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ORIGINAL ARTICLE

# Melatonin effects on Schiff's base levels induced by iodide administration in rats

**Dominika Swierczynska-Machura [1], Andrzej Lewinski[1,2] & Ewa Sewerynek[1,2]**

[1] Department of Thyroidology;
[2] Department of Endocrinology and Isotope Therapy; Institute of Endocrinology, Medical University of Lódz, POLAND

*Correspondence to:* Ewa Sewerynek, M.D., Ph.D.,
Department of Thyroidology,
Institute of Endocrinology,
Medical University of Lodz, Dr. Sterling Str. No. 5
91-425 Lodz, POLAND
FAX/PHONE: (48) (42) 632 25 94
EMAIL: ewa@tyreo.am.lodz.pl

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**Abstract**

**OBJECTIVES:** Administration of iodides to animals, living in iodine-deficient areas, can induce necrosis and fibrosis of the thyroid gland. It is believed that structural and functional changes of the thyroid may be related to oxidative processes. Increased lipid peroxidation levels were reported in murine thyroid glands after high doses of iodine.
Melatonin (MEL) is believed to exert its effects *via* electron donation to directly detoxify free radicals, such as, e.g., the highly toxic hydroxyl radical. In numerous reports related to the antioxidative action of MEL, the authors have considered the protective ability of this hormone against peroxidation of lipids.
The goal of the study was to evaluate oxidative processes and the protective role of MEL in three organs of the rat (the liver, the brain, and the lungs) during treatment with different doses of iodide.
**MATERIAL AND METHODS:** Schiff's bases (SB) concentrations (a parameter of oxidative stress) were measured in liver, lung and brain homogenates of male Wistar rats. The animals received iodides in their diet in the following concentrations, for 2 weeks: Group 1 – Controls (standard normal-iodine diet, containing approx. 0.7 mg of kalium iodide per kg; KI/kg); Group 2 – diet containing 0,25 mg KI/kg; Group 3 – diet with 4,0 mg KI/kg; Group 4 – diet with 8,0 mg KI/kg. Group 5 – standard normal-iodine diet and MEL alone in a dose of 1 mg/kg BW i.p. at 3.00 pm, every day for two weeks. Subsequent three groups (6–8) received KI in their diet in doses as above, respectively, together with MEL.
**RESULTS:** We noted increased levels of SB in the lungs and in the liver, when compared to those observed in controls. We also found decreased SB concentrations in liver and lung homogenates after an administration of MEL but – unexpectedly – the level of SB increased in the group with the highest dose of iodine in diet in lung homogenates. Increased levels of Schiff's bases suggest that iodine is involved in oxidative processes not only in the thyroid but also in other tissues, and MEL protects against the iodine-induced oxidative stress.
**CONCLUSION:** Our results confirm the differences in lipid peroxidation among the examined organs. These alterations can possibly be related to different sensitivity rates of examined tissues to oxidative damage.

## Introduction

It was Follis as early as in 1959, who showed for the first time the toxic effect of iodine in animals, kept in iodine-deficient area [1]. It has also been demonstrated that iodine excess in iodine-depleted thyroid glands can induce necrosis of thyroid follicular cells and may bring about changes comparable to those observed in oxidative stress and lipid peroxidation in other tissues [2,3]. Many et al. [4] have found increased lipid peroxidation levels in the thyroid glands of mice after high doses of iodine; the authors have succeeded to demonstrate partial protection by antioxidants. The free-radical hypothesis, as postulated by Denef et al. [5], confirms those changes.

Melatonin (N-acetyl-5-methoxytryptamine; MEL), the main pineal hormone, plays an essential role in many physiological processes. It is involved in the regulation of circadian rhythms, the growth of tissues, immune functions, sleep, retinal physiology, and functions of endocrine glands in many animal species and in humans [6,7,8].

