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EFFECTS OF MELATONIN ON LIVER OF RATS WITH EXPERIMENTAL HYPERTHYROID

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The aim of this study was to investigate the structural changes that occurred in the liver of rats with experimental hyperthyroidism and the possible effects of melatonin on these changes.

The animals were designated as control group (group I), 3,3', 5-Triiodo-$_L$-Thyronine (T_3) injected group (group II) and T_3+ melatonin injected group (group III). At the end of study, tissue specimens were examined for changes in structure. In the T_3 injected group, dilatation in sinusoids and pale cytoplasm were observed, as well as an increased number of the Kupffer cells and an increased amount of glycogen. In T_3 + melatonin injected group, the amount of glycogen was similar to the T_3 injected groups while the number of Kupffer cells increased but sinusoid largeness and hepatocyte structure were similar to the control. On electron microscopic examination the mitochondria of T_3 injected group were slightly larger than those of the control group. In T_3 + melatonin injected group enlargement in the spaces of Disse, increased number of lipid vacuoles of Ito cells and increased number of microvilli of hepatocytes were observed. Kupffer cells were more active in this group.

The results of this study indicate that T_3 injection causes structural changes in the liver, and melatonin hormone has a small, if any, protective effect on the liver of rats with hyperthyroid.

Key words: Hyperthyroidism, liver, melatonin, rat.

INTRODUCTION

Thyrotoxicosis is a hypermetabolic state that causes the increase in free triiodothyronine (T_3) and thyroxin (T_4) levels. Thyroid hormones accelerate the basal metabolic rate by induction of mitochondrial enzymes in the target tissues. The hypermetabolic state in hyperthyroidism is associated with an increase in free radical production and lipid peroxide levels (Venditti *et al*. 1997). The increase in reactive oxygen species induced by thyroid hormones causes oxidative stress in the liver and results in tissue injury (Fernandez *et al*. 1991).

Recently, melatonin, the major secretory product of the pineal gland, is admitted as both direct radical scavenger and an indirect antioxidant (Reiter 1994, 1998). Melatonin crosses every morphophysiological barrier because of its

extreme lipophilic effect, e.g., the blood-brain barrier, and gets into every bodly fluid and cell (Reiter *et al*. 1993, 1994 (b), Reiter 1994). Thus, it can readily protect cell membranes from lipid peroxidation (Reiter *et al* 1993, Pierrefiche *et al*. 1993, Reiter *et al*. 1996). Melatonin is a very potent scavenger of the highly toxic hydroxyl radical (.OH) (Reiter 1994, Reiter 1996, Reiter *et al*. 1994 (a)). Remarkably, relative to other well-known free radical scavengers, i.e., glutathione and mannitol, melatonin proved to be respectively 5 fold and 14 fold more effective (Reiter 1994, Reiter *et al*. 1996). Also it shows a general inhibitory effect on thyroid gland functions. Reports of this inhibitory effect included data providing the evidence that circulating free T_4 as well as the total circulating free T_4 were depressed by injections of melatonin toward the end of the daily photoperiod. Pinealectomy induces an increase in T_4 secretion and thyroid hypertrophy; in contrast melatonin decreases T_4 secretion, increases serum and pituitary thyroid-stimulating hormone (TSH) levels and hypothalamic content of thyrotropin-releasing hormone (TRH) (Binkley 1988, Viriend *et al*. 1986).

In the present study we investigated the structural changes which occurred in the liver of rats with experimental hyperthyroidism and the possible effects of melatonin.

MATERIAL AND METHODS

Adult male Wistar rats (weighing 250-300 g, n=30) were kept under controlled temperature (21±1°C) and photoperiod (07.00 to 19.00 h). Food (standard pellet diet) and tap water were supplied *ad libitum*.

The animals were divided into 3 groups. Group I (n=10) was designated as control group. They were injected daily 2 ml saline for 20 days intraperitoneally (ip) and then saline (ip) and 0.1 ml 10% ethanol subcutaneously (sc) for 20 days. To induce hyperthyroidism, the rats in group II were daily administrated ip injections of 3,3', 5 triiodo-$_L$–thyronine (T_3) (10μg/100g body weight.) in 2 ml saline for 20 days and then T_3 + 0.1 ml 10% ethanol were injected sc for 20 days. The rats in group III were administrated daily ip injections of T_3 (10 μg/100 g body weight.) in 2 ml saline for 20 days and then T_3 (10 μg/100g body weight) + melatonin (6 mg/kg) in 0.1 ml 10% ethanol were injected sc for 20 days. 3,3', 5 triiodo-$_L$thyronine (T_3) and melatonin were purchased from Sigma Chemical Company.

