PRACA ORYGINALNA

Effect of melatonin supplementation on antioxidant enzyme activities in patients with short- and long-term hypokinesis

Wpływ melatoniny na aktywność enzymów antyoksydacyjnych u pacjentów z hipokinezją krótko- i długoterminową

Małgorzata Mrowicka, Paweł Garncarek, Elżbieta Miller, Katarzyna Malinowska, Jerzy Mrowicki, Ireneusz Majsterek

ABSTRACT

INTRODUCTION

Hypokinesis may contribute to an increase in oxidative stress in muscle. Melatonin has been known as a radical scavenger with the ability to remove reactive oxygen species and also is supposed to stimulate antioxidant enzymes including catalase (CAT) and glutathione peroxidase (GPx). The aim of the work was to determine the effect of melatonin supplementation on CAT and GPx activity in the red blood cells of patients with short- and long-lasting hypokinesis.

MATERIAL AND METHODS

The study group consisted of 33 patients with immobilization, divided into groups depending on hypokinesis duration: short-term immobilization – patients were administered melatonin (5 mg daily) for 10 days and long-term hypokinesis – patients were administered the same dose of melatonin for 30 days. The control group consisted of 17 subjects with normal physical activity, which received the hormone supplement for 10 and 30 days.

RESULTS

It was found that melatonin supplementation of immobilized patients did not affect CAT activity in either of the analysed groups in comparison to the control group. GPx activity in the group with short-lasting hypokinesis was higher than in the patients after 30 days of melatonin supplementation ($p < 0.001$).

CONCLUSION

The results indicate that melatonin supplementation in subjects with normal physical activity increases CAT and GPx activity regardless of the period of administration of the hormone. In the study groups, only in the patients with short-term hypokinesis, 10-day melatonin supplementation may induce increased activity of GPx.

KEY WORDS

hypokinesis, melatonin, antioxidant enzymes

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STRESZCZENIE

WSTĘP

Hipokinezja może przyczynić się do wzrostu stresu oksydacyjnego w mięśniach. Melatonina, jako zmiatacz reaktywnych form tlenu ze zdolnością ich usuwania, być może wpływa na wzrost aktywności enzymów antyoksydacyjnych, w tym katalazy (CAT) i peroksydazy glutationowej (GPx). Celem pracy była ocena wpływu suplementacji melatoniną na aktywność CAT i GPx w krwinkach czerwonych pacjentów z hipokinezją krótko- i długoterminową.

MATERIAŁ I METODY

Badaniem objęto 33 pacjentów poddanych ograniczeniu ruchowemu, podzielonych na grupy w zależności od czasu trwania hipokinezji: krótkoterminowa – pacjenci otrzymali melatoninę w dawce 5 mg/dobę przez 10 dni; długoterminowa – pacjenci otrzymali melatoninę w tej samej dawce przez 30 dni. Grupę kontrolną stanowiło 17 osób z prawidłową aktywnością fizyczną, suplementowanych melatoniną przez 10 i 30 dni.

WYNIKI

Wykazano, że suplementacja melatoniną pacjentów z ograniczeniem ruchowym nie miała wpływu na aktywność CAT w obu badanych grupach w porównaniu z grupą kontrolną. Aktywność GPx w grupie z krótkotrwałą hipokinezją była wyższa niż u pacjentów po 30 dniach suplementacji melatoniną ($p < 0,001$).

WNIOSKI

Wyniki badań wskazują, że suplementacja melatoniną osób z prawidłową aktywnością fizyczną wpływa na wzrost aktywności CAT i GPx niezależnie od okresu podawania hormonu. W grupach badawczych tylko u pacjentów z hipokinezją krótkoterminową przyjmowanie melatoniny mogło wpłynąć na wzrost aktyności GPx.

