The Effect of Salicylate on the Leucocyte Migration Inhibition Test

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SUMMARY

The effect of sodium salicylate on peripheral blood leucocyte migration is described. At a concentration of 3.5 mmol/ml, no effect on leucocyte migration was seen, but this concentration appears to influence the leucocyte migration in the presence of specific antigen, either by increasing or by decreasing leucocyte migration inhibition.


Cell-mediated immune reactions have been implicated in rheumatoid arthritis, and in vitro cell-mediated immune reactions have been defined to a variety of antigens ranging from diphtheroid organisms and mycoplasma cell wall antigens to aggregated IgG and extracts of the rheumatoid synovial membrane. Furthermore, studies of rheumatoid synovial fluid have demonstrated an increased prevalence of T cells over B cells.

Most studies of in vitro cell-mediated immunity have given little consideration to the concurrent drug administration when such studies have been undertaken. Little is known of the mode of action of the anti-inflammatory drugs, and an effect on immune function seems possible. In this article we report on the effect of salicylate incorporation in assay media used in the leucocyte migration inhibition test, in healthy subjects.

MATERIALS AND METHODS

Source of Leucocytes

Heparinised blood, 30 - 40 ml, was drawn from healthy and hospitalised subjects whose Mantoux tests were known to be positive or negative. All these subjects were tested with PPD (first strength) and the reactions were read at 24 and 48 hours. The blood was processed as previously described. None of the subjects was taking salicylates at the time of the experiment.

Tissue Culture Medium

Minimal essential medium (Grand Island Biologicals) was used throughout and was supplemented with 10% sterile horse serum (Wellcome inactivated horse serum).

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Antigen

Intradermal BCG (Glaxo Laboratories, England) was reconstituted with sterile pyrogen-free saline. The reconstituted BCG at a concentration of 0.075 ml/ml of tissue culture medium was used as the antigen throughout the experiments.

Sodium Salicylate

Sodium salicylate (BDH Chemicals) was dissolved in phosphate-buffered saline pH 7.4 to a concentration of 7 mmol/ml. The dissolved salicylate was sterilised by millipore filtration and used in a concentration of 3.5 mmol/ml of tissue culture medium (TCM).

Methods

The packed cells in microcapillary tubes were placed in tissue culture chambers. Two capillary tubes were placed in each chamber, and eight or more capillary tubes were tested with each of the four media shown in Table I.

TABLE I. CONSTITUTION OF MEDIA (IN ml) USED FOR IN VITRO TESTS

<table>
<thead>
<tr>
<th>Saline control</th>
<th>Salicylate control</th>
<th>Antigen control</th>
<th>Antigen and salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM 8,75</td>
<td>8,75</td>
<td>8,75</td>
<td>8,75</td>
</tr>
<tr>
<td>Antigen</td>
<td>0,75</td>
<td>0,75</td>
<td></td>
</tr>
<tr>
<td>Salicylate</td>
<td>0,50</td>
<td>0,50</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>1,25</td>
<td>0,75</td>
<td>0,50</td>
</tr>
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<td></td>
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</tbody>
</table>

The tissue culture chambers were sealed and incubated at 37°C for 24 hours. The migration areas were measured with a planimeter and the migration index was determined by the equation

\[
\text{migration index} = \frac{\text{area of migration in antigen}}{\text{area of migration without antigen}}
\]

In one experiment the BCG was reconstituted with saline and with saline containing sodium salicylate (3.5 mmol/ml). Both these preparations of BCG were dialysed for 24 hours against buffered saline and tested on the blood leukocytes from a subject whose migration index was shown to be less than 0.80.

RESULTS

Twenty-four subjects were tested. This group comprised 15 subjects who had positive Mantoux tests (10 healthy subjects and 5 patients) and 9 subjects whose Mantoux
tests were negative (8 healthy subjects and 1 patient). The 6 patients were hospitalised for the following reasons: tuberculosis (2), chronic rheumatic heart disease (2), chronic non-tuberculous chest disease (1) and nocardiosis (1). With one exception, all the patients were Mantoux-positive.

