Pathomorphological Changes in Organs of Rabbits with Experimental Colibacillosis

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Abstract

The aim of this research was to study pathomorphological alterations in the internal organs of rabbits through experimental reproduction of colibacillosis. The studies were conducted on 10 clinically healthy rabbits at the age of 3 months from a private farm. Animals were infected by intraperitoneal injection of a suspension of a pure culture of *Escherichia coli* isolated from a sand lizard. The disease of infected rabbits was accompanied by cachexia, dehydration and fever. The pathological process in the organism of infected animals was characterized by the violation of hemodynamics, the protein dystrophy of the parenchyma of liver, kidneys, myocardium and the formation of specific granulomas in the lungs and liver. The edema of reticular tissue and the hyperplasia of lymph nodes were observed in the organs of the immune system. The expressive edema of mucous membrane and submucosa, as well as alterative and necrotic processes were detected in the intestine.

Keywords: *Escherichia coli*, rabbits, necrosis, edema, mucous membrane.

Introduction

Escherichia coli is considered a conditionally pathogenic agent of the caudal section of the digestive tube of warm-blooded animals (World Health Organization, 2018). However, recent studies have shown that *E. coli* is the cause of enteritis and enteric diseases (Wasteson, 2002). In many developed countries, the colibacillosis of animals is closely monitored by doctors of veterinary and humane medicine, as well as by the World Health Organization, since *E. coli* plays an important role in human infectious diseases, which releases enterotoxins, the reservoir of which are farm animals (Gomi *et al.*, 2001; Thielman and Guerrant, 2004).

Pathogenic *E. coli* is excreted from sick, as well as from healthy humans, animals (domestic and wild), reptiles, birds (domestic and wild), fish and

other aquatic animals more often than other types of microorganisms throughout the globe (Sinha *et al.*, 2014).

In the diagnostic work of bacteriological laboratories, it is often necessary to resort to infection of experimental animals in order to isolate a pure culture of *E. coli* from a mixture with saprophytic species and to determine the pathogenicity of the microorganism. Laboratory animals such as guinea pigs, mice, and rabbits are sensitive to infection with *E. coli*. In experimental conditions, after infection with *E. coli*, laboratory animals die within 2 days, and sometimes after several hours with signs of severe diarrhea and an increase in body temperature (Zharov, 1994). The pathogenic effect of microbes is manifested both with intraperitoneal and subcutaneous infection. Studies have shown that erosive and necrotizing

enterocolitis, proliferative tubular necrosis and glomerulonephritis have been found in rabbits in experimental infection of *E. coli* (Coussement *et al.*, 1984; García *et al.*, 2002; Swennes *et al.*, 2012).

The aim of our research is to study pathomorphological changes in the rabbits' organism through experimental reproduction of colibacillosis using a suspension of pure culture of *Escherichia coli* isolated from a sand lizard.

Materials and methods

The experiment was performed on 10 clinically healthy rabbits of a private farm aged 3 months weighing 2200 - 2500 g.

Rabbits were infected with CTM - 3 strain of *Escherichia coli* microorganism deposited in the Depository of the State Research and Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine (Skrypka *et al.*, 2017). The CTM - 3 strain of *Escherichia coli* microorganism was isolated from the small intestine content of the sand lizard (*Lacerta agilis*), which was caught in Poltava in the private sector.

Laboratory animals of the experimental group (n = 5) were administered intraperitoneally suspension of CTM - 3 strain pure culture of *Escherichia coli* in the amount of 5×10^9 microbial bodies in 1 ml per 1 kg of body weight (Gosmanov *et al.*, 2017). Animals in the control group (n = 5) were administered 0.9% sterile NaCl solution intraperitoneally. The rabbits were supervised for 21 days. After infection, all laboratory animals were examined daily for clinical signs. At this, the animals' clinical condition, activity, eating food, presence or absence of thirst, diarrhea, hemorrhagic phenomena, hemolytic-uremic syndrome were taken into account.

