EFFECT OF HEPARIN ON BLAST-TRANSFORMATION CAPACITY OF HUMAN BLOOD LYMPHOCYTES

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The effect of heparin on the capacity of human blood lymphocytes to undergo blast transformation in response to stimulation by antilymphocytic γ-globulin and phytohemagglutinin was investigated. Heparin was found to inhibit the transformation of lymphocytes stimulated by antilymphocytic γ-globulin more intensively. A depressive effect appeared if heparin was added 1 h before the mitogen, simultaneously with it, or 1 h thereafter.

KEY WORDS: lymphocytes; blast transformation; heparin; immunodepression.

The object of this investigation was to study the effect of heparin on the ability of human blood lymphocytes to undergo blast transformation (BT) in response to stimulation by antilymphocytic γ-globulin (ALG) and phytohemagglutinin (PHA).

EXPERIMENTAL METHOD

Leukocytes were isolated from donor's blood by the addition of 10% sterile gelatin solution to it to a final concentration of 1%. The suspension of leukocytes was withdrawn from the blood after standing for 30 min in an incubator at 37°C. The lymphocytes were stimulated by the addition of PHA (Wellcome) in a dose of 0.01 ml per culture or of ALG in optimal concentrations, established by preliminary tests for each batch of ALG. Thymidine-H^3 was added in a dose of 2 μCi per culture 48 h after the beginning of cultivation. The incubation time of the cells with labeled thymidine was 24 h. The cells were washed with physiological saline, treated twice with 5% TCA solution, and the precipitates were applied to millipore filters. Activity was counted on a "Unilux-2" counter.

EXPERIMENTAL RESULTS

The experiments showed that the intensity of BT of human lymphocytes falls in proportion to the heparin concentration in the medium. This effect of heparin was exhibited whether the lymphocytes were stimulated by ALG or by PHA. However, heparin inhibited lymphocyte transformation stimulated by ALG more effectively. Small doses of heparin (0.01 mg/ml) were virtually unable to inhibit BT, but with an increase in the heparin concentration in the medium from 0.1 to 1 mg/ml, inhibition of BT increased proportionally to the increase in concentration.

To study how the intensity of BT of the lymphocytes depends on the time of addition of heparin relative to the time of stimulation by the mitogen, heparin was added to the culture 1 h before the mitogen, at the same time, or 1 h thereafter. In every case, heparin had a depressant action: in the cultures with ALG maximal inhibition of BT amounted to 93.1 ±5.9%, in the cultures with PHA it was 74.3 ± 4.1%.

In the experiments to study the relationship between the increase in the number of cells in the S-period after stimulation by the mitogens and without it, the period of contact of the stimulated lymphocytes with thymidine-H^3, in a dose of 2 μCi, was 1 h. In the culture stimulated by ALG the number of cells syn-

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thesizing DNA rose faster than in the culture stimulated by PHA. The inhibitory effect of heparin was stronger when ALG was used as the mitogen. Under the influence of heparin the number of counts per minute in the cultures with ALG fell from 40,780 ± 1115 to 11,375 ± 671; and in the cultures with PHA it fell from 61,187 ± 1812 to 30,456 ± 993.

The inhibitory action of heparin on BT of the lymphocytes was evidently due to the polyanionic character of its molecules. By adsorption on the cell surface, heparin can prevent interaction between the cells and the mitogens and also between each other. Interaction between cells in the process of BT of lymphocytes has been insufficiently studied, but it is known that cellular cooperation takes place during the development of this reaction [1, 2]. The fact that heparin inhibits BT even if added 1 h or more after addition of the mitogen indicated that not all cells participating in BT start to interact simultaneously.

The stronger inhibition by heparin of transformation of lymphocytes stimulated by ALG than of those stimulated by PHA may be explained by the participation of different types of cells in the mechanism of BT under the influence of these mitogens. During the action of PHA only T-lymphocytes are known to be activated [3, 4]. The addition of heparin to the culture with PHA evidently blocks the transformation of T-lymphocytes only. So far as the effect of heparin on BT induced by ALG is concerned, in that case activation of both T- and B-lymphocytes may perhaps be inhibited.

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