At the end of the study, all animals were weighed and blood samples were collected under general anesthesia for biochemical analysis. The specimens of liver tissue of all experimental groups were taken. For light microscopy, the specimens were fixed in 10% neutral formalin, dehydrated in alcohol and embedded in paraffin. Sections 5 μm-thick were stained with Hematoxylen-Eosine and examined by Olympus BH2. For electron microscopy small slices of specimens were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) at 4°C. They were postfixed in phosphate-buffered 1% osmium tetroxide. After dehydration in ethanol, the specimens were embedded in Araldite Cy 212. Semi-thin sections were stained with Toluidin blue and examined by Olympus BH2. Thin sections were cut on ultramicrotome, stained with uranyl acetate and lead citrate, and examined by Carl Zeiss-900 electron microscope.

Quantitative Evaluation of Kupffer Cells

This was achieved by utilizing point counting with a 100-point graticule in the eyepiece of a microscope set at a magnification of X 1000. Ten fields of view for each group were randomly chosen and Kupffer cells were counted.

Statistical Analysis: Statistical analysis was performed for body weight, serum T_3 levels, plasma catalase (CAT) and malondialdehyde (MDA) levels and the number of Kupffer cells. Results were expressed as mean±S.D. Differences between groups were analyzed using One-Way ANOVA.

RESULTS

At the end of the study period, the body weight of animals receiving T_3 injections decreased comparison to the control ($p<0.001$). There was a slight increase in body weight of T_3+ melatonin injected group. The serum T_3 concentration of group II was higher than in both control and T_3+ melatonin injected groups ($p<0.001$). When the free radical (MDA) and antioxidant (CAT) levels were statistically analyzed, a significant difference between CAT levels of groups was not determined ($p>0.05$). MDA level in the T_3 injected group was higher than in the other groups while it was lower in the T_3+ melatonin injected group ($p<0.05$) (Table 1).

Table 1. Body weight, plasma T_3, MDA, CAT levels and the number of Kupffer cells in all groups. Values are mean ± S.D. of groups

	Control	T_3 injected group	T_3+Melatonin injected group
n	10	10	10
Body Weight (gr)	315.10±9.49	296±7.75*	300±5.77**
T_3 (pg/ml)	0.462±0.0312	2.128±0.6008*	0.9830±0.2298**
MDA (μmol/L)	4.225±0.2 9	4.970±0.33*	3.62±0.41**
The number of Kupffer cells	3.46±1.00*	5.64±1.25**	6.31±1.52

Body Weight: *p<0.001 compared to the control **p=0.001 compared to the control
T_3: *p<0.001 compared to the other groups **p<0.05 compared to the control
MDA: *p<0.001 compared to the other groups **p<0.05 compared to the control
CAT: p>0.05
The number of Kupffer cells: *p<0.001 compared to the other groups **p=0.01 compared to the Grup III

The liver tissue of the control group showed a normal structure on light microscopic examination (Figure 1). In the T_3 injected group, dilatation of sinusoids around the central vein was observed. Hepatocytes in this area had a pale cytoplasm (Figure 2). The number of Kupffer cells (Table I) and glycogen amount (Figure 3) in this group increased in comparison to the control. Inflammatory infiltrate of polimorphonuclear leucocytes was also observed

(Figure 4). In T_3+ melatonin injected group, the glycogen amount was similar to the T_3 injected group, the number of Kupffer cells increased, cell infiltration was present in some areas but the prevalence was not so high compared to the T_3 injected group. The width of the sinusoids and the structure of hepatocytes were similar to the control group (Figure 5).

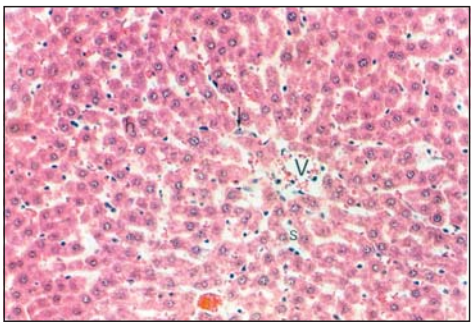

Figure 1. Light micrograph of the liver in control group. V: vena centralis s: sinusoid h: hepatocyte →: Kupffer cell. Hematoxylin and eosin stains x 200

