The effects of inactive toxins of Escherichia coli on hematological parameters in animals

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Materials and methods

Through different cultivation of toxigenic strains of the causative agent of E. coli in test tubes with 10 ml volume of nutrient broth under thermostat conditions, a complex preparation including inactivated exotoxins of E. coli (anatoxin) was acquired. Used a temperature at 37 °C (98.6 F) for 6-8 hours until a slight turbidity appeared, indicating the growth of microorganisms. At the end of the culturing period, the obtained cultures were transferred into 200-300 ml. flasks with nutrient broth and incubated in the growth chamber at 37 °C (98.6 F), while the TL- and TS-producing strains were incubated for 6 days and the STX-producing strain - for 7 days. Thereafter, broth cultures were inactivated by adding formalin to a concentration level of 0.3-0.4%. This procedure was performed for two weeks at 37 °C (98.6 F), and the microbial cultures were stirred twice daily. At the end of inactivation, the cultures were checked for sterility, their equal volumes were combined, and the culture medium and microbial mass were separated by sterilizing filtration. Consequently, the resultant complex solution was a clear liquid, light yellow in color, containing three types of inactivated enterotoxins.

After that, the prepared solution was poured into sterile vials under aseptic conditions and then capped with sterile rubber plugs and sealed with aluminum caps.
Materials and methods

In studying the influence of inactivated toxins on the hematological indices, laboratory white rats, weighing 200±10 g were used. These animals were categorized into five groups of seventy animals each by the paired analysis method. Group 1 rats served as negative controls and were whole. Group 2 animals were positive controls and were administered with sterile nutrient broth. The remaining animals were injected with escherichiosis toxoid in different doses: Group 3 rats received 0.15 cm³; Group 4 - 0.3 cm³; Group 5 - 0.6 cm³. After the drugs had been administered, blood was drawn from the lateral tail vein in 10 animals of each group respectively, 1, 3, 6, 12, 24, 72 and 168 hours using an injection needle and syringe for hematological analysis.

The blood test (the number of red blood cells, white blood cells, and hemoglobin) was performed on an Abacus-3 hematological analyzer. The leukogram was derived separately, according to which the leukocyte index of intoxication (LII) was calculated to assess the intensity of endotoxemia and the extent of inflammatory response.
Results and discussion

As a result, there was a change in the total number of white blood cells as against the registered leukopenia in animals. More specifically, a reduction in lymphocytes and a rise in neutrophil granulocytes were observed, but this process was different for animals from different experimental groups. If 85.0% of all leukocytes and 14.3% of neutrophils accounted for lymphocytes in the rats from the first group then, with the animals into which were injected anatoxin at doses of 0.15-0.3-0.6 ml from groups three through five, these cells amounted to 64.7; 67.0; 66.0 and 29.7; 29.3; 30.6% respectively.

The animals, after 3 hours of starting the experiment, exhibited an alteration in effect of toxoid on leukocytes. The drug dosages were of immense importance. Leukocytes in rats decreased by 0.7×109/l at an immunizing dose of 0.15 ml of toxoid, as compared to the initial study. Increasing the dose of the drug, however, had the opposite effect.

However, the number of lymphocytes in the leukograms of the rats from Groups 2 through 5 was still lower than that of the rats from Group 1. Thus, this difference was 10.2–12.7% in the Group 2 animals, 26.6–28.0% in Group 3, 20.6–30.0 % in Group 4, and 30–42.0 % in Group 5. The number of neutrophils, on the contrary, was higher. If in the Group 1 animals this leukocyte population was 13.7–15.3 %, in the Group 2 through 5 it was 24.3–26.7%, 41.3–43.3 %, 33.0–41.6 %, and 44.0–54.7 %, respectively.

During the first 12 hours of the study, animals of both the experimental and positive control groups were activated by physiologically mature neutrophils in the blood, whose main function is to effect a phagocytic reactions and antigen processing to other cells of the immune system.

Since anatoxin is a stronger antigen than nutrient broth, after its administration to animals, the production of segmented neutrophils in them was more active for 12 hours. Positive control animals had an enormous neutrophil count during the first 3 hours of the experiment.
Results and discussion

In 72 h after administration of anatoxin, the total number of leukocytes increased sharply in all lab animals. Thus, if in control group rats the number was at the level of 7.1±0.98 - 7.3±0.13-109/l, in experimental group rats it was 11.3±0.29-11.9±0.37-109/l. Deductions from the leukogram revealed that the observed leukocytosis occurred due to a rise in the number of lymphocytic cells.

The modifications witnessed during the first 24 hours in the hematological indicators in the experimental animals after they were administered with the inactivated mixture of enterotoxins, indicated a reasonably natural development of pathophysiological processes in the form of short-term moderate leukopenia, followed by leukocytosis with segmented nuclei dominating in the leukogram. After 72 hours, the effect was reversed: animals injected with anatoxin developed leukopenia with the lymphocytes taking prevalence in the white blood cell pool. The leukogram of experimental animals was homogenous to that of the control animals, after 168 hours of administering anatoxin, which may indicate the development of the effector stage of the immune response.

A complex vaccine preparation consisting of formalin-inactivated E. coli exotoxins (anatoxin) retains its antigenic and immunogenic properties and loses its cytotoxic functions. In the first 72 h in the blood of the immunized animals, moderate neutrophilia without nuclear shift was noticed at the initial stage, and then lymphocytosis. A rise in the immunizing dose of anatoxin adversely affected the hematological indicators in rats, which was expressed by an increase in the number of eosinophilic cells in the blood.