The potential role of MEL as an antioxidant was originally shown by Ianas et al [9]. MEL is believed to act *via* electron donation to directly detoxify free radicals, such as the highly toxic hydroxyl radical [10]. The majority of the reports, which relate to the antioxidative action of MEL, consider the ability of this hormone to protect against peroxidation of lipids [11]. It has been shown that MEL protects lipid membranes against oxidative damage induced by a variety of agents, e.g.: bacterial lipopolysaccharide, hydrogen peroxide, L-thyroxine, liver ischemia-reperfusion [12–17]. MEL, as an antioxidant, is effective in protecting nuclear DNA, membrane lipids and, possibly, cytosolic proteins from oxidative damage [12,15].

The goal of the study was to evaluate oxidative processes, measured by Schiff's bases (SB) levels, and the protective role of MEL in three organs of the rat (the liver, the brain, and the lung) during treatment with different doses of iodine.

## Materials and methods

Male Wistar rats, weighing 200 ± 20 g each, were used in the study. The animals were kept in a room with controlled temperature (21–23°C) and illumination (12 h light, 12 h darkness).

The animals were divided into 8 groups, according to the content of iodine in food.

Group 1 – controls [standard normal-iodine diet, containing approx. 0.7 mg of kalium iodide per kg (KI/kg) – Motycz, Poland]
Group 2 – diet containing 0.25 mg KI/kg
Group 3 – diet containing 4.0 mg KI/kg
Group 4 – diet containing 8.0 mg KI/kg
Group 5 – standard normal-iodine diet and MEL in a dose of 1 mg/kg BW i.p. at 3:00 pm
Group 6 – diet containing 0.25 mg KI/kg and MEL (1 mg/kg BW i.p.)
Group 7 – diet containing 4.0 mg KI/kg and MEL (1 mg/kg BW i.p.)
Group 8 – diet containing 8.0 mg KI/kg and MEL (1 mg/kg BW i.p.)

The animals were decapitated after two weeks of the experiment. Then, the organs to be examined were collected. After weighing, the material was frozen in –80°C till homogenisation and biochemical estimations were done.

Concentrations of Schiff's bases – a connection of the aldehydes with amine group – were spectrofluorometrically estimated (the protocol, according to Buege and Aust [18]).

A statistical analysis of the obtained results was performed, using a one-way analysis of variance (ANOVA) and Newman-Keuls' test.

## Results

We found increased SB concentrations in liver homogenates from the rats fed with diet, containing 8.0 mg KI/kg and in lung homogenates from the group of animals fed with diet. containing 4.0 mg KI/kg, as compared to respective values in the controls (Fig.1, 2, 3). MEL administration decreased SB levels in liver homogenates from the groups of animals fed with diet, containing 0.25 mg and 8.0 mg KI/kg, when compared to those remaining on the same diet but without MEL administration (Fig. 4,5). We also noticed that MEL decreased SB concentrations in lung homogenates from Group 7 (diet containing 4.0 mg KI/kg), when compared to those in Group 3 fed only with that diet (Fig. 6) but, unexpectedly, that indoleamine increased SB levels in the group of animals fed with diet, containing 8.0 mg KI/kg, comparing with those in the group of animals on this diet only (Fig.7). MEL did not affect SB concentrations in brain homogenates.

## Discussion

Iodine excess in iodine-depleted animals can induce changes, comparable to those observed in oxidative stress and lipid peroxidation. The differences, observed in the sensitivity of organs to oxidative stress, are due to their different quantity of endogenic antioxidants, both antioxidative enzymes [19], and molecular antioxidants [20].

Numerous findings have shown potent antioxidative effects of MEL. This indoleamine readily enters all subcellular compartments and easily crosses the blood-brain-barrier. Besides scavenging free radicals, MEL also stimulates some endogenous antioxidative enzymes, e.g., glutathione peroxidase [21].

The lungs are organs, which have a close contact with external environment, being at risk of contact with a number of harmful substances from the air. Because of that, the lungs have a very effective system of endogenic antioxidants, which is activated after an excessive production of free radicals in that organ [22,23].

