EFFECTS OF GLYCYRRHIZIN ON THYMOLYTIC AND IMMUNOSUPPRESSIVE ACTION OF CORTISONE

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SYNOPSIS

Glycyrrhizin has been clinically used as an anti-allergic agent, but its mechanism remains unknown. This study was undertaken to examine the effect of glycyrrhizin on thymolytic and immunosuppressive action of cortisone in normal rabbits and adrenalectomized rats. Immunization was performed by injection of bacterial α-amylase emulsified in Freund’s incomplete adjuvant. Antibody titer of the serum was expressed as the enzyme-neutralizing activity. The circulating antibody could be detected in normal rabbits about 10 days after immunization and elevated lineally thereafter. The antibody titer was suppressed to one half and to one fourth of the control by administration of 15 mg/Kg and 30 mg/Kg of glycyrrhizin respectively. Glycyrrhizin had no effect on the antibody titer in the case of adrenalectomized rats, though the titer of the control in these rats was lower than that in normal rabbits. Cortisone treatment resulted in the decrease of the titer. By concomitant administration of glycyrrhizin with cortisone the antibody titer was much more decreased, thus showing an enhancing effect of glycyrrhizin on immunosuppressive action of cortisone. On the other hand, thymolytic action of cortisone was inhibited by concomitant administration of glycyrrhizin on the basis of thymus weight in rats. This effect of cortisone was blocked by glycyrrhizin. Glycyrrhizin by itself had no significant effect on thymus weight. The dissociation of the effect of glycyrrhizin on these cortisone actions suggests that one biological action of cortisone might come through some different mechanism from another. Mechanisms of the action of glycyrrhizin were discussed in relation to various biological actions of cortisone.

It has been reported from our laboratory that glycyrrhizin inhibited the following biological actions of cortisone: anti-granulomatous action (Kumagai et al., 1964), suppression of ACTH biosynthesis in the pituitary (Kumagai et al., 1966 a), liver glycogen deposition and induction of liver tryptophan pyrrolase (Kumagai et al., 1966 b). Anti-inflammatory or anti-exudative action of cortisone, however, was hardly affected by glycyrrhizin (Kumagai et al., 1964).

This study was undertaken to examine how glycyrrhizin influenced such other actions of cortisone as thymolytic and immunosuppressive action.

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MATERIALS AND METHODS

Crystalline bacterial α-amylase (3.2.1.1) was emulsified in an equal volume of Freund's incomplete adjuvant composed of paraffin oil and arlacel A (9:1 v/v). Final concentration of the antigen was 2 mg/ml of the emulsion. Normal female rabbits weighing approximately 2.5 Kg and female adrenalectomized rats of Sprague-Dawley strain (body weight 110-150 g) were immunized by subcutaneous injection of 0.5 ml of this emulsion. In rats, blood was collected through abdominal aorta and the thymus was removed and weighed 20 days after immunization. In rabbits, blood sample was obtained from the auricular vein 10 days after immunization.

Antibody titer of the serum was measured by the method of Onoue et al. (1964) and expressed as the enzyme neutralizing activity.

Rats were divided into the following four groups: 1) control, 2) cortisone, 3) glycyrrhizin and 4) cortisone + glycyrrhizin. Rabbits were divided into two groups: 1) control and 2) glycyrrhizin. The treatments were performed for 8 successive days from the day of immunization. The administered doses of the drugs are described below in the Results.

RESULTS

Effect of cortisone and glycyrrhizin single or in combination on thymus weight in adrenalectomized rats

Table 1 shows no difference of thymus weight between control and glycyrrhizin group in adrenalectomized rats. Thymus weight of cortisone group was significantly less than that of control group, while concomitant administration of 10 mg of glycyrrhizin with 1.25 mg of cortisone did not decrease thymus weight (Table 2). Thus, 10 mg of glycyrrhizin significantly (P<.05) blocked the thymolytic action of cortisone.