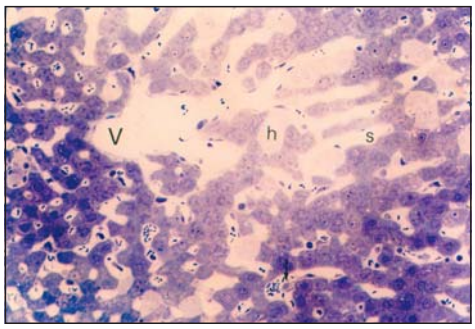

Figure 2. Enlarged sinusoids (s) around central vein (V) and hepatocytes with pale cytoplasm (h) in this area were seen in liver of T_3 injected group. Toluidin blue x 200

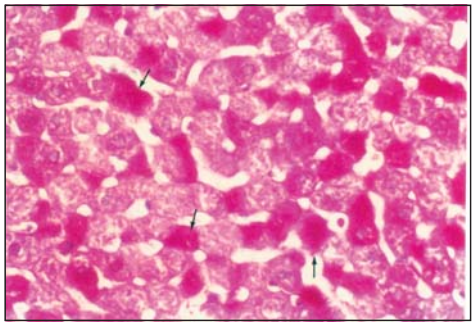

Figure 3. Glycogen (→) amount in hepatocytes of T_3 injected group increased. PAS x 400

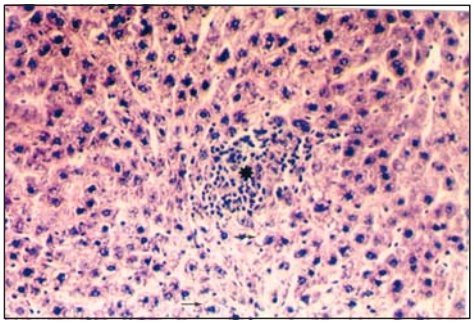

Figure 4. Hepatocyte with pale cytoplasm (→) and infiltrate polymorphonuclear leucocytes (*) was seen in liver of T_3 injected group. Hematoxylin and eosin stains x 200

On electron microscopic examination, the liver ultrastructure of the control group was normal. It was observed that hepatocytes had a marked nucleus and nucleolus, and their cytoplasm included many mitochondria, glycogen particles

and smooth and rough endoplasmic reticulum (Figure 6). In the T_3 injected group, the mitochondria were slightly larger than those of the other groups (Figure 7). When group III was compared to T_3 injected group (Figure 8), it was observed that mitochondria size was similar to the control, some hepatocytes had dense microvilli, the space of Disse was enlarged, Ito cells were bigger and included

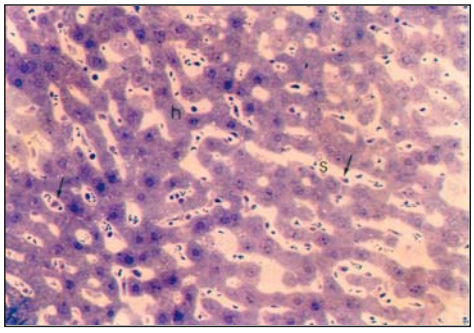

Figure 5. Light micrograph of the liver in T_3 + melatonin injected group. s: sinusoid h: hepatocyte →: Kupffer cell. Toluidin blue x 200

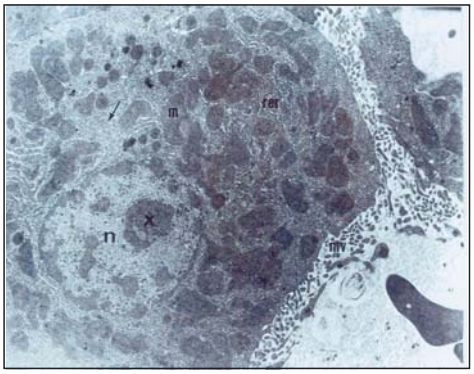

Figure 6. Electron micrograph of the liver in the control group. n: nucleus of hepatocyte, x: nucleolus, m: mitochondria, rer: rough endoplasmic reticulum, →: smooth endoplasmic reticulum, mv: microvillus of hepatocyte. Lead citrate and uranyl acetate, original magnification, x 4400