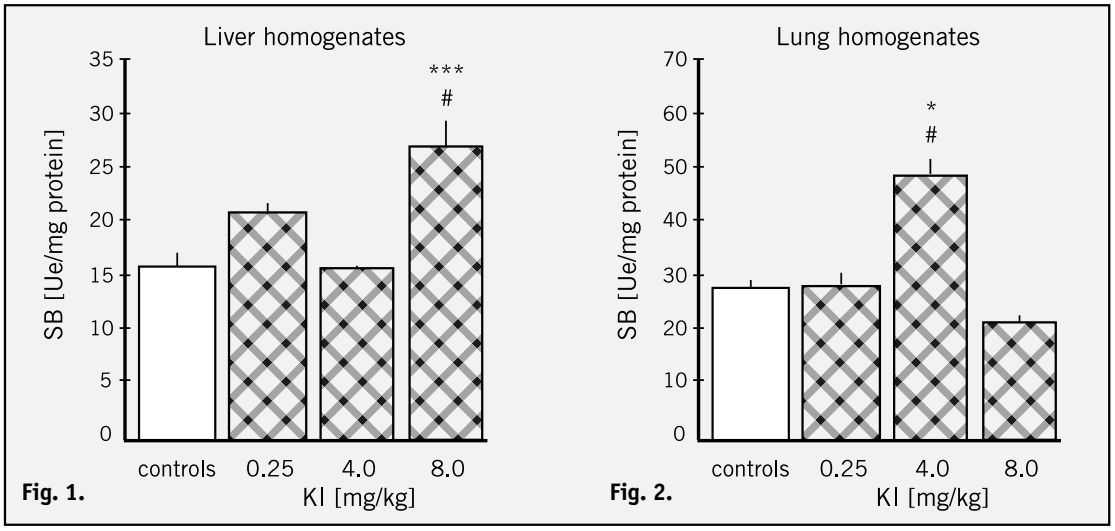


**Fig. 1.** Concentrations of Schiff's bases (SB) in liver homogenates. Mean values ± SEM. Statistical significance: ***p<0.05 vs. group of animals fed with 0.25 mg KI/kg; #p<0.001 vs. Control Group.

**Fig.2.** Concentrations of Schiff's bases (SB) in lung homogenates. Mean values ± SEM. Statistical significance: *p<0.001 vs. group of animals fed with 0.25 mg KI/kg; #p<0.001 vs. Control Group.