A leucocyte migration test was judged positive when the migration index was 0.80 or less. In 14 of the 15 subjects whose skin tests were positive, a migration index of less than 0.80 was obtained, and in all the subjects whose Mantoux tests were negative, a migration index of greater than 0.80 was obtained. No attempt was made to grade the Mantoux reactions and to correlate them with the migration index.

The results of in vitro tests are shown in Figs 1 - 3. The patterns of leucocyte migration in saline or saline + salicylate were nearly identical in most instances. In 10 subjects in whom a migration index of 0.80 or less was obtained the incorporation of salicylate into the antigen-containing medium resulted in a migration index which averaged higher than the migration index obtained from antigen alone. These changes of migration indices were significant in 9 subjects ($P = 0.01$) and the magnitude of change in 5 of these 9 subjects was sufficient to convert a positive migration index into a negative one. There were 4 subjects with positive migration indices in whom the incorporation of salicylate into the antigen-containing medium resulted in further decreases in their migration index. These changes were significant in 2 subjects ($P = 0.01$).

In the Mantoux-negative individuals the antigen-salicylate combination also produced a change of the migration index which was significant in half of the subjects ($P = 0.01$).

**DISCUSSION**

The addition of sodium salicylate in concentrations of 3.5 mmol/ml of tissue culture medium did not influence the migration of cells from capillary tubes and produced patterns of migration which were similar to those en-
countered when cells were allowed to migrate in tissue culture medium which contained saline. In 4 instances the effect of incorporating salicylate into antigen-containing culture media was reversed, and enhanced migration inhibition was seen. The migration pattern in these instances showed cell clumping, which may represent a toxic effect. Since the migration pattern of the same cells in salicylate-containing medium did not differ from that from the control media, it is not possible to infer a toxic effect from the salicylate alone. The reasons for this enhanced effect are not clear. None of the subjects was taking salicylate at the time of the experiment, so that it is not possible to postulate an increased effect due to concurrent salicylate administration.

In contrast to the lack of any effect of salicylate on cell migration in control media, changes were encountered when salicylate and a specific antigen were included together in the assay medium. The antigen-containing medium produced the expected inhibition of leucocyte migration and migration indices of less than 0,80. The assay medium containing antigen and salicylate produced changes in leucocyte migration which differed from those found in both the salicylate-containing and the antigen-containing media. In these media the leucocyte migration areas were increased and when the migration indices for these media were calculated, they were found to be increased. The increased migration indices thus obtained were on average higher than those obtained with antigen alone, and in a few instances they exceeded the accepted upper limit for positive tests. In a few individuals the reverse of this phenomenon occurred, viz. the migration indices in the salicylate-antigen medium were lower than those obtained with antigen alone. In this small group there were no instances where a previously negative (greater than 0,80) result was changed to a positive one.

The effects of the salicylate could have been achieved on action on any of the formed elements of the blood, but since there were changes only in the medium containing antigen and salicylate it seems likely that the salicylate effect was mediated through an action on the specifically sensitised lymphocytes.

Specifically sensitised lymphocytes elaborate lymphokines in response to an encounter with specific antigen, and the effect of one of these lymphokines is to inhibit the migration of peripheral blood leucocytes. The leucocyte-inhibiting factor (LIF) is similar but not identical to the migration inhibiting factor (MIF) but it is probably also dependent on protein synthesis for its elaboration.

Salicylate has been shown to affect lymphocytes in several ways. In a study on the effect of salicylate on peripheral blood lymphocytes, concentrations of 0,5 mg/ml were shown to reduce protein synthesis, and several studies on the effect of salicylate on lymphocyte transformation have shown that concentrations of 0,02 - 3,0 mg/ml have profound effects on the response of lymphocytes to mitogens. One study has also documented the same effect after ingestion of salicylate. These effects on protein synthesis are probably non-specific and affect other cells in a similar way, but are more readily apparent in the immunocompetent cells.

This effect of salicylate on cell migration may have relevance to studies of in vitro cell-mediated immunity in patients with rheumatoid arthritis, and they suggest that prior salicylate administration could influence results, either by mitigating the effects of leucocyte migration, or by enhancing it.

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REFERENCES