each experimental group was significantly higher than that in the control group within 2 hours of intestinal ischemia. After 2 hours, the number of I-FABP positive particles gradually decreased and significantly lower than that in the control group, the difference was statistically significant ($P < 0.05$).

**Table 2.** Expression of I-FABP in ischemic intestinal tissue/$x \pm s$

| Time                        | Experimen-
|-----------------------------|--------------|
| Superior mesenteric artery | 451 ± 34     | 323 ± 36 |
| ligation for 0.5 h (I)      | 705 ± 71     | 328 ± 40 |
| Superior mesenteric artery | 441 ± 35     | 329 ± 23  |
| ligation for 1 h (II)       | 273 ± 37     | 323 ± 23 |
| Superior mesenteric artery | 224 ± 31     | 341 ± 32 |
| ligation for 2h (III)       | 161 ± 15     | 375 ± 23 |

Note: * Comparison of each experimental group and control group, $P < 0.05$; @ comparison of II and I, $P < 0.05$; # comparison of III and II, $P < 0.05$; ※※ comparison of IV and III, $P < 0.05$; ※※※ comparison of V and VI, $P < 0.05$.

3.3 Intestinal histopathology

HE staining of rat intestine at various periods of time of ischemia is shown in Figure 1.

- a: In the control group, the villi of small intestine were arranged orderly without obvious necrosis and abscission. b: After intestinal ischemia for 0.5 h, the villi of small intestine began to fall off. c: After intestinal ischemia for 1 h, there was partial and obvious necrosis of the villus of the small intestine. d: After intestinal ischemia for 2 h, most of the small intestinal villi were necrotic and exfoliated, and the normal villi structure was rare. e: After intestinal ischemia for 4 h, small intestinal villus was almost necrotic, the normal villus structure was rare, and there was no obvious necrosis of the muscular layer. f: After intestinal ischemia for 8 h, small intestinal villus and gland were necrotic, the blood vessels of mesentery were dilated and congested, with obvious bleeding, and the muscular layer was necrotic. g: After intestinal ischemia for 12 h, intestinal tissue cells dissolved.

**Figure 1.** HE staining of rat intestine at each time point (×400).

- 0: Blank group: no positive staining was found. A: In the control group, more positive particles were found in the intestinal tissue. I-FABP was mainly expressed in the intestinal mucosal epithelial villi, and a small amount was also expressed in the intestinal submucosa and even the muscular layer. B: After intestinal ischemia for 0.5 h, the morphology of intestinal wall was normal. I-FABP positive particles increased in intestinal mucosal glands and intestinal wall muscular layers, especially granular fluorescence was obvious in intestinal wall. C: After intestinal ischemia for 1 h, the morphology of intestinal wall was basically normal. I-FABP positive granular fluorescence was found in intestinal mucosal glands and intestinal wall muscular layers, with the most uniform distribution. D: After intestinal ischemia for 2 h, I-FABP positive particles were less than those of C. E: After intestinal ischemia for 4 h, the positive particles of I-FABP were less than those of D. F: After 8 h of intestinal ischemia, I-FABP positive particles were scattered in intestinal tissue and lumen, and the number was lower than that of E. G: After intestinal ischemia for 12 h, intestinal tissue cells dissolved. A small amount of I-FABP positive particles were scattered among necrotic tissue, and the number was significantly lower than that of F.

**Figure 2.** Expression of intestinal tissue in each group (×400).
3.4 Observation of the immunofluorescence staining images for I-FABP

As observed under a 100 x fluorescence microscope, PE produced an emission fluorescence wavelength of 450 nm with positive I-FABP staining in orange. See Figure 2.

4. Discussion

AII is divided into two main categories according to the etiology[6]. The first category is ischemia caused by intravascular factors, such as mesenteric arteriovenous embolism and thrombosis, also known as acute mesenteric ischemic syndrome (AMIS). The second type is the disorder of intestinal blood supply caused by some factors other than blood vessels, such as volvulus, intussusception and incarcerated inguinal hernia, that is, strangulated mechanical small bowel obstruction (SMSBO). Although there are many and different causes of AII, the ischemic changes of the intestinal canal itself are basically the same, that is, a process from ischemia to necrosis. In the early stage of ischemia, the histopathology of intestinal tissue mainly showed the damage of intestinal wall glandular duct, the abscission of intestinal mucosal epithelial cells, edema, inflammatory cell infiltration and so on[7]. After stopping the blood supply for several minutes, the intestinal mucosa will suffer ischemic injury. With the extension of ischemic time, the epithelial cells of intestinal mucosa will fall off obviously, the normal villus structure is rare, and myometrial necrosis will occur, resulting in cell lysis and changes in the tissue structure of intestinal canal.

