THE DETOXICATING ACTION OF ANTIBIOTICS OF THE TETRACYCLINE GROUP IN EXPERIMENTAL DYSENTERY TOXICOSIS

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(Received December 28, 1957. Presented by Active Member AMN SSSR G. P. Rudnev)

Numerous investigations by clinicians have shown that antibiotics have a marked effect in diminishing the toxicosis in a number of infectious diseases. Penicillin is known to have an effective action in infections caused by cocci and chloramphenicol and the tetracyclines in typhoid and typhus fevers and dysentery.

The detoxicating action of antibiotics, and in particular of the tetracyclines, in experimental conditions has been studied less thoroughlythan the various aspects of their influence on microorganisms and on experimental bacterial infections [1-6, 9-11, 13].

There is as yet no single terminology to describe the effect of antibiotics on toxicoses. This effect is called "antitoxic", "antidotal", "disintoxicating" and, finally,"detoxicating". The mechanism of the detoxicating action of antibiotics continues to be uncertain.

The aim of the present investigation was to determine the characteristic features of the detoxicating action of the tetracyclines in experimental dysentery toxicosis.

EXPERIMENTAL METHOD

Toxicosis was induced by injecting white mice, weighing 16-18 g, with complete antigen prepared from Shigella flexneri by the method of tryptic digestion, at at the Moscow Mechnikov Institute of Vaccines and Sera.

The antigen was injected intramuscularly into the hindlimb in a volume of 0.1 ml of physiological saline or intraperitoneally in 0.25 ml. The MLD and the LD were determined for each antigen series and for the different methods of injection. As a rule, the MLD for intraperitoneal injection was $\frac{1}{2}$ to $\frac{1}{3}$ that for intramuscular injection. A single injection of 1 MLD of toxin led to the death of 80-100 % of animals in the course of 24-48 hours.

EXPERIMENTAL RESULTS

Histological examinations showed that in mice, as in certain other laboratory animals, dysentery endotoxin causes the severest changes in the spinal cord. The time of appearance and the degree of these changes depended on the method of injection and the dose of the toxin (see also [7, 8, 12]).

After the intramuscular injection of 1 MLD of toxin, the first changes in the motor neurones appeared after an average of 1 hour, and after intraperitoneal injection, in 30 minutes. The first phase, in which no changes were yet to be found, was thus of very short duration.

The second phase, lasting 4-8 hours, was characterized by a gradual development of reversible reactive changes. At first these consisted of extension of the tigroid bodies, an increase in the dimensions of the nucleoli

TABLE 1

Influence of Tetracyclines on the Course of Experimental Dysentery Toxicosis

Preparation	Method of administration	Dose of antibiotic (mg per mouse)	Number of mice in group	Number of mice dying
Chlortetracycline	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	25	1
By mouth	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	30	3
Intramuscularly	Oxytetracycline	1.0	30	0
	Tetracycline	0.2	20	2
Control	—	—	40	30

TABLE 2

Relationship between the Detoxicating Effect and the Time of Administration of the Antibiotic and the Dose of Toxin

Dose of antigen (mg per mouse)	Dose of tetracycline (mg per mouse)	Interval bet. adminis. of antigen and antibiotic	Number of mice in group	Number of animals dying
2,0	1,0	4 hours	15	0
2,0	1,0	6 hours	15	0
2,0	—	—	10	3
4,0	1,0	4 hours	14	2
4,0	—	—	20	8
6,0	1,0	simultaneuosly	20	3
6,0	—	—	20	11
8,0	1,0	simultaneously	20	3
8,0	—	—	20	20

and a rise in the content of desoxyribonucleic acid in the nucleus. Reactive division of the nucleoli took place subsequently. As the result of partial tigrolysis, the tigroid was arranged mainly around the nucleus (Fig. 1).