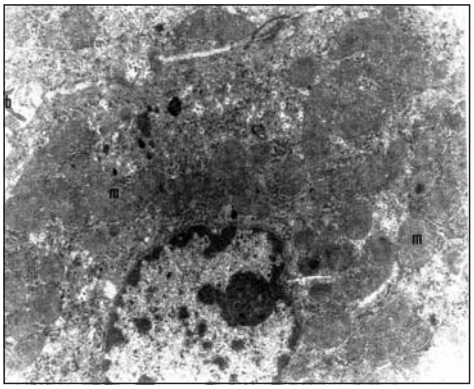

Figure 7. Electron micrograph of the liver in T_3 injected group. m: dilated mitochondria, b: bile duct. Lead citrate and uranyl acetate, original magnification, x 4400

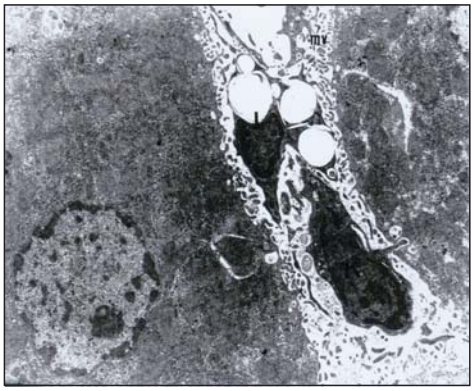

Figure 8. Electron micrograph of the liver in T_3 injected group. I: Ito cell, K: Kupffer cell, mv: Microvilli of hepatocyte. Lead citrate and uranyl acetate, original magnification, x 4400

many lipid vacuoles, Kupffer cells had many cytoplasmic processes and their cytoplasms included many lisosomes and vacuoles (Figure 9).

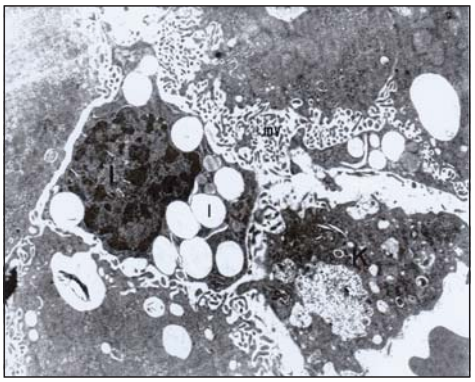

Figure 9. Electron micrograph of the liver in. T_3 +Melatonin injected group. Microvillus (mv) were dense, lipid vacuoles (l) in ito cells (l) increased, kupffer cell (K) was more active. Lead citrate and uranyl acetate, original magnification, x 4400

DISCUSSION

The increase in reactive oxygen species induced by thyroid hormones leads to a condition of oxidative stress with a consequent lipid peroxidative response (Asayama *et al*. 1990). In previous studies, it was reported that MDA (Adalý *et al*. 1999, Seven *et al*. 1996), a marker of lipid peroxidation and CAT levels in the serum of experimental hyperthyroid rats were higher than in the control group. It was observed that MDA and CAT levels reduced after prophythiouracil, vitamin E and propranolol treatment in hyperthyroid rats (Adali *et al*. 1999). In the present study, plasma MDA values in the T_3 injected group were found to be significantly higher than in the control and T_3+ melatonin injected groups. Melatonin treatment reduced MDA levels in plasma. We did not observe a significant difference among CAT levels between groups. Great controversies exist as to whether hyperthyroidism is associated with an increase or a decrease in the activities of antioxidant enzymes (Asayama *et al*. 1987, Fernandez *et al* 1985). Fernandez *et al*. (1991) investigated the oxidative capacity of rat liver with hyperthyroid. They stated that the elevation of the metabolic rate of rats with hyperthyroid occured by T_3 treatment and was accompanied by increased rates of hepatic O_2 consumption and lipid peroxidation. The authors suggested that oxidative stress developed during hormonal dysfunction.

The increase in body weight was reduced in animals receiving T_3 injections despite unchanged or higher food intake (Angeras *et al*. 1985, Callas and Cannon 1974). We observed that the body weight of animals receiving T_3 injections was

reduced as compared to previous studies, and there was a slight increase in the average body weight of the melatonin injected group.

