A REFLEX COMPONENT OF THE MECHANISM OF PRODUCTION OF BLOOD CHANGES BY CHLORTETRACYCLINE (BIOMYCIN)

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During investigations on the effect of chlortetracycline (biomycin) on the physiological processes of the body, carried out in our laboratory, it was found that this antibiotic, when administered orally in certain doses to rabbits, may cause a series of changes in the composition of the blood [8]. The most pronounced changes were in the number of leucocytes — which was increased — and in the proportion of lymphocytes and segmented neutrophils (pseudoeosinophils).

Since chlortetracycline hydrochloride has a stimulating action on organs and tissues, it might be supposed that these changes are the result of both the direct and reflex action of the drug on the hemopoietic organs following stimulation of receptors.

There are several recent reports of work on the nervous regulation of the blood system [1-7, 10-28].

Nevertheless the influence of antibiotics on the blood has received scant attention. G. S. Kan [13] investigated the effect of streptomycin on reflex changes in the composition of the blood resulting from stimulation of interoceptors. This author found that streptomycin depresses leucocytosis due to stimulation of chemoreceptors. No investigations of a similar type have been carried out with chlortetracycline.

In the present communication we give our findings in regard to the role of stimulation of the receptor apparatus in changes in the composition of the leucocytes after intramuscular injection of chlortetracycline, and of its humoral effect on the composition of the leucocytes.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits. Blood was taken from the marginal vein of the ear and the white cell count and leucocytic formula determined (films were stained by Leishman's method).

The rabbits were starved for 18 hours before each investigation. As a preliminary measure, in each rabbit we investigated the effect of the experimental environment on the composition of the white cells of the blood (background investigation). For this purpose blood was taken after 0.5, 1, 2, 3, 6 and 8 hours and in some experiments — after 10, 24, 48 and 72 hours. Animals showing variations in the white cell count, in the tests at these times, greater than ± 22% were not included in the experiment. The leucocytic formula was more stable in the course of the experiment. The percentage of lymphocytes and segmented granulocytes varied on the average by ± 10%, and that of the other cells by a few per cent (1-3). Within the limits of these variations we could observe no particular regular features in the daily variation of the white cell count or composition.

Chlortetracycline was dissolved in physiological saline in a concentration of 10 mg/ml and injected into the muscles of both thighs in a dose of 25 mg per 1 kg body weight. Blood for examination after the injection of

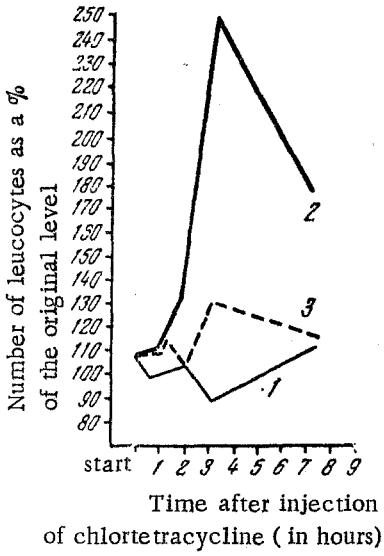

Fig. 1. Changes in the white cell count before and after intramuscular injection of chlortetracycline. 1) White cell count during investigation of the initial background; 2) change in the white cell count after intramuscular injection of 25 mg/kg body weight of chlortetracycline; 3) change in the white cell count after preliminary injection of novocain into the site of injection of chlorotetracycline.

chlortetracycline was taken at the same times as in the background investigation. From 2-3 days after the first investigation, chlortetracycline was again injected in the same dose, but 1-2 minutes before this was done, injections were given at the same points of the right and left thighs of 1.5 ml of a 0.5% solution of novocain.

In view of the fact that the chlortetracycline solution had a pH of 2.9, preliminary control experiments were carried out in which injections were given of physiological saline (5-10 ml) acidified with hydrochloric acid to pH = 2.9 (6 experiments). We also carried out control experiments in the same manner in which 3 ml of a 0.5% solution of novocain was injected intramuscularly (6 experiments). It was established that in each case the daily variations in the composition of the white blood did not exceed those of the background investigation.

EXPERIMENTAL RESULTS

The first series of investigations was carried out on 14 rabbits weighing from 2 to 3 kg; the blood examinations after injection of chlortetracycline or novocain and chlortetracycline continued for 8-10 hours in this series.