The third phase of the process was characterized by rapidly progressive degenerative changes in the motor neurones (pyknosis and hyperchromatosis of the nucleid, increasing tigrolysis, going as far as complete disappearance of the tigroid, and a sharp reduction in the volume of the cell body, Fig. 2). Degenerative changes of this type occurred on a large scala, especially in the lumbar and cervical divisions of the spinal cord. Changes in a large number of motor neurones led to the rapid development of paralysis, mainly of the hindlimbs. In animals dying 24-48 hours after injection of toxin, signs of neuronophagia were found. Similar, but less pronounced changes took place in the medulla oblongata of all the animals which died. Additionally, irrespective of the time of death, the animals showed constantly a massive hyperemia of the internal organs (Fig. 3) and multiple areas of extravasation. In roughly $\frac{1}{3}$ of the mice dying on the 2nd-3rd day, inflammatory lesions developed in the intestine.

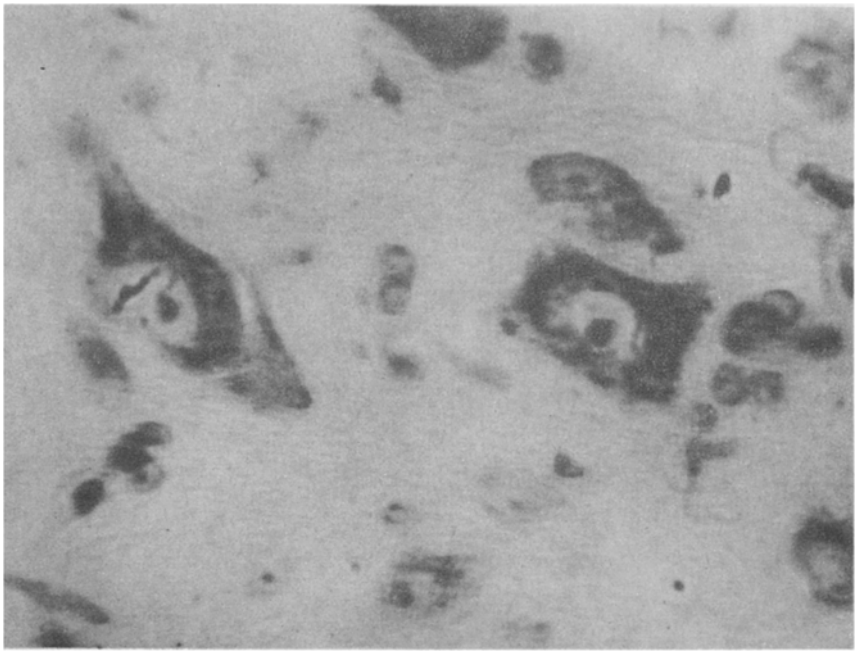

Fig. 1. Changes in the motor neurones of the lumbar division of the spinal cord of a mouse 6 hours after injection of 1 MLD of Flexner dysentery antigen. Tigrolysis of the neurone on the left, extension of the tigroid in the right neurone. Stained by Shabadash's method. Magnification 950 x.

