ACTION OF NOVOEMBIKHINE* ON COMPLEMENT AND PRODUCTION OF AGGLUTININS AND PRECIPITINS IN ANIMALS

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Preparations of the chlorethylamine group (embikhine* and others) are employed in the treatment of proliferative diseases of the hematopoietic system (lymphogranulomatosis, leukosis) and with some malignant tumors. In small doses the chlorethylamines may be employed in nonhyperplastic diseases, for example in bronchial asthma [2], and in other internal, infectious and nervous diseases [1]. Obviously the mechanism of action of chlorethylamines is complicated and varied. The suggestion has been made that chlorethylamines in small doses may exert a stimulating action on the protective and compensatory reaction of the body [3]. However, this aspect of the action of chlorethylamines has been studied very little.

The purpose of the work presented here was to investigate the action of the parent compound of a group of chlorethylamines, novoembikhine [4], on complement and the production of agglutinins and precipitins.

The data on this question in the literature apply only to large doses and boil down to this:

Watkins and Wormall [7, 8] showed that embikhine in large dose (0.5-2.8 mg/kg) exerts an inactivating action on complement. Bukants, Damnin, Wilson, Johnson and Alexander [5] established that periodic intravenous injection of embikhine in large dose inhibits the production of antibodies, as well as the development of the vascular disease of rabbits which was produced by the intravenous injection of a massive dose of horse serum. Spurr [6] came to the conclusion that "a single preliminary injection of embikhine (0.5-1 mg/kg) into rabbits represses the production of antibodies in response to typhoid antigen."

EXPERIMENTAL RESULTS

Influence of novoembikhine. Experiments on normal animals interested us especially, as contrary to the study of this question in the clinic, in normal animals there is excluded the influence of pathological processes, which themselves depress the complement function of the serum in nearly all diseases.

Our preliminary experiments in vivo were carried out on rabbits to which, in the course of other experiments we administered novoembikhine about 1-10 days before determining the titer of complement. Further experiments were carried out with the complement of 25 rabbits, to which we administered, about 24 and 48 hours, about 4 and 10 days before, our usual dose of (1.5 mg/kg). In these experiments the complement titer was higher in a significant portion of the rabbits than in the controls.

After this a special experiment was set up with 5 rabbits, whose complement titer was determined three times during the 9 days before the administration of novoembikhine and four times afterward (Fig. 1).

From Figure 1 it is seen that the absolute complement titers before the administration of embikhine in the first three experiments varied significantly (from 0.16 to 0.08 ml), but in spite of this, in all 5 experiments

* Transliteration of Russian – Probably Russian trade name – Publisher's note.

they were more often average (0.1-0.12 ml) or lower (0.14-0.16). After the administration of novoembikhine the absolute titers of complement in all 5 experiments were more stable and not so high, namely in the range 0.06-0.08 ml (see the first three titrations). Experiment No. 1 (Rabbit No. 961) is an exception, where a high titer of complement in the first three titrations (0.02-0.04) fell to 0.16 in the third titration, which is possibly connected with some special moment.

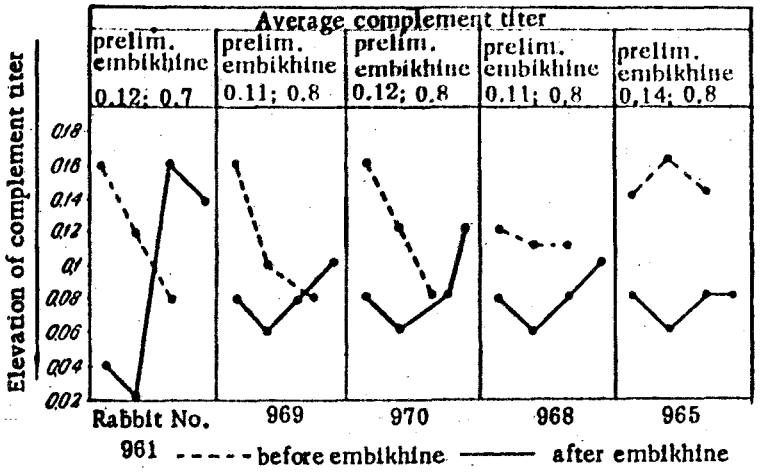

Fig. 1. Effect of novoembikhine on complement liter in rabbits (in vivo).