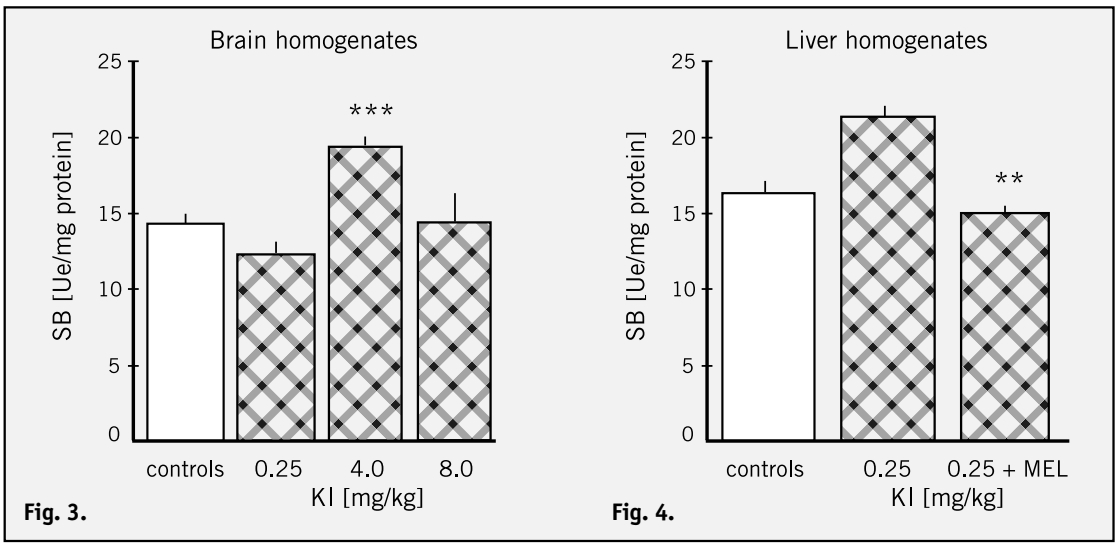


**Fig.3.** Concentrations of Schiff's bases (SB) in brain homogenates. Mean values ± SEM. Statistical significance: ***p<0.05 vs. group of animals fed with 0.25 mg KI/kg.

**Fig.4.** Concentrations of Schiff's bases (SB) in liver homogenates after MEL administration. Mean values ± SEM. Statistical significance: **p<0.01 vs. group of animals fed with 0.25 mg KI/kg.

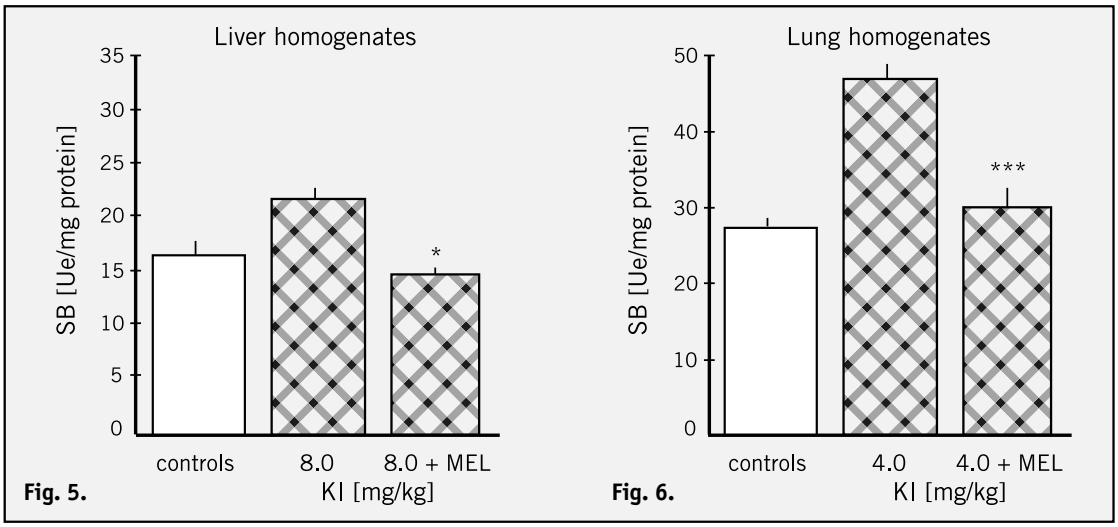


**Fig.5.** Concentrations of Schiff's bases ( (SB) in liver homogenates after MEL administration. Mean values ± SEM. Statistical significance: p<0.001 vs. group of animals fed with 8.0 mg KI/kg.

**Fig.6.** Concentrations of Schiff's bases (SB) in lung homogenates after MEL administration. Mean values ± SEM. Statistical significance: ***p<0.05 vs. group of animals fed with 4.0 mg KI/kg;

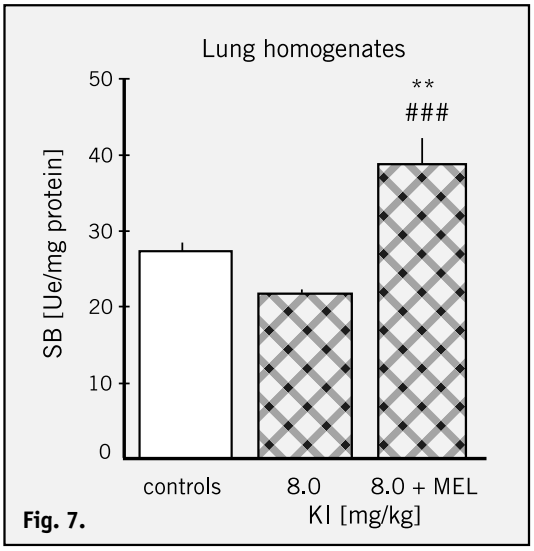


Fig. 7.

