STUDIES OF THE MECHANISM OF GLUCOCORTICOID ON GROWTH RETARDATION

—Effect on Body Growth, Serum Levels of Somatomedin A and Chondrocyte Proliferation in the Rats—

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SUMMARY

Serum levels of somatomedin A, as measured by radioreceptor assay, and body weight gain were 86.5 ± 9.2% and 166.9 ± 7.8% (N=5) of the initial values, respectively, after 18 days administration of 2.5 mg cortisone acetate (CA). These values were significantly lower than those for saline treated rats (p<0.005). Reduced serum somatomedin A and body growth rate were partially restored after halting the injection of CA. A positive correlation was observed between body weight and serum somatomedin A values (N=30, r=0.55, p<0.005). A combined administration of daily doses of 100 μg hGH with CA did not prevent the decrease in somatomedin activity in treated rats. This observation suggests that decreased secretion of GH plays a minor (or no) role in the fall of serum somatomedin A in CA-treated rats.

By utilizing chondrocyte culture the direct inhibitory effect of hydrocortisone for chondrocyte proliferation was observed.

From these data we conclude that the mechanism of glucocorticoids on retarded growth is partly due to reducing serum levels of somatomedin by inhibiting the effect of GH on the generation of somatomedin and partly due to a direct inhibitory effect of cell growth.

INTRODUCTION

Growth is a complicated phenomenon and there are a number of factors which influence growth in children. Several clinical observations show that glucocorticoids have a growth retarding effect in children. Children with Cushing's syndrome suffer retarded growth. Their height increases after surgical treatment. Somatomedin is widely known to stimulate growth of cartilage and is accepted as being one of the factors responsible for human growth. The mechanisms of the effect of glucocorticoids on retarded growth have been suggested to be due partly to a direct inhibitory effect on cell growth and partly to a suppressive effect on the release of growth hormone, which regulates the generation of somatomedins.

In this paper, we studied the effects of glucocorticoids and GH on body growth, serum levels of somatomedin and chondrocyte proliferation in rats. The somatomedin A levels were determined by radioreceptor assay.

MATERIALS AND METHODS

Effect of cortisone acetate in vivo

Male rats of the Wistar strain were used in the following experiments. They were housed in a room kept at a constant temperature (23°C) and lighted from 0800-2000 h, and maintained on laboratory chow (Oriental Yeast Co., Ltd. Tokyo). The injection of each substance was performed subcutaneously.

Group A consisted of 15 rats 28 days old. They were divided into 3 subgroups of 5 rats each; group I was given a daily administration of saline for 18 days; group II was given a daily injection of 2.5 mg cortisone acetate (CA) for 18 days; and group III was given a daily administration of 2.5 mg CA for 7 days followed by the administration of saline for 11 days.

Group B was comprised of 20 rats 21 days old. They were divided into 4 subgroups of 5 rats each receiving daily one of the following; saline, saline...
+100 μg hGH, 2.5 mg CA, or 2.5 mg CA + 100 μg hGH for 6 days.

Body weight and food intake were determined daily. Venous blood samples were drawn from individual rats under ether anesthesia before, 7, and 18 days after injection in group A, and 6 days after injection in group B. The samples were centrifuged and the sera were stored at -20°C.

**Effect of hydrocortisone on chondrocyte culture**

Rat chondrocytes were isolated from normal male Wistar rats, weighing about 60 g, as described by Shimomura et al.7). Briefly, the costochondral junction was removed aseptically from rat and trimmed from adhering soft tissue. The translucent portions of cartilage were taken out, minced and used for culture. They were firstly incubated in Tyrode's solution with 0.1 % EDTA for 20 min at 37°C. Then they were digested in 0.1 % of trypsin for 1 h at 37°C and in 0.2 % of collagenase for following 3 h at 37°C. Isolated chondrocytes were collected by filtering through nylon sieve (120 μm pore size). The cell suspension was centrifuged and the cells were washed in Ham's F-12 medium. This procedure was repeated three times. The cells (1 × 10⁶ cells) were resuspended in 10 ml of Ham's F-12 medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml Streptomycin and 2 μg/ml fungisone and cultivated in 75 cm² flask in 5% CO₂ and air at 37°C for 3 days. After that the cells were grown in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum and antibiotics and fed every other day. When the cells reached confluence the cells were detached from flask by 0.125 % trypsin treatment and the cells were washed with MEM.