SŁOWA KLUCZOWE

hipokinezja, melatonina, enzymy antyoksydacyjne

INTRODUCTION

The adverse effect of hypokinesis can cause the inhibition of anabolic processes in favour of enhancement of lipid, protein and carbohydrate catabolitic reactions, as well as inadequate metabolism of polyunsaturated fatty acids. These alterations can induce increased lipid peroxide synthesis, overproduction of reactive oxygen species (ROS) and acceleration of lipid peroxidation of the cell membrane. These highly reactive oxidants react with virtually all biological molecules, including DNA, RNA, cholesterol, lipids, carbohydrates, proteins and antioxidants. The resulting alterations induced by free radicals lead to molecular dysfunction and eventually, due to the domino effect, to cellular death as a consequence of either necrosis or apoptosis. The oxidative modification of proteins, lipids, nucleic acids, carbohydrates and some molecules by ROS is implicated in the etiology or progression of the panoply of disorders and diseases [1].

When the equilibrium between free radicals (oxidants) and the antioxidant defense system is imbalanced in favor of oxidants, the condition causes what is known as oxidative stress. In normal cells, permanently produced oxygen derivatives are neutralized or eliminated due to the presence of a natural defensive mechanism that involves enzymatic antioxidants: superoxide dismutase (CuZn-SOD), glutathione peroxidase (GPx), catalase (CAT) or fat soluble non-enzymatic antioxidants (glutathione, vitamins C and E, ceruloplasmin, uric acid and other). Antioxidative enzymes provide a major defense mechanism against free radical damage either by metabolizing them to less reactive species or to non-toxic byproducts.

Melatonin is a neurohormone of the pineal gland. Its secretion is rhythmic with high levels occurring at night. This compound of indole structure (N-acetyl-5--methoxytryptamine) is synthesized from serotonin. Melatonin secretion is related to the duration of darkness [2]. The amount of melatonin produced by the pineal gland of mammals changes with age. In humans, melatonin production not only diminishes with the age but is also significantly lower in many age-related diseases including Alzheimer's, Parkinson's disease [3,4] and cardiovascular diseases [5,6].

This hormone has a number of membrane-bound receptor-mediated actions including the regulation of circadian and seasonal rhythms, retinal function, reproduction, immune functions and tumour inhibition [7].
Since 1993, melatonin has been known as a radical scavenger with the ability to remove reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$), hydroperoxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and the lipid peroxide radical ($LOO^{\bullet}$) [8]. Melatonin has redox properties because of the presence of an electron-rich aromatic ring system, which allows the indoleamine to easily function as an electron donor [9]. The beneficial antioxidant effects of melatonin have been recently shown in clinical settings for several chronic diseases, including patients with rheumatoid arthritis, elderly patients with primary essential hypertension and females with infertility [10].
The aim of the study was to determine the effect of melatonin supplementation on the status of antioxidant enzymes activity: catalase (CAT) and glutathione peroxidase (GPx) activity in red blood cells of patients with short- and long-term hypokinesis as compared to the control group of subjects with normal physical activity.

MATERIAL AND METHODS

Study subjects

The study group consisted of 33 patients with immobilization, divided into groups depending on hypokinesis duration: 15 subjects classified to hip joint alloplasty (group of short-term hypokinesis) hospitalized at the Hospital of the Ministry of Internal Affairs and Administration in Lodz in the Traumatic and Orthopedic Department (average age: 71.5 SD 11.5). In most cases, the degenerative disease of the hip joint was of idiopathic, post-traumatic origin; 18 patients suffering from multiple sclerosis or brain stroke (group of long-term hypokinesis) rehabilitated in the Rehabilitation Department of Municipal Hospital No. 3 in Lodz (average age: 58 SD 30.0). The control group consisted of 17 subjects with normal physical activity.
The study groups were supplemented with melatonin for 10 days and for 30 days. Melatonin was applied at a dose of 5 mg per daily, one hour before sleep.
Approval of the Bioethics Committee of the Medical University of Lodz was obtained.

Sample collection

Peripheral blood was collected in test tubes with lithium heparin. The blood was centrifuged at 3000 rpm for 10 minutes. The erythrocyte mass was rinsed 3 times with a 0.9% NaCl solution (+4°C) maintaining the same conditions of centrifugation. After the supernatant was removed, the rinsed erythrocytes were hemolyzed by adding redistilled water ana partes equales and then frozen at the temperature of -18°C. The hemolysate, after being defrosted was used in subsequent studies.

Erythrocyte catalase activity

The catalase activity in the blood was assayed using Beers and Sizer's method [11] and calculated as Bergmeyer units/g Hb. The CAT activity was measured at 25°C by recording H_2O_2 decomposition at 240 nm. One Bergmeyer unit (BU) of this activity is defined as the amount of enzyme decomposing 1 g of H_2O_2 per minute.

