Methylprednisolone Induced Histological and Histochemical Changes in the Adrenal Cortex of Rat

S.A. Sakr, 1 N. I. El-Desouky and 2 S.M. Hanafy

1Zoology Department, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt
2Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
3Zoology Department, Faculty of Science, South Valley University, Aswan, Egypt

Abstract: In this experiment the effect of the synthetic glucocorticoid methylprednisolone on the adrenal cortex of albino rat was investigated by applying histological and histochemical techniques. Animals were orally given methylprednisolone at a dose level of 1.8 mg/kg body weight dissolved in sterile saline, 3 times/week for 3 weeks. Histological results revealed atrophy of zona fasciculata, thickness of adrenal capsule and hypertrophy of zona reticularis. Total carbohydrate content of the zona glomerulosa, zona fasciculata and zona reticularis cells showed an obvious decrease after treatment which was prominent after 3 weeks. Total protein content showed a marked decrease in zona glomerulosa and zona fasciculata. On the other hand, the cells of zona reticularis appeared with an increase amount of total protein.

Key words: Methylprednisolone, adrenal cortex, histology, histochemistry

Introduction
Glucocorticoids are commonly used as therapeutic agents for many diseases (Crosland, 1980). Methylprednisolone is widely used as an anti-inflammatory drug (Harman et al., 1984). On the other hand, the use of glucocorticoids and their synthetic analogues were accompanied by deleterious effects. Prednisolone leads to change in diurnal periodicity of the hypothalamo-hypophysemadrone corticosteroid system in adult rats (Zebina et al., 1990). Oral administration of prednisolone altered endogenous adrenocorticotrophic hormone (ACTH) concentration and adrenocortical response to exogenous ACTH in dogs (Brocas et al., 1988). Methylprednisolone produced diabetes mellitus in dog (Jeffers et al., 1981).

Glucocorticoids were found to induce histopathological alterations in the liver (Bharagav and Deochar, 1988; Bharagav and Fores, 1971; Wassaf and Demian, 1982). Kidney (Langa and Doornbos, 1984) and adrenal gland (Challis et al., 1974; Wassaf and Demian, 1992). The use of glucocorticoids and their synthetic analogues during pregnancy increased fetal mortality (Beitner et al., 1981) and induced cleft palate in the offspring (Fraser and Fens_sh, 1981). So, this study was carried out in order to demonstrate the histological and histochemical effects of the synthetic glucocorticoid, methylprednisolone on the adrenal gland of the albino rats.

Materials and Methods
Adult male albino rats, Rattus norvegicus weighing 100 to 120g were used in the present investigation. They were maintained under the standard laboratory conditions of temperature and were fed on standard rodent chow with water provided ad libitum. After one week of acclimatization to the laboratory environment, the animals were divided into two groups:

Group 1: Animals of this group (65 rats) were orally given methylprednisolone by gastric tube (Urban et al., 1988; Hoefnagel and Rousell, Germany) at a dose level of 1.8 mg/kg body weight dissolved in sterile saline, 3 times/week for 3 weeks.

Group 2: Animals of this group (15 rats) were given saline and were served as control.

The animals were killed by decapitation after two and three weeks. Their adrenal glands were removed and were immediately fixed. For histological examination, the tissues were fixed in Bouin's fluid, while for histochemical study, they were fixed in Carnoy's fluid. Fixed materials were embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained by haematoxylin and eosin for histopathological examination. Total carbohydrates were demonstrated using Periodic Acid Schiff's technique (PAS) (Mashke, 1988). Total proteins were detected using the mercury bismuth blue method (Mada et al., 1963).

Results
Histopathological results: The adrenal gland formed of two distinct parts, the adrenal cortex and the adrenal medulla. Section of the adrenal cortex of control rats showed three zones: zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). The ZG zone is adjacent to a relatively thick capsule of connective tissue and this zone is a relatively narrower one. Its cells are arranged in closely packed rounded oval clusters with deeply stained nuclei. The ZF represents the thickest zone of the cortex and consists of straight cords of vacuolated cells in a radial direction toward medulla. The ZR comprises an innermost portion with anastomotic cords of trabeculae of the adrenal cortex and the cells are generally smaller than in ZF and they have deeply stained nuclei (Fig. 1a, b).