The effect of glucocorticoid on proliferation of rat chondrocytes were studied using these cells. The cells (6 × 10⁵ cells/3 ml MEM) were cultivated in 60 mm dish for 2 days. After that cells were grown in MEM with 10% normal rat serum, 10% cortisone acetate treated rat serum, or 10% normal rat serum plus hydrocortisone (0.5, 2.5, and 12.5 μg/ml). The medium was exchanged with new medium every other day. On the seventh day after adding rat serum and/or glucocorticoid the cell number of chondrocytes was counted.

**Radioreceptor assay for somatomedin A**

Serum somatomedin A was determined by the radioreceptor assay described previously⁸⁻¹⁰. The somatomedin A used for labelling was purified by Dr. L. Fryklund at Recip Polypeptide Laboratory, AB Kabi, Stockholm, and had a biological activity of 1,000 U/mg as determined by radioreceptor assay for somatomedin A. One unit (U) of somatomedin A is defined here as the somatomedin content in one ml of pooled human serum.

**Materials**

Cortisone acetate (cortone®) and hydrocortisone (Hydrocortone®) used for this study were obtained from Merck Banyu (Tokyo, Japan). The growth hormone preparation used for this study was a highly purified human growth hormone, Crescormone®, obtained from AB Kabi/Recip, Stockholm, Sweden. EDTA, trypsin and collagenase (type II) were obtained from Kanto Chemical Co. (Tokyo, Japan), Handai Biseibutsu-byo Kenkyukai (Osaka, Japan) and Sigma (Saint Louis, U.S.A.), respectively. MEM, fetal calf serum, penicillin, streptomycin and Amphotericin B (Fungisone®) were purchased from Gibco (N.Y., U.S.A.), and Ham's F-12 medium from Flow Laboratories (Virginia, U.S.A.).

**Statistics**

Student's t-test was used for statistical analysis. Correlation coefficients between serum somatomedin A and body weight were calculated by the least squares method.

**RESULTS**

The effects of CA and saline administration on body weight and serum somatomedin A were studied (Fig. 1). In saline treated rats the body weight increased with mean increases of 152.3 ± 1.6% and 228.5 ± 3.7% of the initial values on the 7th and 18th days, respectively. In CA treated rats, the body weight increased with mean increases of 206.5 ± 4.2% and 328.5 ± 6.7% of the initial values on the 7th and 18th days, respectively. The differences from saline treated rats are significant at p<0.05 (*) and p<0.005 (**).
and 18 days, respectively. In contrast, the mean increase of 124.4 ± 2.7% and 166.9 ± 7.8% of the initial values, respectively, in CA treated rats. These values were significantly lower than those for saline treated rats (p<0.005). A significant difference in body weight was observed after only 2 days' administration of CA (p<0.005). The body weight increased markedly after changing the injection of CA to saline on the 7th day (group III). There were significant differences among the body weights of the three groups on the 18th day.

The mean increases in serum somatomedin A levels in saline treated rats on days 7 and 18 of the experiment were 113.8 ± 5.3% and 141.5 ± 9.2% of the initial values, respectively. In contrast, somatomedin A levels in CA treated rats decreased to 92.7 ± 7.3% and 86.5 ± 9.2% of the initial values, respectively. A significant difference in serum somatomedin levels was observed only on the 18th day of the experiment (p<0.005). Serum somatomedin A increased after changing from the administration of CA to saline. When the correlations between percent increase in body weight and serum somatomedin A were calculated together in these rats, there was a positive correlation between these two values as shown in Fig. 2 (r=0.55, p<0.005, N=30). The mean total caloric intakes during the 7 days in which the rats treated with saline or CA were 424 and 417 Cal per rat, respectively, and those during the 18 days in the rats treated with saline, CA, or CA + saline were 1179, 1185, and 1218 Cal per rat, respectively. These three values did not differ significantly.

