CARBIMAZOLE INDUCED HISTOLOGICAL AND HISTOCHEMICAL ALTERATIONS IN THE ADRENAL CORTEX OF ALBINO RAT

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ABSTRACT

This study was conducted to investigate the effect of carbimazole on the histology and histochemistry of rat adrenal cortex. Adult male albino rats were used in the present study. They were divided into 2 groups : Group(1) treated with carbimazole at a dose level of 12 mg /kg body weight in drinking water, 3 days /week for six weeks and Groups (2) served as control . At the end of 6 weeks of treatment animals were sacrificed. The morphometric results showed that carbimazole affected the diameter of the cortex and the thickness of ZF and ZR zones. Histological results revealed that carbimazale induced atrophy of zona fasciculata and zona reticularis, thickness of adrenal capsule and vacuolation of zona glomerulosa. Histochemical results revealed a marked depletion of general carbohydrates in zona glomerulosa and zona fasciculata and lacking in zona reticularis. Total protein contents showed a marked decrease in zona glomerulosa and zona fasciculata, while the cells of zona reticularis appeared with an increased amount of total proteins. These results indicated that carbimazole affected the adrenal cortex.

Key words: Carbimazole, adrenal cortex, rat, histochemistry, histology.

INTRODUCTION

The thionamides (carbimazole and its active metabolites methimazole and propylthiouracil) are antithyroid drugs that act by inhibition of iodide oxidation of iodination of tyrosine and of coupling of the iodotyrosines to form the thyronines T3 and T4. These drugs are used in the long – term treatment of hyperthyroidism and to reduce thyroid function before surgery (Grahame and Aronson, 1988). On the other hand, the use of thionamides and their synthetic analoges were accompanied by deleterious effects. Carbimazole cause shrinkage of adrenal cortex (Sarwar and Janjua, 2003). Carbimazole has to be added to the list of drugs capable of inducing acute pancreatitis, hepatic cholestasis and erythema nodosum for Grave’s disease, and should be emphasized the need to discontinue this medication as soon as there is evidence of pancreatic dysfunction (Marazuela et al., 2002). Treatment with carbimazole of hyperthyroid pregnancy with hepatic impairment after propylthiouracil administration resulted in elevated hepatic enzymes (Kontoleon, 2002). It also caused coanal atresia and severe adverse effects such as a granulocytosis (Round and Berg, 2002). After carbimazole treatment, thyroid carcinoma increases with the time and appears to be considerable higher than after radiiodine treatment (Dotsch et al., 2003; Joseph et al., 2003). Acute liver insult was induced by bupropion given concurrently with carbimazole therapy (Lengk et al., 2003). Study was carried out to elucidate the effect of carbimazole on the histology and histochemistry of the adrenal cortex in the adult albino rat.

MATERIALS AND METHODS

Adult male (18) albino rats, 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 I: Animals of this group (12 rats) were given carbimazole orally by gastric tube @ 12 mg /kg body weight in drinking water, 3 days /week for six weeks.

Group II: Animals of this group (6 rats) were used as control.

At the end of six weeks treatment, animals were sacrificed, their adrenal glands were removed and were immediately fixed. The tissues were fixed in Bouin’s fluid for histological examination, while for histochemical study, they were fixed in Carnoy’s fluid. Fixed materials were dehydrated in a series of ethyl alcohol and embedded in paraffin wax and sections of 5 microns thickness were cut. Slides were stained with haematoxylin and eosin for histopathological examination. General carbohydrates were demonstrated using periodic acid Schiff’s technique.
Total protein contents were detected using the mercury bromophenol blue method (Mazia et al., 1953).

**Morphometric techniques**

The changes in thickness of zona fasculata, zona reticularia and zona glomerulosa were measured in haematoxylin and eosin stained section, by using calibrated ocular scale grid. The data was expressed in micrometers and the mean values were calculated for each group. The significance of differences between the means was calculated using the Student’s t-test.

**RESULTS**

**Histopathological results**

In haematoxylin and eosin stained sections, the gland of the control group was surrounded by a connective tissue capsule which was formed mainly of collagenous fibers. The parenchyma was divided into the cortex and medulla, the cortex was subdivided into three zones: zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR). Zona glomerulosa was relatively the narrowest one, its cells were arranged in closely packed rounded oval clusters with deeply stained nuclei surrounded by capillaries. Zona fasciculata represented the thickest zone of the cortex and consisted of straight cords of vacuolated cells in a radial direction towards the medulla, arranged in straight columns of one or two cells thick. The columns anastomosed at different levels and were not parallel along the whole length of the zone. Such columns were separated by radially arranged, elongated, narrow blood spaces. The cells had pale vacuolated cytoplasm and a large vesicular, centrally located nuclei.

