Original Communication

Melatonin reduces high levels of lipid peroxidation induced by potassium iodate in porcine thyroid

Paulina Iwan[1], Jan Stepniak[1], and Malgorzata Karbownik-Lewinska[1,2]

[1] Department of Oncological Endocrinology, Medical University of Lodz, Lodz, Poland
[2] Polish Mother's Memorial Hospital – Research Institute, Lodz, Poland

Abstract: Iodine is essential for thyroid hormone synthesis. Under normal iodine supply, calculated physiological iodine concentration in the thyroid is approx. 9 mM. Either potassium iodide (KI) or potassium iodate (KIO_3) are used in iodine prophylaxis. KI is confirmed as absolutely safe. KIO_3 possesses chemical properties suggesting its potential toxicity. Melatonin (N-acetyl-5-methoxytryptamine) is an effective antioxidant and free radical scavenger. Study aims: to evaluate potential protective effects of melatonin against oxidative damage to membrane lipids (lipid peroxidation, LPO) induced by KI or KIO_3 in porcine thyroid. Homogenates of twenty four (24) thyroids were incubated in presence of either KI or KIO_3 without/with melatonin (5 mM). As melatonin was not effective against KI-induced LPO, in the next step only KIO_3 was used. Homogenates were incubated in presence of KIO_3 (200; 100; 50; 25; 20; 15; 10; 7.5; 5.0; 2.5; 1.25 mM) without/with melatonin or 17ß-estradiol. Five experiments were performed with different concentrations of melatonin (5.0; 2.5; 1.25; 1.0; 0.625 mM) and one with 17ß-estradiol (1.0 mM). Malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA) concentration (LPO index) was measured spectrophotometrically. KIO_3 increased LPO with the strongest damaging effect (MDA + 4-HDA level: ≈1.28 nmol/mg protein, $p < 0.05$) revealed at concentrations of around 15 mM, thus corresponding to physiological iodine concentrations in the thyroid. Melatonin reduced LPO (MDA + 4-HDA levels: from ≈0.97 to ≈0,76 and from ≈0,64 to ≈0,49 nmol/mg protein, $p < 0.05$) induced by KIO_3 at concentrations of 10 mM or 7.5 mM. Conclusion: Melatonin can reduce very strong oxidative damage to membrane lipids caused by KIO_3 used in doses resulting in physiological iodine concentrations in the thyroid.

Keywords: Melatonin, potassium iodate, lipid peroxidation, thyroid, antioxidant

Introduction

Reactive oxygen species (ROS) and free radicals participate in metabolic processes [1]. Under physiological conditions, there is a balance between the production and detoxification of ROS. Thyroid gland is an organ of "oxidative nature" [2], in which oxidative processes are necessary, e.g. for thyroid hormone biosynthesis. In turn, an enhanced oxidative stress, defined as an imbalance between oxidants and antioxidants, may result in different thyroid diseases [2–5].

Iodine is a micronutrient that is essential for the synthesis of thyroid hormones [6]. The only natural source of iodine is the diet. Salt iodization is one of the safest and most effective methods of achieving iodine sufficiency across a population [6, 7]. Either potassium iodide (KI) or potassium iodate (KIO_3) are used for salt iodization [8].

It has been documented that two main iodine compounds, i.e. KI and KIO_3, have different chemical properties, resulting in different pro-/anti-oxidative properties [9]. KI is less reactive whereas KIO_3 reveals stronger oxidizing properties. This difference may be associated with main chemical properties of these two compounds, namely KI is the reductant whereas KIO_3 is the oxidant and, as one of halogenate salts, it may react very easily with oxidisable substances. Before IO_3^- can be effectively used in human body it should be reduced to I^- [10]. Potassium iodide may prevent oxidative damage to membrane lipids in the thyroid gland when used in doses recommended in iodine prophylaxis, although in higher concentrations (>50 mM) it increased lipid peroxidation [11]. Additionally, in the process of inducing oxidative stress, KI stimulates simultaneously a well-known antioxidative enzyme, i.e. it increases peroxiredoxin 3 protein expression, in Fischer rat thyroid cell line [12]. In contrast, KIO_3 increased lipid peroxidation in concentrations > 2.5 mM with the strongest damaging effect at the concentration of 10 mM [11], which corresponds to the physiological concentration of iodine in the thyroid.

