· Advances in Medical Sciences · Vol. 53(2) · 2008 · pp 234-239 · DOI: 10.2478/v10039-008-0035-7

Effect of melatonin on the blood oxygen transport during hypothermia and rewarming in rats

Hlutkin S, Zinchuk V*

Department of Physiology, Grodno State Medical University, Belarus

* CORRESPONDING AUTHOR:
Department of Physiology,
Grodno State Medical University,
80 Gorky str.
230015 Grodno, Belarus.
fax: +375 152 435341
e-mail: zinchuk@grsmu.by (Victor Zinchuk)

Received 18.02.2008
Accepted 10.07.2008
Advances in Medical Sciences
Vol. 53(2) · 2008 · pp 234-239
DOI: 10.2478/v10039-008-0035-7

ABSTRACT

Purpose: We aimed to study effect of melatonin on the blood oxygen transport during hypothermia and rewarming in rats.
Material/methods: Cold exposure was performed on male rats (body weight 220-270 g, n = 48) for 120 minutes under the box water temperature of 19°C; rewarming took the next 120 min, with a mean rate of 0.06°C/min. Melatonin was administered intraperitoneally 30 min before the cold exposure (bolus doses of 0.1, 1 or 10 mg/kg, or 1 mg/kg*day for 4 days). Haemoglobin-oxygen affinity was evaluated by p50 (blood pO_2 at its 50% O_2 saturation) determined by the "mixing" method at 37°C, pH 7.4 and pCO_2 40 mm Hg ($p50_{stand}$) and at actual pH, pCO_2 and temperature ($p50_{act}$).
Results: After hypothermia and rewarming, the values of $p50_{stand}$ and $p50_{act}$ were 31.5±0.28 and 30.2±0.61 mm Hg, respectively. The 0.1 mg/kg of melatonin virtually did not change these values, whereas the larger doses increased them. This effect was maximal after the prolonged (4 days) melatonin administration: $p50_{stand}$ rose by 5.4% ($p<0.05$) and $p50_{act}$ - by 12.9 ($p<0.05$) compared with rats without the melatonin treatment. Melatonin affected the mechanisms of O_2 transport by decreasing the haemoglobin-oxygen affinity (shifting the oxygen dissociation curve of haemoglobin rightwards) and promoting the tissue oxygenation, thereby enhancing the body's resistance to cold.
Conclusions: The melatonin effect mediated by haemoglobin-oxygen affinity change may be used for the correction of metabolic disorders and the improvement of the body's resistance to low environmental temperature.

Key words: hypothermia, rewarming, melatonin, blood

INTRODUCTION

The study of processes and functional regulation in a body under the changed (including the lower) body temperature is the important medical problem [1]. An increase of the body's resistance to low environmental temperature is especially important for the patient reanimation after exposure to cold, and the assessment of mechanisms creating the tissue O_2 flux may help the development of ways for reanimation of a cooled body [2].

Haemoglobin-oxygen affinity (HOA) largely determines the capillary O_2 transfer being confirmed with mathematical model calculations for the various conditions [3]. In the hamster window chamber model tissue pO_2 at 1 h after an 80% isovolemic blood exchange with polymerised bovine haemoglobin (Hb) was 27±3 mm Hg, which was significantly higher than baseline (22±2 mm Hg, $p<0.50$) [4]. The rise of p50 by 1 mm Hg increased the arteriovenous pO_2 difference by 3.2 mm Hg, thereby enhancing the tissue oxygenation and cardiac output under the constant O_2 delivery decreased by 5.8% for the unit of p50 change [5]. Under the hypoxia, O_2 tissue delivery is finely regulated by HOA changes: even a small HOA shift can maximally increase the arteriovenous O_2 difference and optimise the O_2 tissue delivery, maintaining the relatively low hemodynamical load. The physiological Hb properties are determined by the protein structure and the effects of physicochemical factors (pH, temperature and concentrations of cofactors modulating the O_2 binding) [6]. The functional adaptation of Hb depends on the homotropic (cooperativity of O_2 binding by various hemes that determines the sygmoidal shape of O_2 equilibrium curve) and heterotropic interactions between the hemes and effector-binding sites.