Zona reticularis comprised of an innermost portion with anastomosing cords of polygonal cells which were separated by irregular wide blood spaces. The cells were more crowded smaller in size than that of the zona fasciculata. The cytoplasm was more or less acidophilic, containing small rounded, deeply stained nuclei (Fig. 1a, b).

Examination of the adrenal glands of rats treated with carbimazole for six weeks showed that the adrenal capsule appeared thicker and the inner layers showed proliferation of the cells with deeply stained flattened nuclei. Zona glomerulosa cells were crowded and irregularly arranged with distinct boundaries and different size of cells. Balloonning of some cells with very pale vacuolated cytoplasm and pyknotic nuclei were observed. Cells of the zona fasciculata showed less regular arrangement than those of the control and interferes with either zona glomerulosa or zona reticularis. The cytoplasm of the cells showed reduction of the acidophilia and marked increase of the vacuoles, so the cells became hypertrophy, very pale with reticular appearance. The nuclei were large in size, vesicular and pyknotic. Zona reticularis cells were increased in thickness and was markedly hypertrophied. Acidophilia was decreased and some degenerated cells with pyknotic nuclei were observed (Fig. 2a, b).

**Histochemical result**

**Total carbohydrates**

Sections of control rats stained with PAS method showed general carbohydrate contents and distribution in the form of deeply stained reddish granules in the cytoplasm of cortical cells. The adrenal capsule exhibited a moderate stainability. The cytoplasm of zona glomerulosa (ZG) cells showed a moderate reaction with PAS. The cells of zona fasciculata (ZF) showed a relatively moderate PAS reaction, while the cells of zona reticularis (ZR) revealed strong reaction (Fig. 3a, b). Examination of adrenal cortex of carbimazole treated rats showed that the adrenal capsule was weakly stained with PAS – reaction. A marked depletion in general carbohydrate contents was observed in the cells of ZG and ZF zones. In most cells of ZR zone, the PAS inclusions were lacking and in some cells the cytoplasm showed diffuse stainability (Fig. 4).

**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 structures were intensely stained. The adrenal capsule showed a high content of total proteins. The cells of ZG and ZF zones showed a moderate amount of total proteins while ZR
zone displayed a strong stainability (Fig. 5 a, b). Animals treated with carbimazole for six weeks showed that total proteins were depleted in the cells of ZG and ZF zones, while the cells of ZR showed a moderate amount of proteins (Fig. 6).

**Fig. 1a, b.** Section of adrenal cortex of a control rat showing capsule (C) and the three zones: zona glomerulosa (ZA) was arranged in clusters and zona fasciculata (ZF) was formed of outer part contain acidophilic vacuolated cells arranged in columns and inner part contain less regularly arranged cells indicate blood sinusoids, zona reticularis (ZR) and medulla (M). (H & E X400).

**Fig. 2a, b.** Section of adrenal gland of a rat treated with carbimazole for six weeks showing the reticular appearance of the cortical cells and ballooning of some cells, atrophied ZF and hypertrophy of ZR indicates the deeply stained flattened and oval nuclei, thick capsule (C), (H & E X400).
Fig. 3a, b. Section of adrenal cortex of control rat showing PAS. Positive inclusions in the cells of the three zones (ZG, ZF, ZR) (PAS X400).

Fig. 4. Section of adrenal cortex of a rat treated with carbimazole for 6 week showing a marked depletion of total carbohydrates in the capsule (C)
and the three zones. (PAS X400).

**Fig. 5a, b.** Section of adrenal cortex of control rat showing moderate amount of total protein contents in ZG and ZF, while ZR displayed a strong stainability (Bromophenol blue X400).

**Fig 6.** Section of adrenal cortex of treated rat with carbimazole for 6 weeks showing a marked reduction of the total proteins in ZG and ZF, while ZR showed moderate amount of total proteins (Bromophenol blue X400).
The numerical data obtained in the current study are presented in Table 1 as mean ± standard deviation. The results showed that the diameter of the cortex as well as the thickness of the ZF and ZR were significantly (p<0.05) decreased in carbimazole-treated rats.

Table 1. Effect of carbimazole on the diameter of the cortex and thickness of the three zones during the experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Diameter in µm. (Mean ± S.D.)</th>
<th>Thickness of the three zones in µm. (Mean ± S.D.)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Z.G.</td>
</tr>
<tr>
<td>Control</td>
<td>182 ± 9.3</td>
<td>74.3 ± 2.3</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>133 ± 9.4</td>
<td>61.4 ± 6</td>
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(*) Significant at P<0.05.