Examination of the adrenal of rats treated with methylprednisolone for two weeks showed that ZF zone was
Fig. 2 a,b: Section of adrenal gland of a rat treated with methylprednisolone for 2 weeks showing thin capsule (c), vacuolated ZG, atrophied ZF and hypertrophy of ZR. Note the interference of ZF with either ZG or ZR (H&E, X 400).

Fig. 3 a,b: Section of adrenal gland of a rat treated with methylprednisolone for 3 weeks showing thick capsule (c), vacuolated ZG, atrophied ZF and hypertrophy of ZR. Note the interference of ZF with either ZG or ZR (H&E, X 400).

Fig. 4 a,b: Section of adrenal control rat showing PAS-positive inclusions in the cells of the three zone (ZG, ZF, ZR). (PAS, X400).

Fig. 5: Section of adrenal cortex of a rat treated with methylprednisolone for 2 weeks showing a moderate amount of total carbohydrates in the cells of the three zone, (PAS, X400).

Histological results:

Total carbohydrates: Sections of control rats stained with PAS method showed total carbohydrate content and distribution in the form of deeply stained reddish granules in the cytoplasm of cortical cells. The adrenal capsule exhibited a moderate red colour. The cytoplasm of ZG cells showed a moderate reaction with PAS. The cells of ZF zone showed a relatively moderate PAS reaction while the cells of ZR zone revealed strong reaction. (Figs. 4 a, b)

Table 1 showed the change in total carbohydrate content in the adrenal cortex of rats treated with methylprednisolone. Adrenal cortex of rats treated with methyl prednisolone for 2 weeks showed that the adrenal capsule was weakly stained with PAS. The cells of all the zones (ZG, ZF, ZR) appeared with a moderate amount of total carbohydrates (Fig. 5). After 3 weeks of treatment with this drug, the cells of ZG and ZF revealed a marked depletion of their PAS inclusions. In most cells of ZF zone, the PAS inclusions were lacking and in some cells the cytoplasm showed
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Table 1: Changes in total carbohydrates and total proteins in adrenal cortex of rats treated with methylprednisolone

<table>
<thead>
<tr>
<th>Weeks after treatment</th>
<th>Total carbohydrates</th>
<th>Total proteins</th>
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<tbody>
<tr>
<td></td>
<td>ZG</td>
<td>ZF</td>
</tr>
<tr>
<td>Control</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Two weeks</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Three weeks</td>
<td>+</td>
<td>+</td>
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ZG: zone glomerulae; ZF: zone fasciculata; ZR: zone reticularis. 1+++: strong, 1++: moderate, 1+: slight, 1-: depleted

Fig. 6: Section of adrenal cortex of a rat treated with methylprednisolone for 3 weeks showing marked depletion of total carbohydrates in the capsule, ZG and ZF. Cells of ZR showing moderate amount of carbohydrates (PAs, X400).

Fig. 7a, b: Section of adrenal cortex of a control rat showing total protein content of the capsule and cells of ZG, ZF and ZR (Bromophenol blue, X400).

Fig. 8: Section in the adrenal cortex of a rat treated with methylprednisolone for 2 weeks showing slight amount of total proteins in ZG&ZF, and moderate amount of total proteins in the cells of ZR (Bromophenol blue, X400).

Fig. 9: Section in the adrenal cortex of a rat treated with methylprednisolone for three weeks showing a marked decrease of total proteins in ZG & ZF, while ZR showing an increase amount of total proteins. (Bromophenol blue, X400).
diffuse stainability (Fig. 6).

**Total proteins:** Total proteins appeared in the adrenal cortical cells of control rats in the form of small, bluish granular bodies in the cytoplasm. The cell membrane and the nuclear membrane were intensely stained. The chromatin particles and the nucleoli were also stained. The adrenal capsule showed a high content of total proteins. The cells of 2Z and 2F zones showed a moderate amount of total proteins, while 2R zone displayed a strong stainability (Fig. 7 a,b). Animals treated with methylprednisolone for 2 weeks showed that total proteins were decreased in the cells of 2Z and 2F zone, while the cells of 2R showed a moderate amount of proteins (Fig. 8). A marked reduction of proteins was observed in the cells of 2Z and 2F zones after 3 weeks of treatment. On the other hand, the cells of 2R showed a moderate to strong stainability (Table 1, Fig. 9)

