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ORIGINAL ARTICLE

Effects of melatonin on the oxidative stress induced by thyrotoxicosis in rats

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Abstract

High concentrations of thyroid hormones can affect the metabolism of oxygen in aerobic conditions and stimulate free radicals generation in mitochondria. Reactive oxygen species are toxic to biomembranes and lead to peroxidation of lipids unless they are not removed by free radical scavengers. A few years ago melatonin (MEL), a pineal hormone, was described as a scavenger of free radicals, especially of hydroxyl and peroxyl radicals.

The goal of the study was to examine the parameters of oxidative stress [the levels of conjugated dienes (CD), malondialdehyde (MDA) and Schiff bases (SB)] in male rats with thyrotoxicosis, induced by the injection of L-thyroxine (L-T4) in a dose of 100 μg/kg B.W. After one week of treatment with L-T4, the animals received MEL in a dose 5 mg/kg B.W., i.p., daily, for 7 days. Diagnosis of thyrotoxicosis was confirmed, using hormonal assays.

We observed the increased concentration of free T4 (fT4) and free T3 (fT3) after L-T4 injections. The concentration of fT3 was diminished after MEL in L-T4-injected animals. In the liver, we did not observe any changes in the content of both CD and MDA after L-T4 injections but the content of SB was higher after L-T4 injections when compared to the control group. In the liver, melatonin did not change any parameters of oxidative stress, either alone or after L-T4. The changes in the parameters of oxidative stress were better expressed in the kidney, compared to the liver. Melatonin decreased the concentration of CD and SB in the kidneys of a group of animals with thyrotoxicosis. Additionally, MEL decreased the basal concentrations of CD and SB, when compared to the control group.

Introduction

In the course of hyperthyroidism, the oxidative stress and peroxidation of lipids can be generated [1]. Additionally, the high concentration of thyroid hormones may change the metabolism of oxygen in the cells and generate the production of free radicals [2]. In hyperthyroidism, the thyroid gland is infiltrated by mononuclear cells and macrophages which, after stimulation, may release cytokines. Intrathyroid cytokine production during hyperthyroidism is not only restricted to the thyroid-infiltrating mononuclear cells, but it may also involve thyroid follicular cells [3]. The activated mononuclear cells and macrophages are able to produce cytokines, e.g., interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor (TNF) [4], which have prooxidant and proinflammatory properties.

A few years ago melatonin was shown to be a highly efficient free-radical scavenger [5, 6]. Melatonin is believed to exert its effect via electron donation to directly detoxify free radicals, such as the highly toxic hydroxyl radical [5, 7] or peroxyl radical [6]. Additionally, in both in vitro and in vivo experiments, MEL has been found to protect cells, tissues and organs against oxidative damage, induced by a variety of free radicals generating agents and processes, e.g., the carcinogen safrole, lipopolysaccharide, kainic acid, Fenton reagents, potassium cyanide, L-cysteine, excessive exercise, glutathione depletion, carbon tetrachloride, ischemia-reperfusion, MPTP, amyloid β protein [25–35 amino acid residue), and ionizing radiation [8–19]. Melatonin, as an antioxidant, is effective in protecting nuclear DNA, membrane lipids and, possibly, cytosolic proteins from oxidative damage [12–14, 20]. It has been shown that melatonin can synergistically react with other antioxidants [21–22]. Melatonin has also been reported to alter the activities of enzymes which improve the total antioxidative defense capacity of the organism, e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and nitric oxide synthase [8, 23].

The goal of the study was to examine the effect of MEL on the oxidative stress in rats with thyrotoxicosis induced by the injections of L-T4. The following parameters were measured: conjugated dienes, malondialdehyde, and Schiff bases in the homogenates of the liver and the kidney.

Materials and Methods

In the study, 38 male Wistar rats, weighing 150–175 g each, were used. The animals were fed with a standard diet and had free access to both food and water. The rats were kept in normal light conditions (14 h light; 10 h darkness). The administered solutions were sterile and were i.p. injected with disposable syringes and needles in the following concentrations: L-T4 (100 μg/kg, B.W.) as a single injection for 2 weeks, and melatonin (5 mg/kg B.W; at 3 p.m.), given for 7 days, starting after one week of L-T4 administered, with or without the last one. The animals were decapitated and blood was collected to measure the concentrations of thyroid hormones. The liver and kidney were stored at -80°C until the products of lipid peroxidation were measured in their homogenates.

