EFFECT OF STEROID HORMONES ON THE SILICA-INDUCED GRANULATION TISSUE IN THE RAT

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It has recently been confirmed that systemic administration of cortisone markedly inhibits the regeneration of connective tissues (1, 2), and also that this compound prevents the development of granulation tissues around turpentine-induced abscesses, when it is administered simultaneously with the provocative turpentine (3). Hydrocortisone has independently reported to have a local effect of inhibition on tuberculin reaction, when this compound was previously administered under the skin, and similarly to show antiinflammatory effect on mosquito bits and chemical compound application (4, 5). Fibroblast destruction by histamin injection is successfully prevented with combined administration of either one of cortisone or hydrocortisone, and, at the same time, polymorphonuclear leukocytes are suppressed to infiltrate into the histamin-produced foci (6). Contrary to the antiphlogistic effect of gluco-corticoids, desoxy-cortico-sterone, one of the most potent mineralo-corticoids, has been observed to have a stimulating effect on wound healing (7). This evidence entirely agrees with the concept of prophlogistic mineralo-corticoids and antiphlogistic gluco-corticoids (8). By a brilliant method to test granuloma-inducing effects of various irritants and of their combination with steroid hormones, it was found that desoxycorticosterone and \( \Delta^5 \)-pregnenolone failed to inhibit inflammatory reaction with the similar dose to hydrocortisone (9).

It is well evidenced that silica particles are markedly potent to develop local fibrosis when they are intraperitoneally and subcutaneously administered as well as inhaled through the respiratory tract. The latter mode of administration offers a serious problem on miner's silicosis. Therefore, the effect of steroid hormones on experimental silicosis strongly attracts our attention in order to obtain the final indication to human silicosis. When a finely ground quartz powder was introduced into the intraperitoneal cavity of animals combined with systemic treatment of cortisone, the fibrosis was inhibited in the mouse and depressed in the rat (10, 11). However, there was no influence to the previously developed fibrosis (2, 12). Although this fact is somewhat discouraging to secure a successful measure to prevent human silicosis, there is still left some probability to suppress its further development and more interest in connection with so-called “constitutional” participation in the aggression of silicosis. Along this line, we have examined the effect of four kinds of steroids
available at present, namely, hydrocortisone, progesterone, estradiol and desoxycorticosterone, on the granulation tissue induced by silica dusts.

**EXPERIMENTAL METHOD**

The silica particles which were subcutaneously administered in mixing with relevant steroid hormones, were composed of natural quartz with 99.2% of purity and finely ground in less than 3μ of diameter. Four kinds of steroid hormones were hydrocortisone (HYDROCORTONE, Merck), desoxycorticosterone (CORTATE, Schering), estradiol and progesterone. Each cubic centimeter of vial had been prepared so as to contain 25 mg of steroid, except for desoxycorticosterone. Desoxycorticosterone was suspended in sesame oil with 5 mg/cm³ (a commercial preparation). The rest three compounds were suspended in a vehicle composed of 0.9% benzyl alcohol, 0.9% NaCl, 0.4% polyoxyethylene sorbitan monooleate, 0.5% sodium carboxymethyl cellulose, and finally distilled water.

Three aqueous suspensions were individually mixed with quartz powder so that 0.1 cc suspension might be administered for one injection with addition of 25 mg of the latter. Oil suspension of desoxycorticosterone was similarly prepared so that 0.3 cc. of suspension mixed with 25 mg of quartz powder might be administered for one injection. Control suspension of silica particles with the same vehicle as for steroid preparations was prepared in the similar way. Quartz powder had been carefully sterilized prior to its mixture.

Thirty adult male rats were divided into five groups with equal number of six animals. One was a control group and the rest deserved as treated groups. Among six animals in each treated group, three received two subcutaneous injections paired on both sides of the abdominal region with silica-and-steroid mixed suspension. The rest three rats were subcutaneously injected with a mixed suspension and a control simple suspension into the similar regions of the abdomen; the mixed suspension was administered on the left side, and the simple suspension with silica on the right side.

Two animals of each group thus treated were sacrificed in the different three periods, that is, on the 2nd, 5th, and 15th days. Specimens were taken from the regions of various treatments, fixed with formalin, and stained by both hematoxylin-eosin and Mallory methods.

**RESULTS**

On the second day of treatment, a cluster of quartz powder was formed at the site of injection. On its peripheral zone, there were found the infiltration of leukocytes and the proliferation of histiocytes*, and on its inner zone, a lot of destroyed nuclei (plate 1). On the fifth day after the initiation of treatments, there were found more pronounced proliferation of histiocytes and active vas-

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* Prof. K. Akazaki proved that this cell is the histiocyte by neutral-red staining.
cularization into the foci (plate 2). On the fifteenth day, the foci showed the most active proliferation of histiocytes and slight intercellular fiber formation (plate 3).

In the group of hydrocortisone treatment, on the contrary, there was found neither infiltration nor proliferation at any stage of fifteen days' experiment (plates 4–6). There was, of course, no picture of fiber formation. In the animals who were injected with both mixed and control suspensions, the left focus treated with the mixed suspension showed no granulation tissue formation, whereas the other focus treated with the control suspension had the same picture as that of the control group with pronounced granulation tissue formation. Therefore, it is clearly shown that the remarkable inhibitory effect of hydrocortisone is not systemic but essentially topical.

Progensterone showed a slight inhibition to granulation tissue formation throughout the period of experiment (plate 7), while estradiol showed no effect on the reaction. The result of desoxycorticosterone was variable; several foci showed an increased production and the others showed a decreased production (plates 8, 9).