As previously mentioned, thyroid gland has the "oxidative nature", therefore the antioxidative defence system

should prevent the build-up of excessive ROS [2, 4]. Different exogenous antioxidants were founded to prevent experimentally-induced oxidative damage to macromolecules in the thyroid [2], with melatonin being the first antioxidant tested [13]; it is worth mentioning others, e.g. indole-3-propionic acid (IPA) [14, 15], propylthiouracil [14, 16], estrogens [17].

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine produced mainly, but not exclusively, in the pineal gland and possesses excellent antioxidative properties [18–26]. Its protective effects against oxidative damage to macromolecules have been confirmed in numerous studies [13–15, 18–20, 27–29]. However, melatonin effects are concentration dependent. As it was documented recently, this indoleamine may also reveal at certain concentrations some prooxidative effects; melatonin at concentrations lower than 100 μM induced lipid peroxidation, Band 3 protein expression, and cell shape alterations in human erythrocytes [29]. However, according to the point of view of most experts working on melatonin, this indoleamine reveals almost exclusively antioxidative effects, and single observations, such as mentioned above [29], should not confirm its prooxidative nature.

It is worth mentioning that melatonin has been documented to reveal inhibitory effects on thyroid growth and function [30–32].

The aim of the study was to evaluate potential protective effects of melatonin against oxidative damage to membrane lipids (lipid peroxidation) induced by either KIO_3 or KI in porcine thyroid homogenates.

Materials and methods

Chemicals

Potassium iodide (KI), potassium iodate (KIO_3), melatonin and 17ß-estradiol were purchased from Sigma (St. Louis, MO, USA). The ALDetect Lipid Peroxidation Assay Kit was obtained from Enzo Life Sciences, Inc. (Zandhoven, Belgium). All the used chemicals were of analytical grade and came from commercial sources.

Animals

Porcine thyroids were collected from twenty four (24) animals at a slaughter-house, frozen on solid CO_2 and stored at -80° until assay. Each experiment was repeated three to four times. Therefore, three to four tissue pools were prepared, with six (6) thyroid glands used for each homogenate pool.

Assay of lipid peroxidation

Thyroid tissue was homogenized in ice cold 20 mM Tris-HCl buffer (pH 7.4) (10%, w/v) and then incubated for 30 min at 37° in the presence of either KI (500; 250; 100; 50 mM) or KIO_3 (200; 100; 50; 25; 10; 5.0; 2.5 mM) without or with addition of melatonin (5 mM).

As melatonin did not reveal any protective effect against KI-induced lipid peroxidation, in the next step only KIO_3 was used.

In the next step thyroid homogenates were incubated in the presence of KIO_3 (200; 100; 50; 25; 20; 15; 10; 7.5; 5.0; 2.5; 1.25 mM) without/with addition of melatonin or 17ß-estradiol. Five experiments were performed with different concentrations of melatonin, i.e. 5.0; 2.5; 1.25; 1.0; 0.625 mM and one experiment with 17ß-estradiol in concentration of 1.0 mM.

The concentrations of KI and KIO_3 [11], 17ß-estradiol [17] and melatonin [14] were chosen on the basis of the results of our previous studies.

The reactions were stopped by cooling the samples on ice. Each experiment was run in duplicate.