In the present study, the following products of lipid peroxidation were examined after treatment with MEL in the homogenates of those two tissues: the concentration of malondialdehyde (MDA), conjugated dienes (CD), and Schiff bases (SB) [24–26]. The results were expressed as amount of MDA, CD and SB per gram of the wet tissue. The analysis of variance (ANOVA) was applied to estimate the statistical significance. All the calculations were performed with the use of Microsoft-EXCEL 5.0 computer software.

Results

We observed the increased concentrations of fT3 and fT4 in the group of rats treated with L-thyroxine (L-T4). Melatonin diminished the concentration of fT3 in the L-T4-injected animals (Table 1). In the liver, we did not observe any changes in the content of either CD or MDA after LT4 injections but the content of SB was higher after the L-T4 injection, compared to that in the control group (Table 2).

Table 1. Effect of melatonin on the concentration of thyroid hormones in the serum of animals with thyrotoxicosis: free T3 and free T4.

	Control (0.9% NaCl)	**L-thyroxine** (100 µg/kg B.W.)	**L-thyroxine** (100 µg/kg B.W.) **+ Melatonin** (5mg/kg B.W.)	**Melatonin** (5mg/kg B.W.)
Free T3 (pg/ml)	2.24 ± 0.12	3.7 ± 0.32*	1.8 ± 0.15+	2.63 ± 0.05
Free T4 (pg/ml)	4.0 ± 0.5	6.56 ± 0.34*	7.58 ± 0.29	3.48 ± 0.1

The mean values ± SEM are presented.
Levels of significance: * p< 0.001 vs control group; + p< 0.001 vs L-T4 group.

Melatonin in the liver did not change the parameters of oxidative stress, either alone or after L-T4. The changes in the parameters of oxidative stress were better expressed in the kidney, when compared to the liver (Table 3). Melatonin decreased the concentration of CD and SB in the group of animals with thyrotoxicosis in the kidney. Additionally, MEL decreased the basal concentrations of CD and SB, compared to the control group.

Table 2. Effect of L-thyroxine and melatonin on the content of conjugated dienes (CD), malondialdehyde (MDA) and Schiff bases (SB) in the homogenates of liver, measured per gram of the wet tissue.

	Control (0.9% NaCl)	**L-thyroxine** (100 µg/kg B.W.)	**L-thyroxine** (100 µg/kg B.W.) **+ Melatonin** (5mg/kg B.W.)	**Melatonin** (5mg/kg B.W.)
MDA nmol/g of	25.85 ± 3.53	20.22 ± 1.43	19.01 ± 2.10	30.51 ± 5.95
wet tissue				
CD units of absorbance at 233 nm/g of wet tissue	1.03 ± 0.03	0.96 ± 0.10	1.06 ± 0.13	1.51 ± 0.19
SB emission units/g of wet tissue	163.62 ± 5.25	208.75 ± 9.10**	186.92 ± 8.76	238.46 ± 9.13^{*++}

The mean values ± SEM are shown. Levels of significance * p< 0.001; ** p< 0.02 vs control group; $^{++}$ p< 0.05 vs L-T4 group.

Table 3. Effect of L-thyroxine and melatonin on the content of conjugated dienes (CD), malondialdehyde (MDA) and Schiff based (SB) in the homogenates of kidney, measured per gram of the wet tissue.

	Control (0.9% NaCl)	**L-thyroxine** (100 µg/kg B.W.)	**L-thyroxine** (100 µg/kg B.W.) **+ Melatonin** (5mg/kg B.W.)	**Melatonin** (5mg/kg B.W.)
MDA nmol/g of	73.77 ± 1.44	73.42 ± 2.4	70.67 ± 2.27	67.45 ± 1.8
wet tissue				
CD units of absorbance at 233 nm/g of wet tissue	2.30 ± 0.16	2.24 ± 0.20	1.48 ± 0.13^{*+}	1.24 ± 0.18^{*+}
SB emission units/g of wet tissue	72.18 ± 4.72	74.43 ± 3.50	61.67 ± 2.13^{**++}	56.16 ± 1.99^{*+}

The mean values ± SEM are presented. Level of significance: * p<0.005; ** p,0.05 vs control group; + p<0.001; ++ p<0.02 vs L-T4 group.