The role of absolute temperature among the various factors determining the oxygen dissociation curve of Hb (ODC) position is unclear [5]. Our previous investigations had shown, in rats, the decrease of p50 for actual pH, pCO_2 and temperature from 33.23±0.70 to 20.63±0.47 mm Hg after the body temperature fell, in average, to 23.7±0.24^{0}C [7], and HOA decrease is favourable during the deep hypothermia due to the optimised O_2 flux to tissues and its lower fraction spent for the free radical reactions that is associated with the less marked distresses of prooxidant-antioxidant balance.

In accordance with its protective function, substantial amounts of melatonin are found in tissues and organs that have high oxygen consumption such as the brain [8]. Effects of melatonin on bovine embryonic development in vitro depended on the oxygen tension during the treatment being harmful under normoxia and protective under hyperoxia [9]. The melatonin effect on the O_2-transporting mechanisms, including the red blood cell (RBC) deformability, was shown under the lipopolysaccharide-induced oxidative stress [10,11]. The pineal gland also displayed the morpho-functional changes during the dynamic adaptation to hypothermia (+4 ^{0}C for 3 h; melatonin-forming function enhanced during the first 15 min and then dramatically fell) [12]. We aimed to study the effect of melatonin on the blood oxygen transport during hypothermia and rewarming in rats.

MATERIAL AND METHODS

Animals

Animals were anaesthetised by sodium thiopental (50 mg/kg intraperitoneally). The male rats (body weight 220-270 g, n = 48) were kept for 2 weeks in a constant-climate (temperature and humidity) environment with a light/dark cycle of 12/12 h. Animals were fed on a laboratory diet with water and food *ad libitum* until use and fasted overnight with free access to water before the operation. Operation procedures were performed between 8.00 hr and 12.00 hr to avoid the chronobiological variations. All the experimental procedures described in this paper are in accordance with the Guiding Principles for the Care and Use of Animals accepted by the Ethical Committee of Grodno Medical University.

Procedure

We used the combined method of artificial hypothermia and the following rewarming. During cooling and rewarming rats were placed into the special boxes without direct contact with water. Cold exposure lasted 120 min under the box water temperature of 19°C; rewarming was performed during the next 120 min with a mean rate of 0.06°C/min. Melatonin was injected intraperitoneally 30 min before the hypothermia in 1 ml of 1% ethanol. Depending on the treatment, rats were subdivided into 6 groups. Rats of the 1st group received 1.0 ml 1% ethanol (as a usual solvent for melatonin) intraperitoneally and served as a control. Rats of the 2nd group received 1.0 ml 1% ethanol and then were exposed to hypothermia and the following rewarming. Animals of the 3rd, 4th and 5th groups received the bolus injection of melatonin (0.1, 1 and 10 mg/kg, respectively) immediately before the hypothermia. Rats of 6th group were pre-treated with melatonin (1 mg/kg*day, 4 days), and then were subjected to hypothermia and rewarming. Rectal temperature was measured with 10-min intervals using the electric thermometer. Mixed venous blood was taken from right atrium at the end of rewarming period.

Blood oxygen transport

The pO_2 and acid-base balance (pH, venous carbon dioxide pressure (pCO_2), concentration of bicarbonate (HCO_3^-), the concentration of total carbon dioxide (TCO_2), the actual excess of buffer bases (ABE), the standard excess of buffer bases (SBE) and the standard bicarbonate (SBC)) were measured with a micro gas analyser "Synthesis-15" (Instrumentation Laboratory) at 37°C with the following correction to the actual temperature value. HOA was evaluated by p50 (blood pO_2 at its 50% O_2 saturation) determined by the "mixing" method at 37°C, pH 7.4 and pCO_2 40 mm Hg ($p50_{stand}$) [13]. The p50 at actual pH, pCO_2 and temperature ($p50_{act}$) was calculated from $p50_{stand}$ with Severinghaus' formula [14] using the temperature coefficient of 0.024. ODCs were calculated with Hill's equation using n=2.8. The amounts of Hb and metHb were determined spectrophotometrically.

Statistics

The results obtained were analysed statistically using Statistica. Data are presented as mean ± standard error of mean (SEM). Since the data were not normally distributed, Mann-Whitney U-tests for unrelated results were used to compare differences between the groups. A p value of <0.05 was accepted as statistically significant.

