THE EFFECTIVENESS OF SORBED TRIANATOXIN IN COMBATING GAS GANGRENE IN MONKEYS (PRELIMINARY COMMUNICATION)

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During the last few years, a new preparation, sorbed trianatoxin for use in anaerobic infections has been developed in the Department of Traumatic Infection of the N. F. Gamaleya Institute of Epidemiology and Microbiology, AMN SSSR. Experiments on small laboratory animals and on volunteers have shown that this substance produces an effective immunity against gas gangrene.

A further study has been undertaken to determine the prophylactic effect of this preparation on apes, because they are animals which are the most closely related to human beings both anatomically and physiologically.

METHOD

The experiments were carried out on 18 macaque rhesus monkeys. Twelve of them were immunized with sorbed trianatoxin series No. 13, obtained from the Department of Traumatology of the N. F. Gamaleya Institute. One ml of trianatoxin contained 30 antigen units of Cl perfringens, 40 of Cl oedematiens, and 150 units of tetanus antigens. Two 1-ml injections were given into the left thigh at an interval of one month. After six months, a further injection of 1 ml of trianatoxin was given. Experiments were made to determine the change in the amount of antitoxin in the blood. On the 21st day after the last injection, the extent of the immunity was tested. After a single injection of trianatoxin, the amount of antitoxin present in the serum of the immunized monkeys on the 30th day was 0.1 antitoxic units (and in one case 0.25 units). On the 14th day, after the second injection, the titre of the antitoxin had increased from 0.25 to 2.5 antitoxic units. Six months after immunizing, it had fallen to 0.1 units or less. A further inoculation caused a renewed increase in the amount of antitoxin in the blood, and by the 14th day after this reinoculation it had reached 0.5 - 4 units; by the 21st day the titre of antitoxin had fallen to 0.25 - 2 units. A test of the induced immunity in 12 monkeys was made by infecting them with 3, 4, and 6 times the lethal dose of a culture. The lethal dose was first determined by injecting an 18-hour culture grown on Tarrotsi's medium into six animals. The culture was activated by the addition of 0.1 ml of a 50% solution of calcium chloride per ml, and was injected into the soft tissue of the thigh.

RESULTS

All the six nonimmunized animals infected with a culture of the gas gangrene organism sickened; five of the six which received from 0.5 to 2 ml of the culture died within 1 to 4 days, and the one which received 0.25 ml survived.

The affected animals showed a grave general deterioration, they were inert, and their temperature fell to 36 - 34°. At the site of the injection, there was a marked edema of the tissue, which became tense, and in the blood there was an increase in the number of leukocytes and in the red cell sedimentation rate. The animals finally died from heart failure. A post-mortem study at the site of the injection showed a considerable edema of the subcutaneous fat of the limbs, and a necrosis of the muscles of the thigh, parts of which were pultaceous.

Thus the absolute lethal dose for monkeys was 0.5 ml of an 18-hour culture of perfringens.

All the immunized monkeys reacted to infection by the development of generalized and local signs of gas gangrene. There was a transient failure of appetite, and the body temperature, unlike that of the control animals, was either temporarily raised, or else remained normal. In two animals there was a leukocytosis, and in all twelve the red cell sedimentation rate was increased. The site of the injection was infiltrated, and edema developed to various extents. By the 3 - 5th day the condition of the monkeys improved, the generalized symptoms had disappeared, the sedimentation rate had become normal, the infiltrated areas had softened, the edema had disappeared, and arteriovenous fistulas had developed. However, whatever the extent of the damage, all the ulcers were well granulated and healed within 2 - 3 weeks. All of the immunized animals recovered without treatment within 2 - 4 weeks from the time of infection. The most seriously affected animals were those infected with six times the lethal dose.

The process of infection and the subsequent development of the illness constituted a new and powerful antigenic stimulus which resulted in the production of considerable amounts of antitoxin in the immunized monkeys. Thus, by the seventh day, the amount of antitoxin produced increased to 9 units, and in some of the animals it rose to 25 antitoxic units between the 10th and the 19th days. It must be emphasized that the more severe the course of the illness, the greater was the production of the antitoxin.

Thus, after the development of generalized and local symptoms, all the immunized monkeys infected with gas gangrene recovered, whereas five of the control group died when infected with a dose of only 0.5 - 2 ml of a culture of Cl. perfringens. Of the six controls the only one to survive had received only half the lethal dose.

The degree of severity of illness which developed in the immunized monkeys was directly related to the size of the lethal cultures which were administered.

At the same time, the experiments showed that the presence in the blood of only 0.5 antitoxic units was sufficient to afford protection against an infection with a culture representing 3 to 6 times the lethal dose.

The results indicate that sorbed trianatoxin is an effective prophylactic preparation against gas gangrene induced by Cl. perfringens.

SUMMARY

Monkeys infected with 0.25 to 2 ml of an 18-hour culture of Cl. perfringens developed the general and local symptoms characteristic of gas gangrene. A dose of 0.5 ml of an 18-hour Cl. perfringens culture was absolutely lethal for monkeys. Immunization by sorbed trianatoxin, and subsequent reinoculation led to the accumulation of 1 to 4 units of Cl. perfringens antitoxin in the blood. Monkeys which were twice immunized with sorbed trianatoxin and subsequently reimmunized six months later were then able to survive infection with 3 to 6 times the lethal dose of the culture.