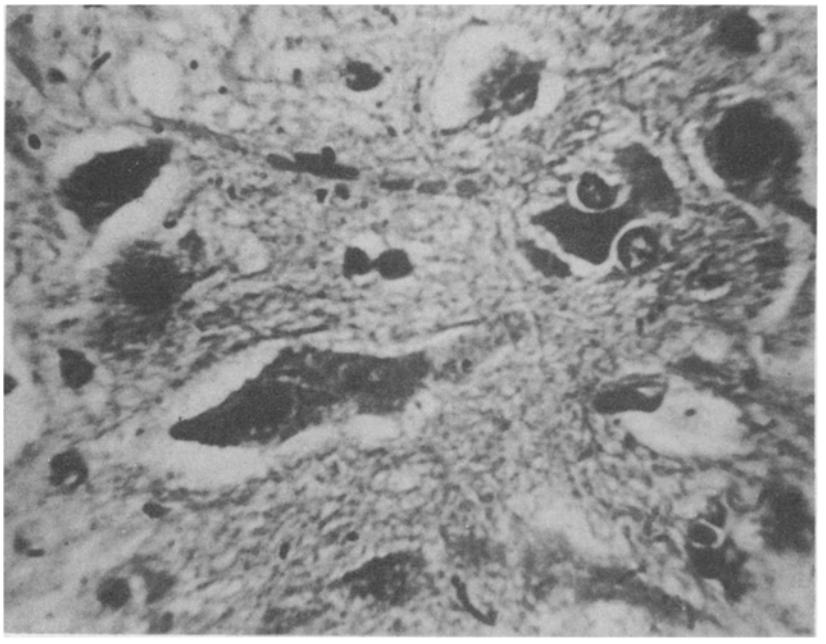

Fig. 2. Two deformed, degenerating motor neurones of the lumbar division of the spinal cord 24 hours after injection of 1 MLD of Flexner dysentery antigen. Magnification 1000 x. Fixation by alcohol formalin, stained by Hansen's iron trioxyhematein method.

Antibiotics (chlortetracycline, oxytetracycline, tetracycline, penicillin and streptomycin) were given as a single dose either intramuscularly or by mouth, before or after injection of the antigen. Observations were continued for 7 days. At various times a number of control and experimental animals were sacrificed for the purpose of histological examination and blood culture on different media.

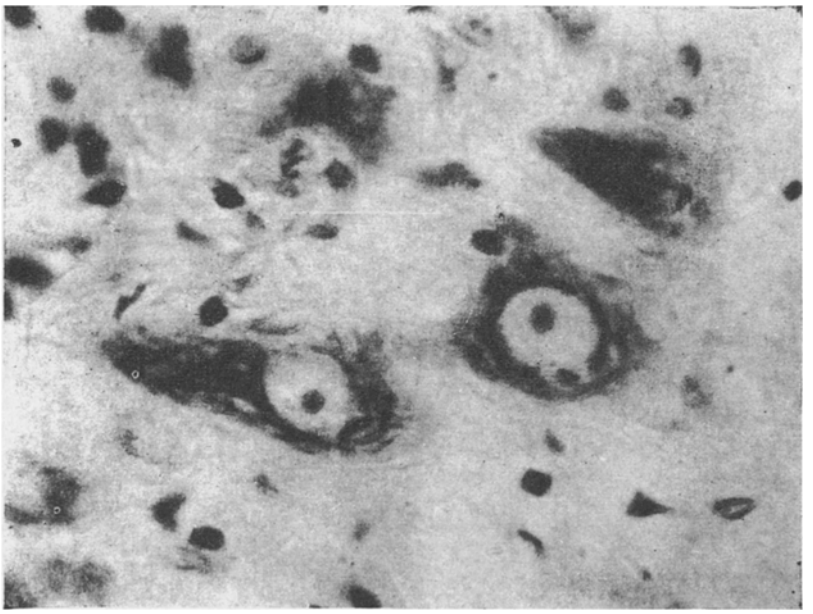

Fig. 3. Gross engorgement of a vein in the submucosal layer of the intestinal wall of a mouse during the development of dysentery toxicosis. Stained by Lepine's method. Magnification 600 x.

Using chlortetracycline as an example, it was shown that after intramuscular injection of 0.1 mg of antibiotic into a mouse in the course of one hour before injection of 1 MLD of toxin, the mortality among mice in the group receiving the antibiotic was 10%, whereas that among the control group of mice was over 80%.

This well-marked action of the tetracyclines on the course of the toxicosis was apparent when the antibiotic was given in the course of the hour, or immediately after injection of the antigen.

In Table 1 are shown the results of the action of the tetracyclines in toxicosis caused by the intramuscular injection of 1 MLD of whole antigen of Shigella flexneri. The antibiotics were given immediately after the antigen, by mouth or intramuscularly (Table 1).

As will be seen from the results in Table 1, both methods of treatment were effective: all the antibiotics prevented development of a fatal toxicosis in the animals. With oral administration all the animals survived, and when one fifth of this dose of antibiotic was injected intramuscularly, only solitary mice died.

In the majority of animals receiving antibiotics of the tetracycline group immediately after injection of dysentery toxin, degenerative changes (3 phases) were not present in the nerve cells. The reactive changes soon disappeared, and after 2-4 days were no longer found (the exceptions were those few cases in which the animals died in spite of administration of the antibiotic). Treatment with the tetracyclines prevented the development of any marked hemodynamic disturbances or of an inflammatory reaction in the intestine.