RESULTS

High doses of melatonin (1 and 10 mg/kg and 1 mg/kg*day for 4 days) resulted in the weaker fall of body temperature (*Tab. 1*). Rats treated by high doses of melatonin displayed the higher rectal temperatures at the end of rewarming than the animals treated by 1% ethanol + hypothermia/rewarming.

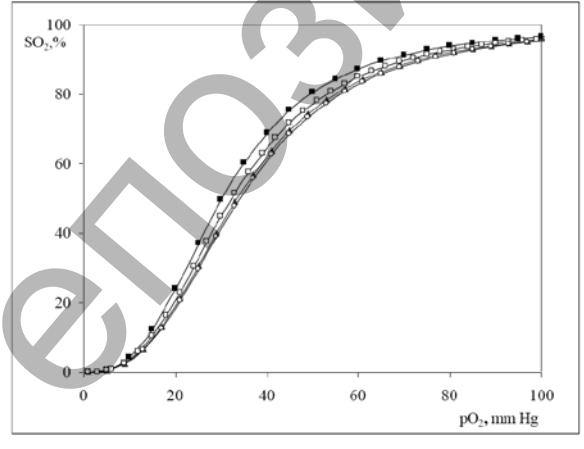
Tab. 1 shows the indices of blood oxygen transport. Cold exposure and the following rewarming decreased the values of pO_2 by 2.5±0.55 mm Hg ($p<0.05$), and SO_2 - by 1.5±0.27% ($p<0.05$) vs. control. Bolus melatonin injection in doses of 1 and 10 mg/kg resulted in a rise of pO_2 (by 3.3±0.42, $p<0.05$, and by 3.0±0.26 mm Hg, $p<0.05$, respectively) and SO_2 (by 5.2±1.09, $p<0.05$, and by 2.9±0.93%, $p<0.05$, respectively) compared with the hypothermia/rewarming group. Chronic melatonin administration (1 mg/kg*day, 4 days) increased pO_2 by 3.0±0.26 mm Hg ($p<0.05$) vs. the hypothermia/rewarming group. Prominent changes in acid-base balance values were absent.

Table 1. **Effect of melatonin on indices of blood oxygen transport and acid-base balance during hypothermia and rewarming in rats.**

Parameters	Control	Hypothermia /rewarming	Melatonin (0.1 mg/kg)+ hypothermia /rewarming	Melatonin (1 mg/ kg)+ hypothermia /rewarming	Melatonin (10 mg/ kg)+ hypothermia /rewarming	Melatonin (1 mg/kg*day, 4 days) + hypothermia /rewarming
n	9	8	7	8	8	8
$p50_{stand}$, mm Hg	30.1±0.55	31.5±0.28*	30.8±0.58	32.3±0.52 *	33.0±0.24 *#	33.2±0.30 *#
$p50_{act}$, mm Hg	29.0±0.36	30.2±0.61*	31.4±0.74 *	32.3±0.48 *#	33.5±0.35 *#	34.1±0.65 *#
pO_2, mm Hg	27.9±0.35	25.4±0.50*	26.6±0.37 *	28.6±0.42 #	28.4±0.26 #	28.4±0.26 #
pCO_2, mm Hg	51.6±1.01	53.8±1.15	52.7±0.78	50.3±0.48 #	52.4±0.61	50.9±0.83
Hb, g/dl	10.91±0.14	10.70±0.27	10.57±0.16	10.69±0.15	10.70±0.15	10.54±0.14*
SO_2, %	27.1±0.64	25.6±0.27	26.3±0.53	30.8±1.09 *#	28.0±0.73 #	28.6±1.10
pH, units	7.352±0.01	7.344±0.01	7.322±0.01	7.349±0.01	7.341±0.01	7.334±0.01
HCO_3^-, mM	30.3±1.21	29.2±0.67	28.4±0.54	29.4±0.99	29.9±1.02	29.5±0.88
TCO_2, mM	32.0±1.32	30.8±0.72	30.1±0.57	31.0±1.00	31.6±1.06	31.2±0.92
ABE, mM	3.21±0.70	3.18±0.57	1.93±0.52	3.54±1.03	3.90±0.79	2.69±0.24
SBE, mM	3.21±0.75	3.18±0.67	1.83±0.59	3.59±1.15	4.18±0.92	2.63±0.31
SBC, mM	26.4±0.75	26.0±0.45	25.1±0.39	26.2±0.79	26.7±0.58	26.4±0.59
Rectal temperature (hypothermia), ^{0}C		28.4±0.2	28.3±0.1	29.1±0.2#	29.2±0.2#	29.2±0.2#
Rectal temperature (rewarming), ^{0}C	35.5±0.2	35.3±0.3	35.9±0.1	36.2±0.1*#	36.3±0.2*#	36.3±0.2*#