**Discussion**

These results revealed that methylprednisolone caused histopathological changes in the adrenal cortex of rats. Among these changes was the atrophy of zone fasciculata. Similarly, Okazaki et al. (1992) and Nagashima et al. (1992) reported that prednisolone farnesylate decreased the weight of adrenal gland and caused atrophy of zone fasciculata in rats and beagle dogs. Glucocorticoids were found to affect adrenal gland of different animals (Tuchmann-Duplessis, 1970; Chalis et al., 1974; Wassel et Deman, 1992). Adrenocortical hypofunction was recorded upon administration of glucocorticoids (Brooks et al., 1999). Thus, in this experiment, it is speculated that methylprednisolone has a degenerative effect on the adrenal cortex of rats and this effect in secondary to the adrenocortical hypofunction. This is supported by the work of Mopy and Kolanovsk (1986) who found that in adrenocortical insufficiency, the excretion of epinephrine is reduced proportionally to the decrease in adrenocortical activity.

In this study, it has been observed that methylprednisolone induced a decrease of total carbohydrates in the adrenal cortex of the rats and this decrease was marked after 3 weeks. These results are similar to those reported in different tissues under the effect of glucocorticoids. Olejniczak and Lee (1984) reported that treating rats with methylprednisolone and methyl 17-deoxy prednisolone caused significant decrease in liver glycogen content, plasma corticosterone level and relative adrenal gland weight. Furl et al. (1976) observed that metopirone caused decline of glucose and pyruvate concentrations in plasma and drop of liver glycogen in pigs. Rooney et al. (1988) examined the effect of dexamethasone and triiodothyronine (T3) alone and in combination on glycogen and fatty acids in fetal rat lung. The hormones were administered to the mothers on the 2 days before delivery and on days 17-22 of gestation. Their results showed that there is increase in lung glycogen on days 17-20 with a decrease thereafter and an increase in the rate of fatty acid synthesis between days 20-21.

Total proteins showed noticeable decrease in the zone glomerulosa and zone fasciculata of methyl prednisolone-treated rats similarly. Bezdrobny and Germaniuk (1976) reported that treating rats with the glucocorticoid hydrocortisone induced inhibition of protein synthesis in skeletal muscles. Baxter et al. (1972) mentioned that the catabolic actions of glucocorticoids result in decreased synthesis and increased degradation of protein and RNA in the adrenal gland, skin, muscle and connective tissue. Clark et al. (1986) investigated the effect of dexamethasone on cardiac protein metabolism of rats. They found that body weight was significantly lowered in the treated animals in comparison with controls and a 13% increase in protein degradation was occurred. England and Jurkowitz (1992) observed that protein degradation in skeletal muscle is accelerated in rats with chronic renal failure. Exogenous glucocorticoids do not alter protein degradation, but inhibit protein synthesis in BC 3H1 myocytes. Silbermann and Maor (1979) examined the influence of triamcinolone hexacetonide, a long-acting synthetic analogue of cortisol, on nucleic acid and protein synthesis in condylar cartilage of neonatal mice. Following a single injection of the drug, RNA and protein contents were significantly reduced. Significant changes become apparent by 24 hours and persisted for 72 hours after administering the hormone. Correlative relationship was noted between the inhibitory effects on the uptake and the subsequent incorporation of the above precursors into their respective nucleic acid. This study clearly indicates that corticosteroid hormones possess a significant inhibitory effect on the proliferative activity of neonatal chondrocytes and upon the latter's protein synthetic pathways, thereby affecting the normal process of endochondral bone growth.

Total proteins increased in the cells of zona reticularis of methylprednisolone-treated animals. This is due to the hypertrophy of this zone observed in the present work. Welsh et al. (1982) found that administration of the synthetic glucocorticoid, dexamethasone inhibited testosterone production and this inhibition was accompanied by marked decreases in androstenedione and 17-alpha-hydroxypregosterone with reduction of gonadal function. Thus, the hypertrophy of zona reticularis, recorded in the present work, may be due to the involvement of the cells of this zone in secretion of androgens.

From these results, it is speculated that one or more metabolites of methylprednisolone are responsible for induction of adrenal hypofunction in rats and resulted in the observed histological and histochemical changes.

**References**


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