Measurement of lipid peroxidation products

The concentrations of malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA), as an index of lipid peroxidation, were measured in thyroid homogenates, with the ALDetect Lipid Peroxidation Assay Kit. The homogenates were centrifuged at 5,000 x g for 10 min at 4°. After obtaining supernatant, each experiment was carried out in duplicate. The supernatant (200 μl) was mixed with 650 μl of a methanol: acetonitrile (1:3, v/v) solution, containing a chromogenic reagent, N-methyl-2-phenylindole, and vortexed. Following the addition of 150 μl of methanesulfonic acid (15.4 M), the incubation was carried out at 45° for 40 min. The reaction between MDA + 4-HDA and N-methyl-2-phenylindole yields a chromophore, which is spectrophotometrically measured at the absorbance of 586 nm, using a solution of 10 mM 4-hydroxynonenal as the standard. The level of lipid peroxidation is expressed as the amount of MDA + 4-HDA (nmol) per mg protein. Protein was measured using Bradford's method [33], with bovine albumin as the standard.

Statistical analyses

Results are expressed as means ± SE. The data were statistically analyzed, using a one-way analysis of variance (ANOVA) followed by the Tukey's test or unpaired t-test. Normality of distribution was confirmed by the use of

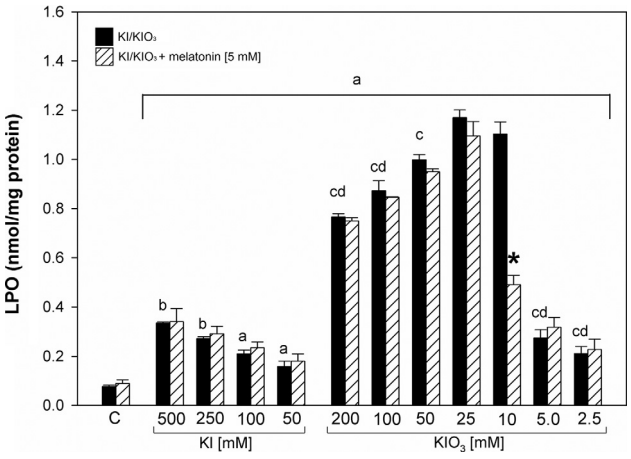

Figure 1. Lipid peroxidation, measured as MDA + 4-HDA level, in homogenates prepared from twenty four (24) porcine thyroids, incubated for 30 min in the presence of either KI (500; 250; 100; 50 mM) or KIO_3 (200; 100; 50; 25; 10; 5.0; 2.5 mM) with (striped bars) or without (black bars) melatonin (5.0 mM). The experiment was repeated three to four times. Therefore, three to four tissue pools were prepared, with six (6) thyroid glands used for each homogenate pool. Data are expressed as nmol/mg protein. Values are expressed as mean ± SE (error bars). *$p < 0.05$ vs. KIO_3 in the same concentration without melatonin. $^a p < 0.05$ vs. respective control. $^b p < 0.05$ vs. any other KI concentration. $^c p < 0.05$ vs. KIO_3 at the concentration of 25 mM. $^d p < 0.05$ vs. KIO_3 at the concentration of 10 mM. C – control.

Shapiro-Wilk test, whereas the equality of variance was confirmed by the use of Levene's test. The level of $p < 0.05$ was accepted as statistically significant.

Results

In the present study we have chosen those concentrations of KI (500; 250; 100; 50 mM) or KIO_3 (200; 100; 50; 25; 10; 5.0; 2.5 mM) which revealed stimulatory effects on lipid peroxidation in thyroid homogenates in our previous study [11]. Similarly to results of this previous study [11], potassium iodide, in all used concentrations, did increase lipid peroxidation in concentration-dependent manner (Figure 1). In turn, potassium iodate did increase lipid peroxidation in all used concentrations with the strongest damaging effect to membrane lipids at concentrations of 10 mM and 25 mM (Figure 1).

When thyroid homogenates were incubated in the presence of either KI or KIO_3 plus melatonin (5.0 mM), significant reduction of lipid peroxidation was observed only when KIO_3 was used at the concentration of 10 mM (Figure 1).

As in the above experiment melatonin did not protect against KI-induced lipid peroxidation, in next steps we used only KIO_3.

In the subsequent experiment we decided to use additional concentrations of KIO_3 (i.e. 20; 15; 7.5; 1.25 mM) to clarify unexpected results obtained in the first step of experiments and in our previous study [11].