Autopsy and histological examination were performed according to generally accepted methods (Zon *et al.*, 2010; Goralskiy *et al.*, 2011). Fragments of internal organs were fixed in 10% neutral formalin solution. The material was then dehydrated in ethyl alcohols of increasing strength and impregnated with consolidating solutions. After that, the pieces of organs were embedded into paraffin and paraffin blocks were made. Paraffin sections were cut to a thickness of 3–10 μ m with MS - 2 sliding microtome. Histological sections were stained with hematoxylin and eosin. Study of microscopic changes in tissues was performed using Micromed XS - 5520 microscope. Histologi-

cal specimens were photographed with CCD Micromed 5.0 Mpix video camera.

After pathological autopsy of the experimental group animals, bacteriological studies were performed to detect *Escherichia coli* in the internal organs. Sampling of the material for bacteriological studies was performed according to conventional methods (Birger *et al.*, 1982). Bacterial inoculation was made of the heart, lungs, liver, kidneys, spleen, lymph nodes, and the contents of the gastrointestinal tract was also inoculated. For studies, the conventional nutrient media meat-peptone broth (MPB) and meat infusion agar (MIA) were used with subsequent transfer of colonies to selective nutrient media (Endo agar, bile glucose agar). Smears were prepared of daily broth and agar cultures, stained by the Gram method and examined in a light microscope field. The culture properties were studied by culturing in liquid and dense nutrient media at 37°C for 24 - 48 hours. Biochemical properties were determined by culturing in Phenol Red Broth Base liquid nutrient medium with addition of carbohydrates. The incubation was performed at 37 °C for 48 hours.

Experimental animals were treated in compliance with the "European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes" (Strasbourg, 1986) and the Directive of the European Parliament and of the Council of Europe (/2010 / 63 / EU of 22.09.2010).

Results and discussions

Infection of the experimental group rabbits with *Escherichia coli* CTM - 3 strain was accompanied by the development of severe generalized infection clinical signs: the animals were starving, there was weight loss, apathy, opaque urine, frequent urination, shortness of breath, diarrhea, fever ($40.05 \pm 0.21^\circ\text{C}$) were observed. High pathogenicity of the pathogen led to the death of 2 animals on the 2nd day of the experiment, 3 animals perished on the 4th day.

Signs of dehydration, dryness of visible mucous membranes, contamination of hair around the anus and hind limbs with watery-mucous feces were registered in the experimental animals.

The pathological autopsy revealed that in laboratory animals the lungs had an uneven color, contained foci of edema colored dark red. Light pink foamy liquid (transudate) flowed from the

surface of the edema areas incision (from the lumen of alveoli and bronchi).

The heart was enlarged in volume due to dilation of the right ventricle or all parts lumen. The heart muscle had a dull, uneven light red color with gray-pink foci, and spotted and striped hemorrhages were recorded under the endocardium in two animals.

The liver was moderately enlarged, flabby, clay-colored, the incision surface was dull, with spotting hemorrhages under the capsule. In some animals, with acute dilation of the heart, the liver became dark red colored.

The intestinal mucosa was thickened, unevenly red colored with hemorrhages. In the intestinal lumen of two animals serous-hemorrhagic exudate was found, and in others it was catarrhal.

Cranial mesenteric lymph nodes were enlarged, uneven in color, full-blooded, the incision surface was highly moistened, unevenly red colored.

Vessels of the omentum, mesentery, intestinal serous membrane, liver, kidneys and spleen had blood filling above moderate.

Histological examination of the myocardium, kidneys and liver registered signs of granular dystrophy. In the heart muscle, focal necrosis of cardiomyocytes was found, the nuclei of the latter

having signs of pyknosis, some of them having with signs of lysis. Minor hemorrhages were registered.

In the liver, dystrophic processes were more pronounced in the central areas, which led to an impairment of the hepatocytes' lobular-radial localization. In large areas, hepatocytes showed signs of hydropic and granular dystrophy: the cytoplasm acquired a diffuse eosinophilic color or cobweb-like appearance, cells and their nuclei being increased in volume. Individual cells had the form of hollow structures with a nucleus (Figure 1.). Rounded infiltrates were formed in different parts of the organ (Figure 1 A.). Blood filling in vessels of various calibers, small infiltrates which mainly consisted of granulocytes (Figure 1 B.) were revealed. A pronounced edema of the portal tracts' connective tissue was registered in the infiltration foci, which led to a volume increase of these areas.

