Growth Hormone Increases the CD4/CD8 Ratio in Splenic Lymphocytes in Glucocorticoid-Treated Rats

Hiroaki DOBASHI, Makoto SATO, Terukazu TANAKA, Hiroki MITSUNAKA, Genji YAMAOKA*, Michiaki TOKUDA and Jiro TAKAHARA

First Department of Internal Medicine 1750-1, Ikenobe, Miki-Cho, Kita-Gun, Kagawa 761-0793, Japan
* Central Laboratory, Kagawa Medical University, 1750-1, Ikenobe, Miki-Cho, Kita-Gun, Kagawa 761-0793, Japan

Key words: Growth hormone, Lymphocytes, Glucocorticoid

GLUCOCORTICOIDs (GC) are widely used to treat a variety of clinical conditions, including certain collagen diseases and bronchial asthma, but the therapeutic use is hampered by severe adverse effects related to the catabolic effects of GC. Growth hormone (GH) is a potent anabolic hormone and it might counteract some catabolic effects of GC. Several clinical studies suggest that GH can counteract the impairment of growth due to long-term GC treatment in children with juvenile rheumatoid arthritis [1] and bronchial asthma [2]. It has to be noted, however, that GH may interfere with the immunosuppressive effect of GC, because GH is thought to have an important role in the immune system [3]. Informations on the effect of GH in the immune system under chronic GC excess must be therefore be obtained before using GH.

Positive effects of the GH-insulin-like growth factor (IGF-I) axis in the immune function are supported by a number of in vivo and in vitro studies [3]. GH reverses thymic atrophy and deficiency in the number of T cells and their function in aged rats [4], aged monkeys [5] and GH-deficient mice [6]. In vitro, GH stimulates the proliferation of thymic epithelial cells [7]. These effects of GH may be mediated by autocrine and paracrine IGF-I, because many lines of evidence indicate that lymphoid tissues and lymphocytes produce IGF-I [3]. Proliferation of T-cell lines [8] and thymic epithelial cells [7] stimulated by GH is inhibited by antibodies specific to IGF-I and IGF-I receptor in vitro. IGF-I also directly stimulates the proliferation and inhibits apoptosis in hemopoietic cells [9-11]. These reports raise the possibility that GH counters the immunosuppressive effects of GC. In the present study we tested whether GH affects the subsets (CD4+/CD8+) of splenic lymphocytes in GC-treated rats.

Materials and Methods

Six-week-old male Wistar rats (Clea Japan) were used in this study. The rats were divided into four different groups. An osmotic minipump (Model 2002, Alzet Co., Palo Alto, CA), previously filled with dexamethasone (Dex) or saline, was implanted in the subcutaneous tissue of the rat's back. Since drug delivery by these minipumps is limited to 14 days, they were replaced 14 days later. Dex was infused into the rats continuously at the rate of 10 g/day for 28 days. Recombinant human GH (0.5 U/rat) or saline was administered sc every day for 28 days. The spleens were removed and splenic lymphocytes were collected by passing them through a sterilized mesh. The lymphocytes were cultured overnight in RPMI 1640 medium containing 10%
FCS. Subpopulations of the lymphocytes were determined by flow cytometric analysis with anti-CD3, anti-CD4 and anti-CD8.

The serum corticosterone and IGF-I levels were measured in the trunk blood obtained by decapitation as previously described [18].

Results

Four-week treatment with Dex (10 µg/day) significantly decreased the body growth in rats, and the changes in body weight, spleen weight, femur length and food intake are shown in Table 1. Daily sc administration of GH (0.5 U/day) failed to prevent Dex-induced impairment of body growth in the rats. Food intake was significantly reduced by Dex and slightly increased by GH administration. The changes in serum albumin, glucose, cholesterol, calcium and phosphate are shown in Table 2. Serum albumin, glucose, calcium and phosphate levels were similar in all four treatment groups. GH did not change Dex-induced hypercholesteremia. Figure 1 shows the serum corticosterone and IGF-I levels in the four groups. Endogenous corticosterone levels were noticeably suppressed by Dex, suggesting that the dose of Dex used (10 µg/day) was sufficient to

![Fig. 1. Serum corticosterone and IGF-I levels in the four groups. Dexamethasone (Dex) was continuously administered to the rats with an osmotic minipump at a dose of 10 µg/day for 28 days. GH was administered to the rats by means of a single sc injection every day for 28 days. Serum corticosterone levels were less than 20 ng/ml in all of the Dex-treated rats. Vertical lines represent the SEM. **P <0.01 (versus Saline + Saline). Numbers in parentheses indicate the number of animals in each group.](image)

