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Melatonin Enhances Hepatic Glutathione-peroxidase Activity in Sprague-Dawley Rats

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Effects of melatonin on hepatic glutathione-peroxidase (GSH-Px) and glutathione-reductase (GSH-reductase) activities were studied in Sprague-Dawley (SD) rats administered i.p. (10 mg/kg body weight) with melatonin during 15 days. The activity of cytosolic GSH-reductase in the liver was not changed by melatonin. However, melatonin injection increased significantly the activity of liver cytosolic GSH-Px activity compared with those in saline-treated rats. At the same time, plasma GSH-Px was also increased significantly in melatonin-treated rats. Since GSH-Px, a major antioxidative enzyme, removes H_2O_2 and lipid peroxides which are formed during lipid peroxidation from cellular membrane, such elevation of heptatic GSH-Px activity may contribute to the improvement of antioxidative effects under oxidative damage in the liver.

Key Words: Melatonin, Hepatic glutathione-peroxidase, Plasma

INTRODUCTION

Oxygen free radicals and lipid peroxides, which are produced by a free radical chain reaction, have been implicated in the pathogenesis of tissue injury in a variety of diseases including cancer, degenerative adult diseases, chronic inflammatory diseases and aging (Hasegawa et al, 1992). There exists ultimately a critical balance between free radical generation and antioxidant defenses. Free radical generation is reduced by the action of several detoxifying enzymes such as superoxide dismutase (SOD), GSH-Px, GSH-reductase, and catalase (Yu, 1994). In particular, the hydroxyl radical which abstracts a hydrogen atom, i.e., initiates lipid peroxidation, from cellular lipid membrane in a chain reaction.

The extremely reactive hydroxyl radical is reported to directly be scavenged by melatonin (Reiter et al, 1995; Poeggeler, et al, 1993). Melatonin, a neuro-endocrine hormone, is secreted by the pineal gland, is transported via the blood to all tissues where it enter cells and seemingly accumulates intracellularly, since it is highly lipophilic, easily crosses cell membranes. (Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al, 1993). It has been demonstrated that nuclear binding site for melatonin exists in the cell nuclei of rat liver and it may be a physiological melatonin receptor (Acuna-Castroviejo et al, 1994).

The main pools for GSH-Px was the liver (Behne and Wolters, 1983), and thus heptatic GSH-Px may largely contribute to antioxidative effects against oxidative damage in the biological systems. However, alteration of GSH-Px by melatonin has not yet clarified in the rat liver.

Therefore, in the present study, we designed the experiment to ascertain whether melatonin affects hepatic GSH-Px activity in rats administered i.p. with melatonin or not. Alterations of liver cytosolic GSH-reductase of reducing oxidized GSH (GSSG) to reduced GSH, and plasma GSH-Px activity were determined as well.

MATERIALS AND METHODS

Chemicals

All reagents were of the highest quality available.

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Melatonin, oxidized glutathione (GSSG), GSH-reductase (yeast), NADPH, and EDTA were purchased from the Sigma Chemical Co. (St. Lous, MO, USA).

Animal grouping and melatonin treatment

Male SD rats (4 weeks old, average b.w., 53~57 g) were purchased from Dae Han Experimental Animal Co. (Seoul, Korea). They were housed in plastic cages with *ad libitum* access to food and water. The light cycle was 12h:12h (light period, 08 : 00~20 : 00), and the room temperature was maintained at 21~23°C. Sixteen rats were alloted to 2 groups; 8 melatonin-treated group and 8 saline-treated (control) group. Melatonin was intraperitoneally injected (10 mg/kg b.w./10 ml/day) for 15 days. Melatonin was dissolved in absolute ethanol (the alcohol concentration in the final solution: 1%). Control rats received an equivalent volume of physiological saline. They were sacrificed 24 hr after last injection and the liver and plasma were collected for assays. Measurement of body weight gains was performed periodically through the course of experiment.

Enzyme assay

The liver tissue was homogenized in 0.25M sucrose. The supernatant fraction was prepared by centrifugation at 105,000 x g for 1 hour, and then the supernatant was stored at −80°C until use. The activities of GSH-Px and GSH-reductase assays were determined by measuring the disappearance of NADPH using spectrophotometer at 340 nm (Lawrence and Burk, 1976).