Note: Data are expressed as mean ± SEM
* - significant difference from control group;
\# - significant difference from hypothermia/rewarming group.

Figure 1. **Oxyhemoglobin dissociation curves at actual pH, pCO_2 and temperature during the hypothermia and the following rewarming combined with various doses of melatonin: hypothermia/rewarming (■), melatonin (1 mg/kg) + hypothermia/ rewarming ( ), melatonin (10 mg/kg) + hypothermia/rewarming (▲), melatonin (1 mg/kg*day, 4 days) + hypothermia/rewarming (○).**



Melatonin in a dose of 0.1 mg/kg did not substantially affect these values, and higher doses increased them (*Tab. 1*). This effect was maximal after the chronic (4-day) melatonin administration: $p50_{stand}$ rose by 5.4% ($p<0.05$), and $p50_{act}$ – by 12.9% ($p<0.05$) vs. hypothermia/rewarming group. ODCs at actual pH, pCO_2 and temperature were shifted to the right by various melatonin doses (*Fig. 1*).

DISCUSSION

Melatonin shifted ODC to the right, thereby increasing the O_2 flux to tissues and reducing the effects of O_2 deficiency. Melatonin prevents the temperature from falling during hypothermia by its thermogenic effect on nocturnal animals. As a result, groups that received melatonin (bolus doses of 1 or 10 mg/kg, or 1 mg/kg*day for 4 days) had a statistically significant higher rectal temperature after hypothermia/rewarming. The lower pO_2 during the hypothermia/rewarming reflected the worse tissue O_2 delivery and the development of hypoxia.

HOA decreases with temperature rise because of the exothermic nature of oxygenation. This effect may be adaptive, if it enhances O_2 unloading in parallel to increasing metabolic

rate, or maladaptive, if it inhibits O_2 loading during the hypoxia. Endothermic processes counteracting the exothermia of this reaction include O_2-associated release of protons (Bohr effect) and anions, just as the oxygenation-induced conformational changes. Intraspecies adaptation of Hb function to exogenous factors, such as hypoxia and temperature, is usually mediated by the changes of intraerythrocytic effector concentration [15].

The ODC position during hypothermia of the entire body is largely determined by the influences of temperature, pH and pCO_2. The ODC position under real values of temperature, pH and pCO_2 is a compromise between these differently acting determinants. The evaluation of acid-base state should take in account the intrinsic temperature effect (in our present investigations pH and pCO2 changes under standard conditions (370C) correspond to acidosis and hypercapnia, but their values at real temperatures were different) [7]. The temperature is the most significant determinant. Hb oxygenation is an exothermic reaction; hence, oxyhemoglobin dissociation consumes the heat [16]. This pattern of temperature influence on Hb-oxygen interaction is typical for the majority of Hbs [17]. Willford and Hill [18] considered the dependence between p50 and temperature as the mechanism for the prevention of misbalance between O_2 demands and delivery. The temperature influences may be compensated, either due to the higher synthesis of thermotolerant Hbs or because of changes in HOA modulator synthesis [19]. The last mechanism appears to be more realistic for the homeothermic animals. Rats are known to have the higher ATP and 2.3-diphosphoglycerate concentrations during the hypothermia [20], resulting in a weaker temperature influence on the ODC position (weaker shift leftwards). In our experiments, melatonin administration was accompanied by the increase of $p50_{act}$, revealing the development of compensatory processes to the effects of low temperature.