After using additional concentrations of KIO_3, the strongest damaging effect to membrane lipids was observed for KIO_3 concentration of around 15 mM (Figures 2–4) with the highest LPO level confirmed for concentrations of 15 mM and 20 mM (Figures 3 and 4).

Melatonin reduced, in concentration-dependent manner, KIO_3-induced lipid peroxidation, but only when this prooxidant was used at concentrations of 10 mM or of 7.5 mM. Namely, melatonin, at concentrations of 5.0 mM or 2.5 mM, decreased lipid peroxidation induced by KIO_3 in concentrations either of 10 mM or of 7.5 mM (Figure 2).

Lower concentrations of melatonin, i.e. 1.25 mM and 1.0 mM, decreased KIO_3-induced lipid peroxidation but only when this prooxidant was used at the concentration of 7.5 mM (Figure 3). The lowest concentration of melatonin used in our study, i.e. 0.625 mM, was not protective in our model (Figure 3).

The incubation of porcine thyroid homogenates in the presence of melatonin only (in concentrations of 5.0; 2.5; 1.25; 1.0; 0.625 mM) did not change the basal lipid peroxidation (Figures 1–3).

In the present study we decided to compare protective effects of melatonin with a well-known endogenous antioxidant – 17ß-estradiol. 17ß-estradiol, used at the concentration of 1.0 mM, being the highest possible concentration to be used in our model (due to its limited solubility), did not cause any protective effects against KIO_3-induced lipid peroxidation (Figure 4), whereas melatonin, used in the same concentration of 1.0 mM, reduced lipid peroxidation induced by KIO_3 (7.5 mM) (Figure 3).

Discussion

On the basis of experimental findings the concentration of inorganic iodine in human or rat thyroid was calculated to be approx. 9 mM [34–36]. Due to similarity between human and porcine thyroid (volume, hormone synthesis, etc.) [37], it may be estimated that iodine concentration in porcine thyroid is at similar level. Such relatively high level of iodine in the thyroid is expected, especially that much lower iodine levels have recently been found in human placenta, i.e. 1.38 μg/g equal to approx. 10 μM [38] or in human blood serum, i.e. 99.1 μg/L equal to almost 1 μM [39]. It is worth emphasizing that the highest lipid peroxidation caused by KIO_3 was observed in the present study for the concentration of around 15 mM, which is of the same order of magnitude as physiological concentration of iodine in the thyroid.