**DISCUSSION**

From these results it is obvious that the hydrocortisone simultaneously introduced with quartz powder has a pronounced potency to inhibit the granulation tissue formation that is ordinarily produced by the administration of silica particles. This inhibitory effect of hydrocortisone is strictly local in spite of that of cortisone which has been found to be effective by systemic administration (10, 11). Shapiro (3) and Selye (9) have made similar observations that hydrocortisone is potent to inhibit topical inflammatory reactions induced by several kinds of irritant. It is also reported that no infiltration of polymorphonuclear leukocytes occurs during histamin-induced inflammation, if hydrocortisone is simultaneously administered (6). The inhibitory effect of the compound on their infiltration is correspondingly characteristic in the earlier stage of this experimental observation with silicosis. A remarkable inhibition to the histiocyte proliferation is also found in the early stage. Therefore, proliferative vascularization and fiber formation were continuously prevented at least until the fifteenth day after the initiation of treatment when the last specimens were taken. The mechanisms of these inhibitory effects are still obscure and worthy of further studies.

Although hydrocortisone is now considered to affect the inflammatory reaction at the cellular level (3, 6, 13), one of the authors has recently evidenced an ischemic zone around the wheal following the intracutaneous administration of hydrocortisone in humans, and suspected a possible role of peripheral vascular system in affecting inflammation (14). In respect to the local effect of hydrocortisone, an interesting report by Goldman and his associates (15) should be
referred. They have evidenced that the local injection of free alcohol of hydrocortisone, plate crystal type, produces less pronounced reactions.

As for the variable results of desoxycorticosterone, conditioning factors should be considered. Since the mineralo-corticoids are conditionally-acting hormones, it is more probable that their prophlogistic effect upon the foci greatly depends on the sensitizing factors.

Table 1 shows tentative gradings of antigranulomatogenic potency in four steroids here studied in regard to silicotic granuloma. Lipschutz and his associates have made a scheme exclusively based upon experimental findings in regard to

<table>
<thead>
<tr>
<th>Name of Compound</th>
<th>Grade</th>
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<tbody>
<tr>
<td>Hydrocortisone Acetate (aqueous suspension)</td>
<td>++++</td>
</tr>
<tr>
<td>Progesterone (&quot; )</td>
<td>+</td>
</tr>
<tr>
<td>Estradiol (&quot; )</td>
<td>0</td>
</tr>
<tr>
<td>Desoxycorticosterone Acetate (oil suspension)</td>
<td>- or +</td>
</tr>
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antifibromatogenic action of cortical steroids (16). They have made a comparison as to chemical structures of those steroids, and shown that progesterone is the most potential antifibromatogenic compound, desoxycorticosterone and desoxocorticosterone are less potent, and cortisone is the least potent. This finding partly agrees with our results obtained by the silica-induced granuloma which is generally expected to develop itself to fibrosis in the long run, while their results are exclusively taken from the estrogen-induced abdominal fibroma. Silica particles are very stable as an inorganic compound. This character is particularly convenient to study antigranulomatogenic action with a variety of steroids, because the granulomatogenic and fibrogenic potencies of quartz are remained at a constant level during prolonged period of treatment. Therefore, quartz powder seems to be excellent to compare the antigranulomatogenic and/or antifibrogenic potencies of various steroid hormones as such.

**SUMMARY**

Four kinds of steroids (hydrocortisone, progenerone, desoxycorticosterone, and estradiol) were comparatively studied in the rat to evaluate their antigranulomatogenic potencies when they were subcutaneously administered in mixing with quartz powder. The histo-pathological results showed a pronounced antigranulomatogenic action (suppression of leukocytes infiltration, histiocyte proliferation, active vascularization, and fiber formation usually induced by silica particles) of hydrocortisone, the less marked potency of progesterone, the variable effects of desoxycorticosterone, and the ineffectiveness of estradiol, so far as examined for fifteen days following the initiation of treatment. Possible mechanisms were discussed.
The authors gratefully acknowledge the generous supply of hydrocortisone by Merck & Co., U. S. A., estradiol by Sankyo Co., desoxycorticosterone by Nakamura-Taki Co., and progesterone by Prof. Y. Ito of Tokyo University as well as the technical assistance of Mr. T. Horiuchi.

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REFERENCES


Plate 1. Medium magnification of a focus simply treated with silica particles for two days. Note slight infiltration of leukocytes.

Plate 2. A section with similar treatment to plate 1 for 5 days. Note the granulation tissue formation.

Plate 3. A section with the same treatment as plate 1, but after 15 days. Note a pronounced granulation tissue formation invading toward the center of a dust cluster.

Plate 4. Medium magnification of a focus treated for 2 days with a mixed suspension of hydrocortisone acetate and silica particles. No reaction is revealed.

Plate 5. Focus treated for 5 days with hydrocortisone and silica particles. Note no reaction.

Plate 6. Focus treated for 15 days with hydrocortisone and silica. Still no reaction is seen.

Plate 7. Focus treated for 15 days with a mixed suspension of progesterone and silica particles. Note less pronounced reaction compared to the control section (plate 3).

Plate 8. Focus treated for 5 days with a oil suspension of desoxycorticosterone acetate and silica particles. Note rather pronounced reaction. Several vacuols are seen in the lower part of section. They result from the administered sesame oil.

Plate 9. Focus treated for 5 days with a oil suspension mixed with desoxycorticosterone acetate and silica particles. Note the suppressing effect of desoxycorticosterone acetate.