Alteration of the Liver in Rats with Experimental Dysbiosis

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We evaluated the relationship between pathological changes in the liver and the state of intestinal microflora in rats with experimental dysbiosis. Changes in the intestinal microflora were accompanied by alteration of the morphological structure in the liver. Enhanced proliferation of Ito cells served as an indirect evidence of damage to the liver. Ito cells did not undergo transformation into myofibroblasts that excluded the possibility of fibrosis.

Key Words: *dysbiosis; kanamycin; liver; Ito cells; regeneration*

Close anatomical and functional relationship between the liver and intestine suggests that damage to the liver of different etiology would be accompanied by functional changes in the intestine. Acute hepatic failure resulting from toxic damage, hepatitis of various etiologies, and resection is followed by qualitative and quantitative changes in the intestinal microflora and bacterial translocation from the intestine. Under these conditions bacteria are found in the portal and systemic circulation, mesenteric lymph nodes, and liver tissue [6,8].

At the same time, qualitative changes in the intestinal microflora and excessive growth of bacteria can provoke bacterial translocation and liver dysfunction [3,9]. The development of hepatobiliary complications after intestinal inflammation is related to high permeability of the intestinal wall and endotoxemia [9].

Here we evaluated the relationship between liver functions and the state of intestinal microflora in rats with experimental dysbiosis. We determined the composition of the microflora in the small and large intestine and estimated morphological characteristics of the liver in rats with experimental dysbiosis.

MATERIALS AND METHODS

Experiments were performed on 18 Wistar rats. Intestinal dysbiosis in experimental animals was induced by kanamycin sulfate (1.4 mg/100 g body weight, though a gastric tube, daily for 2 weeks). Samples were taken 24 h after the last administration and 2 weeks after the course of treatment. The control group included intact rats (n=6) receiving placebo (physiological saline) through a tube.

We found no published data on hepatotoxicity of kanamycin and hypothesized that this substance directly affects intestinal microflora [1].

The rats were decapitated under ether anesthesia. We examined the microflora of the small and large intestine, mesenteric lymph nodes, liver, and portal and systemic circulation (heart chambers) and evaluated morphological characteristics of the liver.

The blood from the heart and liver homogenate (taken from the portal zone) was inoculated into a double medium (glucose agar slants with meat-peptone broth (1:10) and incubated at 37°C for 10 days with periodic reinoculations into 5% blood agar. Homogenates of the liver, mesenteric lymph nodes, and content of the small and large intestine were emulsified 1:40 in physiological saline. After 15-min sedimentation at 20°C the supernatant was placed on dense nutrient media (5% blood agar and Endo medium). The content of the small and large intestine in increasing dilutions (1:10-1:1000, 0.1 ml) was maintained in tubes with regenerated Wilson—Blair medium. Inoculate were grown at 37°C for 18-24 h. The cultures were identified by routine methods. The sensitivity of microorganisms from the intestine of healthy rats to kanamycin was determined by the disc method.

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Liver samples from various regions were taken for morphological examination. Paraffin sections were stained with hematoxylin and eosin. The presence and severity of periportal damage, centrolobular necroses, and inflammatory infiltration were evaluated and the index of histological activity (IHA) was calculated as described by Knodell *et al*. (1981). Immunohistochemical staining (LSAB-Kit, Dako) was performed with antibodies to desmin (resting Ito cell marker), α-isoform of smooth muscle antigen (α-SMA), proliferating cell nuclear antigen (PCNA, proliferating cell marker, Dako).

RESULTS

The small intestine in healthy rats contained *E. coli* (10^3-10^5 CFU/g), *E. agglomerans* (0-10^4 CFU/g), bacteria of genera *Moraxella* (10^5-10^8 CFU/g) and *Alcaligenes* (0-10^6 CFU/g), *Staphylococcus saprophiticus* (10^2-10^3 CFU/g), and *Streptococcus hemolyticus* (10^4 CFU/g). The large intestine contained *E. coli* (10^3-10^7 CFU/g), *E. agglomerans* (10^5-10^9 CFU/g), bacteria of genera *Moraxella* (10^8-10^{10} CFU/g), *Citrobacter* (10^4-10^5 CFU/g), *Alcaligenes* (10^6 CFU/g), *Corynebacterium* (10^6 CFU/g), and *Clostridium* (10^6 CFU/g), and staphylococci (10^2-10^7 CFU/g).

In vitro culturing confirmed sensitivity of isolated cultures to kanamycin.

Study of the microflora from healthy animals did not reveal bacteria in the systemic circulation, blood of the portal vein, liver parenchyma, and mesenteric lymph nodes.

