ACTION OF HEPARIN AND HEPARIN-PRECIPITATED FRACTION OF HUMAN BLOOD PLASMA ON ANTIBODY-PRODUCING CELLS in vitro

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The effect of heparin and heparin-precipitated fraction of human blood plasma (HPF) on the ability of spleen cells of mice immunized with sheep's red blood cells to form plaques in vitro was studied. Heparin and HPF were found to inhibit plaque formation as a result of interaction with lymphocytes. It is postulated that the possible point of application of the action of heparin and HPF may be surface cell membranes of the antibody-forming cells.

KEY WORDS: antibody-forming cells; heparin; heparin-precipitated fraction of human blood plasma.

The search for new therapeutic preparations for the treatment of autoimmune diseases is a very urgent and relevant problem at the present time. This is particularly true of substances which, being constantly present in the human and animal body, are able to inhibit the development of immunological reactions such as blast transformation of lymphocytes in response to phytohemagglutinin, the cytoplastic action of lymphocytes on target cells, and antibody synthesis [1, 2, 4]. The list of these substances evidently includes the α_2-globulin fraction of blood serum, glycoproteins, seromucoid substance, heparin, and the heparin-precipitated fraction of human blood plasma (HPF), a protein precipitated by heparin in the cold [1, 2, 4, 5].

The action of heparin and HPF on antibody production in vitro was investigated.

EXPERIMENTAL METHOD

Mice of strains CBA, C57BL, and DBA/2y and F_1(CBA × C57BL) hybrids, males and females, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, were used.

The principle of the experimental method was as follows: Animals were immunized intraperitoneally with a 2% suspension of sheep's red cells. On the fourth to fifth day the mice were killed and a suspension prepared from their spleen cells, in which the number of antibody-producing cells was determined by the method of local hemolysis in semiliquid medium [7], by counting the number of plaque-forming cells (PFCs). To do this, spleen cells of immune mice together with an equal volume of sheep's red cells (10% suspension) and diluted guinea pig complement (1:5) were incubated at 37°C for 45-60 min in special glass chambers.

To study the effect of heparin and HPF on PFCs, the spleen cells of immune animals were incubated at 37°C for 15-20 min together with heparin (140 IU/ml) or with HPF (10 mg/ml), and then washed three times in Hanks's solution, before being mixed with the sheep's red cells and complement. Since HPF is a complex multicomponent substance, containing heparin, fibrinogen, and γ-globulin [5], further experiments were carried out to rule out the effect of γ-globulin on the reaction. For this purpose, before addition to

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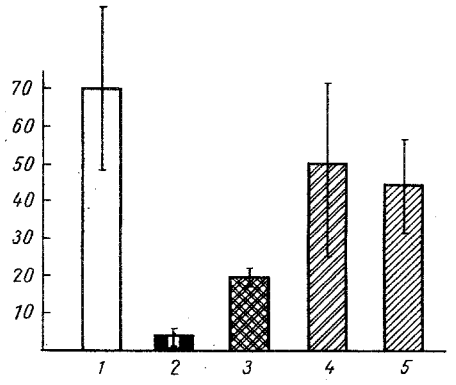

Fig. 1

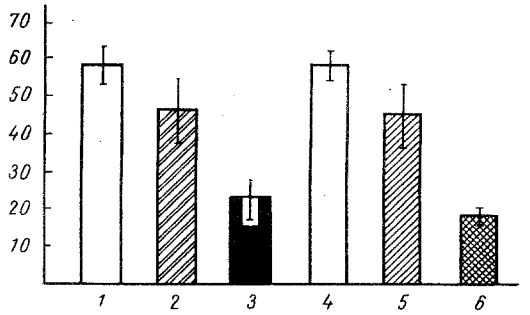

Fig. 2

Fig. 1. Effect of heparin, HPF, and γ-globulin on number of PFCs in CBA mice immunized with sheep's red cells. Abscissa: 1) control, 2) heparin, 3) HPF, 4) γ-globulin control, 5) γ-globulin; ordinate, number of PFCs (per 10^6 lymphocytes).

Fig. 2. Character of plaque formation following isolated treatment of red cells and lymphocytes used in the in vitro tests with heparin and HPF. Abscissa: 1 and 4) control, 2) red cells treated with HPF, 3) lymphocytes treated with HPF, 5) red cells treated with heparin, 6) lymphocytes treated with heparin; ordinate, number of PFCs (per 10^6 lymphocytes).

the plaque formation test, the spleen cells were incubated with human γ-globulin, with strict observance of the same conditions of incubation temperature and sequence of operations during treatment of the lymphocytes with heparin and HPF. The quantity of globulin added during the reaction corresponded to the protein content in HPF.

A standardized solution of heparin (Richter), heparin (Richter) purified with ether, and commercial dry heparin were used in the experiments.

The experimental results were subjected to statistical analysis by the Student–Kolmogorov method.

EXPERIMENTAL RESULTS

The results of investigations of the action of heparin and HPF on antibody-producing cells in vitro are given in Fig. 1. Clearly the relative number of PFCs in suspension treated with heparin and HPF was much lower than the number in the control; when heparin was used, moreover, the decrease was greater than with HPF.

To discover the mechanism inhibiting the action of heparin and HPF on the reaction of formation of local zones of hemolysis in semiliquid medium, a special series of experiments was carried out on DBA mice. As Fig. 2 shows, a considerable decrease in the number of PFCs was observed only when lymphocytes used in the test, but not red cells, were given preliminary treatment with heparin and HPF.

A study of the character of the action of different types of heparin [standardized solution of heparin (Richter), standardized heparin (Richter) purified with ether, and commercial dried heparin] on the degree of plaque formation showed that all types of heparin equally reduced the total number of PFCs.

Comparison of the effects of high and low doses of heparin on antibody-producing cells with the control values gave the following results. High doses of heparin (160-200 IU/ml) of splenic suspension) led to a decrease in the total number of PFCs from 50-60 cells/10^6 lymphocytes in the control to 0.5-1 cell/10^6 lymphocytes in the experiment. Small doses of this substance (10-30 IU/ml) had a weaker action, reducing the number of PFCs to 10-15/10^6 lymphocytes.

It must be noted that treatment of PFCs with human γ-globulin did not affect their number in spleen suspensions obtained from mice immunized with sheep's red cells.

On the basis of these experiments, it can evidently be postulated that heparin and HPF have a blocking effect on PFCs if treated with the particular substance. The mechanism of action of heparin and HPF under the experimental conditions used is not clear. Only the following suggestion can be put forward: Heparin and HPF evidently can stabilize the surface membranes of many cells [3, 6], including lymphocytes.

By changing the physicochemical properties of the membranes, they create an obstacle to the liberation of antibodies. This is reflected in the decrease in the number of PFCs in the experiments described above. However, the possibility cannot be ruled out that heparin and HPF may bind with complement to block its hemolytic action. This view is confirmed by results obtained by other owrkers.

However, in the present investigations cells incubated with heparin and HPF were washed three times and the dose of complement added was relatively high. Consequently, the mechanism of action suggested above evidently cannot be regarded as playing the leading role in this situation. The hypotheses expressed above naturally require careful future experimental verification.

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