I-FABP is a water-soluble protein that is relatively stable to heat. Its molecular weight is small, about 12–15 KD[8]. It is abundant in the intestine and mainly exists at the top of intestinal mucosal epithelial villi[9]. Its main function is to regulate fatty acid metabolism. Intestinal bile acid salts and trypsin emulsify and decompose food derived lipids into medium and short chain fatty acids and long chain fatty acids. The former is absorbed by intestinal epithelial cells in a diffusion manner. Long chain fatty acids (C16–20) combining with I-FABP are targeted and transported to intracellular mito-

chondria, endoplasmic reticulum and other places to participate in lipid synthesis and decomposition in the body[10–12]. The main metabolic pathway of I-FABP is glomerular filtration and it is removed from the body by the kidney. Its half-life period is 11 min. Therefore, I-FABP can also be detected through urine[13]. Under normal circumstances, the concentration of I-FABP in peripheral blood is very low and can hardly be detected. Because the blood flow at the intestinal villi is a countercurrent exchange mechanism, it is most difficult to tolerate ischemia, hypoxia and other injuries. In the early stage of intestinal ischemia, the oxygen partial pressure at the top of intestinal villi is significantly reduced, resulting in ischemic villi at the top of intestinal villi, and the I-FABP in it can pass through the cell membrane earlier, capillaries, lymphatic capillaries and portal veins to enter the blood circulation, as well as the intestinal cavity and abdominal cavity[14].

It was found that the concentration of serum I-FABP increased significantly at 0.5 h of intestinal ischemia, reached the peak at 1 h of intestinal ischemia, and then decreased gradually with the extension of ischemia time. It is mainly due to the continuous destruction of intestinal mucosal epithelial villi and the release of a large amount of I-FABP to peripheral blood in the early stage of intestinal ischemia (<1 h). 1 h after intestinal ischemia, the epithelial villi of intestinal mucosa have been largely destroyed, and the I-FABP released to peripheral blood gradually decreases. At the same time, due to the short half-life period of I-FABP and its metabolism through the kidney, it is continuously consumed, so that the concentration of I-FABP in peripheral blood continuously decreases with the extension of ischemia time. At the same time, immunofluorescence showed that after PE labeling with I-FABP antibody, I-FABP was mainly expressed in intestinal mucosal epithelial villi, and a small amount was also expressed in intestinal submucosa and even the muscular layer. Within 1 h of intestinal ischemia, the expression of I-FABP positive particles in intestinal tissue gradually increased. Considering that in acute intestinal ischemia, intestinal tissue mainly mobilizes and uses fatty acids for energy supply, the transport and metabolism of fatty
acids need to be combined with FABP, so as to indirectly activate FABP in tissue. At the same time, the fatty acids entering the cell are transported to the nucleus after binding with FABP, and then bind with the fatty acid activation receptor in the nucleus to activate the downstream nuclear factor signal transduction pathway, so as to regulate the synthesis and expression of intracellular FABP. Further increase. When ischemia reached 1 h, the number of I-FABP positive particles in intestinal tissue was the largest, which further verified that the damaged intestinal mucosal tissue released the most I-FABP. After 1 h, with the extension of intestinal ischemia time, the number of I-FABP positive particles in intestinal tissue gradually decreased, the normal villi gradually decreased or even disappeared, the intestinal mucosal epithelial villi had been largely destroyed, and the mucosal epithelial cells necrotized and dissolved.

In conclusion, I-FABP mainly exists in intestinal mucosal epithelial cells at ordinary times. In the early stage of acute ischemia, I-FABP is rapidly expressed, released into intestinal wall tissue and intestinal cavity, and absorbed into blood. When the serum I-FABP concentration reaches the peak, the intestinal mucosal epithelial villi have been seriously damaged. At this time, the ischemic damage may have reached the submucosa. Clinicians should consider surgical treatment. This suggests that I-FABP can not only be used for the early diagnosis of intestinal ischemia, but also play a guiding role in the treatment of intestinal ischemia. Therefore, I-FABP has certain clinical significance in the early diagnosis and treatment of acute intestinal ischemia.

**Conflict of interest**

The authors declare no potential conflicts of interest.

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