The combined effects of hGH and saline and/or CA administration on body weight and serum somatomedin A for 6 days are displayed in Fig. 3. The mean body weights of saline + hGH treated rats and CA + hGH treated rats were 86.0 ± 3.6 g and 62.7 ± 2.4 g, respectively. These values did not differ significantly from those for saline or CA treated rats, respectively. The mean levels of serum somatomedin A in saline and saline + hGH treated rats were 2.8 ± 0.2 U/ml and 3.2 ± 0.2 U/ml, respectively. These two values were not statistically different. The mean levels of serum somatomedin A in CA and CA + hGH treated rats were 1.7 ± 0.2 U/ml and 1.9 ± 0.2 U/ml, respectively. There was a positive correlation between the serum somatomedin level and body weight in the four groups of rats on day 6 of the experiments (Fig. 4) (r=0.79, p<0.001, N=20). The total caloric intake during 6 days in the rats treated with saline, saline + hGH, CA, or CA + hGH were 197, 188, 171, and 169 Cal per rat, respectively.

The direct effect of CA in the somatomedin receptor assay was examined. CA, at very high concentrations up to 300 µg/ml, did not disturb this assay. To examine the possibility of some factors which might be present in CA-treated sera and interfere in the assay, the following experiments were performed. Serum from a normal rat was mixed...
Table 1  Somatomedin A levels in sera from normal and cortisone acetate (CA) treated rats and in mixtures of normal and CA treated sera

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CA</th>
<th>Mixture of normal and CA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
</tr>
<tr>
<td>Somatomedin A (U/ml)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal</td>
<td>5.74</td>
<td>1.48</td>
<td>3.61</td>
</tr>
<tr>
<td>CA</td>
<td>5.44</td>
<td>1.52</td>
<td>3.48</td>
</tr>
<tr>
<td>Mixture of normal and CA</td>
<td>5.00</td>
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<td>3.24</td>
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with an equal volume of serum from a CA-treated rat, and the concentrations of somatomedin A in the three mixtures were determined by radioreceptor assay (Table 1). The values obtained were exactly those expected from the concentrations of somatomedin A present in the serum from the normal rat plus that from the CA-treated rat.

In order to study the direct effect of glucocorticoid on proliferation of cartilage, the chondrocyte culture was used. The number of chondrocyte incubated for 7 days with normal rat serum was $15.2 \pm 1.04 \times 10^5$/dish while those incubated with CA-treated rat serum and 0.5 µg/ml, 2.5 µg/ml and 12.5 µg/ml of hydrocortisone were $8.5 \pm 1.58 \times 10^5$, $7.8 \pm 0.49 \times 10^5$, $9.0 \pm 0.74 \times 10^5$ and $8.8 \pm 1.21 \times 10^5$, respectively. These values were significantly lower than that obtained from normal rat serum ($p<0.01$).

**DISCUSSION**

The results of our study demonstrate that, in CA treated rats, the serum levels of somatomedin A as well as body weight are significantly lower than those in saline treated rats. These levels are partially restored by ceasing the injection of CA. A positive correlation was obtained when the body weight was compared with the serum somatomedin A level. Previously, we reported that serum levels of somatomedin A decreased after fasting. In the present study a similar quantity of calories was consumed by CA treated rats and saline treated rats, suggesting that shortage of caloric intake alone cannot account for the difference in somatomedin A levels. A sufficient recovery of somatomedin A in mixed serum of a normal rat with a CA treated rat revealed that the low levels of somatomedin A found in CA treated rats cannot be ascribed to the presence of substances which affect the radioreceptor assay directly.

There are some discussions of the effect of glucocorticoids on the serum levels of GH. Morris et al.
and others studied serum GH concentration in children treated with corticosteroids and found that GH levels were similar to those of normal children both during fasting and after insulin-induced hypoglycemia. However, in children with Cushing's syndrome a subnormal growth hormone in insulin-induced hypoglycemia was reported by Strickland et al. and others. In adult patients undergoing glucocorticoid treatment and with Cushing's disease basal level of GH and GH response to hypoglycemia decreased. In animal studies, Pecile & Müller measured the GH content of rat pituitary glands and reported that the suppressive action of cortisol on GH secretion was due to an impairment of the release mechanism for GH. In this connection, we examined whether sufficient amounts of simultaneously administered GH restored the decreased levels of somatomedin A induced by CA injection. A slight increase in serum somatomedin A occurred when hGH was administered with saline; however there is no increase in somatomedin A when hGH was administered with CA. These observations suggest that GH plays a minor (or no) role in the fall of serum somatomedin in CA treated rats.

