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METHIMAZOLE-INDUCED HYPOTHYROIDISM IN RATS: EFFECT OF METHIMAZOLE-INDUCED CELLULAR DAMAGE ON HEART, LUNG AND OVARY

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ABSTRACT

Great efforts are ongoing in understanding and management of thyroids, the disease and disease related complications are increasingly unabated. It is known that thyroid hormones have an effect on tissue damage. The objective of this study was to determine hypothyroidism causes cellular damage in the heart, lung, thyroid gland and ovary. In the present investigation an attempt is made to study the cyto-morphological changes in the heart, lung, thyroid glands and ovary caused by methimazole (an antithyroid drug) or hypothyroidism. Twelve female Sprague Dawley rats were divided into 2groups: euthyroid (control) and methimazole-induced hypothyroidism (20mg/kg body weight in 1ml water/day). At the end of the treatments (28 days for each group), the animals were sacrificed. The heart, lung, thyroid glands and ovary were removed and were processed for embedding in paraffin wax and also measure the serum concentrations of thyroid hormones. Coronal sections were stained with hematoxylin–eosin. At the end of treatment, animals with the methimazole hypothyroidism had a significant reduction of serum concentration of thyroid hormones. Only methimazole-induced hypothyroidism causes cellular disturbances in the lung, heart, thyroid glands and ovary. These results indicate that tissue damage found in hypothyroidism is caused by methimazole and thyroid hormones have an effect on cyto-morphological alteration of heart, lung, thyroid glands and ovary tissue.

KEYWORDS: Cyto-Morphological Changes, Hypothyroidism, Methimazole

INTRODUCTION

Thyroid gland is specialized for production, storage and release Thyroid hormones, such as tri-idothyronine (T_3) and thyroxine (T_4), are important for the regulation of variety of biochemical reactions in virtually all tissues. They are important for growth, development and metabolism of humans and animals (Hulbert, 2000). Apart from general metabolic disturbance, impairment of thyroid hormone production causes serious intellectual and behavioral abnormalities that may affect patient's daily functioning and result in additional stress and depression. Therefore, the research on the thyroid gland has not only significant medical but also social implication. One of the most frequent thyroid disorders in humans is hypothyroidism, defined as a condition when the production of the thyroid hormones decreases below the normal need of the body (Farwell AP et al., 1996). Hypothyroidism can result from thyroid dysfunction, from impediment in mechanisms that control thyroid function, or may arise as the complication during treatment of hyperthyroidism (Silva JE, 1986). Numerous animal models of experimental hypothyroidism have been developed in effort to elucidate mechanisms underlying hypothyroidism and to contribute to resolve accompanied problems. Some investigators have noted that anti-thyroid induced hypothyroidism can cause cellular damage (Bergman et al., 1999). In the present study we artificially induced hypothyroidism in otherwise healthy and metabolically stable rats in order to elucidate the existing controversies related to effects of experimentally induced hypothyroidism on energy storage/dissipation in rats, as well as

in attempt to give acceptable explanation for discrepancies which exist in that regard between human and animal models of hypothyroidism. Hypothyroidism was induced by anti-thyroid drug methimazole that is frequently and preferentially used in the treatment of human hyperthyroidism (Homsanit M et al., 2001). This study objective was to determine morpho-functional alterations of the heart, lung, ovary and thyroid gland induced by methimazole.

MATERIALS AND METHODS

Animals and Housing

Twelve female Sprague Dawley rats; body weight 130-150g from animal care facilities were used. Animals were housed singly in metal cages with food and water *ad libitum*. The cages were located together in racks in a light (12:12 h light-dark), temperature (22±2°C) and humidity (50 ± 10 %) controlled room. The rats were allowed to acclimatize to the room conditions for at least one week before starting the experiments.

Experimental Design

The adult female rats were divided into 2 groups. **I) Euthyroid /Control (n=6):** which received no drugs or treatment **II) Methimazole-induced hypothyroidism (n = 6):** which received 20mg/kg body weight in 1ml water/day of the anti-thyroid drug methimazole (Sigma Chemical Co.). During treatment, the body weight and rectal temperature were evaluated to indirectly determine the thyroid status. At the end of the treatment (28 days) we determined the serum concentration of the thyroid hormones (T_3 and T_4). The tissues were fixed in 4% para-fromaldehyde solution for histological observation.

