

The effect of melatonin on glutathione and glutathione transferase and glutathione peroxidase activities in the mouse liver and kidney *in vivo*

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Abstract

OBJECTIVES: The changes in reduced glutathione (GSH), activity of glutathione transferase (GST) and glutathione peroxidase (GSPx) after administration of exogenous melatonin (N-acetyl-5-methoxy-tryptamine) at a dose 20 mg/kg b.w. were investigated for five and ten days in the liver and kidney of male and female mice.

METHODS: The liver and kidney were homogenized in 0.1 M phosphate buffer. Glutathione level and activity of studied glutathione enzymes were determined in the supernatants.

RESULTS: Melatonin caused an increase in glutathione level, the activity of glutathione peroxidase as well as glutathione transferase in the examined organs.

CONCLUSION: Melatonin may exert some effects on the activity of enzymes engaged in the metabolism of thiol compounds in the cell. Glutathione may be useful for monitoring melatonin's protective role on cell damage.

Introduction

Melatonin, the main secretory product of the pineal gland, was recently found to be a free radical scavenger and antioxidant [1–5]. In both *in vitro* and *in vivo* investigations, melatonin protected healthy cells from radiation-induced and chemotherapeutic drug-induced toxicity [6]. Furthermore, several clinical studies have demonstrated the potential of melatonin, either alone or combined with traditional therapy, to yield a favorable efficacy to toxicity ratio in the treatment of cancers [7–8].

Reduced glutathione is a major intracellular nonprotein sulphhydryl compound. Glutathione has many biological functions, including maintenance of membrane protein sulphhydryl groups in the reduced form, the oxidation of which can otherwise

cause altered cellular structure and function [9–12]. Glutathione is also a cofactor for many enzymes involved in the detoxification [12–16].

This study examined the relationship between cellular glutathione level, activity glutathione enzymes and the effect of exogenous melatonin in the liver and kidney of male and female mice.

International literature provides little information on differences in female and male reactivity under the influence of melatonin and the expressed changes of concentration glutathione and the activity of glutathione enzymes. We wanted to show female reactivity differences and male identical conditions from the experiment.

Material and methods

Animals

Mice came from the Institute of Genetics and Animal Breeding Polish Academy of Sciences in Jastrzębiec. Male and female Swiss mice, all two months of age, were housed in standard cages and maintained in environmentally controlled rooms ($22 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ relative humidity) with a 12:12-h light-dark cycle. They were fed standard "Murigran" feed – 16% of protein, (Animal Food Company, from Łomna near Warsaw) and had constant access to water. All animals received proper veterinary care. This experiment was approved by the Ethic Commission for Animal Research in Jastrzębiec.

Scheme of experiment

Mice were randomly divided into six groups (I–III males and IV–VI females; $n=15$ in each group) and received according to the following scheme:

- I control 0.9% NaCl
- II melatonin 20 mg/kg b.w. 5 days
- III melatonin 20 mg/kg b.w. 10 days
- IV control 0.9% NaCl
- V melatonin 20 mg/kg b.w. 5 days
- VI melatonin 20 mg/kg b.w. 10 days

The male (I) and female (IV) of the control groups received everyday 100 μl of 0.9% NaCl solution per os by micropipette, between 10:00–11:00 a.m. At the same time, the experimental mice (II, III, V, VI) received 20 mg/kg b.w. of exogenous melatonin (N-acetyl-5-methoxy-tryptamine Sigma –aldrich-Chemie GmbH, Steinheim, Germany) dissolved in physiological salt analogously.

Tissue preparation

After a suitable time, the mice were slaughtered by breaking the spinal cord and decapitation. Livers and kidneys were immediately isolated. Livers were subjected to perfusion by a solution of physiological salt cooled to 4°C . Tissues were homogenized in a Potter type homogenizer with a teflon piston at 200 rot./min. in 0.1M phosphate buffer (pH 7.4) supplemented with 10 mM EDTA. Homogenates were centrifuged in Janetzki K-24 centrifuge at 12.000 rot./min. for 15 minutes. The glutathione level was determined by [17] method, glutathione transferase according to [18], glutathione peroxidase by [19] and total protein by the method [20] in modification of [21] The enzymatic substrates used were purchased from Sigma.

Statistical analyses

All data are expressed as means \pm SE and presented in bar graphs in Figures 1–6. The data were analyzed with ANOVA and Student- Fisher test.

Results

As can be seen from Figure 1, glutathione concentration in the liver of males increased significantly after five and ten days of melatonin administration to 7.39 ± 0.96 and 7.84 ± 0.84 , respectively. In the kidney, it increased markedly after five and ten days to 4.09 ± 0.57 and 4.48 ± 0.41 respectively.