Table 1. Changes in body weight, spleen weight, femur (right) length and food intake after 28-day treatment with Dex (10 µg/day) and GH (0.5 U/day) in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>Spleen weight (g)</th>
<th>Femur length (mm)</th>
<th>Food intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + Saline</td>
<td>(5)</td>
<td>155.2±1.5</td>
<td>314.4±2.5</td>
<td>0.82±0.02</td>
<td>34.22±0.31</td>
<td>18.0±0.3</td>
</tr>
<tr>
<td>Saline + Dex</td>
<td>(6)</td>
<td>159.8±1.1</td>
<td>208.2±3.6**</td>
<td>0.37±0.02**</td>
<td>29.82±0.16**</td>
<td>13.3±0.3**</td>
</tr>
<tr>
<td>Dex + GH</td>
<td>(4)</td>
<td>156.8±1.4</td>
<td>202.3±3.4**</td>
<td>0.36±0.01**</td>
<td>29.80±0.12**</td>
<td>14.2±0.3**</td>
</tr>
<tr>
<td>Saline + GH</td>
<td>(5)</td>
<td>153.8±1.3</td>
<td>319.4±6.6</td>
<td>0.79±0.05</td>
<td>34.08±0.30</td>
<td>19.4±0.4</td>
</tr>
</tbody>
</table>

BW; body weight, **P<0.01 vs Saline + Saline

Table 2. Changes in serum albumin, glucose, cholesterol, calcium and phosphate levels after 28-day treatment with Dex (10 µg/day) and GH (0.5 U/day) in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Albumin (g/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Calcium (mg/dl)</th>
<th>Phosphate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + Saline</td>
<td>(5)</td>
<td>2.64±0.06</td>
<td>146.6±3.2</td>
<td>80.4±4.0</td>
<td>11.18±0.16</td>
<td>9.48±0.25</td>
</tr>
<tr>
<td>Saline + Dex</td>
<td>(6)</td>
<td>2.83±0.10</td>
<td>133.0±2.2</td>
<td>128.8±5.1**</td>
<td>10.70±0.12</td>
<td>8.93±0.10</td>
</tr>
<tr>
<td>Dex + GH</td>
<td>(4)</td>
<td>2.95±0.07</td>
<td>121.8±4.6</td>
<td>153.5±6.4**</td>
<td>11.13±0.15</td>
<td>8.78±0.34</td>
</tr>
<tr>
<td>Saline + GH</td>
<td>(5)</td>
<td>2.62±0.04</td>
<td>137.8±3.2</td>
<td>88.2±3.2</td>
<td>11.42±0.11</td>
<td>9.56±0.05</td>
</tr>
</tbody>
</table>

BW; body weight, **P<0.01 vs Saline + Saline
EFFECT OF GH ON CD4/CD8 RATIO IN RATS

Inhibit the CRH-ACTH-adrenal axis in the rats. Although the serum IGF-I level was significantly inhibited by Dex, GH administration partially reversed the decrease (Fig. 1). The results of flow cytometric analysis are shown in Figure 2. The CD4/CD8 ratio was not altered by Dex treatment, but GH administration increased the CD4/CD8 ratio in Dex-treated rats, although it did not change the ratio in saline-treated rats.

Discussion

In the present study the CD4/CD8 ratio in splenic lymphocytes was not changed in the rats treated with Dex. On the other hand, the spleen weight was noticeably reduced by Dex treatment and the splenic cellularity was also decreased (data not shown). Dex treatment appears to kill CD4- and CD8-positive T lymphocytes equally in the rats. Interestingly, daily administration of GH increased the CD4/CD8 ratio in Dex-treated rats. This suggests that GH might inhibit the Dex-induced apoptosis in CD4-positive T lymphocytes. The observation that administration of GH alone had no effect on the CD4/CD8 ratio indicates that the immunological effect of GH occurs predominantly in chronic GC excess. Previous reports suggest that GH affects the function, proliferation and subsets of T lymphocytes [3]. Proliferation of normal and leukemic T lymphocytes is stimulated by GH in vitro [8, 13]. These effects could not be achieved with T cells derived from individuals with the Laron syndrome [8], suggesting that GH receptors are involved. LeRoith et al have reported that infusions of GH and IGF-I for 7 weeks affected the phenotype of lymphocytes in blood, spleen and lymphnodes as measured by flow cytometry [5]. The CD4/CD8 ratio fell with GH and IGF-I in blood but increased in the spleen. The authors suggest that the paradox of different effects on lymphocyte subsets in different body compartments may be due to the anabolic hormones affecting lymphocyte trafficking [5]. There is so far no report indicating a direct effect of GH on CD4-positive lymphocytes. The different effects of GH on the subsets of T lymphocytes need to be elucidated.

The effects of GH on the CD4/CD8 ratio in splenic lymphocytes might be mediated by IGF-I. Indeed, serum IGF-I levels were increased by GH in Dex-treated rats. IGF-I is generated in many tissues including hematopoietic systems as well as in the liver, and autocrine or paracrine activity of IGF-I is thought to be very important in the immunological activities of GH [3]. GH-induced stimulation of T cells and thymic epithelial cell proliferation are replaced by IGF-I and inhibited by antibodies to IGF-I and IGF-I receptor [7, 8]. IGF-I receptors are found in both CD4- and CD8-positive T lymphocytes [3]. Furthermore, one report suggests that IGF-I regulates the apoptosis of hematopoietic cells [9]. Taken together, it is possible that GH prevents the apoptosis of CD4-positive T lymphocytes through the autocrine or paracrine action of locally generated IGF-I in GC-treated rats. Clinical studies suggest that GH can counter the impairment of growth due to long-term GC treatment in children with juvenile rheumatoid arthritis [1] and bronchial asthma [2]. Our present results suggest that GH might interfere with the immunosuppressive effect of GC. This must be considered before recommending GH therapy for many diseases due to GC excess.
References