Acute and Subacute Effects of Dexamethasone on the Number of White Blood Cells in Rats

Yasuhiko Ohkaru, a Natsuko Arai, a Hitoshi Ohno, a Shogo Sato, a Yuko Sakakibara, a Hiroko Suzuki, a Shoko Aritoshi, a Shunta Akimoto, a Takamasa Ban, a Jun Tanihata, b Kaoru Tachiyashiki, c and Kazuhiko Imaizumi*, a

Laboratory of Physiological Sciences, a Laboratory of Rehabilitation Biomedical Sciences, Faculty of Human Sciences, Waseda University, 2–579–15 Mikajima, Tokorozawa, Saitama 359–1192, Japan and b Department of Living and Health Sciences, Joetsu University of Education, 1 Yamayashiki, Joetsu, Niigata 943–8512, Japan
(Received November 10, 2009; Accepted December 11, 2009; Published online December 14, 2009)

The purpose of this study was to elucidate the effects of dexamethasone, a synthetic glucocorticoid, on the immune system by analyzing the number of white blood cells (WBCs) over the course of hours and days of dexamethasone administration. Dexamethasone was given as either a single dosage [1.0 mg/kg body weight (BW); subcutaneous injection (s.c.)] or as a daily dosage (1.0 mg/kg BW per day; s.c.) for 10 days for the hourly and daily assessment of changes in the number of white blood cells, respectively. A single administration of dexamethasone markedly decreased the number of total WBCs, as well as the number of lymphocyte, monocyte, neutrophil and eosinophil subsets with a nadir at 8 hr post-injection. The number of these cells recovered to the control levels at 24 hr. The numbers of total WBCs, lymphocytes, monocyte, eosinophil and basophil were reduced by the daily administration of dexamethasone. However, the number of neutrophil was significantly higher at days 2 and 8 after the injection. These results suggest that glucocorticoid-mediated immuno-suppressions are at least partly attributable to quantitive changes in the number of circulating WBCs.

INTRODUCTION

Prolonged hypokinesia (i.e., reductions in limb movement) and/or hypodynamia (i.e., reductions in muscle loading) arising in the context of prolonged bed rest, restriction to a wheel chair, limited muscular function or a microgravity environment lead to skeletal muscle atrophy and a reduced capacity to do work.1–4) These conditions induce activation of the sympahto-adrenal axis and are associated with increased circulating levels of adrenal medulla-produced noradrenalin and adrenalin.5) Similarly, activation of the hypothalamo-hypophyseal-adrenocortical axis leads to the release of corticotropin releasing factor (CRF) from the pituitary and glucocorticoids from the adrenal cortex into the peripheral blood.2,6) These factors are known as modulators of immune system function.7, 8)

Physical exercise is also associated with changes in neuroendocrine and immune functions,5) and it can affect host defense mechanisms including the number of circulating granulocytes and secretory immunoglobulin levels.9–11) However, these induced changes are small in magnitude and brief in duration, and their physiological significance is uncertain.5–11) Therefore, a greater understanding of stress-induced immunesuppression derived from social and/or physical activity is needed.12–14)

Conditions leading to reduced physical activity and decreased mechanical muscle loading induce changes in the responses of physiological host defense systems including the hypothalamic-pituitary-adrenocortical axis, the sympathetic nervous system and the immune system. Host defenses are suppressed by glucocorticoids under stressful conditions.15–17) Glucocorticoids are generally known for their immunosuppressive effects, and widely used as a potent immunosuppressive agent.13–16) On the other hand, there is a little information on the effects of stress-hormones, glucocorticoids on host defense mechanisms such as under reduced physical activity conditions. Because patients suffering from immobility are more prone to infections, it is important to document the factors that physiologically modulate this response in...
In the present study, we examined the acute and subacute effects of a synthetic glucocorticoid, dexamethasone, on the number of total white blood cells (WBCs), lymphocytes, monocyte, neutrophil, eosinophil and basophil in rats.

**MATERIALS AND METHODS**

**Experimental Protocols and Animal Care**

All experiments had two parts: the acute and the subacute effects of dexamethasone on the number of total WBCs, lymphocytes, monocyte, neutrophils, eosinophils and basophils were examined in rats. The analyses of the number of red blood cells (RBCs), hemoglobin concentration and hematocrit value were also carried out by flow cytometry. The experimental protocols are shown in Fig. 1 (A and B).

Male 7-week-old Sprague Dawley rats (n = 38, CLEA Japan, Inc., Tokyo, Japan) were prefed for 5 days to allow adaptation to their new environment (Fig. 1). The rats were housed in cages at temperatures between 23–25°C and relative humidity of 50–60%. Lighting was automatically provided from 8:00–20:00. Animal chow (CE-2 cubic type, CLEA Japan, Inc.) and distilled water were given to the rats ad libitum. After the adaptation period, the rats were randomly divided into two groups in each experiment. Experiments were designed to minimize pain and discomfort for the rats. Both experiments were approved by the Committee on Animal Care Use at Waseda University.