In all experiments the initial dilution of complement for the titrations was a 1:1 dilution of rabbit serum in physiological saline. The titration of complement was carried out in a volume of 1 ml according to the usual scheme. We added complement to a series of test tubes in steps of 0.02, that is 0.02, 0.04, 0.06, 0.08, etc.; hemolysin in triple titer, erythrocytes 1:40. The complement titer was determined 30 minutes after 30 minutes exposure in the thermostat to 37°.

Effect of novoembikhine on the production of agglutinins. Experiments were set up on 48 chinchilla rabbits weighing 2.5 and 3 kg. In the majority of experiments we administered the novoembikhine in a single dose of 1.5 mg/kg (intravenously). Each series of experiments consisted of three groups of animals: into one group we injected novoembikhine and antigen simultaneously; into the second, first we injected novoembikhine and after 4 days, antigen; into the third, first antigen, then after about 4 days novoembikhine.

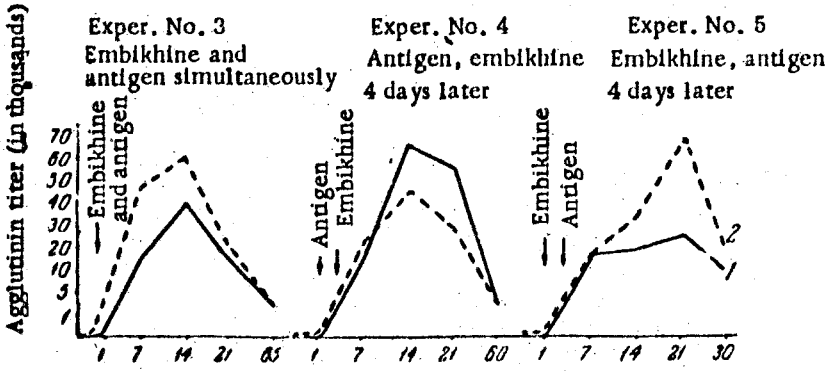

Fig. 2. Influence of embikhine on the production of agglutinins in variously arranged experiments.
1) experiment, 2) control.

For the production of agglutinins we used typhoid diagnostic antigen, injected once subcutaneously, and to determine the resulting agglutinins we added 0.1 ml in each test tube to the corresponding dilution of serum. The agglutination reaction was carried out in a volume of 1 ml. The reaction was estimated according to the characteristics of the precipitate at the bottom of the test tube, without shaking after the racks had been for two hours in the incubator and then up to an hour in the refrigerator.

In each experiment there were 5 experimental and 4 control rabbits. The results of the experiments are presented in Figure 2.

From Figure 2 it is seen that on injecting antigen (typhoid diagnostic antigen)simultaneously with novoembikhine or about 4 days after the injection of novoembikhine there is observed an inhibition of the production of agglutinins for the administered antigen. The interval of 4 days between injection of novoembikhine and antigen was chosen because in that time (on the 4th day) there is generally observed the maximum depression of hematopoiesis, shown by a decrease in the number of leukocytes.