We have previously shown [1] that the administration of an antibiotic at later stages, for example 1 hour after the injection of 1 MLD of antigen, gives far inferior results and prevents the death of only a comparatively small proportion of animals.

Histological examination shed some light on the causes of this phenomenon. It was found that a detoxicating effect was possible if the antibiotic was given before the appearance of marked, morphologically detectable changes in the animals, and primarily in the central nervous system. The duration of this initial phase of the toxicosis determined the time interval during which the antibiotic was able to prevent death of the animals.

The shorter this first phase, the more quickly the antibiotic had to be given after injection of the toxin in order to show a detoxicating effect. Conversely, with a longer first phase, good results could be obtained with relatively late administration of the tetracyclines. Thus with an insignificant reduction in the dose of toxin, a detoxicating action is demonstrated when tetracyclines were given in the course of the first two hours,

and when the dose of toxin was halved, of the first 6 hours or more (Table 2). Meanwhile, in animals untreated with tetracyclines, even with half the dose of toxin, degenerative changes developed in time in the motor neurones of the spinal cord, although not on such a large scale as when 1 MLD was injected. The failure of the tetracycline series of antibiotics to show a detoxicating action in cases of far advanced toxicosis was due to the rapidly progressive and intensively expressed lesions in the more important systems of the body.

In identical conditions of dysentery toxicosis, penicillin, streptomycin and colimycin had no effect on the survival of the animals.

Concurrently with the histological examinations, at various phases of the toxicosis, blood from the animal's heart was cultured in meat-peptone broth and in Ploskirev's medium. In no case, as also in the healthy mice of the same batch, was bacteriemia discovered.

It was thus shown that chlortetracycline, oxytetracycline and tetracycline possess a marked detoxicating effect in experimental dysentery toxicosis of white mice. The degree of this detoxicating effect is dependent on the time and method of administration of the antibiotic. Under the same conditions other antibiotics — penicillin, streptomycin and colimycin — did not possess such an action.

We have shown [1] that the detoxicating effect of the tetracyclines is not due to neutralization of dysentery toxin as a result of direct fixation, and is only manifest in experiments in vivo.

The present investigation has shown that the tetracyclines prevent death of animals from toxicosis by preventing the development of severe degenerative changes in the central nervous system and other organs. Since this effect develops in the body in the absence of living microorganisms, there are no grounds for associating it with only the antibacterial action of the antibiotic.

Some authors suggest that the mechanism of detoxication is connected with the action of antibiotics on members of the so-called "normal" microflora, since toxicosis provides favorable conditions for proliferation of these organisms and for their penetration into the blood stream. Geller [6], on the basis of his observations, considers that this possibility is unlikely. In our own investigation, we did not find a single case of bacteriemia in mice into which antigen had been injected. In the conditions of these experiments the detoxicating effect could not, therefore, be due to the action of the antibiotics on the bacteriemia.

The establishment of the fact that a certain antibiotic has a detoxicating action makes a step forward in the explanation of the mechanism of its action in the body. The study of the detoxicating properties of antibiotics indicates new possibilities in the development of methods of rational treatment of those infections in which the toxicosis is of primary importance.

SUMMARY

Experiments were performed on white mice poisoned with the total antigen of Flexner dysentery bacilli. The detoxicating effect of chlortetracycline, oxytetracycline and tetracycline was demostrated both in oral and in intramuscular administration. A suitable dose of antibiotics prevented the degeneration of the cells of the central nervous system as well as the inflammatory changes in the intestines and the severe hemodynamic disturbances observed in the majority of untreated animals in dysentery intoxication. In treatment with tetracyclines the action is detoxicating manifested in the animals before the development of pronounced morphological changes in the central nervous system. This effect of tetracyclines is not caused by the neutralization of the toxin as a result of direct binding, nor by the action of the antibiotics on the bacteriemia (the latter, as shown by the authors' experiments does not developed in these conditions). Other antibiotics (penicillin, streptomycin and colimycin) had no detoxicating effect in analogous conditions.

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