**Administration of Dexamethasone to Rats**

Dexamethasone 21-phosphate (Sigma, St. Louis, MO, U.S.A.) was first dissolved in 0.9% NaCl solution as a vehicle to obtain a final dexamethasone concentration of 0.1%. In the dexamethasone-treated group, dexamethasone was administered at the cervical region of the back via a subcutaneous injection (s.c., 9:00–9:30 a.m.). In the control group, an equivalent volume of dexamethasone-free solution was administered in the same manner.

**Acute Effects of Dexamethasone on the Numbers of WBCs**

After 5 days of prefeeding, the rats were divided into a dexamethasone group [dose = 1.0 mg/kg body weight (BW), n = 9, the initial BW = 284 ± 3 g, mean ± SEM] and control group (n = 8, the initial BW = 286 ± 2 g, mean ± standard error of the mean (SEM)).

Count analyses of WBCs and RBCs were carried out by the hematology analyzer (Model SF-3000, Sysmex Co., Kobe, Japan) based on a flow cytometry technique with light-emitting diode. The SF-3000 type analyzer is known to fractionate lymphocytes, monocyte, neutrophil, eosinophil...
and basophil.\textsuperscript{17} The numbers of WBCs (lymphocytes, monocyte, neutrophil, eosinophil and basophil) and RBCs were analyzed by flow cytometry (Fig. 1A).	extsuperscript{5, 17, 22–25}

Plasma immunoglobulin G (IgG) concentrations were assayed by our routine method of enzyme-linked immunosorbent assay (ELISA) until 24 hr after dexamethasone administration.

Subacute Effects of Dexamethasone on the Number of WBCs —— After 5 days of prefeeding, the rats were divided into dexamethasone (dose = 1.0 mg/kg BW per day, \( n = 11 \), the initial BW = 259 ± 3 g, mean ± SEM) and control groups (\( n = 10 \), the initial BW = 260 ± 2 g, mean ± SEM). Dexamethasone was administered to rats for 10 days. Blood samples were collected from tail veins of rats. The numbers of WBCs (lymphocytes, monocyte, neutrophil, eosinophil and basophil) and RBCs were analyzed as already described (Fig. 1B).	extsuperscript{5, 23–26}

Statistical Analyses —— Experimental data were presented as mean ± SEM. The effects of dexamethasone on the number of WBCs were evaluated by a two-way analysis of variance (ANOVA) for repeated measures. Subsequent post hoc analyses to determine significant differences between two groups and from day 0 or hour 0 in each group were performed by Fisher’s protected least significant difference (PLSD) test. These analyses were carried out by the SPSS computer software (SPSS Japan Inc., Tokyo, Japan). The differences were considered significant when \( p < 0.05 \).

RESULTS

Significant Acute Changes in WBCs after Dexamethasone Administration

There were no significant changes in the number of RBCs, hemoglobin concentration and hematocrit value between the two groups at 0–24 hr after dexamethasone administration (data not shown). These results suggest that a single administration of dexamethasone does not alter the extracellular volume and short-term hematopoiesis. The administration of dexamethasone also did not alter plasma IgG concentrations at 0–24 hr (data not shown). However, as shown in Fig. 2, the administration of dexamethasone significantly decreased the number of total WBCs, lymphocytes, monocyte, neutrophil and eosinophil. The numbers reached a nadir at 8 hr after injection, but these cell populations returned to control levels at 24 hr. No essentially changes in the number of basophil in both groups were observed during the experiment (Fig. 2F).

Significant Subacute Changes in WBCs after Dexamethasone Administration

There were significant increases in the number of RBCs, hemoglobin concentration and hematocrit at days 8 and 10 following dexamethasone injection (data not shown). These results suggest that the administrations of dexamethasone for 10 days decreased the extracellular volume.

On the other hand, after a single administration of dexamethasone, the number of total WBCs decreased in the immediate post-injection period, but it returned control level by 24 hr (Fig. 2A). Despite these phenomena, when dexamethasone was administered to rats for several days, the number of total WBCs was significantly reduced to approximately 40% that seen in the control-treated animals at days 2, 8 and 10 (Fig. 3A). The kinetics in the reduction of lymphocytes were similar (Fig. 3B), but the num-
Fig. 3. Subacute Effects of Dexamethasone on the Number of WBCs
Values: mean ± SEM. A: total WBCs, B: lymphocytes, C: monocyte, D: neutrophil, E: eosinophil, and F: basophil. ⋄: control group (n = 10) and ●: dexamethasone group (n = 11). Statistics: * p < 0.05, "p < 0.01 and ""p < 0.001 (vs. control group).