Thyroid Hormones (T_3 and T_4) Assay

To observe the changes in serum thyroid-hormone concentration of the rats, blood samples from the rat-tail vein were taken at the end of the treatment. Serum was separated and stored at -20°C until the day of they were assessed for. T_3 and T_4 concentrations were determined by enzyme immunoassay (Immunometrics UK Ltd.).

Histology

After the end of the treatment time, all animals were anesthetized with sodium pentobarbital (35mg/kg i.p.) and they were then trans-cardially perfused with 0.9% saline. The heart, lung, thyroid glands and ovary were removed and they were processed by routine protocol for embedding in paraffin wax. Coronal sections of 5µm thick were prepared. Each section was stained with hematoxylin–eosin, according to standard protocols. At least 25 tissue sections for each organ were assessed.

DAPI staining

DAPI (4′, 6-diamidino-2-phenylindole) staining and counterstaining was processed for determination of a nuclear morphology indicator, based on nuclear size and roundness, which we call the nuclear area factor. The nuclear area factor is an early indicator of cell death (significant after 4 h post treatment). Commercially available image analysis software used to indicate early as well as delayed cellular damage processes.

Statistical Analysis

All the data were presented as mean ± SEM. The data were analyzed by independent sample *t*-test. Results with P<0.05 was considered statistically significant.

RESULTS

Body Weight and Rectal Temperature

At the end of treatment, the methimazole induced hypothyroid groups showed differences in the rectal temperature and bodyweight compared to the euthyroid/control group (Table 1).Although at the beginning of the experiment mean body mass in both control and experimental group of animals was practically equal (Control group: 141.2 ± 2.67 g; Methimazole induced hypothyroid group: 141.5 ± 2.36 g) but at the end of the treatment the rats from the experimental group were significantly lighter (Control group: 276.0 ± 4.87 g; Methimazole induced hypothyroid group: 211.09 ± 5.53 g; p<0.001), namely they gained approximately ~50% less mass than the control animals.

Table 1: Changes in Body Weight and Rectal Temperature after Methimazole Treatment

Variables	Euthyroid /Control Group (n=6)	Methimazole Induced Hypothyroid Group (n=6)	P Value
Body weight gain (gm)	136.73 ± 4.871	69.51±4.306*	<0.001
Rectal temperature (°C)	38.633±0.439	37.567±0.411*	<0.05

Values are expressed as mean ±SEM. *P< 0.05 vs. euthyroid/control group

Histological Analysis and DAPI Staining

Heart

Normal heart tissue is formed by striated branched muscle fibers. All the fibers have a central nucleus. This architecture was present in the control groups. But in the methimazole-induced hypothyroid heart was observed loss of striation and nuclei (Figure 1).

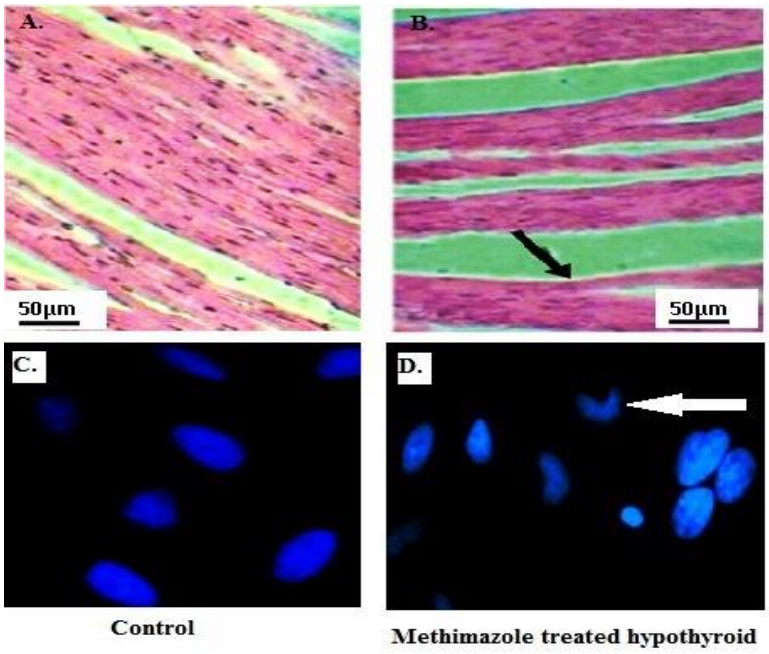

Figure 1: Representative Images of Heart Stained by Hematoxylin–Eosin (A. & B.) and DAPI (C. & D.) Methimazole-Induced Hypothyroidism Caused Loss of Striation and Changes of Nucleus Shapes (Arrows)