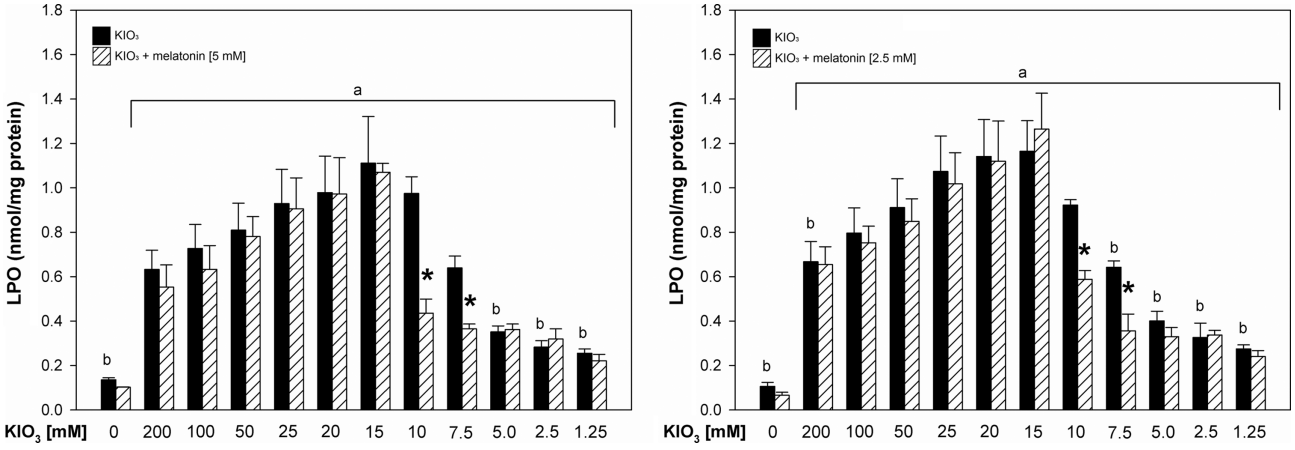

Figure 2. Lipid peroxidation, measured as MDA + 4-HDA level, in porcine thyroid homogenates, incubated in the presence of KIO_3 (200; 100; 50; 25; 20; 15; 10; 7.5; 5.0; 2.5; 1.25 mM) with (striped bars) or without (black bars) melatonin (5.0 mM or 2.5 mM). The experiment was repeated three to four times. Therefore, three to four tissue pools were prepared, with six (6) thyroid glands used for each homogenate pool. Data are expressed as nmol/mg protein. Values are expressed as mean ± SE (error bars). *$p < 0.05$ vs. KIO_3 in the same concentration without melatonin. [a]$p < 0.05$ vs. respective control. [b]$p < 0.05$ vs. KIO_3 at the concentration of 15 mM.

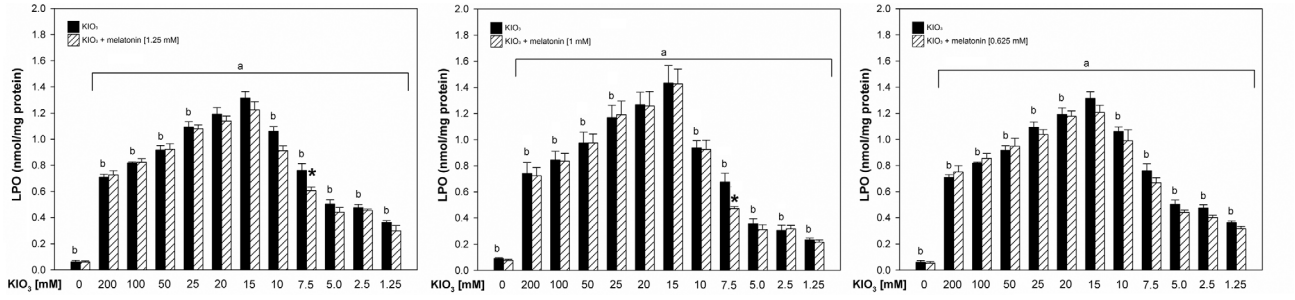

Figure 3. Lipid peroxidation, measured as MDA + 4-HDA level, in porcine thyroid homogenates, incubated in the presence of KIO_3 (200; 100; 50; 25; 20; 15; 10; 7.5; 5.0; 2.5; 1.25 mM) with (striped bars) or without (black bars) melatonin (1.25 mM, 1.0 mM or 0.625 mM). The experiment was repeated three to four times. Therefore, three to four tissue pools were prepared, with six (6) thyroid glands used for each homogenate pool. Data are expressed as nmol/mg protein. Values are expressed as mean ± SE (error bars). *$p < 0.05$ vs. KIO_3 in the same concentration without melatonin. [a]$p < 0.05$ vs. respective control. [b]$p < 0.05$ vs. KIO_3 at the concentration of 15 mM.

It should be stressed that at this concentration of iodine, KI did not increase the level of lipid peroxidation in porcine thyroid homogenates [11].

Because iodate is more stable than iodide (iodide is easily oxidized to I_2 and then lost by evaporation), some health authorities preferentially recommend iodate as an additive to salt for correcting iodine deficiency [9]. On the other hand, the superiority of KI over KIO_3 may rely on its stronger protective effects against oxidative damage to mtDNA [40]. Although iodate has been conferred GRAS ("generally recognized as safe") status by the FDA [9], available publications show "dual nature" of KIO_3.