GSH-Px activity was measured by the method of Lawrence and Burk (1976). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 1 mM NaN_3, 0.2 mM NADPH, 1 E.U./ml GSSG-reductase, 1 mM GSH, and 0.25 mM H_2O_2 in a total volume of 1 ml. All ingredients except enzyme source and cumene hydroperoxide or hydrogen peroxide were combined at the beginning of each day. Enzyme source (0.1 ml) and cumene hydroperoxide or hydrogen peroxide solution (0.1 ml) were added to 0.8 ml of the mixture mentioned above.

The assay procedure used for the determination of GSH-reductase activity was based on the method of Worthington and Rosemeyer (1976). The assay medium (1 ml) contained enzyme source, 100 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 20 mM potassium chloride, 0.1 mM NADPH, and 1 mM GSSG. The reaction was started by the addition of GSSG. Protein was determined by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistical analysis

All data are presented as the mean±SD. Statistical analyses were performed by Students *t*-test.

RESULTS

As shown in Fig. 1, body weight gains in melatonin-treated rats were not changed compared with that in saline-treated rats (saline, 170.8±15.3 g; melatonin, 168.9±13 g, at final measurement).

Melatonin injection did not alter the activity of cytosolic GSH-reductase in the liver (Table 1). However, the activity of liver cytosolic GSH-Px was significantly increased in melatonin-treated rats compared with that in saline-treated rats (melatonin, 362.4±53.3 versus saline, 261.2±39.5 mU/mg protein) (Fig. 2). At the same time, in plasma, the activity of GSH-Px in melatonin-treated rats was also increased compared with that in saline-treated rats (melatonin, 37.0±6.7 versus saline, 28.6±6.4 mU/mg protein, p=0.023).

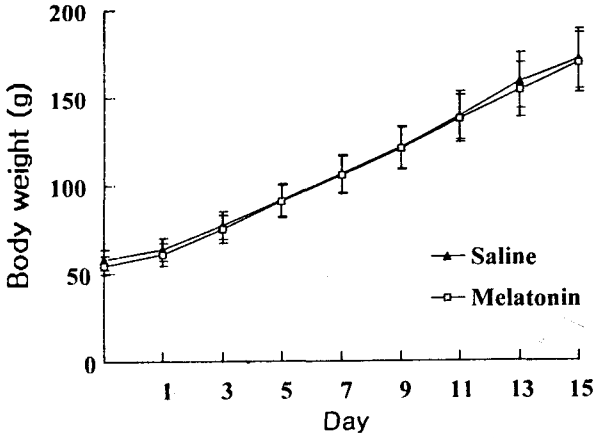

Fig. 1. The effect of melatonin on change of the body weight. Melatonin (10 mg/kg/10 ml) or an equivalent volume of saline were injected (i.p.) to SD rats for 15 days. Vertical line represents mean±S.D. (n=8).

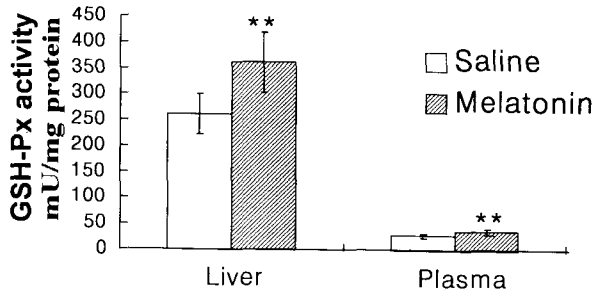

Fig. 2. The effect of melatonin on the activity of glutathione-peroxidase (GSH-Px) in the liver and plasma of SD rat. Each column represents mean±S.D. (n=8). **Significant difference at $p < 0.01$.

Table 1. The effect of melatonin on the activity of liver cytosolic GSH-reductase

	Saline (mU/mg protein)	Melatoin (mU/mg protein)
Liver	80.6±8.3	81.9±13.6

Values represent the means±S.D. (n=8).