Erythrocyte glutathione peroxidase activity

The glutathione peroxidase activity was measured by means of Sedlak and Lindsay's method [12] modified by Little and O'Brien [13] and presented as U/g Hb. To determine GPx activity, the difference in the rate of enzyme reaction with glutathione and kumen in the sample test and the control test was used, which manifests itself in the absorbance difference in the above mentioned solutions. For one unit of GPx activity, an amount of enzyme which causes a decrease of 10% of the amount of reduced glutathione as compared to the baseline within 1 minute at 25°C and pH 7.0 was used.

Hemoglobin assay

The hemoglobin (Hb) concentration in the erythrocyte hemolysate, necessary to determine enzyme activity was estimated at 540 nm after conversion into cyanmethemoglobin using the Drabkin reagent (Aqua-Med, Poland).

Statistical analysis

The data were expressed as a mean value ± standard division (SD) and the statistical differences between were evaluated by Student's t-test. A probability of $p = 0.05$ was set as the level of statistical significance. All the statistical calculations were performed using the STATISTICA 6.0 package (StatSoft, Tulsa, OK, USA).

RESULTS

Figures 1–4 present the results of CAT and GPx activities in the patients with short- and long-lasting hypokinesis in comparison to subjects with normal physical activity with or without melatonin supplementation.

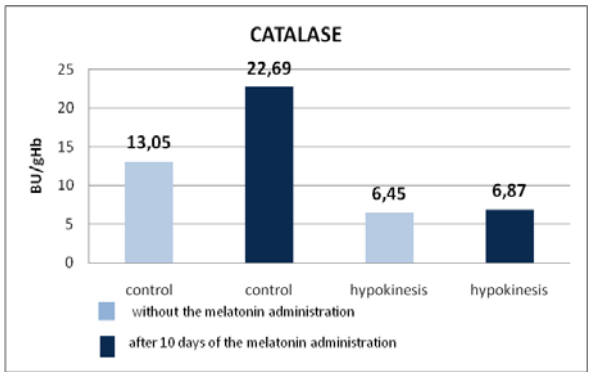

Fig. 1. Effect of melatonin supplementation on catalase (CAT) activity in patients with short-lasting hypokinesis in comparison to control group.
Ryc. 1. Wpływ melatoniny na aktywność katalazy (CAT) u pacjentów z krótkoterminową hipokinezją w porównaniu z kontrolą.

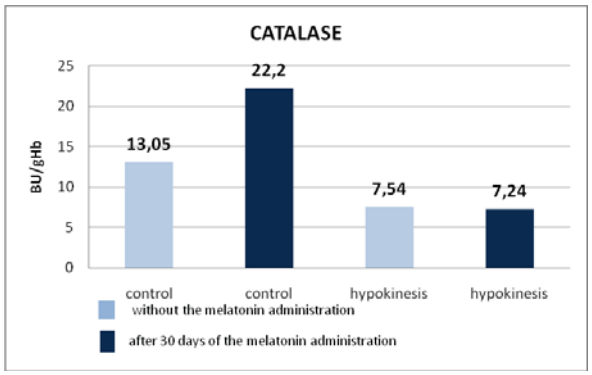

Fig. 2. Effect of melatonin supplementation on catalase (CAT) activity in patients with long-lasting hypokinesis in comparison to control group.
Ryc. 2. Wpływ melatoniny na aktywność katalazy (CAT) u pacjentów z długoterminową hipokinezją w porównaniu z kontrolą.

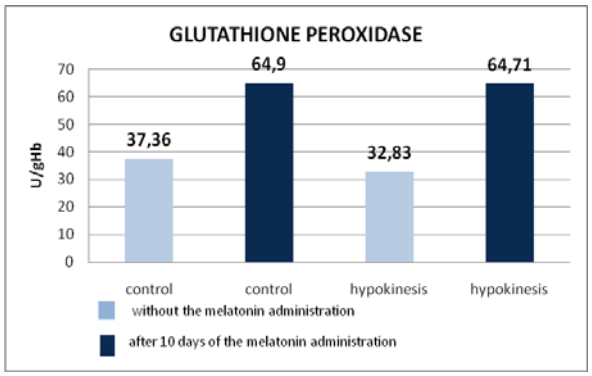

Fig. 3. Effect of melatonin supplementation on glutathione peroxidase (GPx) activity in patients with short-lasting hypokinesis in comparison to control group.
Ryc. 3. Wpływ melatoniny na aktywność peroksydazy glutationowej (GPx) u pacjentów z krótkoterminową hipokinezją w porównaniu z kontrolą.