After the injections of chlortetracycline all the animals developed leucocytosis. In the majority of the experiments this was apparent between the 1st and 2nd hour after injection of the drug; the white cell count rapidly increased and reached a maximum in 4 rabbits at the 2nd hour, in 6 rabbits at the 3rd hour, and in the remaining 4 at the 5th-6th hour. The increase in the number of leucocytes varied from 30 to 220% of the original count, but in the majority it was between 75 and 220%. After reaching a maximum increase in 9 rabbits, the leucocytosis fell and regained its normal value at the 5th, 6th, 8th or 10th hour. In the 5 rabbits with the greatest leucocytosis, the white cell count after 8-10 hours either remained at a high level (3 rabbits) or was just beginning to fall (2 rabbits). In 3 rabbits, after the white cell count had returned to its original level, it showed a tendency to rise again.

Thus the intramuscular injection of chlortetracycline in a dose of 25 mg/kg regularly caused in all the animals a rapidly developing leucocytosis, the magnitude and duration of which varied in accordance with the individual features of the animals. Different results were found when the chlortetracycline was given after a preliminary injection of novocain at the same point. Of the 14 rabbits, 3 showed a slight leucopenia (to 25-50%) in the first 2-3 hours in place of the leucocytosis, followed by a return of the leucocyte count to its original value after 6-8 hours. In 6 rabbits which had developed a marked leucocytosis in response to the injection of chlortetracycline alone showed a considerably reduced leucocytosis after novocain, and its development was delayed by 1-2 hours. Only in 5 rabbits was the reaction to injection of chlortetracycline unchanged after novocain, although in these experiments the development of maximum leucocytosis was delayed by approximately 1 hour. Furthermore in 3 rabbits the leucocyte count returned to its original value 4-5 hours earlier than after injection of chlortetracycline alone.

Thus in the majority of cases the preliminary injection of novocain either prevents or reduces the degree of leucocytosis, or delays its development and it comes to an end more rapidly (Fig. 1).

The explanation of these findings may be that anesthesia of the nerve endings in the muscles abolishes or reduces the stimulating action of chlortetracycline. Hence it follows that the leucocytosis developing immediately after injection of chlortetracycline includes a reflex component.

In 11 animals of the first series the leucocytic formula was examined in blood taken at the same times as that for the white cell counts. It was found that regular changes took place in the numbers of all forms of leucocytes, mainly in the proportions of the lymphocytes and pseudoeosinophils. In all 11 rabbits a fall was observed

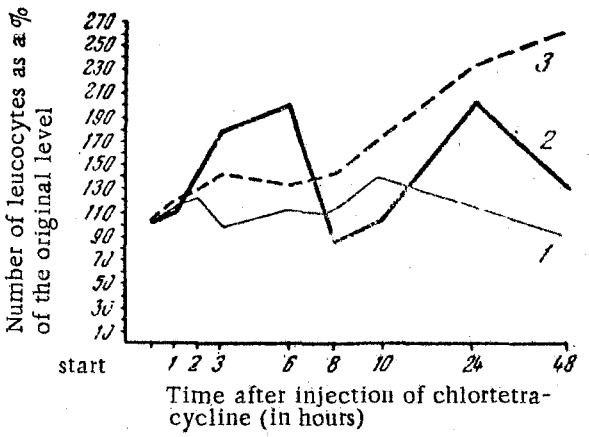

Fig. 2. Changes in the number of leucocytes before and after injection of chlortotracycline for a period of 48 hours. Legend as in Fig. 1.

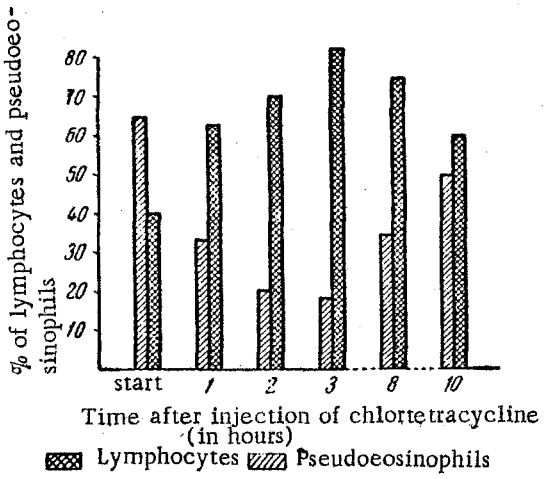

Fig. 3. Changes in the proportions of pseudoeosinophils and lymphocytes after intramuscular injection of chlortetracycline.

in the number of lymphocytes in favor of an increase in the pseudoeosinophils. This fall began from 2-3 hours after injection of the preparation and reached its maximum in 4 rabbits after 3-4 hours and in the rest 5-6 hours. The proportion of lymphocytes fell by 24-44%, on the average by 33.6%, i.e. the absolute lymphocyte count fell by $1\frac{1}{2}$-2 times.