Kanamycin treatment affected the composition of the intestinal microflora: after two weeks the population diversity of bacteria decreased. Microorganisms of 1-3 taxonomic groups were found in the small intestine and 1-2 taxonomic groups in the large intestine. After administration of kanamycin *Enterobacter agglomerans* were revealed in the liver tissue (10^7 CFU/g, 1 rat) and mesenteric lymph nodes (10^4 CFU/g, 1 rat). These data indicate that kanamycin treatment for 2 weeks produces qualitative and quantitative changes in the intestinal microflora. The appearance of microorganisms in the liver tissue and mesenteric lymph nodes reflects bacterial translocation through the intestinal wall and development of bacteremia.

Histological characteristics of liver tissue from control rats corresponded to normal. IHA was 1-3 points. Immunohistochemical staining with antibodies against desmin revealed single desmin-positive Ito cells (Fig. 1, *a*). Staining with anti-PCNA antibodies demonstrated the presence of single proliferating cells (Fig. 1, *b*). The myofibroblast marker α-SMA was detected in cells of portal vessels, but not in the parenchyma (Fig. 1, *c*).

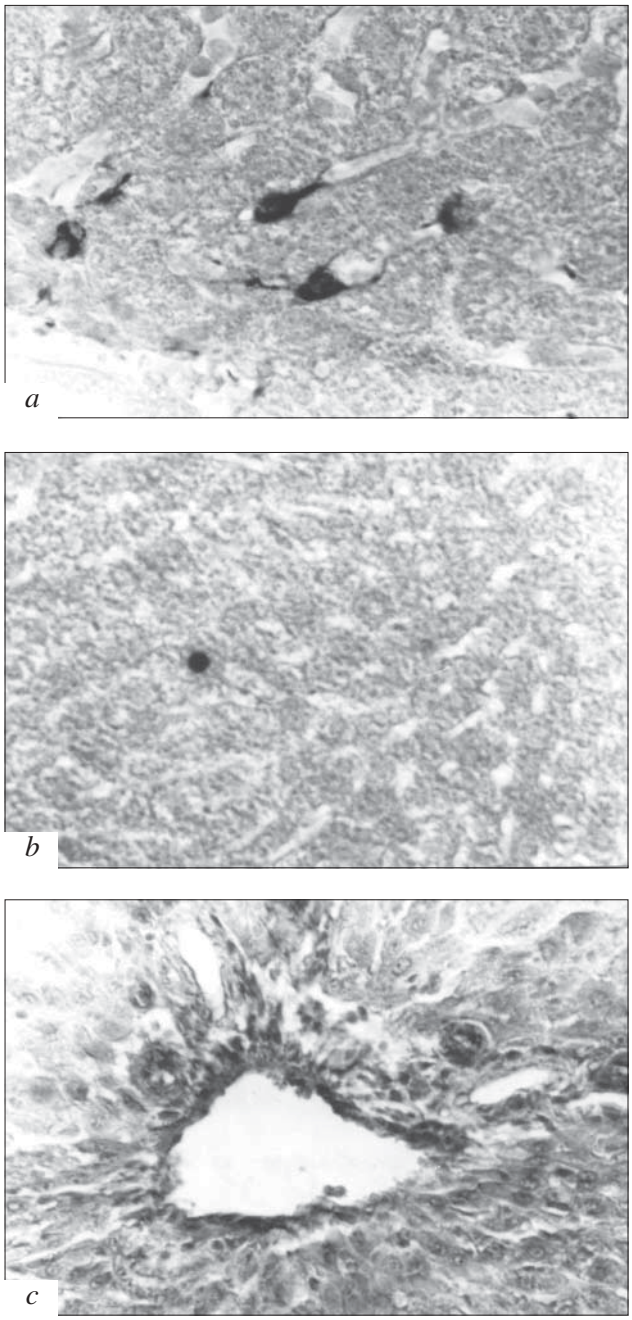

Fig. 1. Rat liver under normal conditions. Single desmin-positive Ito cells with periportal localization, staining with antibodies to desmin (×400, *a*). Single PCNA-positive cells, staining with antibodies to PCNA (×200, *b*). Smooth muscle cells in portal vessels, staining with antibodies to α-SMA (×200, *c*).

Two-week treatment with kanamycin led to the development of intralobular degeneration of hepatocytes, inflammation in portal tracts, and periportal piecemeal and bridging necroses. IHA was 3-4 points (average 3.7 points). It was primarily related to changes in the pericentral zone. Histological characteristics of the liver remained practically unchanged 2 weeks after a 2-week course of kanamycin administration.