Effect of glycyrrhizin on circulating antibacterial α-amylase antibody titer in normal rabbits

Anti-bacterial α-amylase antibody of the control rabbits could be detected about 10 days after immunization and elevated linearly thereafter for the following 20 days (Fig. 1). The antibody titer was suppressed by the administration of 15 mg/Kg and 30 mg/Kg of glycyrrhizin respectively to one half and to one fourth of the control. This finding suggests that glycyrrhizin has suppressive

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Glycyrrhizin 0.1 mg/d</th>
<th>Glycyrrhizin 10 mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus weight per 100 g B.W.</td>
<td>418±58.1*</td>
<td>416±22.6</td>
<td>419±24.0</td>
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<td>(N=4)</td>
<td>(N=4)</td>
<td>(N=5)</td>
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</tbody>
</table>

* mean±S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Cortisone 1.25 mg/d</th>
<th>Cortisone 1.25 mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+ Glycyrrhizin 0.1 mg/d</td>
<td>+ Glycyrrhizin 10 mg/d</td>
</tr>
<tr>
<td>Thymus weight per 100 g B.W.</td>
<td>470±35.7</td>
<td>384±55.8</td>
<td>463±75.9**</td>
</tr>
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<td>(N=4)</td>
<td>(N=7)</td>
<td>(N=7)</td>
<td>(N=8)</td>
</tr>
</tbody>
</table>

* mean±S.D.

** Difference between the last two columns is significant (P<.05)
Effect on the level of circulating antibody production in normal rabbits.

Effect of cortisone and/or glycyrrhizin on circulating anti-bacterial α-amylase antibody titer in adrenalectomized rats

In adrenalectomized rats, it was found that the antibody titer was about 20 units in control group 20 days after immunization (Fig. 2). Treatment with cortisone resulted in the decrease of the titer. By administration of glycyrrhizin combined with cortisone the antibody titer was much more decreased. In another experiment, there could not be found any significant difference in antibody titer between control (14.9±3.7 units) and glycyrrhizin group (13.6±4.1 units). These data indicate that glycyrrhizin has an enhancing effect on immunosuppressive action of cortisone.

DISCUSSION

It has been accepted that cortisone has thymolytic and immunosuppressive action, which was confirmed by the present studies. Glycyrrhizin, when given with cortisone, inhibited thymolytic action of cortisone, while it enhanced immunosuppressive action of cortisone.

The dissociation of the effects of glycyrrhizin on the two actions of cortisone is very interesting and this dissociation suggests that one biological action of cortisone might come through some different mechanism from another. The finding that glycyrrhizin had no significant effect in adrenalectomized rats suggests that glycyrrhizin was effective only in the presence of cortisone. And the lowering effect of glycyrrhizin on antibody titer in normal rabbits might be considered due to the interaction with endogenous glucocorticoids.

It has been generally accepted that glycyrrhizin has some anti-allergic effect, (Kuroyanagi et al., 1961) but the mechanism remains unknown. From the present study, this anti-allergic effect of glycyrrhizin might be partly due to the suppression of circulating antibody production by enhancing the action of cortisone.

In the previous report (Kumagai et al., 1957), the authors demonstrated that glycyrrhizin enhanced the therapeutic effect of
cortisone and ACTH in patients with systemic lupus erythematosus and rheumatic fever, which was considered to be ascribed to the inhibition of steroid metabolism in the liver by glycyrrhizin. The suppressive effect of glycyrrhizin on circulating antibody titer might be explained by the inhibition of steroid degradation by glycyrrhizin.

In regard to the inhibitory effect of glycyrrhizin on other cortisone actions like thymolytic or anti-granulomatous action, it is supposed that glycyrrhizin may be acting at the site of cortisone action.

It is considered from our data that glycyrrhizin is effective in the presence of cortisone by influencing the action and metabolism of glucocorticoids.

SUMMARY

1. Concomitant administration of glycyrrhizin with cortisone to rats inhibited cortisone action in reducing thymus weight.
2. Glycyrrhizin, however, enhanced cortisone action to suppress circulating antibody titer in adrenalectomized rats.
3. Glycyrrhizin suppressed circulating antibody titer in normal rabbits.
4. Mechanism of the effect of glycyrrhizin on thymolytic and immunosuppressive action of cortisone were discussed.

REFERENCES