Effect of Heparin on Erythrocyte Filtration by the Popliteal Lymph Node in the Dog

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SUMMARY

In 12 experiments the effect of heparin on lymph node filtration efficiency was tested, utilizing perfusion of $^{51}$Cr-tagged erythrocytes through a popliteal lymph node preparation in the dog.

Heparin does not exert any detectable effect on the filtration efficiency of the dog lymph node for red blood cells.

INTRODUCTION

Fisher and Fisher (3) and Agostino and Clifton (1, 2) have shown that the metastatic “take” of tumor cells given intravenously to animals can be reduced by prior heparinization. However, if heparinization were to reduce the filtration efficiency of the first echelon lymph nodes for lymphatic metastases from a localized tumor, this useful action would be counterbalanced.

To study the effect of heparin on lymph node filtration, the previously described popliteal lymph node preparation in dogs (4) was perfused with $^{51}$Cr-tagged erythrocytes. One hind limb was first perfused as a control, then the other limb was perfused after heparinization of the animal.

MATERIALS AND METHODS

The perfusion of an isolated popliteal lymph node as previously described (4) allows for quantitative results and is therefore suitable for studying the possible effects of heparin in lymph node filtration.

Suitable preparations were attempted in 16 dogs, but were successful in 12. Both hind limbs of each dog were prepared; an afferent lymphatic as well as a single efferent lymphatic was cannulated, while all other efferents were ligated. In one hind limb, 25,000 RBC/cu mm suspended in 3 ml of modified lymph were injected. Modified lymph consisted of 1:1 mixture of plasma and buffered saline. The injection rate was 2.5 ml/hr. Efferent lymph was collected. After completion of this injection, the lymph node was “washed” by injecting 1 ml of modified lymph. At this point the popliteal lymph node was excised. The control side having been completed, the second limb was perfused. Ten mg of heparin were injected intralymphatically immediately preceding the perfusion of the RBC; 1 mg of heparin also was added to the red blood cell perfusate.

Measurements were made of the total radioactivity injected, and of the radioactivity in the efferent collections and in the excised lymph node. The percentage of the injected radioactivity present in the lymph node was determined. The counting procedure has been described in detail previously (4).

Hematoxylin-and-eosin-stained sections were made from each of the excised popliteal nodes.

TABLE 1

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Nonheparinized control side</th>
<th>Heparinized side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% in node</td>
<td>% in efferent lymph</td>
</tr>
<tr>
<td>1</td>
<td>63</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
</tr>
<tr>
<td>12</td>
<td>76</td>
<td>9</td>
</tr>
</tbody>
</table>

Mean value: 67 ± 15 in node, 21 ± 16 in efferent lymph, 88 ± 17 total.

Student’s t values: 2.04 for the % radioactivity of the nodes, 1.64 for the % radioactivity of the efferent lymph. The critical value of t at 5% level is 2.2. Since the observed values are less than the critical value, no true difference is demonstrated.

RESULTS

The results obtained in the 12 animals are shown in Table 1. The mean lymph node filtration was 67 ± 15% on the nonheparinized side, and 76 ± 17% on the heparinized side.

The significance of the difference in results between the two sides was evaluated by the Student t test for paired values. The t value is 2.04, and the critical value for significance of t at the 5% level is 2.2. Therefore, the heparinized side was not significantly different in filtering function from the nonheparinized side. Also there was no difference in the percentage of radio-

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activity collected in the efferent flow between the 2 sides (t value of 1.64).

Histologic study of the heparinized and nonheparinized lymph nodes did not show any significant difference. The perfused red cells were mostly phagocytosed by reticulum cells, or were adherent to the endothelial walls of the lymph sinuses.

DISCUSSION

The purpose of these experiments was to see if heparin exerted any effect on the filtration efficiency of the lymph node. Our previous study (4) had shown that when 25,000 RBC/cu mm in a volume of 3 ml are injected into the lymph node preparation, the node behaves principally as a biologic filter, and to a lesser extent as a mechanical “settling chamber.” The effect of heparin, therefore, would most likely be detected at this dose range.

Erythrocytes were selected because of their suitable size, uniformity, ready availability, and ease of tagging. The lymph node was assumed to filter the erythrocyte in a similar fashion to a tumor cell.

The way in which heparin reduces the “takes” of disseminated cells is to prevent thrombus formation (3). Conceivably, heparin might interact with the walls of the lymph node sinuses to reduce stickiness and thereby prevent the sieving of the RBC in their passage through the node. Our results indicate that there was apparently no such effect because the filtration efficiency of the heparinized node was not decreased. Also, the amount of RBC passing through the node and collected in the efferent flow was not changed by heparinization.

Heparinization apparently does not decrease the “barrier” function of the lymph node under these experimental conditions.

REFERENCES
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