DISCUSSIONS

Body weight in melatonin-treated rats was not changed compared with that in saline-treated rats. No clinical signs were observed in rats treated with melatonin.

As shown in Fig 2, liver cytosolic GSH-Px activity in melatonin-treated rats was increased by 38.4% of the control. A *in vivo* study using chicks reported that melatonin increased the activity of GSH-Px in the liver (Pablos et al, 1995).

Melatonin, highly lipophilic, is transported via the blood to all tissues where it enters cells and especially accumulates in the nuclear fraction (Menendez-Pelaez and Reiter, 1993; Menendez-Pelaez et al, 1993). It has been reported that nuclear binding site for melatonin exists in the cell nuclei of rat liver (Acuna-Castroviejo et al. 1994; Menendez-Pelaez and Reiter, 1993). Thus, it seems that the possible existence of a protein receptor for melatonin in the cell nucleus is similar to that for the steroid receptor family. Furthermore, it is suggested that melatonin may be involved with enzyme induction (Barlow-Walden et al, 1995), although GSH-Px gene expression by melatonin has not been investigated. On the basis of these findings described above, the present finding, an increase in hepatic GSH-Px activity, is possibly due to that melatonin may stimulate GSH-Px activity via a genomic action in the liver. The increase of liver cytosolic GSH-Px activity also indicated that protein content of GSH-Px was increased in liver cytosol, since the level of GSH-PX protein was paralleled with the GSH-Px activity (Takahashi et al, 1986).

In this study, plasma cytosolic GSH-Px activity in melatonin-treated rats was increased by 29.4% of the control. An *in vitro* study reported that plasma GSH-Px was synthesized by the hepatocyte in culture, suggesting that hepatic GSH-Px is secreted into the plasma (Sunde, 1980; Hill et al, 1987). Thus, the present finding can be explained from that more protein content of liver cytosolic GSH-Px increase was caused by secretion into plasma, resulting in an increase in plasma GSH-Px activity. It also suggests that the plasma GSH-Px activity is dependent upon the liver GSH-Px activity.

For the relationship between liver cytosolic and plasma GSH-Px, another study reported that selenium deficiency caused a decrease in liver cytosolic GSH-Px activity, followed by the fall in plasma GSH-Px activity, suggesting that plasma GSH-Px activity depends upon liver cytosolic GSH-Px activity. Thus, those results mentioned above may support our present findings, the increases in liver cytosolic GSH-Px activity concomitant with plasma GSH-Px activity.

It was observed that when hepatic GSH concentration was decreased by paraquat, melatonin injection recovered the decreased hepatic GSH level. (Melchiorri et al, 1995). Therefore, we tried to examine the possibility that melatonin elevates hepatic GSH level through stimulating the activity of liver cytosolic GSH-reductase of reducing oxidized GSH (GSSG) to reduced GSH. As shown in Table 1, melatonin injection did not alter the activity of cytosolic GSH-reductase in the liver. It suggest that melatonin doesn't directly participitate to regulate the level of GSH through the activity of GSH-reductase.

In a free radical chain reaction, GSH-Px, a major endogenous antioxidative enzyme, removes H_2O_2 which is converted partially to hydroxyl radical in a Fenton-type reaction catalyzed by transition metals as

well as lipid peroxides which are formed during lipid peroxidation from cellular membrane (Reiter, 1995; Reiter et al, 1995). An enhanced GSH-Px activity could slow the progress of neurodegenerative diseases, which are associated with lower levels of GSH-Px activity as well as enhanced oxidative stress due to the highly reactive and toxic hydroxyl radical (Beal, 1993; Hann et al, 1992). In the liver damaged with oxidative stress, the level of lipid peroxides, to be removed by GSH-Px, was reported to be significantly increased (Hasegawa et al, 1992; Ozdemirler et al, 1994).

Therefore, in the present study, such elevation of heptatic GSH-Px activity, may contribute to the improvement of antioxidative effects under oxidative damage in the liver. Further study is necessary to ascertain the level of GSH-Px protein in the liver of melatonin-treated rats.

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