Lung

Control animals showed the normal appearance of the alveoli and epithelial lining of the bronchioles. Methimazole-induced hypothyroid had abnormal lung architecture. The alveoli and epithelia lining of the bronchioles had altered cyto-architecture (Figure 2)

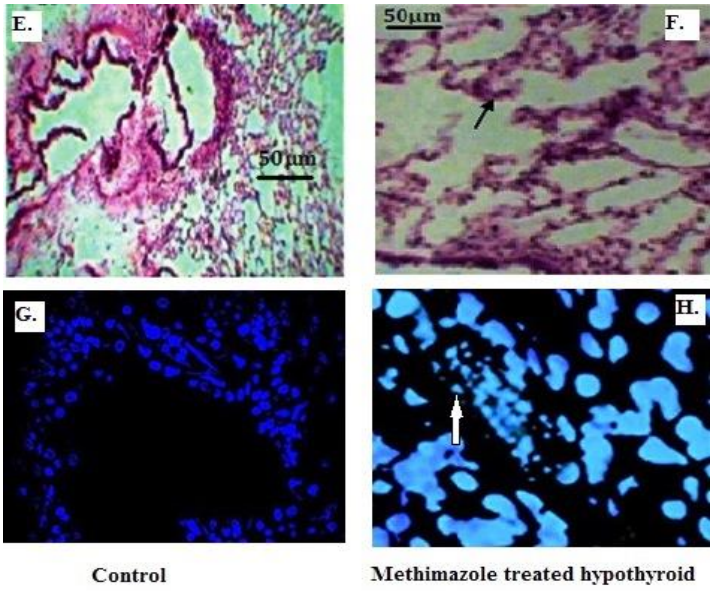

Figure 2: Representative Images of Lung Stained by Hematoxylin–Eosin (E. & F.) and DAPI (G. & H.) Methimazole induced Hypothyroidism Caused Cellular and Nuclear Damage (Arrows)

Thyroid Gland

There were no significant degenerative or necrotic changes were showed in the control group. However, dwindled follicles and increased number of follicular epithelial cells were found in methimazole treated hypothyroid group (Figure 3).

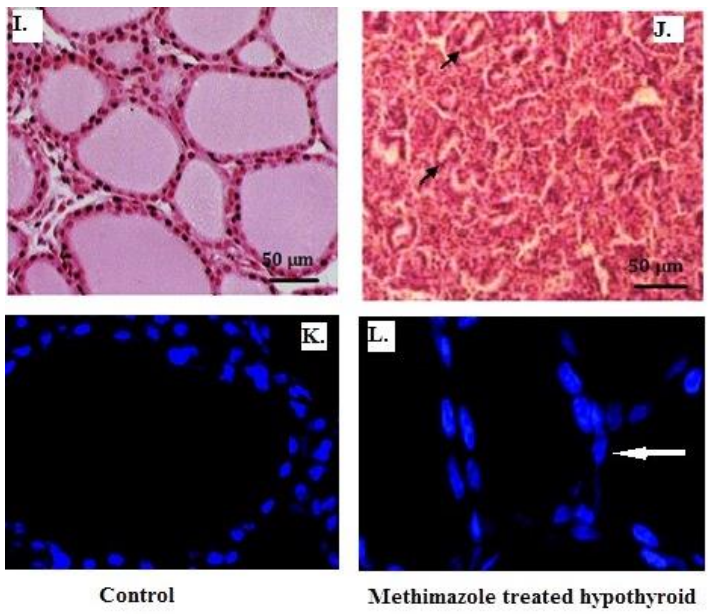

Figure 3: Representative Images of Thyroid Gland Stained by Hematoxylin–Eosin (I. & J.) and DAPI (K. & L.) Methimazole-Induced Hypothyroidism Caused Dwindled Follicles and Nuclear Shape Changes (Arrows)

Ovary

Ovarian morphology was normal in the control, showing normal compartmentalization of germ cells and visible ovum in normal sized lumen. In contrast, methimazole-induced hypothyroid groups showed no compartmentalization of germ cells or visible ovum (Figure 4).