It can be expected that the alterations of the thyroid hormones influence the hepatic function with different intensity (Sola *et al*. 1991). Many authors have reported liver injury in hyperthyroidism The observed histopathological changes might be summarized as follows: intrahepatocytic cholestasis (Sola *et al*. 1991, Yao *et al*. 1989), inflammatory infiltrate of polymorphonuclear leukocytes, eosinophils and lymphocytes, nuclear polymorphism with enlarged hyperchromatic nuclei in some hepatocytes, Kupffer cell hyperplasia, sinusoidal centrilobular dilatation and centrilobular macrovesicular fatty changes in liver (Sola *et al*. 1991). In the ultrastructure of liver with hyperthyroid glycogen depletion, stacked cisternae of the rough endoplasmic reticulum, growth and dilatation of smooth endoplasmic reticulum, dilatation of mitochondria, vacuoles opened from the periphery of hepatocytes into the spaces of Disse, increased number of microbodies and lysosomes were seen. Also, respiration, urination and defecation in rats with hyperthyroid increased (Callas and Cannon 1974). Nieri *et al*. examined the primary biliary cirrhosis associated with Graves' disease. On liver biopsy, they defined necrosis, rupture, and hyperplasia in the epithelium of intrahepatic biliary ducts. In addition, they observed that the ducts were often surrounded by lymphocytes aggregated into dense follicles (Nieri *et al*., 1985). Gay *et al*. investigated the changes that occurred in the liver as a result of amiodarone hydrochloride treatment which is an antiarhythmic agent widely used for the treatment of ventricular arrhytmias. It's undesirable side effects include hypothyroidism or hyperthyroidism. During treatment with the drug damage of parenchyma with isolated and focal necrosis as well as moderate microvesicular steatosis were observed. In electron microscopic examination, hepatocellular injury with dispersed chromatin and lamellar inclusion bodies in the lysosomes were seen (Gay *et al*., 1986). Yao *et al*. (1989) reported that liver and spleen enlarged in patients with hyperthyroidism. A wedge biopsy of the liver showed only centrilobular cholestasis.

In the T_3 injected group, we observed dilatation in sinusoids around the central vein, inflammatory infiltrate of polimorphonuclear leukocytes in some areas, hyperplasie in Kupffer cells, and dilatation in mitochondria similar to the above described alterations. We observed hepatocytes with pale cytoplasm. Glycogen amount of hepatocytes by Periodic acid Shiff staining increased contrary to the study of Callas and Cannon (1974).

The studies concerning the effects of antioxidant treatment in liver of rats with hyperthyroid were at a biochemical level. In the present study we investigated effects of melatonin recently proven as an antioxidant on structural changes concerning rat liver with hyperthyroidism. In the T_3 + melatonin injected group the glycogen amount was similar to the T_3 injected group. Sinusoid width, hepatocyte structure and size of mitochondria were similar to the control. It was observed that the space of Disse enlarged, lipid vacuoles in Ito cells increased, Kupffer cells were more active, hepatocytes had dense microvilli thus differing from the T_3 injected group.

On the basis of our findings, we suggest that hyperthyroidism causes reduction in body weight by increasing the metabolic rate and structural changes in the liver. Melatonin has little, if any, protective effect on rat liver with hyperthyroid.

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EFEKTI MELATONINA NA ĆELIJE JETRE PACOVA SA IZAZVANIM HIPERTIREOIDIZMOM

ÖNER J i OZAN E

SADRŽAJ

Cilj ovog rada je bio da se ispitaju strukturne promene u hepatocitima pacova sa eksperimentalno izazvanim hipertireoidizmom i mogući efekti melatonina. Ogledne životinje su bile podeljene u tri grupe: kontrolnu (bez tretmana), grupu tretiranu trijod-tironinom (T_3) i grupu tretiranu istovremeno trijod-tironinom i melatoninom. Svetlosnom mikroskopijom, u grupi tretiranoj sa T_3 uočene su dilatacije sinusoidnih prostora jetre, svetlija citoplazma hepatocita, povećan broj Kupferovih ćelija i povećana količina glikogena. U grupi tretiranoj sa T_3 i melatoninom takođe je zapažena veća količina glikogena ali su stepen dilatacije sinusoidnih prostora i broj Kupferovih ćelija bili slični kao kod kontrolne grupe. Elektronskom mikroskopijom je utvrđeno da su mitohondrije u hepatocitima pacova tretiranih sa T_3 nešto veće u odnosu na kontrolnu grupu. U grupi tretiranoj sa T_3 i melatoninom zapažena su proširenja Disse-ovih prostora, povećan broj lipidnih vakuola u Ito ćelijama i povećan broj mikrovila hepatocita. Kupferove ćelije su u ovoj grupi bile aktivnije.

Naši rezultati ukazuju da hipertireoidizam ima za posledicu strukturne promene u hepatocitima dok melatonin ima mali ili čak zanemarljiv protektivni efekat.