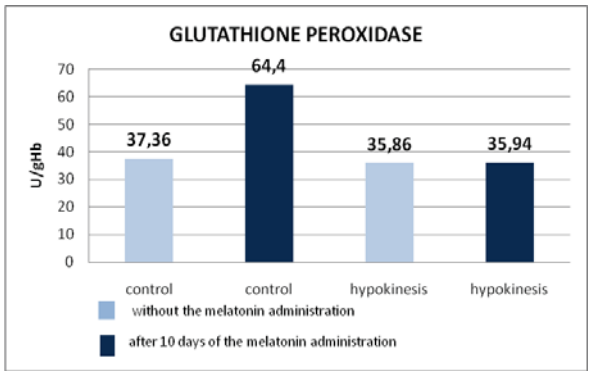

Fig. 4. Effect of melatonin supplementation on glutathione peroxidase (GPx) activity in patients with long-lasting hypokinesis in comparison to control group.
Ryc. 4. Wpływ melatoniny na aktywność peroksydazy glutationowej (GPx) u pacjentów z długoterminową hipokinezją w porównaniu z kontrolą.

The CAT and GPx activities of the control groups after 10 days and 30 days of melatonin supplementation were higher than without hormone administration. The CAT activity did not change in the immobilized patients supplemented with melatonin for 10 and 30 days. However, statistically significantly lower CAT activity was observed in patients of both the examined groups without and with the melatonin administration as compared to the control group (CAT for short--lasting hypokinesis without the hormone supplementation: 6.45 BU/g Hb SD 0.78 *vs* 13.05 BU/g Hb SD 3.7, $p < 0.001$; for short-term hypokinesis after 10 days of melatonin administration 6.87 BU/g Hb SD 0.99 *vs* 22.67 BU/g Hb SD 6.4, $p < 0.001$; for long--lasting hypokinesis without the hormone supplementation: 7.54 BU/g Hb SD 1.42 *vs* 13.05 BU/g Hb SD 3.7, $p < 0.001$; for long-term hypokinesis after 30 days of melatonin administration 7.24 BU/g Hb SD 1.69 *vs* 22.2 BU/g Hb SD 3.9, $p < 0.001$). Moreover a statistically significant difference in CAT activity was observed in the patients with long-lasting hypokinesis after 30 days in comparison to the control group without melatonin supplementation (7.24 BU/g Hb SD 1.69 *vs* 13.05 BU/g Hb SD 3.7, $p < 0.001$). The comparison of short- and long-term hypokinesis showed statistically significantly lower CAT activity in patients with a short period of immobilizationn without melatonin administration (6.45 BU/g Hb SD 0.78 *vs* 7.54 BU/g Hb SD 1.42, $p < 0.05$).

The GPx activity in the patients with hypokinesis after 10 days of melatonin supplementation was statistically significantly higher than in the group after 30 days (64.71 U/g Hb SD 7.3 *vs* 35.94 U/g Hb SD 5.14, $p < 0.001$). A statistically significant difference in GPx activity was determined in the patients with short-lasting hypokinesis without melatonin supplementation as compared to the control group (32.83 U/g Hb SD 6.23 *vs* 37.36 U/g Hb SD 5.33, $p < 0.05$).

Moreover statistically significantly higher GPx activity was observed in patients after 10 days of the hormone supplementation in comparison to the reference group without melatonin (64.71 U/g Hb SD 7.3 *vs* 37.36 U/g Hb SD 5.33, $p < 0.001$). In the group with long-term hypokinesis, a statistically significant difference in GPx activity was observed in the patients after 30 days of melatonin supplementation as compared to the control subjects (64.4 U/g Hb SD 10.5 *vs* 35.94 U/g Hb SD 5.14, $p < 0.001$).