Clarke et al., utilizing a chick cartilage bioassay, observed a greater than 50% reduction in serum somatomedin activity in young rats receiving daily doses of 20 mg/kg cortisol for 10 days. The administration of CA impeded the rise in serum somatomedin levels in hypophysectomized rats treated with marginal doses of bGH. They suggested that glucocorticoid inhibition of growth involved antagonism of GH-induced somatomedin generation. Our study further confirmed his speculation by measuring somatomedin by radioreceptor assay, which is not influenced by inhibitory factors affecting sulphate uptake into cartilage.

It is well recognized that glucocorticoids at very high concentrations inhibit sulphate incorporation into chick embryonic cartilage and pig cartilage. Glucocorticoids also inhibit the conversion of proline-U-C14 to hydroxyproline. We observed that 0.5 µg/ml of hydrocortisone inhibits cell proliferation of growing cartilage in cell culture. It was reported that glucocorticoids morphologically caused the decrease of cell number and size and the change of ultrastructure of chondrocyte. Several investigators have also demonstrated the decrease of proteoglycan in cartilage. Therefore, there is no doubt that one cause of the retarded growth by glucocorticoids is due to direct inhibition of bone metabolism.

Somatomedin activity in Cushing's syndrome and children treated with glucocorticoids has been reported to be low by bioassay. Using radioreceptor assays for somatomedin A and C, normal levels of somatomedin were reported in Cushing's syndrome and Cushing's disease by radioimmunoassay. Recently, Thorén et al. reported that serum levels of somatomedin A by radioimmunoassay were normal in Cushing's disease and that the values increased significantly after administration of 8 IU (4 mg) hGH per day for 3 days. However, we did not observe increased levels of somatomedin A when hGH was simultaneously administered with CA. This difference might be due partly to the difference in the dosage of glucocorticoids and GH used, or partly to the difference in species.

The mechanism of the inhibitory effect of glucocorticoids on somatomedin generation is unknown. However, we speculate that glucocorticoids might compete with the effect of GH on somatomedin generation. The precise mechanism of this suppressive effect needs to be clarified.

ACKNOWLEDGMENTS

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References

4) Copinschi, G., P. Nève, R. Wolter and P.A.


グルココルチコイドの成長抑制作用の機序に関する研究
―ラットにおける体重、血清ソマトメジン値及び軟骨細胞への影響―

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小児にグルココルチコイドを投与すると、成長が抑制されることが臨床的に認められている。このグルココルチコイドの成長抑制作用の機序として、成長因子のひとつである血清ソマトメジン値への影響と、軟骨細胞への影響をラットを用いて検討した。血清ソマトメジンの測定は、ヒト胎盤細胞膜分画を用いるラジオレセプターアッセイにて行った。
ラットにコーシゾン・アセテート（CA）を1日2.5mgを18日間連続皮下注射したところ、体重増加率は116.9±7.8％と、CA投与群の増加率228.5±3.7％に比し著明に減少し、成長抑制作用を認めた。血清ソマトメジンA（SMA）値は、CA投与群では前値の86.5±9.2％に減少し、非投与群に比し著しく低値であった。CA投与7日目でCA投与を中止すると、体重及び血清SMA値の増加を認めた。これらのラットの血清SMA値と体重増加率との間に正の相関関係を認めた（r = 0.55, p < 0.005）。CA投与による血清SMA値の減少が、下垂体のGH分泌減少を介して起こったものかを検討するために、hGH 100μg/日をCAと同時に6日間投与して検討したところ、血清SMA値は依然として低値であった。従って、CAはSMの産生を直接抑制することが考えられた。

更に、グルココルチコイドの軟骨細胞への直接作用を研究するために、ラットの軟骨細胞培養を行い、培養液中にハイドロコルチゾン0.5μg/mlを加えたところ、細胞増殖作用の抑制が認められた。

以上の結果より、グルココルチコイド過剣による成長障害の機序の一部には、グルココルチコイドがSM合成過程を障害すること及び、軟骨細胞に対して細胞増殖を抑制することが考えられた。

―1871―