Discussion

Reactive oxygen species may initiate pathological changes by the mechanism of lipid peroxidation, leading to a development of many diseases. Free radicals, especially the very toxic and reactive hydroxyl radical, can destroy proteins, lipids and DNA in cells [27]. In physiological situations in the body, there is a system of endogenous antioxidants which protect against the effects of the oxidative damage, e.g., glutathione, mannitol, vitamin C [28], vitamin E [29], proteins: transferrin [30], ceruloplasmine [31], or antioxidative enzymes: catalase [32], superoxide dismutase [33], or glutathione peroxidase (GSH-Px) [34].

It is well known that thyroid hormones accelerate the basal metabolic rate, especially oxidative metabolism, as evidenced by the increased mitochondrial respiration and induction of certain mitochondrial enzymes in target tissues [35, 36]. In aerobic conditions, free radicals are generated as by-products of oxidative metabolism in mitochondria [37]. Reactive oxygen species are also generated in tissues by activated mononuclear cells and macrophages [38] which are able to produce the cytokines, such as IL-1, IL-6 and TNF [4]. These cytokines are the mediators for both the prooxidant and the proinflammatory properties [39, 40].

It is assumed that free radicals play a certain role in tissue damage in hypermetabolism induced by thyrotoxicosis [41, 42]. In our study, thyrotoxicosis was induced by the injections of L-thyroxine in a dose of 100 μg/kg B.W. daily for 2 weeks. In the group after L-T4 injections, the significantly elevated concentrations of both fT3 and fT4 were shown. Melatonin diminished the concentration of fT3 in the L-T4-injected rats. This is in agreement with the results of other authors. Melatonin, injected in late afternoon

daily injections, inhibited the secretion of thyroid hormones in hamsters [43, 44].

In the present study, L-T4 injection increased the SB content in the liver. None of the other parameters were changed, either in liver or kidney homogenates. In the liver, a tendency was shown to decreasing the content of MDA but the difference did not reach a border of statistical significance.

Our results are in agreement with other papers. Namely, Fernandez et al. [45] demonstrated in hyperthyroid rats that there was an increased generation of oxygen free radicals in the liver microsome fraction. Ademoglu et al. [46] have observed an enhancement of the lipid peroxide content during hyperthyroidism, an increased activity of glutathione S-transferase and glutathione peroxidase, and increased levels of vitamin E and ascorbic acid, accompanied by the same changes in plasma. The achievement of euthyroidism brought about an improvement in those parameters. Mano et al. [47] have observed the opposite effect—depletion of lipid peroxides, determined indirectly by the measurement of thiobarbituric acid reactants in thyrotoxicated rats. On the other hand, they have shown increased concentrations of some endogenous antioxidative enzymes, e.g., catalase, Mn-superoxide dismutase and glutathione peroxidase in the cerebral cortex of hyperthyroid aged rats. The authors have suggested that free radicals and lipid peroxides are scavenged to compensate for the changes induced by hyperthyroidism.

The level of products of lipid peroxidation depends on the dose of the agent used and the time of measurement. High rates of metabolism during thyrotoxicosis stimulate the oxidative process, leading to an increase in mitochondrial respiration and in the production of free radicals. The latter ones are toxic to membranes and bring about the peroxidation of lipids, unless they are not removed by scavengers, such as SOD, GSH-Px, catalase, and vitamins E and C [37, 48, 49]. The level of products of lipid peroxidation depends on the balance between the prooxidant and antioxidant status in the organism.

We have showed that MEL decreased the content of the CD and SB in the kidney, either after L-T4 injections or in the basal conditions. This is in agreement with other results. Melatonin is thought to reduce the oxidative stress by several means, e.g., it inhibits an oxidative damage that is a consequence of paraquat toxicity, potassium cyanide administration, lipopolysaccharide treatment, kainic acid injection, carcinogen administration, carbon tetrachloride poisoning, as well as reduces the oxidation of macromolecules that occurs during swimming exercise or liver and brain ischemia-reperfusion and radiation [8–14, 16–18, 49]. Melatonin crosses morphophysiological barriers and easily enters subcellular compartments. In order to keep the balance between the oxidative and antioxidative status, MEL is able to stimulate some important antioxidative enzymes, i.e., SOD, PSH-Px and GSH reductase and, additionally, it may act synergistically with other antioxidants [21, 22, 50].

In conclusion, the results of this study show that MEL can protect against oxidative stress induced by thyrotoxicosis. In fact, this is the first observation related to the effect of MEL in the model of thyrotoxicosis-induced hypermetabolism.

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