DISCUSSION

A decrease in a patient's physical activity may result from a basic disease, surgical intervention, as well as frequently overlooked or underestimated immobilization of various durations. A period of decreased physical activity can cause a disturbance in the balance between the systemic processes of oxidation and reduction, which leads to a rise in reactive oxygen species (ROS) and oxidative stress generation. Hypokinesis may contribute to an increase in the lipid peroxidation process in muscle and an excess in thrombocyte aggregation and a significant decrease in antioxidant enzymes activity [14].

In the available literature [15,16,17], the authors who studied the effects of hypokinesis, mostly short-term, on the pro-antioxidant status, in most cases observed prooxidative effects. The status of antioxidant defense in erythrocytes that are particularly exposed to harmful ROS activity is very important for proper functioning of the organism. However, oxygen in the normal course of metabolism produces ROS, some of which are highly toxic and deleterious to cells and tissues. The largest number of of ROS generated during cellular metabolism are superoxide radicals (O_2^-). Dismutation of O_2^- produces hydrogen peroxide (H_2O_2). In addition, H_2O_2 is generated, *in vivo*, by several enzyme systems including xanthine, urate and D-amino acid oxidases [18].

Two major enzymes – peroxidase and catalase participate in the removal of H_2O_2 from the cellular environment. The most abundant peroxidase is glutathione peroxidase, which is present in both the mitochondrion and cytosol. Both the enzymes detoxify H_2O_2 by reducing it into water and oxygen. In most mammalian cells, CAT is found within the peroxisomes where it participates in removing H_2O_2 generated by the β-oxidation of long-chain fatty acids. CAT is reported to convert two molecules of H_2O_2 into one molecule of water and diatomic oxygen [19,20].

In our study, patients with hypokinesis who were supplemented with melatonin for 10 days showed a higher activity of GPx. The CAT activity in subjects with hypokinesis did not change in any study period in contrast to the control groups. Glutathione peroxidase has a higher affinity than catalase to hydrogen peroxide. This indicates that increased GPx activity during hypokinesis may influence the protection of membrane phospholipids of erythrocytes by inhibiting the peroxidation process. Our data also suggests that GPx is a more effective enzyme for protecting cells against oxidative damage than catalase.

Moreover, the cause of increased glutathione peroxidase activity in the blood of patients on the 10^{th} day following surgery and melatonin supplementation may be the excessive production of hydrogen peroxide. Overproduction of H_2O_2 may occur due to the increased activity of superoxide dismutase (CuZn-SOD) resulting from increased generation of the superoxide anion [21]. The above argument may confirm the results of catalase activity after 10 and 30-day supplementation with melatonin.

Melatonin is also supposed to stimulate antioxidant enzymes including SOD, CAT, GPx [6,22]. Thus, both the inflammation, and the period of reduced physical activity are factors that might affect the increase in oxidation processes and the corresponding increase in GPx activity. Moreover, melatonin, through a synergic effect on stimulating enzyme activity, might contribute to an increase in glutathione peroxidase activity.

It should be taken into account that patients with short-term hypokinesis consist of subjects after total alloplastic surgery due to osteoarthritis of the hip. The applied surgical treatment of the patients was a total interference and breach of continuity of body tissues which causes activation of the coagulation cascade as well as the first phase of immune system factors. The process of phagocytosis is coupled with the rapid consumption of oxygen by phagocytic cells (respiratory burst) in which the oxygen consumed is converted into hydrogen peroxide, superoxide anion, hypochlorous acid and hydroxyl radical.

According to literature data, it is also suggested that the impact of post-traumatic stress, patient's age as well as the inflammatory process should be considered as modifying factors in the group of patients with hypokinesis. Previous data showed that the degradation of articular cartilage might result from the inflammatory pathway [23,24]. It is known that melatonin exerts a strong anti-inflammatory effect and can regulate the maintenance of optimal immune response [25,26].

In conclusion, our results suggest that the limitation of physical activity leads to a decrease in catalase activity, but there is no effect on the activity of glutathione peroxidase in the erythrocytes of the patients. Melatonin supplementation increases the activity of both enzymes only in the subjects with normal physical activity.

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