After Fig. 3B, and the number of monocyte was nearly eliminated from the peripheral circulation after 10 days of dexamethasone injection (Fig. 3C). The numbers of eosinophil and basophil were also significantly reduced (Fig. 3E and 3F). However, the number of neutrophil increased at all time points measured (Fig. 3D).

DISCUSSION

In the present study, we investigated the acute and subacute effects of dexamethasone on the number of WBCs and WBC subsets in rats (Figs. 2 and 3). Briefly, a single dose of dexamethasone significantly reduced the numbers of circulating total WBCs, lymphocytes, monocyte, neutrophil and eosinophil by 8 hr after administration, but the numbers of these cells recovered by 24 hr. The number of basophil was not essentially affected. These results suggest that the short-term dexamethasone-induced changes in the number of total WBCs are possibly due to redistribution of cells; there may be some degree of cell death and decreased regeneration. In contrast, the administration of dexamethasone daily for 10 days led to significant decreases in the numbers of total WBCs, lymphocytes, monocyte, eosinophil and basophil, but the number of neutrophil was significantly increased.

Dhabhar et al.27) showed that numbers of total WBCs, lymphocytes and neutrophil were significantly reduced by corticosterone effects under restraint stress conditions of 2 hr. In the present study, we examined that the effect of synthesized glucocorticoid, dexamethasone, on the number of WBCs at 24 hr after injection and at consecutive injection for 10 days.

The observed decrease in circulating WBCs in hours following a single injection of dexamethasone with recovery of counts at 24 hr post-injection suggests some degree of altered leukocyte localization from the blood to the margins of blood vessels and interstitial space. Alternatively, induction of cell death by dexamethasone with subsequent restoration of cell populations by de novo hematopoiesis can not be excluded. Although we did not examine cells for markers of apoptosis such as annexin V, DNA fragmentation and morphologic changes, glucocorticoid-induced apoptosis has been well described for lymphocytes, monocyte and eosinophil.15 28) Heslet29) showed that apoptotic cells are engulfed by macrophages without the release of potential histotoxic contents, and the induction of apoptosis is an important mechanism by which glucocorticoids exert their anti-inflammatory properties.16 30) While glucocorticoids are known to induce apoptosis in dendritic cells, eosinophil, monocyte and T lymphocyte, they potently delay the rate of apoptosis in neutrophils.15 16 30 31) Glucocorticoids are released from the adrenal cortex in response to stress, and they can induce apoptosis of immature thymic epithelial cells. Studies of patients with prolonged bed rest, restriction to a wheelchair, restricted movement, limited muscular function and exposure to microgravity environment have demonstrated thymic atrophy, a decrease in the number of Cluster of Differentiation (CD)4+/CD8+ thymocytes, and DNA fragmentation seen in apoptosis.5 14 28) Different cell types are differentially susceptible to glucocorticoid-induced apoptosis. The combination of lymphocytes, neutrophil and monocyte together accounts for more than 98% of the number of total peripheral WBCs in.
In the present study, dexamethasone administration markedly decreased the number of WBCs, lymphocytes and monocyte at 1–3 hr after injection (Fig. 2A–2C). However, the subsets of granulocyte responded differently to dexamethasone treatment. The numbers of eosinophil and neutrophil were decreased, but the number of basophil was not essentially affected (Fig. 2D–2F). Additionally, the kinetics of these changes differed from these cell subsets such that the number of total WBCs was reduced by 1 hr post-injection, but changes in the number of neutrophil became apparent at only 4 and 8 hr nor for the number of basophil (Fig. 2D). The behavior of neutrophil differed from the two different phases of this study. Glucocorticoids are known to induce neutrophil mobilization from the bone marrow and inhibit apoptosis in circulating neutrophil while promoting migration of neutrophil into the tissues. Thus, glucocorticoids appear to exert different effects on neutrophil and basophil, and may differentially affect their migration and/or vascular margination (Fig. 2D).

In contrast to the short-term findings, the administration of dexamethasone for 10 days significantly decreased the number of circulating basophil (Fig. 3F), and significantly increased the number of circulating neutrophil (Fig. 3D) during the experimental period. Indeed, glucocorticoids are reported to enhance apoptosis of eosinophil and basophil, but they inhibit neutrophil apoptosis. In both short-term and long-term experiments, the administration of dexamethasone significantly decreased the number of circulating lymphocytes and monocyte (Figs. 2B, 2C, 3B, and 3C). Thus, the ability of dexamethasone to induce these cytopenias is likely directly related to its ability to modulate apoptosis and underlies the anti-inflammatory effects of glucocorticoids. These results suggest that the administration of glucocorticoid for 10 days decreased the number of WBCs, and dexamethasone-induced suppression of WBCs are maintained for 10 days. However, these properties remain uncertain, and future studies are needed to address and resolve these questions.


REFERENCES