Considerations concerning potential toxicity of KIO_3 are as follows

Iodic acid (HIO_3), together with chloric acid and bromic acid, belongs to the class of oxohalogen acids. Halogenate salts are stable under most conditions, but due to their oxidative properties they may react rapidly with easily oxidisable substances. As previously mentioned, KIO_3 belongs to the group of GRAS, but due to its similarity to $KBrO_3$ (known potential carcinogen belonging to the group 2B according to IARC [41] it is justified to check their mutagenic and cancerogenic potential. On the other hand, iodate has a lower oxidative potential than bromate has, and it did not induce toxic effects under conditions in which bromate did [9, 15].

It is probable that KIO_3-caused lipid peroxidation in porcine thyroid results from direct oxidative effects of this compound on cellular membranes. However, it should be stressed that probably also other macromolecules in thyroid cells can be directly affected by KIO_3, as it has been documented for nDNA and mtDNA in our earlier studies [40]. Additionally, similar direct effects can be exerted by KIO_3 in other tissues, but that should be experimentally proven.

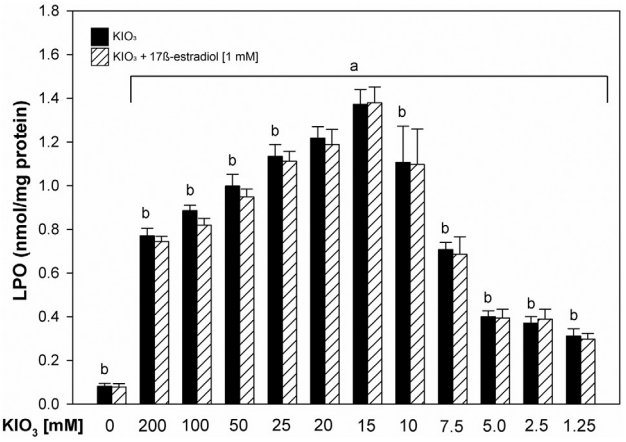

Figure 4. Lipid peroxidation, measured as MDA + 4-HDA level, in porcine thyroid homogenates, incubated in the presence of KIO_3 (200; 100; 50; 25; 20; 15; 10; 7.5; 5.0; 2.5; 1.25 mM) with (striped bars) or without (black bars) 17ß-estradiol (1.0 mM). The experiment was repeated three to four times. Therefore, three to four tissue pools were prepared, with six (6) thyroid glands used for each homogenate pool. Data are expressed as nmol/mg protein. Values are expressed as mean ± SE (error bars). $^{a}p < 0.05$ vs. respective control. $^{b}p < 0.05$ vs. KIO_3 at the concentration of 15 mM.

Iodate was tested for its potential toxicity, but that was not confirmed till now in humans.

However, taking into account chemical properties of iodate and its prooxidative effects documented in our previous [11] and present studies, it cannot be excluded that this compound is potentially dangerous.

For this reason it is advisable to search for new potential protective tools against prooxidative nature of KIO_3.

In the present study we have shown that melatonin, in concentrations usually used in in vitro studies (1.0 mM–5.0 mM), significantly reduced lipid peroxidation induced by KIO_3, when this compound was used at doses corresponding to physiological concentrations of iodine in the thyroid. The concentrations of melatonin used in the current study should be treated as corresponding to pharmacological doses, as they exceed even by three orders of magnitude the highest physiological concentrations of the indoleamine [42]. However, on the basis of the present results it is still advisable to maintain high concentrations of melatonin to prevent oxidative damage in the thyroid gland. It is suggested to avoid factors, which decrease melatonin concentrations in organisms, such as strong light at night [43, 44]. Furthermore, it may be beneficial to use exogenous melatonin by elderly, because physiological concentration of melatonin decreases with age [45].

We chose melatonin for our research, because protective effects of this compound against oxidative stress have been known for a long time [18, 20–24, 46]. These effects were observed in both in vivo and in vitro experiments. In the thyroid gland for example, melatonin reduced lipid peroxidation caused by Fenton reaction substrates ($Fe^{2+} + H_2O_2$) [13] and potassium bromate [14].