Melatonin is synthesised mainly in the pineal gland, and it regulates mammalian circadian and seasonal rhythms [21]. It has a broad spectrum of physiological functions. For example, it is an effective free radical scavenger and thereby defends the tissues against an oxidative injury [22]. This suggestion about the melatonin mechanism of action is confirmed by the cytoprotective effects of this molecule during both acute and chronic hypoxic cellular stress [23].

Melatonin effect on the mechanisms of blood oxygen transport is largely unclear. Chronic administration was shown to result in larger amounts of Hb and RBCs [24]. In experiments in vitro addition of melatonin to human or animal RBCs prevented the hemolysis induced by various cytotoxic agents (paraquat, malonic dialdehyde, hydrogen peroxide, heavy metal salts etc.), with almost doubling of 100% hemolysis time, increasing of RBC osmoresistance simultaneously with rise of intracellular potassium, slowing of membranous protein modification, Hb denaturation and hemin release [25,26]. Melatonin was shown effectively to reduce perferryl-Hb to methemoglobin [27]. During the oxidative stress, melatonin actively enters in RBCs and is used for cellular defence by means of the slower Hb denaturation and of the inhibited hemin release [28]. It affects the erythrocyte deformability [11]. During oxidative stress, it can defend RBCs against the stiffening factors [29]. After the preincubation with RBCs melatonin was shown to decrease the level of thiobarbituric acid reactive substances and enhance the erythrocyte membrane fluidity (reduce the membrane rigidity) [30]. This substrate reduced the lipid peroxidation activity and improved the RBC deformability in rats during the lipopolysaccharide-induced sepsis [10,31]. However, the intraperitoneal melatonin administration for 5 days weakened the oxidative injury induced by diabetes mellitus but did not affect the RBC deformability or aggregation [32]. In the present study, we observed the increase of $p50_{act}$ with melatonin dose including the most prominent change after its chronic administration. Worth noting is the similar change in p50 at standard conditions – HOA is obviously changed due to other modulatory factors than pH, pCO_2 and temperature.

Melatonin has the well-known antioxidant activity in specific conditions of reactive oxygen species-dependent cellular damage [23]. Melatonin functions via multiple receptors, both membrane and nuclear, and it scavenges free radicals by processes that do not require the receptor/binding site [33]. Antioxidant effects were described, not only for melatonin itself, but also for its metabolites (N^1-acetyl-N^2-formyl-5-methoxykynuramine, cyclic 3-hydroxymelatonin and others) [8]. In addition, HOA may be considered as one of the factors participating in the maintenance of body prooxidant-antioxidant balance. During the oxidative stress, the HOA changes mediated by nitric oxide (NO)-dependent mechanisms (in first turn, endothelial) can affect the O_2 flux to tissues and body prooxidant-antioxidant balance as a whole [34]. Effects of melatonin are mediated by its specific receptors (MT 1, MT 2) [35,36]. Melatonin efficiently interacts with various reactive O_2 and reactive nitrogen species (receptor-independent actions). It also regulates upward the antioxidant enzymes and regulates downward the pro-oxidant enzymes (receptor-dependent actions) [37]. It may change the NO synthesis activity [38]. It inhibits and inducible NO syntheses, thereby decreasing NO production [39,40]. The positive effects of NO and melatonin may have the similar mechanisms.

These results showed that melatonin administration affected the O_2 transporting mechanisms, weakening the HOA (shifting ODC rightwards) and promoting the tissue oxygenation, thereby enhancing the body's resistance to cold. These effects might be due to its direct action (through its specific receptors) and might be mediated by changes in blood oxygen transport and prooxidant-antioxidant balance. Effects of melatonin mediated by HOA changes may be used for correction of the metabolic disorders and enhancement of the body's resistance to low environmental temperatures.

ACKNOWLEDGMENT

This work was supported by a grant from the Fund of Fundamental Investigations of the Republic of Belarus (№ Б07-023).

REFERENCES

1. Maeng M, Kristiansen SB, Mortensen UM, Andersen HR. Myocardial cooling for reperfusion injury protection achieved by organ specific hypothermic autologous perfusion. Scand Cardiovasc J. 2003 Sep;37(5):297-303.