**Fig.7.** Concentrations of Schiff's bases (SB) in lung homogenates after MEL administration. Mean values ± SEM. Statistical significance: ***p<0.01 vs. group of animals fed with 8.0 mg KI/kg, ###p<0.05 vs. Control Group

In the present study, the increased SB concentrations in lung homogenates were demonstrated after high doses of iodine (4.0 mg KI/kg), when compared to the values in the control group and to those in the group with 0.25 mg KI/kg in diet. Besides, we noticed that MEL decreased SB concentrations in lung homogenates of animals from the group, administered 4.0 mg KI/kg but that indoleamine increased SB levels in the group with the highest dose of iodides. To our knowledge, so far there have been no reports on oxidative processes in the lung after high doses of iodine. There are some reports, concerning the stimulation of LPO by xenobiotics. We have shown that MEL administration to rats prevented the oxidative changes in rat lungs after injections of paraquat, which is a known herbicide [24]. Although the mechanism of cell membrane damage by paraquat is complex, its major role is connected with free radicals [25].

The liver is an organ, which plays an important metabolic function in the organism, having a well-developed antioxidative defence mechanism. In our previous study [12], we demonstrated increased MDA and 4-HDA levels in the liver after 6 hours of lipopolysaccharide administration to rats, but, after subsequent 16 hours, that parameter normalised. A stimulation of LPO was observed in this organ after an administration of many free radical-induced substances, e.g., doxorubicin, paraquat, and ionising radiation [26–29]; the effects of administered toxic substances were dependent on their doses. The paraquat-induced LPO in the rats was analysed after 3 doses of herbicide: 20, 50 and 70 mg /kg B.M. [30–31]. Only higher doses increased LPO. In the liver after doxorubicin administration, a similar effect was observed [26,27]. We found that MEL effectively protected against lipopolysaccharide-induced oxidative damage not only in the liver but also in the brain and in the lungs [14].

Our study confirms the increasing detoxication processes in the liver and considerable regenerative abilities of this organ [12]. SB concentrations increased only after the highest dose of KI. Melatonin decreased SB levels in liver homogenates from the group with 0.25 KI mg/kg and from that with 8.0 KI mg/kg.

The brain is especially sensitive to free radical damage [32–34]. There is a high intensity of metabolic processes in this organ and utilization of oxygen, which leads to overproduction of free radicals. Moreover, the brain has a high metabolic rate, when compared to the metabolic rates of rat lungs and liver, and a much lower activity of the antioxidative enzyme systems [19,20]. Increased lipid peroxidation levels in brain homogenates were, for example, demonstrated after an incubation with kainic acid (a neurotoxic agent) [35] and with quinalinic acid [36]. It has also been noticed that hydrogen peroxide ($H_2O_2$), in the presence of $Fe^{2+}$ ions, is a source of $^{\bullet}OH$ and stimulates LPO in rat brain homogenates [37]. In our study, we found increased SB concentrations in brain homogenates after the diet with 4 mg KI/kg; MEL did not affect that effect of iodine.

Our results demonstrate that iodides are involved in oxidative processes not only in the thyroid but also in other organs. According to our knowledge, so far no studies have been reported, concerning oxidative processes in peripheral tissues after an administration of high doses of KI and protection by MEL. The results of our experiments confirm the differences in organ LPO production, which may be related to different sensitivity rates of tissues to oxidative processes and to different amounts of endogenous antioxidants.

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