According to Figure 2, the level of glutathione increased after five and ten days of melatonin administration into the liver to 7.68 ± 0.61 and 8.14 ± 0.73 . In the kidney it increased to 3.68 ± 0.40 and 3.97 ± 0.44 respectively.

There were no significant differences in the glutathione concentration between male and female mice.

Figures 3 and 4 demonstrate that glutathione transferase activity in the liver increased significantly after five and ten days of melatonin administration in the males to 6.88 ± 0.76 and 7.17 ± 0.93 and females 6.41 ± 0.58 and 6.09 ± 0.49 . In the kidney it increased to 4.05 ± 0.36 and 4.24 ± 0.31 respectively in males and 2.93 ± 0.38 and 3.31 ± 0.46 in females.

As can be seen in Figures 5 and 6, the glutathione peroxidase activity in the liver increased significantly to 59.99 ± 7.85 after five days and to 64.74 ± 5.18 after ten days. In the female, activity increased analogously to 43.61 ± 5.67 and 48.44 ± 5.33 . In the kidney differences statistically confirmed only after ten days of melatonin administration, that activity increased to 22.83 ± 2.74 in male and 22.04 ± 3.31 in female.

In the activity of glutathione enzymes there were no significant differences between males and females as well.

Discussion

Our results indicate that glutathione concentration in control animals is higher in the liver than in the kidney. The liver serves as a glutathione-generating factor which supplies the kidney and intestine with other constituents of glutathione resynthesis. The principal mechanism of hepatocyte glutathione appears to be cellular efflux. The kidney also plays an important role in organismic GSH homeostasis. The results from our study indicate that melatonin increased the concentration of reduced glutathione in both examined tissues. Our data are in agreement with earlier [22–23] and current results that reduced glutathione (GSH) is a key component of the cellular defense cascade against injury caused by reactive oxygen forms. Antioxidative enzymes provide a major defense mechanism against free radical damage either by metabolizing them to less reactive species or to non-toxic byproducts.

In our experiment melatonin increased the activity of glutathione enzymes. The initial reports documenting melatonin's stimulatory effect on glutathione peroxidase appeared almost ten years ago [24–25] when it was shown that melatonin given to rats and chicks *in vivo* resulted in a marked increase of GSPx activity. The regulating of the GSH/GSSG balance by modulating enzyme activities

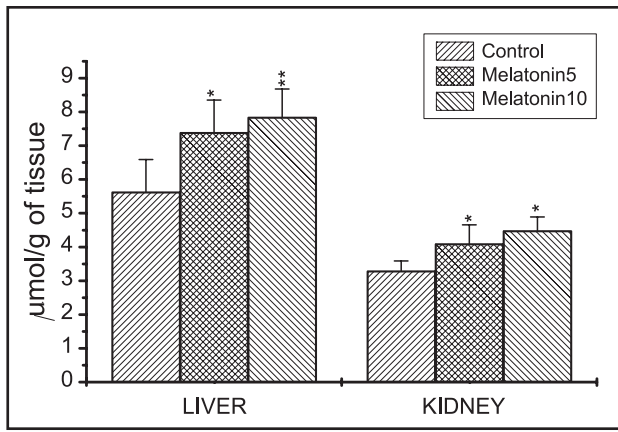


Fig. 1. Changes of glutathione concentration in the liver and kidney of male mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$

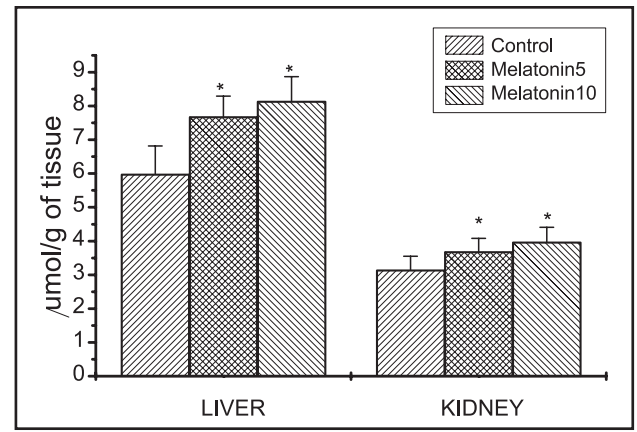


Fig. 2. Changes of glutathione concentration in the liver and kidney of female mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