2. Soeda M. Antegrade selective cerebral perfusion combined with deep hypothermic circulatory arrest on cerebral circulation: comparison between pulsatile and nonpulsatile blood flows. Ann Thorac Cardiovasc Surg. 2007 Apr;13(2):93-101.

3. Brighenti C, Gnudi G, Avanzolini G. A simulation model of the oxygen alveolo-capillary exchange in normal and pathological conditions. Physiol Meas. 2003 May;24(2):261-75.

4. Cabrales P, Tsai AG, Intaglietta M. Increased tissue PO2 and decreased O2 delivery and consumption after 80% exchange transfusion with polymerized hemoglobin. Am J Physiol Heart Circ Physiol. 2004 Dec;287(6):H2825-33.

5. Samaja M, Crespi T, Guazzi M, Vandegriff KD. Oxygen transport in blood at high altitude: role of the hemoglobin-oxygen affinity and impact of the phenomena related to hemoglobin allosterism and red cell function. Eur J Appl Physiol. 2003 Oct;90(3-4):351-9.

6. Kavanagh BD, Khandelwal SR, Schmidt-Ullrich RK, Roberts JD, Shaw EG, Pearlman AD, Venitz J, Dusenbery KE, Abraham DJ, Gerber MJ. A phase I study of RSR13, a radiation-enhancing hemoglobin modifier: tolerance of repeated intravenous doses and correlation of pharmacokinetics with pharmacodynamics. Int J Radiat Oncol Biol Phys. 2001 Mar 15;49(4):1133-9.

7. Zinchuk VV, Dorokhina LV, Maltsev AN. Prooxidant-antioxidant balance in rats under hypothermia combined with modified hemoglobin-oxygen affinity. J Therm Biol. 2002; 27: 345-52.

8. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res. 2007 Jan;42(1):28-42.

9. Papis K, Poleszczuk O, Wenta-Muchalska E, Modlinski JA. Melatonin effect on bovine embryo development in vitro in relation to oxygen concentration. J Pineal Res. 2007 Nov;43(4):321-6.

10. Yerer MB, Yapislar H, Aydogan S, Yalcin O, Baskurt O Lipid peroxidation and deformability of red blood cells in experimental sepsis in rats: The protective effects of melatonin. Clin Hemorheol Microcirc. 2004;30(2):77-82.

11. Berker M, Dikmenoglu N, Bozkurt G, Ergönül Z, Ozgen T. Hemorheology, melatonin and pinealectomy. What's the relationship? An experimental study. Clin Hemorheol Microcirc. 2004;30(1):47-52.

12. Bondarenko LA, Gubina-Vakulik GI. Morphofunctional changes in the pineal gland during dynamic adaptation to hypothermia. Neurosci Behav Physiol. 2003 May;33(4):405-9.

13. Scheid P, Meyer M. Mixing technique for study of oxygen-hemoglobin equilibrium: a critical evaluation. J Appl Physiol. 1978 Nov;45(5):818-22.

14. Severinghaus JW. Blood gas calculator. J Appl Physiol. 1966 May;21(3):1108-16.

15. Weber RE, Fago A. Functional adaptation and its molecular basis in vertebrate hemoglobins, neuroglobins and cytoglobins. Respir Physiol Neurobiol. 2004 Dec 15;144(2-3):141-59.

16. Hsia CW. Mechanisms of disease: respiratory function of hemoglobin. New Engl J Med. 1998; 338: 239-47.

17. Samaja M. Blood gas transport at high altitude. Respiration. 1997;64(6):422-8.

18. Willford DC, Hill EP. Modest effect of temperature on the porcine oxygen dissociation curve. Respir Physiol. 1986 May;64(2):113-23.

19. Wood SC. Adaptation of red blood cell function to hypoxia and temperature in ectothermic vertebrates. Am Zool. 1980; 20: 163-72.

20. Mairbäurl H, Humpeler E. Diminution of the temperature effects on the oxygen affinity of hemoglobin after prolonged hypothermia. Pflugers Arch. 1980 Feb;383(3):209-13.

21. Reiter RJ. The melatonin rhythm: both a clock and a calendar. Experientia. 1993 Aug 15;49(8):654-64.

22. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. J Pineal Res. 2003 Jan;34(1):1-10.