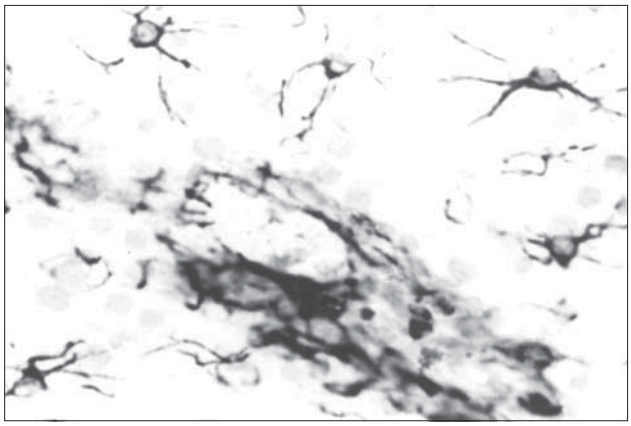

Fig. 2. Rat liver after 2-week treatment with kanamycin. The count of desmin-positive Ito cells slightly increased. Staining with antibodies to desmin, ×400.

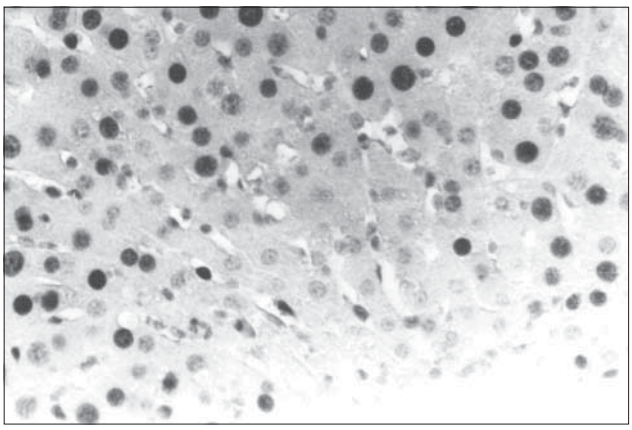

Fig. 3. Rat liver after 2-week treatment with kanamycin. Increased count of proliferating cells. Staining with antibodies to PCNA, ×200.

IHA was 2-5 points (average 3.3 points) due to pericentral damage. We revealed permanent damage to the liver tissue. Probably, a 2-week period is insufficient for normalization of the intestinal microflora and structural recovery of the liver during intestinal dysbiosis.

The count of desmin-positive Ito cells surpassed the control after 2-week treatment with kanamycin and remained unchanged over the next 2 weeks (Fig. 2). We found no cells stained with antibodies to α-SMA. These data indicate that Ito cells did not undergo transdifferentiation into myofibroblasts.

Staining with anti-PCNA antibodies showed that kanamycin administration for 2 weeks sharply increases proliferative activity of liver cells (Fig. 3). The number of PCNA-positive cells did not differ from that observed after treatment with hepatotoxins (*e.g.*, CCl_4). The count of proliferating cells did not decrease over the next 2 weeks.

Thus, kanamycin administration for 2 weeks induced damage to the liver parenchyma and led to activation of Ito cells. The increase in the count of desmin-positive cells is essential for liver recovery and reflects the development of compensatory and repair reactions. Ito cells are the main source of components of the intercellular matrix [10], stem cell factor [6], and hepatocyte growth factor [11,12]. Moreover, they play a role in the reparation [5] and differentiation of epitheliocytes in the liver [13].

Previous studies showed that single treatment with endotoxin activates Ito cells, but does not cause their transdifferentiation into myofibroblasts [2]. Endotoxin also can play an important role in the damage to the liver under conditions of kanamycin-induced intestinal dysbiosis. Signs of fibrosis were absent after single treatment with endotoxin [2] and after long-term endotoxemia caused by intestinal dysbiosis. Endotoxemia is a physiological process and of important pathogenetic value is activity of the endotoxin—antiendotoxin system, rather than blood endotoxin level.

Endotoxemia probably determines the release of cytokines necessary for liver regeneration from phagocytizing macrophages and monocytes [4]. Taking into account that hepatocyte proliferation is a typical reaction of the liver to damage, we hypothesize that intestinal dysbiosis leads to alteration of the liver parenchyma and activation of cell proliferation. Under conditions of liver damage associated with experimental dysbiosis, its regeneration occurs via proliferation of hepatocytes, while activation of Ito cells plays a less important role in this process. This excluded the possibility of rapid fibrosis.

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