Inhibition of Human Neutrophil Chemotaxis by Corticosteroids

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ABSTRACT

A direct inhibitory effect of hydrocortisone on granulocyte chemotaxis has been demonstrated with an increasing inhibition between concentrations of 5 μg to 12.5 μg per ml. Washing the granulocytes after incubation with hydrocortisone did not reverse the inhibitory effect on chemotaxis indicating a direct cellular effect. Bacteriocidal capacity of the hydrocortisone treated cells was not reduced. These studies indicate corticosteroids alter motility of granulocytes irreversibly possibly by incorporation into the cell membrane.

Introduction

Corticosteroids increase the circulating blood granulocyte level by two possible mechanisms. First, Rebuck7 and later Boggs4 showed that granulocytes fail to appear at sites of abrasion, so called Rebuck skin windows, in patients who are receiving corticosteroids, suggesting that the granulocytes are kept in the circulation by either a direct effect on the granulocyte or an indirect effect on the capillary endothelium. Second, an effect of steroids to increase marrow release has been suggested by studies of Bishop et al3 when they demonstrated by DFP32 tagging studies that the total blood granulocyte pool was increased within hours after the administration of corticosteroids. It is not clear which of these mechanisms is predominant. On the basis of computer simulation studies, Bishop et al suggest marrow release is predominant.

However, it is important to consider the possibility that the increase in circulating granulocytes after steroid administration might be due to a direct effect on the granulocyte, decreasing chemotaxis and preventing the 12 μ cell from undergoing the contortion necessary to squeeze through a 2 to 3 μ pore out of the circulation. This may contribute to the understanding of the increased susceptibility to infection of persons given long term steroid administration and to the modern treatment of infections with transfusion of granulocytes obtained from donors stimulated by the short term administration of steroids.

Methods

In the present study, granulocytes, collected in heparinized whole blood, freed of red cells by dextran sedimentation and washed twice in Hanks buffered salt solu-
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Granulocytes were layered on top of a filter placed in a lucite chamber and held in place by a plastic ring. Approximately $2.5 \times 10^6$ granulocytes were placed in each chamber. Chemotactic agent, serum activated with *Escherichia coli* endotoxin, was added to the lower portion of the chamber and the chamber was incubated at $37^\circ C$ for three hrs. After incubation, the filters were removed, rinsed and stained in a holding rack. The filters were then placed upside down on a microscope slide and covered for counting. Under low power, the filter showed many granulocytes had traversed the $70 \mu$ thickness of the filter which contains pores of $3 \mu$ diameter. Under oil emersion, the intact characteristics of the granulocytes were clearly visible and easily recognized indicating these cells had undergone remarkable shape change and recovery. They were quantitated by counting the cells in ten high powered fields.

**Results**

Granulocytes were incubated with hydrocortisone sodium succinate in concentrations from 2.5 to 50 $\mu$g per ml for one hr at $37^\circ C$, then layered in chemotaxis chambers in identical manner to controls. The number of cells migrating in hydrocortisone treated preparations was subtracted from the number of cells migrating in control chambers and the difference expressed as a percent of controls to give the percent inhibition. Inhibition was related to the concentration of hydrocortisone to which the cells were exposed (figure 1). The bar lines indicate the standard error of the mean. At 5 $\mu$g per ml the migration is statistically significantly different from controls with inhibition increasing to 60 percent at 12.5 $\mu$g per ml with a plateau thereafter.

To determine if the inhibitory effect of hydrocortisone on granulocyte chemotaxis was a direct effect on the granulocytes or an indirect effect on chemotactant molecules, some granulocyte preparations were washed after exposure to hydrocortisone for one hour to remove free hydrocortisone. The granulocytes were then layered in the chemotactic chambers and compared to similarly treated control preparations without hydrocortisone. Both

![Figure 1. Inhibition of chemotaxis by hydrocortisone.](image-url)
TABLE I

Inhibition of Chemotaxis

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<table>
<thead>
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<tbody>
<tr>
<td>Hydrocortisone Present (50 µg/ml)</td>
<td>63 ± 7% (10)</td>
<td>P NS</td>
</tr>
<tr>
<td>Hydrocortisone Removed</td>
<td>66 ± 5% (17)</td>
<td>+ SEM</td>
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washed and unwashed hydrocortisone treated granulocytes showed marked inhibition of chemotaxis compared to control cells indicating an irreversible effect of hydrocortisone directly on cells (table I).

In contrast, studies of bacteriocidal capacity of hydrocortisone treated granulocytes by incubation with E. coli for one hr, gentle centrifugation at 170 g to remove the granulocytes and determination of growth of organisms in supernatant broth by automated technique using the Autobac I8 showed no difference from non-steroid treated control granulocytes (table II). Thus, while hydrocortisone markedly affected the response of granulocytes to chemotactic products, it did not inhibit the ability of the granulocytes to phagocytize and kill bacteria when the bacteria were directly mixed with granulocytes.

Discussion

It appears human granulocyte chemotaxis is specifically and directly inhibited by exposure to hydrocortisone while bacteriocidal capacity is not altered. The mechanism of hydrocortisone action is not clear.6,9 It has been shown by Becker and coworkers2 that certain n-formyl-peptides which are found in bacterial cell walls, are highly chemotactic in purified form and that granulocytes contain a peptidase which may be responsible for the pseudopod movement of the granulocyte membrane toward the increasing concentration gradient of the peptide chemotactant. In addition, Boucek and Snyderman5 have recently reported that calcium influx of sufficient magnitude to trigger contractile protein systems is required for human neutrophil chemotaxis and that both calcium influx and chemotaxis are inhibited by lanthanum chloride.

It is possible that hydrocortisone interacts with the receptor site for chemotactic peptides on the granulocyte membrane and prevents steriochemically the chemotactic response to the peptide molecule. Becker and his colleagues2 have estimated that there are 2,400 receptor sites per cell. Preliminary studies by the present authors using tritiated hydrocortisone show uptake of approximately 3000 molecules per granulocyte, which would favor the receptor site theory.

On the other hand, the hydrocortisone could nonspecifically incorporate into the granulocyte membrane1 as cholesterol, a similar molecular compound, does into the red cell membrane. This molecular rearrangement could interfere with membrane contractability or with calcium flux.1,5 Our present observations that maximum inhibition of chemotaxis occurs at 12.5 µg per ml suggest that maximum inhibition of chemotaxis is produced by presence of approximately 5 billion molecules per cell. These findings favor a nonspecific membrane effect which interferes with the complex motility of the granulocyte, but not with bacteriocidal capacity. Further investigation is needed in this interesting area of research.

References