The mechanisms by which melatonin protects against lipid peroxidation involve direct or indirect antioxidative effects and free radical scavenging activities of this indoleamine [18, 21, 47, 48]. Melatonin, which is highly lipid soluble, is believed to be widely distributed in cellular membranes, where it may intercalate between the polar heads of fatty acids – this property simplifies melatonin to diminish prooxidative damage to lipids [21].

One of the most important issues is that melatonin, when used in very high pharmacological doses to either humans [49] or animals [19], has never revealed any undesirable effects.

In the present study melatonin did not reduce KI-induced lipid peroxidation. Although melatonin is usually highly protective against a prooxidant-induced damage, that indoleamine rarely does not prevent oxidative damage, especially under in vitro conditions. At the same time the lack of in vitro antioxidative effects caused by melatonin does not exclude such protective effects in in vivo conditions. However, taking into account that KI is absolutely safe in living organisms, it is not necessary to look for any protective action against this compound. It should be stressed again, that at concentrations close to physiological concentrations of iodine, KI did not induce lipid peroxidation in thyroid homogenates [11].

The observation from the present study, which should be discussed, is that melatonin was effective only when KIO_3 was used at concentrations of 10 mM and 7.5 mM; these concentrations correspond to physiological iodine concentration in the thyroid. Although the iodine concentration in the thyroid differs depending on the age, such differences are presumably not huge; therefore it can be stated that these two effective concentrations of KIO_3 correspond to physiological iodine concentration in the thyroid at any age. Prooxidative effects of KIO_3 were not reduced by melatonin when the prooxidant was used either in higher or in lower concentrations than 10 mM and 7.5 mM. We are not able to present clear explanation of these rather unexpected results. However, it can be hypothesized that during phylogenetical development in mammals, protective mechanisms have been developed to protect against well recognized toxic agents, to which organisms are potentially exposed for a long period of time. That can be the reason why melatonin reduced lipid peroxidation induced by KIO_3 in concentrations corresponding to physiological concentration of iodine in the thyroid. Although the thyroid and the whole organism can be exposed to much higher concentrations of iodine (e.g. resulting from pharmacological treatment), that is not a common epidemiological or any other individual situation. Thus it can be hypothesized that

protective mechanisms have not been developed against these rare conditions.

In the present study we compared potential protective effects of 17ß-estradiol used in the concentration of 1.0 mM (the highest achievable concentration) with protective effects of melatonin used in the same concentration. We have found that 17ß-estradiol, well known endogenous antioxidant, is not protective at all in our model. Thus, melatonin appeared to be better potential protective agent.

As previously mentioned, salt iodization is one of the most effective methods to achieve iodine sufficiency across a population. However, KIO_3 frequently used for salt iodization, reveals oxidizing properties.

Major strengths of our study are as follows. We documented that KIO_3 causes oxidative damage to membrane lipids, with strongest effects observed at KIO_3 concentrations, resulting in physiological concentration of iodine in the thyroid. This finding is of great importance as KIO_3, frequently used for salt iodization, should be absolutely safe. Additionally, we observed protective effects of melatonin against prooxidative nature of KIO_3. This finding is again of great importance, as exogenous melatonin is confirmed to be absolutely safe even in very high pharmacological doses, either in humans or in animals. The weakness of our study is that we used only in vitro model; however, this is always the first step of any study.

Conclusions

Melatonin is able to reduce very strong oxidative damage to membrane lipids caused by potassium iodate when this compound is used in doses resulting in physiological concentrations of iodine in the thyroid. Our study is the first one to show protective effects of melatonin against prooxidative effects of iodates.

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The procedures, used in the study, were approved by the Ethics Committee of the Medical University of Lodz, Poland.

ORCID
Karbownik-Lewinska Malgorzata
https://orcid.org/0000-0002-3045-5669

Prof. Malgorzata Karbownik-Lewinska
Medical University of Lodz
7/9 Zeligowski St.
90-752 Lodz
Poland
mkarbownik@hotmail.com