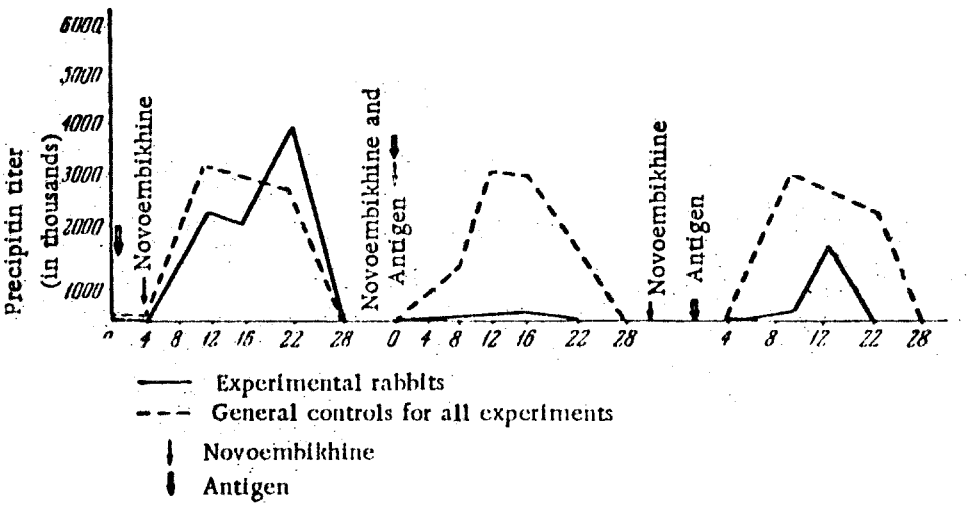

Fig. 3. Influence of novoembikhine the production of precipitins following injection of single doses of novoembikhine, 1.5 mg/kg rabbit weight, and immunization with horse serum (2 injections), 1.5 ml per rabbit. On the left-antigen, after 4 days novoembikhine; center, novoembikhine and antigen simultaneously on the right, novoembikhine, after 4 days antigen.

We obtained different results in the other animals into which we first injected antigen and then (also 4 days later) novoembikhine. In these rabbits the agglutinin titer on the 14th and 21st day after injection of antigen was higher than in rabbits to which we gave no novoembikhine.

In two other experiments in which we injected antigen at weekly intervals, on the 14th day there was also observed some rise in antibody titer in the experimental animals, compared with the titer of the controls.

Influence of novoembikhine on the production of precipitins. To produce precipitins we injected rabbits with unpreserved horse serum as antigen.

For the determination of precipitins we used the ring precipitin reaction. We heated the experimental sera to 56° for 3 minutes and diluted them 1 : 2 with physiological saline. Readings were made after incubating 30 minutes at 37°.

The results of the experiments are presented in Fig. 2, from which it can be seen that following simultaneous injection of antigen and novoembikhine there is observed a very sharp inhibition of antibody production; following injection of antigen about 4 days after novoembikhine there was noted an inhibition of precipitin

production, but it was definitely weaker. In an experiment in which novoembikhine was injected about 4 days after the antigen, after a period of some inhibition of precipitin production, on the 22nd day the precipitin titer was higher than in the controls.

The data obtained confirm that when novoembikhine is injected after the antigen there is observed a temporary repression, then an increase in antibody production, consequently under certain conditions novoembikhine may exert a stimulating action of the protective reaction of the body. These data, perhaps, may possess practical interest for the production of immune sera, although for this supplementary experiments are necessary. Inhibition of antibody production, obtained at first by simultaneous injection of antigen some days after administration of novoembikhine, evidently, is explained by a general inhibition of body function for a few days after administration of the preparation.

SUMMARY

The effect of novoembikhine, i.e., di-(2-chlorethyl)-2-chlorpropylamine, on the complement function of the serum as well as on production of agglutinins and precipitins was studied. Experiments were performed on healthy rabbits. Novoembikhine was injected intravenously, 1.5 mg per kilogram of body weight, usually in a single dose. It was established that within 9 days following the introduction of novoembikhine there is an increase and stabilization of the titer of the complement. This was compared with its variations and activity in the same animals previous to introduction of this preparation. Depending on conditions of experiment novoembikhine either inhibited or stimulated the production of agglutinins and precipitins.

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* In Russian.