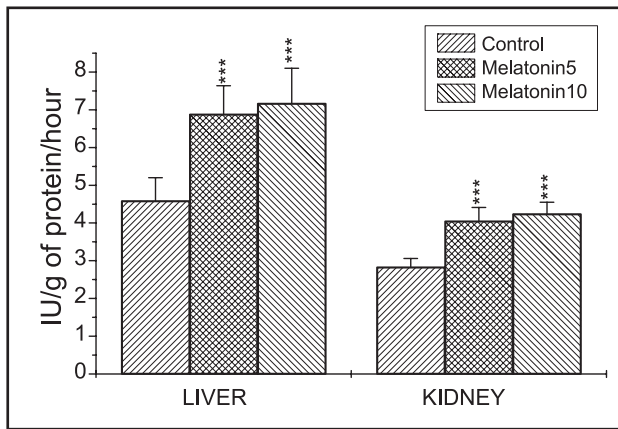


Fig. 3. Changes of glutathione transferase activity in the liver and kidney of male mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

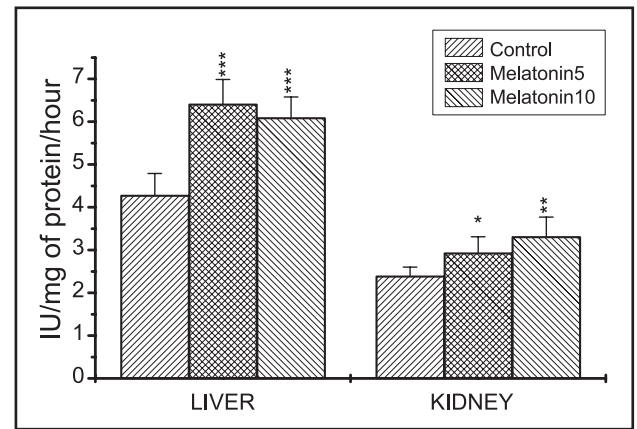


Fig. 4. Changes of glutathione transferase activity in the liver and kidney of female mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

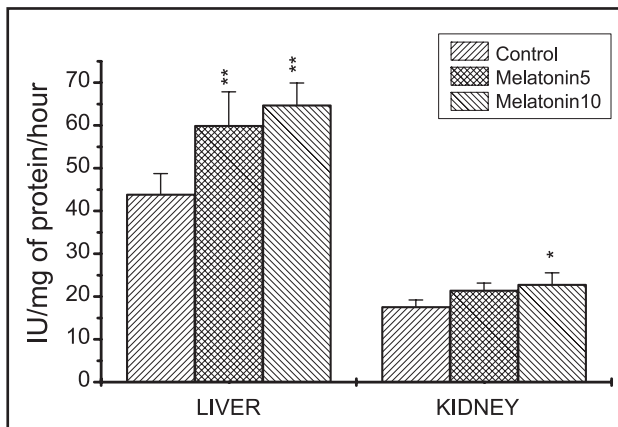


Fig. 5. Changes of glutathione peroxidase activity in the liver and kidney of male mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

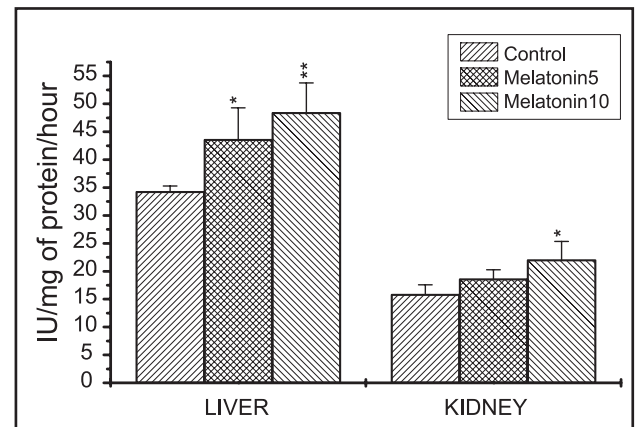


Fig. 6. Changes of glutathione peroxidase activity in the liver and kidney of female mice after melatonin administration for 5 and 10 days. The differences statistically confirmed; * $p<0.05$, ** $p<0.01$, *** $p<0.001$

appears to involve the action of melatonin at a nuclear binding site [26].

Functions of melatonin may include the synergistic actions of classic antioxidants [27], stimulating the synthesis of the important intracellular effective scavengers [28], at the mitochondrial level [29], as well as yet another undefined actions. Mayo et al [30] found that the depression in gene for GSPx and SOD that occurred after treatment of rats with the neurotoxin 6-hydroxydopamine was prevented by melatonin.

Our experiment showed no significant differences in reactivity between male and female mice despite that larger deviations in males appeared at the control level after melatonin administration. Our earlier investigations showed, that males are less resistant to stress factor. Large doses of melatonin will also be such factor.

We think that the revealed changes in the concentration glutathione and activity glutathione transferase and glutathione peroxidase in the liver and kidney of male and female mice may be one of the indicators of the specific biochemical stress reactivity caused by exogenous melatonin.

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