COMPARISON OF THE EFFECTS OF EXPERIMENTALLY INDUCED HYPOTHYROIDISM IN ADRENAL GLANDS MORPHOLOGY IN PRE AND POSTNATALLY TREATED ALBINO RATS

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ABSTRACT
To compare the effects of experimentally induced hypothyroidism on the morphology of adrenal glands in prenatally treated immature and 6 weeks postnatally treated albino rats. 18 immature and 18 adult albino rats were taken for this study. The immature rats were obtained from 3 sets of pregnant female rats that were treated during gestation with carbimazole, distilled water and carbimazole with thyroxine. They were grouped as A1, A2 and A3 respectively. The mature rats were similarly divided into groups B1, B2 and B3 and were treated with carbimazole distilled water and carbimazole with thyroxine for six weeks. Upon completion of treatment the animals were sacrificed and their adrenals removed, fixed and embedded in paraffin. Sections were cut and stained with H&E. The adrenal histology in terms of width of cortex, width of its different zones and number of cells of these zones was studied. The result of this study revealed that experimentally induced hypothyroidism caused adrenal atrophy in mature as well as immature albino rats.

Keywords: Carbimazole, Adrenal cortex, Albino rat.

INTRODUCTION
Thyroid gland was first described by Galen and was named “glandulae thyroideae” by Wharton in 1656. Its endocrine function however was not proposed until almost 200 years later (Gilman 1980). Since then different milestones were crossed slowly and it is now an established fact that thyroid hormones are essential, optimal and critical for normal growth and maturation of skeletal, neuronal and reproductive tissues. They are the important determinants of genetically coded development programme (Ganong 1999).

The biological importance of thyroid gland has been recognized for years. It is also known that reduced levels of thyroid hormones shrink adrenal cortex and decrease its weight where as excess amounts of thyroid hormone enlarge its size and increase the weight.

The effects of thyroid hormones on the body are due to stimulation of oxygen utilization, which is known as calorigenic action.

The purpose of this study was
(i) to determine comparative effects of experimentally induced hypothyroidism (Carbimazole) in 10 days prenatally treated immature rats and postnatally treated adult albino rats of six weeks and
(ii) to determine the effect of simultaneous administration of thyroxine (thyroid hormone analogue) with Carbimazole in 10 days prenatally treated immature rats and postnatally treated adult albino rats of six weeks.

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MATERIALS AND METHODS

Drugs used in the Study

Carbimazole

This is an anti-thyroid drug of thioamide group which acts by inhibiting the enzyme thyroid per-oxidase thereby blocking the iodination of thyroxine and preventing the formation of T3 and T4 (Farewell and Braveman, 2001).

Thyroid Preparation

Thyroxine (levo-thyroxine sodium) is the hormone of choice for thyroid hormone replacement. Simultaneous administration of thyroxine with Carbimazole was used in this study, i.e. synthetic levo-thyroxine (Greenspan and Dong 2001).

A total of 36 animals (18 ten days pups and 18 six weeks mature rats) were used in this study. The 10-day pups constituted Group-A, and adult mature rats of 6 weeks of age constituted Group-B.

Group-A animals were obtained from three sets of pregnant female rats that were treated during gestation with carbimazole, normal saline and carbimazole with thyroxine.

From carbimazole treated mothers 6 pups were randomly selected and named as A-1 group. From normal saline treated mothers 6 pups were randomly selected and named as A-2 group. From carbimazole along with thyroxine treated mothers 6 pups were randomly selected and named as A-3 group. They were cleaned, weighed, marked with punch holes in their ears and left with their mothers' up to 10th postnatal day. On the 10th postnatal day they were sacrificed by an overdose of ether anaesthesia in a glass jar and through the midline abdominal incision the adrenals were removed, weighed and fixed in 10% FORMALIN.

Group-B animals were similarly divided into B-1, B-2 and B-3 groups containing six animals each. Sub-group B1 received the Carbimazole (Neomercazole) as an anti-thyroid agent subcutaneously at a dose of 6 µgm/G (2,400 µgm/cc) body weight daily for six weeks. Sub-group B2 received 0.5 cc (volume equal to thyroxine volume) of Normal Saline (0.9% NaCl) intra-peritoneally daily for six weeks and acted as Control. Sub-group B3 in addition to Carbimazole, received Thyroxine 5 µgm (0.5 cc) intra-peritoneally daily for six week. All the animals of this group were also sacrificed by an over dosage of ether anaesthesia in a glass jar.

The animals were then dissected through midline abdominal incision and the adrenals were removed, weighed, and fixed in 10% FORMALIN.