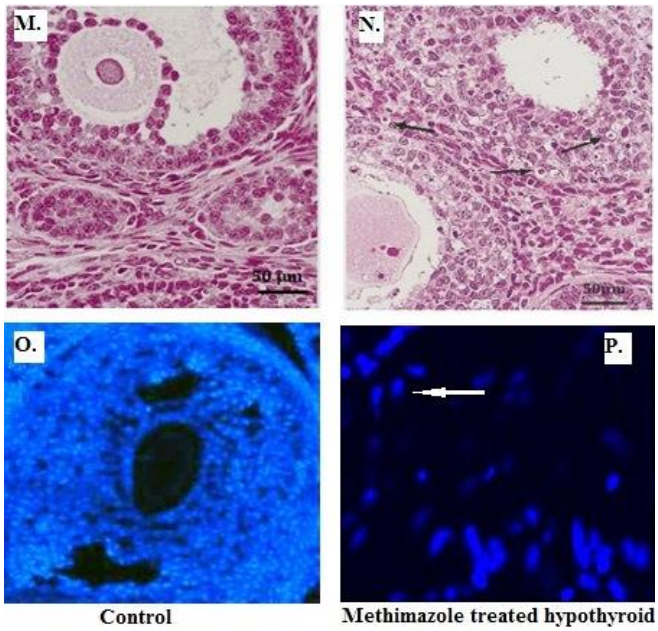

Figure 4: Representative Images of Ovary Stained By Hematoxylin–Eosin (M. & N.) and DAPI (O. & P.) Methimazole-Induced Hypothyroidism Caused Necrosis of follicular Cells and Nucleus (Arrows)

Circulating Hormones (T_3 and T_4)

The plasma concentrations of serum T3and T4 were significantly decreased by 28 days after the methimazole treatment (Table 2).

Table 2: Serum Concentrations of T_4 and T_3 of the Control and Hypothyroid Rat 28 Days after Methimazole Treatment

Variables	Euthyroid/Control Group (n=6)	Methimazole Induced Hypothyroid Group (n=6)	P Value
Serum T4 concentration(nmol/L)	103.24 ± 7.72	33.20 ± 1.109*	<0.001
Serum T3 concentration (nmol/L)	1.17 ± 0.17	0.69 ± 0.08*	<0.05

Values are expressed as mean ±SEM. *P< 0.05 vs. euthyroid/control group

DISSCUSSIONS

It has been reported that the basal metabolic rate reduced by hypothyroidism has two effects on the intracellular redox environment and cellular damage (Elfarra et al., 1994; Oren et al., 1996). The majority of research has been done in pharmacological models of hypothyroidism induced by using thionamides (Bandyopadhyay et al., 2002). In our work, we investigated if methimazole-induced hypothyroidism causes cellular damage in different organs. Histologically, we demonstrated that only methimazole induced hypothyroidism causes cellular damage in the heart, lung, thyroid gland and ovary. Methimazole can participate in cellular damage by different mechanisms because of its chemical structure or the

interaction of this chemical structure and the physiological changes caused by the hypothyroid state. This drug irreversibly inactivates different peroxidases with a hemegroup active center (Bandyopadhyay et al., 1995, 2002). It is possible that methimazole causes an inactivation of the hemegroup of peroxidases involved in scavenging H_2O_2, as catalase (EC1.11.1.6). Pulmonary damage was observed in hypothyroid animals. We suggest that decrease of the thyroid hormones reduced the activity of Na^+–K^+-ATPase (EC3.6.3.9), modified the apical Na^+ conductance and accumulated extra- cellular fluid that caused alveolar damage (Lei et al., 2007). In addition, our histological results demonstrated that there was no oogenesis in the ovary of the hypothyroid rat compared with the control rat. These results suggest that hypothyroidism is associated with dysfunction of the heart, lung, thyroid and ovary in adult female rat, although the mechanisms for these changes are not clear.

The present study also demonstrated that methimazole induced d hypothyroidism caused remarkable decreases in body weights and in the plasma levels of T_3 and T_4 significantly lower in the hypothyroid rats compared with the control rats. The therapeutic effects of the anti-thyroid drug methimazole have been ascribed to its ability to decrease thyroid hormone production (Cooper DS., 1984).In this study, the plasma concentrations of T_3 and T_4 were obviously suppressed by 28 days after methimazole treatment and dwindled follicles and increased numbers of follicular epithelial cells in the thyroid glands were found in the treatment group.

CONCLUSIONS

The results show that cyto-morphological damages found in hypothyroidism is caused by methimazole. Therefore, hypothyroidism may be an important cause for various cellular damages.

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