DISCUSSION

In the present work the histological and histochemical changes observed in the cortical cells of the carbimazole treated rats indicated that adrenals were in a state of increased secretory activity. The adrenals hyperactive produced by increased ACTH secretion and this high level produced hyperplasia and hypertrophy of cortical cells especially zona fasciculata and zona reticularis. Also, a number of changes including an increased of cytoplasmic vacuoles, decreased in acidophilia and enlargement of pyknotic nuclei in both zona fasciculata and zona reticularis was seen. Similarly Saber et al., (1997) reported that in stressfull condition such as under nutrition, ACTH increased and this high level produced hyperplasia and hypertrophy of cortical cells in the three cortical zones. As a result to secretory function of the cortex increased, Carrillo and Herbet (1998) reported that the adrenal gland of ginseng – treated animals showed an obvious enlargement of the cells of zona fasciculata which was confirmed by the morphometric study using the image analyzer. Also, the cells of zona fasciculata showed a number of changes including an increase cytoplasmic vacuoles, succinic dehydrogenase enzyme activity and a decrease in cytoplasmic lipid content and ascorbic acid granules while the cells of zona glomerulosa showed an apparant enlargement and increased cytoplasmic vacuolations. Also, they concluded that ginseng induces its effect through an increase steroidogenic activity of zona fasciculata. This increase in glucocorticoid causes an increase in epinephrine secretion. Ganong (1999) found that synthetic adrenocorticotrophic hormone synacthan was able to induce hypertrophy in adrenal gland in rabbits, particularily in the region of zona fasciculata and zona reticularis. The present results revealed that carbimazole caused histopathological changes in the adrenal cortex. Among these changes was the atrophy of zona fasciculata and reduction of cytoplasmic vacuoles. Marked congestion and atrophy of zona reticularis and highly vacuolated of zona glomerulosa with pyknotic nuclei and degenerated cells were also observed. Thus in this experiment, it is speculated that carbimazole has a degenerative effect on the adrenal cortex of rats. Similar results were obtained by Sarwar abd Janjua (2003) who reported that carbimazole affect the morphology of rat adrenals by decreasing the total width of the cortex and its zones especially the zona fasciculata with decrease in number of cells, it also showed an increase in fat content and they concluded that carbimazole caused shrinkage of adrenal cortex. In agreement of these results, Okazaki et al., (1992), Nagashima et al., (1992) and Sakr et al., (2002) reported that predenisolone decreased the weight of adrenal gland and caused atrophy of zona fasciculata in rats.

In this study, it has been observed that carbimazole administration induced a decrease of total carbohydrates in the cortical cells of zona glomerulosa and zona fasciculata while zona reticularis was lacking to the PAS inclusions. These results are similar to those reported in different tissues under the effect of different glucocorticoids. Olejniczak and Lee (1984) reported that treating rats with methyl prednisolone and methyl 17-deoxy prednisolone caused significant decrease in liver glycogen content, plasma corticosterone level and relative adrenal gland weight. Rooney et al., (1986) 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 delivery and on days 17-22 of gestation. Their results revealed that there is an increase in lung glycogen on days 17-20 with a decrease.
thereafter and increase in the rate of fatty acid synthesis between days 20-21. Sakr et al., (2002) reported that methylprednisolone induced a decrease of total carbohydrates in the adrenal cortex of the rats and this decreases was marked after 3 week treatment.

Total proteins showed noticeable decrease in the zona glomerulosa and zona fasciculata while the cells of zona reticularis showed a moderate amount of total proteins of carbimazole treated rats. This result was similar to those reported by some investigators. Mori (2000) reported that carbimazole caused a significant decrease of the proliferation, nucleic acid and protein synthetic activity of the thyroid follicular and adrenocortical cells. In addition carbimazole has been shown to decrease the functional activity of the thyroid follicular, adrenocortical and chromaffin cells as estimated by a significant decrease of the mean nuclear volume of these cells, 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 control and a 13% increase in protein degradation was occurred. England and Jurkovits (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. Sakr et al., (2002) reported that methylprednisolone induced a decrease of total proteins in zona glomerulosa and zona fasciculata and an increase in the cells of zona reticularis in rat adrenal gland.

It is concluded from the present study that treatment with carbimazole produced a degenerative effects in adrenal cortex which resulted in the observed histological and histochemical changes.

REFERENCES


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