The tissues of A and B groups were processed routinely and sectioned at 5 micron thickness. H&E staining was done to see the morphology of adrenal cortex. Micrometry was done by calibrating through ocular micrometer with reticule under the high power and oil immersion objective lenses. The statistical analysis (bland 1987) was then carried out. The statistical significance of difference between two means of various parameters in different groups was evaluated by student 't' test (Hill 1977) and p-value was calculated. The difference was regarded as highly significant if p-value was <0.001, significant if p-value was <0.05, and non-significant if p-value was >0.05.

RESULTS

Carbimazole Treated Groups-A1 & B1

In both groups, marked changes in the morphology of adrenal cortex were observed. The capsule was thickened with increased infiltration of fibroblasts. The cells of all three zones were hypertrophied and vacuolated showing the congestion especially in zona fasciculata.

The mean total width of Adrenal cortex as well as that of Zona Fasciculata in A1 group was decreased when compared with age matched controls but this decrease was not significant statistically (Table-1). The mean width of Zona glomerulosa however was increased and this increase was also not
significant statistically when compared with age matched controls (Table-1). There were no changes in Zona reticularis of the two groups. A significant decrease was observed in the width of Zona glomerulosa, Fasciculata and Reticularis in rats of group B1 (Table-1) in comparison to control group animals.

A significant decrease was observed in the mean number of cells of Zona fasciculate and Zona reticularis of group A1 when compared with age matched control (Table-2), whereas in zona glomerulosa a statistically non significant increase in number of cells was observed when compared with age matched controls.

A highly significant decrease was observed in mean number of cells in Zona glomerulosa and fasciculata in group B1 when compared with control group animals. Number of cells in Zona reticularis were also less but the decrease was not significant statistically when compared with age matched controls (Table-2).

**Control Groups-A2 and B2**

H&E stained sections were examined under light microscope. Adrenal capsule was infiltrated with fibroblasts and was moderately cellular. The cells of zona glomerulosa showed normal morphological pattern with ovoid and rich network of blood vessels. The cells in the zona fasciculata were arranged in parallel cell cords running radially towards medulla and were cuboidal in shape, whereas zona reticularis was composed of network of cells.

**In the animals of group-A2**, the mean total width of the adrenal cortex was 266.00±7.20 µm as shown in Table-1. The mean total widths of zona glomerulosa, fasciculata and reticularis were 44.00±3.99 µm, 148.00±6.69 µm and 74.00±5.72 µm respectively (Table-1). The mean total number of cells per unit area studied were 57.00±3.80, 48.83±2.30 and 67.50±1.87 in zona glomerulosa, fasciculata and reticularis respectively (Table-2).

**In the animals of group B2**, the mean width of adrenal cortex was 636.00±11.46µm (Table-1). The mean widths of zona glomerulosa, fasciculata, and reticularis were 46.00±3.68 µm, 364.00±5.05 µm, and 266.00±9.50 µm respectively.

The mean total number of cells per unit area in zona glomerulosa, fasciculata, and reticularis were 64.16±4.88, 48.00±9.47, and 63.33±3.33 respectively.

**Carbimazole plus thyroxine treated groups A3 & B3**

In these groups the capsule showed infiltration with relatively less fibroblasts as compared to that of carbimazole treated group, and no gross morphological change occurred i.e. congestion and hypertrophy of the cells were not observed in any of the zones of adrenal cortex.

The width the adrenal cortex and zona fasciculata in A3 group showed statistically non-significant increase, whereas an insignificant decrease was observed in the width of zona glomerulosa and zona reticularis when compared with carbimazole treated group A1 (Table-1). An insignificant decrease was also observed in the mean number of cells in the zona glomerulosa, fasciculata and reticularis when compared with carbimazole treated group A1 (Table-2).

In the B3 group a statistically significant increase was observed in the total width of adrenal cortex, zona glomerulosa, fasciculata and reticularis as compared to carbimazole treated group B1 (Table-1). A statistically significant increase was also observed in the number of cells in zona fasciculata and zona reticularis whereas there was a significant decrease in the number of cells in zona glomerulosa when compared with the carbimazole treated group B1 (Table-2).