In the kidneys there were processes of hemodynamic and protein metabolism disorders. Acute dilation of the heart was characterized by blood filling in the capillaries of the stroma in the cortical and medullar parts of the organ. The cytoplasm of epitheliocytes in the proximal parts of the convoluted tubules contained a fine granular protein mass, having a rich red-pink color. The cells were enlarged in size and advanced

Figure 1. Fragment of rabbit liver histological specimen in experimental colibacillosis; hematoxylin-eosin stain; A: white arrow – protein dysfunction of hepatocytes; yellow arrow – granuloma; 400x; B: blue arrow – blood filling of the vessel; orange arrow – infiltrates of granulocytes. 640x.

into a narrowed lumen of the tubules, a violation of the cell membrane integrity was recorded. In the wall of the cross-sectioned tubules, in 2-3 nephrocytes the nuclei acquired a dark blue color; in other cells the karyoplasm had a moderate basophilia. The cytoplasm of vascular glomerular

endotheliocytes acquired signs of hydropic dystrophy, eosinophilicity of endotheliocytes in individual loops of vascular glomeruli, formation of microthrombi was observed.

Histological examination of the lungs revealed an above-moderate filling of blood vessels and

Figure 2. A fragment of the rabbit's lungs histological specimen in experimental colibacillosis; hematoxylin-eosin stain; blue arrow – blood filling of a medium caliber blood vessel; green arrow – perivascular infiltration; red arrow – blood filling in the aero-hematic barrier capillaries. 640x.

Figure 3. A fragment of the rabbit's lungs histological specimen in experimental colibacillosis; hematoxylin-eosin stain; A: red arrow – hyperplasia of the bronchus lymphoid nodule; 400x; B: blue arrow – blood filling of a medium caliber blood vessels; violet arrow – granuloma. 100x.

capillaries with migration of blood cells' nuclear forms outside the vessels, which led to the formation of perivascular infiltrates (Figure 2). Granulocytes, namely neutrophils, monocytes, macrophages and eosinophils were observed in the infiltrates.

In addition, hyperplasia of the bronchi lymphoid nodules was characteristic (Figure 3 A). Due to the capillaries blood filling, as well as to edema and cellular infiltration, there was thickening of the interalveolar septa. The thickening degree is different in various areas. The lumens of the alveoli were reduced in volume, from round to slit-shaped ones (Figure 3 B). In the lumen of the alveoli and bronchi, a small amount of serous exudate with macrophages was observed, macrophages being located singly and in groups of 3 - 5 cells. Thinning of the alveoli walls, signs of alveolar emphysema were found around the lesions foci.

In the spleen the pronounced division into red and white pulp was registered. Lymph nodules were densely populated with lymphocytes, clearly contoured. In areas with signs of serous edema in the lymph nodules there were small foci in which there were no lymphocytes, skeleton of reticular tissue being traced. The red pulp in the edema areas had a sparse localization of erythrocytes, accumulation of granulocytes (neutrophils, macrophages, single eosinophils). Reticulocytes with signs of vacuolation and lysis were found in both white and red pulp. In other areas of the spleen, large, indistinct conglomerates of lymphoid nodules and inflammatory infiltrates consisting of lymphocytes, neutrophils, macrophages, and single eosinophils were recorded.

Examination of the lymph nodes revealed well-developed lymph nodules of the cortex, the latter were enlarged, densely populated with lymphocytes. Hyperplasia of the lymph nodules led to their merging into large conglomerates on the periphery of the organ. Closer to the central part of the organ, the immune formations were clearly separated, the germinal centers were not expressed. In the medullar substance, stromal edema led to the loss of tissue, histoarchitectonics, reticulocytes had signs of vacuolation, medullary cords in the areas of edema were not observed. B-lymphocytes, macrophages and plasma cells acquired a sparse chaotic localization, clusters of granulocytes (neutrophils, eosinophils, macrophages), edema of trabeculae, blood vessel

walls, vacuolation of endothelial cells were registered.