23. Giacomo CG, Antonio M. Melatonin in cardiac ischemia/reperfusion-induced mitochondrial adaptive changes. Cardiovasc Hematol Disord Drug Targets. 2007 Sep;7(3):163-9.

24. Arushanian EB, Beĭer EV, Mastiagina OA, Mastiagin SS, Sidorenko ZhI. [Melatonin effect on the hematological indices of healthy humans] Eksp Klin Farmakol. 2006 Sep-Oct;69(5):36-8.

25. Allegra M, Gentile C, Tesoriere L, Livrea MA. Protective effect of melatonin against cytotoxic actions of malondialdehyde: an in vitro study on human erythrocytes. J Pineal Res. 2002 Apr;32(3):187-93.

26. Wróbel A, Łukaszyńska B, Kedzierska J. The effect of peroxynitrite and some antioxidants on the rate of osmotic hemolysis of bovine erythrocytes. Cell Mol Biol Lett. 2003;8(2):455-60.

27. Tesoriere L, Allegra M, D'Arpa D, Butera D, Livrea MA. Reaction of melatonin with hemoglobin-derived oxoferryl radicals and inhibition of the hydroperoxide-induced hemoglobin denaturation in red blood cells. J Pineal Res. 2001

Sep;31(2):114-9.

28. Tesoriere L, D'Arpa D, Conti S, Giaccone V, Pintaudi AM, Livrea MA. Melatonin protects human red blood cells from oxidative hemolysis: new insights into the radical-scavenging activity. J Pineal Res. 1999 Sep;27(2):95-105.

29. Aydogan S, Yerer MB, Yapislar H. In vitro effects of melatonin on the filtrability of erythrocytes in SNP-induced oxidative stress. Clin Hemorheol Microcirc. 2004;30(3-4):317-22.

30. Ochoa JJ, Vílchez MJ, Palacios MA, García JJ, Reiter RJ, Muñoz-Hoyos A. Melatonin protects against lipid peroxidation and membrane rigidity in erythrocytes from patients undergoing cardiopulmonary bypass surgery. J Pineal Res. 2003 Sep;35(2):104-8.

31. Yerer MB, Aydogan S, Yapislar H, Yalcin O, Kuru O, Baskurt OK. Melatonin increases glutathione peroxidase activity and deformability of erythrocytes in septic rats. J Pineal Res. 2003 Sep;35(2):138-9.

32. Gumustekin M, Tekmen I, Guneli E, Tugyan K, Topcu A, Ergonen AT, Ozdemir MH, Uysal N, Bediz CS. Short-term melatonin treatment improved diabetic nephropathy but did not affect hemorheological changes in diabetic rats. Pharmazie. 2007 Sep;62(9):693-8.

33. Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnocki Z. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. Acta Biochim Pol. 2007;54(1):1-9.

34. Glebov A, Zinchuk V. Blood oxygen-carrying function during the oxidative stress induced by lipopolysaccharide with a modification of the L-arginine-NO pathway. Rocz Akad Med Bialymst. 2005;50:247-51.

35. Dubocovich ML. Melatonin receptors: role on sleep and circadian rhythm regulation. Sleep Med. 2007 Dec;8 Suppl 3:34-42.

36. Fischer TW, Slominski A, Tobin DJ, Paus R. Melatonin and the hair follicle. J Pineal Res. 2008 Jan;44(1):1-15.

37. Tengattini S, Reiter RJ, Tan DX, Terron MP, Rodella LF, Rezzani R. Cardiovascular diseases: protective effects of melatonin. J Pineal Res. 2008 Jan;44(1):16-25.

38. Acuña-Castroviejo D, Escames G, López LC, Hitos AB, León J. Melatonin and nitric oxide: two required antagonists for mitochondrial homeostasis. Endocrine. 2005 Jul;27(2):159-68.

39. Escames G, León J, Macías M, Khaldy H, Acuña-Castroviejo D. Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. FASEB J. 2003 May;17(8):932-4.

40. Mei Q, Xu JM, Xiang L, Hu YM, Hu XP, Xu ZW. Change of nitric oxide in experimental colitis and its inhibition by melatonin in vivo and in vitro. Postgrad Med J. 2005 Oct;81(960):667-72.