**DISCUSSION**

The present study was undertaken to see the comparative effects of experimentally induced hypothyroidism on the morphology of rat adrenals in 10 days prenatally treated
### Table 1

Mean Width of Cortex and its Zones (μm) (Mean ± S.E.M)

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>10 Days Prenatally Treated</th>
<th>Groups (n=6)</th>
<th>6 Weeks Age Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TWC</td>
<td>TWZG</td>
<td>TWZF</td>
</tr>
<tr>
<td>Ca (A1)</td>
<td>250.00±7.84</td>
<td>48.00±6.19</td>
<td>128.00±7.37</td>
</tr>
<tr>
<td>C (A2)</td>
<td>266.00±7.20</td>
<td>44.00±3.99</td>
<td>148.00±6.69</td>
</tr>
<tr>
<td>Ca+Th (A3)</td>
<td>262.00±3.44</td>
<td>40.00±2.52</td>
<td>156.00±4.38</td>
</tr>
</tbody>
</table>

Statistical Analysis of difference in mean width of Cortex and its different Zones

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TWC</td>
<td>TWZG</td>
<td>TWZF</td>
</tr>
<tr>
<td>Ca (A1)</td>
<td>P&gt;0.32</td>
<td>NS</td>
<td>P&gt;0.25</td>
</tr>
<tr>
<td>C (A2)</td>
<td>P&gt;0.16</td>
<td>NS</td>
<td>P&gt;0.60</td>
</tr>
<tr>
<td>Ca+Th (A3)</td>
<td>P&gt;0.72</td>
<td>NS</td>
<td>P&gt;0.57</td>
</tr>
</tbody>
</table>

Key: C = Control; Ca = Carbimazole; Th = Thyroxin; TWC = Total Width of Cortex; TWZG = Total Width of Zona Glomerulosa; TWZF = Total Width of Zona Fasciculata; TWZR = Total Width of Zona Reticularis; NS = Non-significant; HS = Highly Significant; S = Significant
### Table 2

Mean Number of Cells in different Zones of Cortex (per area of reticule)

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>10 Days Prenatally Treated</th>
<th>Groups (n=6)</th>
<th>6 Weeks Age Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZG</td>
<td>ZF</td>
<td>ZR</td>
</tr>
<tr>
<td>Ca (A1)</td>
<td>58.16±1.93</td>
<td>35.66±2.70</td>
<td>41.00±1.77</td>
</tr>
<tr>
<td>C (A2)</td>
<td>57.00±3.80</td>
<td>48.833±2.30</td>
<td>67.50±1.83</td>
</tr>
<tr>
<td>Ca+Th (A3)</td>
<td>52.66±4.65</td>
<td>31.16±7.31</td>
<td>37.16±4.70</td>
</tr>
</tbody>
</table>

Statistical Analysis of difference in mean width of Cortex and its different Zones

<table>
<thead>
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<tr>
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<td>ZF</td>
<td>ZR</td>
</tr>
<tr>
<td>Ca (A1)</td>
<td>A1 vs A2</td>
<td>P&gt;0.30 NS</td>
<td>P&gt;0.30 NS</td>
</tr>
<tr>
<td>C (A2)</td>
<td>A1 vs A3</td>
<td>P&gt;0.78 NS</td>
<td>P&lt;0.001 HS</td>
</tr>
<tr>
<td>Ca+Th (A3)</td>
<td>A3 vs A2</td>
<td>P&gt;0.30 NS</td>
<td>P&lt;0.001 HS</td>
</tr>
</tbody>
</table>

Key: C = Control; Ca = Carbimazole; Th = Thryoxin; ZG = Zona Glomerulosa; ZF = Zona Fasciculata; ZR = Zona Reticularis; NS = Non-significant; HS = Highly Significant; S = Significant
immature rats and 6 weeks postnatally treated adult albino rats.

In the present study it was observed that there was a marked reduction in the total width of adrenal cortex in the carbimazole treated animals as compared with that of control animals of mature age groups. In 10 days old group the shrinkage was not marked. In carbimazole plus Thyroxin treated group also there was less shrinkage of cortex. The reason of decreased cortical shrinkage in 10-days prenatally treated animals may be attributed to the shorter length of treatment in contrast to the mature animals of 6 weeks age. If the length of treatment had been increased in 10-days prenatally treated animal groups the shrinkage in the width might have been increased.

The shrinkage of zona fasciculata was observed in the carbimazole treated animals of both age groups as compared to controls. The zona glomerulosa was broader in the carbimazole treated animals of both age groups as compared to controls. This is in complete agreement with the study of Dean and Greep (1947) who observed adrenal atrophy resulting into shrinkage of cortex particularly of zona fasciculata where as zona glomerulosa appeared broader than normal.

Regarding the number of cortical cells a marked decrease was observed in the zona fasciculata in both the age groups as compared to that of age matched controls. This may be attributed to the hypothyroidism either due to absence of thyroid hormone or effect of antithyroid drugs.

The response of adrenal glands in both the groups i.e. prenatally treated and adult rats was almost same as both the groups of animals were showing changes of adrenal atrophy by showing the thickness of capsule with increased infiltration of fibroblasts. The cells of all the three zones were showing hypertrophy and congestion especially in zona fasciculata.

CONCLUSION

Experimentally induced hypothyroidism causes the adrenal atrophy in immature as well as adult albino rats.

REFERENCES