Histological examination of the gastric wall revealed edema with impairment of the histoarchitectonics in all layers of the mucous membrane. Edema of the submucosal base was more pronounced, and edema of the connective tissue in the muscular layer was moderately pronounced. The connective tissue components were sparsely localized, the cells have lost their structure. The epithelium had the form of a shapeless homogeneous mass, in which fragments of nuclei were traced. The glands were enlarged and had the appearance of indistinct, translucent formations due to vacuolation of secretory cells.

In the small intestine there was a pronounced edema of the mucous membrane, destruction of microvilli in the apical part of the epitheliocytes, and complete destruction of the villi epithelium. In large areas, the epithelium showed signs of dystrophy and necrosis, eosinophilic dendritic masses were registered, in the periphery of which inflammatory infiltrates were located. Specific weight of infiltrates was occupied by granulocytes.

Significant alteration processes took place in the wall of the large intestine, the cytoplasm of epitheliocytes became eosinophilic, nuclei had signs of pyknosis, karyorexis, many cells were destroyed, macrophages were found in areas of necrosis. In some areas, the destruction of the mucous membrane reached the muscle plate. Hyperplasia of lymphoid nodules, leukocyte infiltrates of the mucous membrane were revealed. The edema covered the entire thickness of the mucous membrane.

Bacteriological examination revealed massive ingress of the experimental animals' internal organs with *Escherichia coli*. The isolated culture grew well on simple MPB and MIA media and caused their diffuse turbidity. *Escherichia coli* caused a small gray precipitate in the MPB medium, which was easily destroyed. There was no film on the broth surface. When culturing in the MIA, the colonies were transparent with a grayish-blue tinge and easily merged with each other. Flat red colonies with a gray sheen were observed in the Endo medium. Rounded mucus colonies with areas of hemolysis were found in blood agar. Gram-negative, small, straight rods that formed indole, did not break down urea, fermented mannitol, and fermented lactose were found in the

stained smears. The biological properties of the microorganism's isolated culture coincided with the test culture.

Thus, the results of our previous studies and the results of current studies show that in the white mice and in the rabbit body, in bioassays using a suspension of pure culture of the CTM - 3 *Escherichia coli* strain isolated from the sand lizard, pathomorphological changes had a similar manifestation. Thus, in the lungs of the both species animals, hyperemia of the alveolar-capillary block, formation of vascular sheathing around large vessels, formation of specific granulomas were registered. It should be noted that in rabbits, in contrast to mice, the processes of alveoli walls and interalveolar tissue cellular infiltration were more pronounced, while in mice there were more pronounced exudative processes with the formation of exudate in the bronchial lumen. In both groups of animals, granulocytes predominated in inflammatory infiltrates during the bioassay (Tul *et al.*, 2016).

In the literature, there are reports of granulomatous lesions of parenchymal organs in colibacillosis in both poultry and wild birds, including during the bioassay (Pan American Health Organization, 2001; Panikar *et al.*, 2017; Panikar *et al.*, 2018).

According to other scientists, in rabbits, due to the toxic effects of *E. coli* on the body, inherent are protein dystrophy of the liver and kidney parenchyma, hemodynamic disorders, namely: hyperemia of internal organs, hemorrhages (Dvadnenko, 2012; Lateef *et al.*, 2018). We consider it necessary to add the edema of the connective tissue elements of the middle and posterior walls of the digestive tube to the above list, which coincides with the results of studies by other scientists (Pai *et al.*, 1986; Vachkov *et al.*, 2004).

Conclusion

The results of bioassay in rabbits using a pure culture suspension of *Escherichia coli* CTM - 3 strain isolated from the sand lizard, indicate high pathogenicity of the pathogen for laboratory animals and indicate that hemodynamic impairments, protein dystrophy of the liver, kidneys, myocardium parenchyma are the result of animal body's intoxication. In the immune system's organs (spleen, lymphoid nodes, lymphoid forma-

tions in the intestinal wall and bronchi) showed hyperplasia, reticular tissue edema, infiltrates of granulation cells (neutrophils, macrophages, eosinophils).

Migration of leukocytes outside the blood vessels led to the formation of perivascular infiltrates in the lungs and liver morphologically similar to granulomas. In the middle and posterior parts of the digestive tube, pathological processes were accompanied by edema and inflammatory infiltration of the mucous membrane. Alternative necrotic processes were more pronounced in the large intestine.

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