There was an equivalent increase in the content of pseudoeosinophils and no essential change in the other forms of leucocytes.

In 3 rabbits reversal of the changes in the proportions of the leucocytes began from 6-8 hours after the injection of chlortetracycline. In all the other animals this reversal could not be seen even after 10 hours. Consequently these changes were more stable than the leucocytosis.

During the action of chlortetracycline after intramuscular injection of novocain the changes observed in the leucocytic formula were approximately the same as those after injection of chlortetracycline alone. In only 3 rabbits was the fall in the number of lymphocytes less marked than before injection of novocain (by 10-15%).

In the second series of experiments carried out on 8 rabbits, the blood was examined for longer periods after injection of chlortetracycline, as follows: after 1, 2, 3, 6, 8, 10, 24 and 48 and sometimes after 72 hours. In the first 8-10 hours the same results were obtained in the rabbits of this series as in the first series of experiments: in the majority of the animals novocain either prevented or reduced the development of leucocytosis resulting from the action of chlortetracycline.

However the subsequent examinations of the blood picture in this series of experiments showed that after the fall in the leucocytosis after 8-10 hours, the leucocyte count may begin to rise again. Usually from 10-12 hours after the injection of chlortetracycline the white cell count begins to rise again, and reaches a maximum after 24-48 hours. The white cell count is increased by 50-150% over its original value; at the 48th-72nd hour the leucocytosis begins to fall, but it mostly does not regain its original level. It was found that the preliminary injection of novocain does not affect this secondary leucocytosis (Fig. 2).

Despite the small number of animals in this series, the fact that a secondary leucocytosis appears and that it is unaffected by injection of novocain cannot be regarded as accidental, since in the first series of experiments we also observed in some rabbits a tendency for the white cell count to rise again after 10-12 hours.

In the second series of experiments we investigated the leucocytic formula of all the animals in blood taken at the same times. The same changes were found as in the animals of the first series, i.e. a relative fall in the lymphocytes and rise in the pseudoeosinophils (Fig. 3). Their proportions were restored only after 24 to 48 hours or even later, independently of any previous injection of novocain.

The results obtained thus indicate that the leucocytosis arising in animals after intramuscular injection of chlortetracycline is brought about in two ways: in the first few hours it is the result of a reflex act from stimulation

of the receptors of the muscle tissue; later, as absorption of chlortetracycline takes place, it evidently causes a second wave of leucocytosis by a humoral mechanism, and this is maintained much longer than the first.

Chlortetracycline also produces changes in the proportions of lymphocytes and segmented neutrophils (pseudoeosinophils in rabbits) in the form of a fall in the first and a rise in the second; these changes are also maintained for a longer time. It is still possible, however, that the secondary leucocytosis and the shift in the formula are connected with reflex effects from the interoceptors of the blood vessels, but further and more detailed evidence is required on this problem.

There is no doubt that in addition to stimulating the hemopoietic tissue by the humoral route, chlortetracycline may also cause reflex changes in the composition of the leucocytes.

Differences in the action of chlortetracycline alone and with novocain may also serve, in particular, to explain clinical observations during the use of chlortetracycline in combination with ecmolin and novocain, as suggested by Z. V. Ermol'eva and her co-workers [9].

SUMMARY

Leukocytosis appears in rabbits after intramuscular administration of chlortetracycline in the dose of 25 mg/kg of body weight. It reaches its maximum in 2-4 hours and decreases or disappears completely in 5-10 hours. Then a second rise of the quantity of leukocytes occurs which reaches its maximum in 24-48 hours following the administration of chlortetracycline. The ratio of the lymphocytes and the segmented neutrophiles (pseudoeosinophils) is altered simultaneously with leukocytosis, with a decrease of the number of the lymphocytes and increase of pseudoeosinophils.

The first rise in the number of leukocytes depends on the reflex action of chlortetracycline on the blood system due to the excitation of the receptor apparatus of the muscle tissue. The second rise of leukocytes and the shift of the blood formula apparently depends upon the humoral action of the absorbed chlortetracycline on the hemopoietic organs.

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