Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 540 (2006) 107–114
www.elsevier.com/locate/ejphar

Role of melatonin in reducing hypoxia-induced oxidative stress and morphological changes in the liver of male mice

Gamal H. El-Sokkary *, Bothaina M. Khidr, Hala A. Younes

Department of Zoology, Faculty of Science, Assiut University, Assiut, 71516, Egypt

Received 30 January 2006; received in revised form 8 April 2006; accepted 18 April 2006
Available online 30 April 2006

Abstract

Oxygen deficiency during critical illness may cause profound changes in cellular metabolism and subsequent tissue and organ dysfunction. Thus, the present study was designed to determine the effects of hypoxia and reoxygenation on the levels of lipid peroxidation and the morphological changes in the liver of male mice as well as the protective role of melatonin as an antioxidant. Two experiments were carried out in this study. Experiment I includes three groups of mice (control, hypoxic, and hypoxic+melatonin) while the experiment II includes two groups (reoxygenated and reoxygenated+melatonin). The levels of oxidized lipids were measured and the morphological changes were investigated using light and electron microscopy. In experiment I, hypoxia strongly stimulated lipid peroxidation levels (88%) while melatonin administration inhibited this increase (69%). Severe morphological changes (necrosis, dilated congested blood vessels, collection of inflammatory cells, condensed heterochromatic with irregular outlines nuclei, and mitochondrial degeneration) were detected in the liver of hypoxic mice. In experiment II, reoxygenation inhibited the levels of oxidized lipids (42%) versus hypoxic mice and some morphological changes were detected. When melatonin was given before reoxygenation, it inhibited the levels of lipid peroxidation by 66% versus hypoxic mice. Also, melatonin enhanced the recovery profile by 41% when compared with mice that reoxygenated with room air only. All morphological alterations that detected in both hypoxic and reoxygenated mice were repaired when melatonin administered. These results indicate that hypoxia and reoxygenation induce severe alterations in the liver and that melatonin exerts beneficial role in restoring tissue alterations after subjection to hypoxia.

Keywords: Hypoxic hypoxia; Melatonin; Oxidative stress; Lipid peroxidation; Liver

1. Introduction

Hypoxia is caused by a failure of oxygen supply to reach the blood, resulting in low blood oxygen tension in the arterial blood. The tension in the venous as well as capillary blood is also reduced below the normal value. The rate of diffusion of oxygen from the blood to the tissues is, thus, reduced causing the symptoms of oxygen lack.

Prolonged living in a low oxygen atmosphere such as high altitude induces a variety of adaptive responses in different animals. Hypoxia is well known to produce oxidative stress in organisms leading to tissue injury (Sarada et al., 2002). Various oxyradicals if not neutralized, cause damage to bio-membranes, which are reflected by lipid peroxidation. Supplementation of antioxidants was found to inhibit the cellular damage induced by oxidative stress (Burton and Traber, 1990; Simon-Schnass, 1992; Palozza et al., 1997). The hypoxic cells are particularly susceptible to oxidative stress, a phenomenon commonly known as "Oxygen Paradox" (Goldfarb and Sen, 1994) leading to free radical generation. Free radicals are known to be directly involved in pathogenesis of various diseases and aging (Jackson et al., 1998). The hypoxia stress leads to lipid peroxidation resulting in membrane damage (Simon-Schnass, 1992).

The liver is more vulnerable than other organs to oxidative stress under hypoxia (Nakanishi et al., 1995). It is an important site for glutathione synthesis (Leeuwenburgh and Ji, 1995). Many studies claimed that the levels of glutathione and glutathione reductase activity in the liver decreased due to hypoxia (Shan et al., 1992; Ohkuwa et al., 2004). The mechanism responsible for hypoxic liver injury has been the focus of much research with the broader goals of preventing such injury and improving treatment

* Corresponding author. Tel.: +20 88 2401724; fax: +20 88 2342708.
E-mail address: Elsokkary2000@yahoo.com (G.H. El-Sokkary).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.04.036

should it occur (Marotto et al., 1988; Suematsu et al., 1992a,b). The previous authors claimed that in the low-flow hypoxic liver, an intralobular oxygen gradient is present that gives rise to hydrogen peroxide production, which in turn induces cell death. Motoyama et al. (2003) described a process whereby hypoxia-induced, xanthine oxidase-dependent hydrogen peroxide production in hepatocytes caused ischemic liver injury by selectively inducing apoptosis in the sinusoidal endothelial cells.

During ischemia/reperfusion, damage induced by hypoxia seems to be related to subsequent reoxygenation, which induces hydroxyl radical (OH) responsible for the initiation of an apoptotic program opening permeability transition pore (Crompton, 1999). Nitric oxide (NO) generated during reperfusion due to nitric oxide synthase (NOS) activation, and the peroxynitrite ($ONOO^-$) produced when NO couples with superoxide anion (O_2^-) seems to be the primary cause of damage to cytochrome-I (C-I) and C-II during reperfusion.

In recent years the function of melatonin as an antioxidant and free radical scavenger has been extensively studied (El-Sokkary, 2000, 2002; Reiter et al., 2000; El-Sokkary et al., 2003, 2005; Abd-Elghaffar et al., 2005). Melatonin's mechanisms of action seem to fall into three categories: receptor-mediated, protein-mediated, and non-receptor-mediated effects. Receptor-mediated melatonin events involve both membrane and nuclear receptors. The membrane melatonin receptors have been identified and are well characterized in humans (Conway et al., 2001).

A series of experiments have provided strong evidence for the anti-excitotoxic properties of melatonin both in vivo and in vitro (Acuña-Castroviejo et al., 1995). Both experimental and clinical anticonvulsant activity of melatonin were reported (Lapin et al., 1998). In a model of ischemia/reperfusion (hypoxia/reoxygenation) in gerbil, nitric oxide synthase/nitric oxide (NOS/NO) system was inhibited after melatonin administration, resulting in a reduction of neuronal damage associated with reperfusion Guerrero et al., 1997). Recently, Li et al. (2005) reported for the first time that melatonin directly interacts with malondialdehyde to form a new product. The findings suggest that melatonin may detoxify unsaturated carbonyls and protect against cellular damage induced by oxidative stress.

The importance of melatonin as an antioxidant depends on several characteristics: its lipophilic and hydrophilic nature, its ability to pass all bio-barriers with ease, and its availability to all tissues and cells. It distributes in all cell compartments, being especially high in the nucleus and mitochondria (Menendez-Pelaez et al., 1993; Martin et al., 2000). The aim of this work is to study the effect of oxygen shortage and reoxygenation on the liver and to test the melatonin's ability to modify these actions.

2. Materials and methods

2.1. Animals

A total number of 32 adult male Swiss albino mice weighing about 30±2 g were used in the present work. The mice were purchased from Assiut University Joint Animal Breeding Unit. It was noticed that when two mice were placed in a well closely fitted 2.35-l desiccator, signs of hypoxia appeared after about 3 h. The appropriate animal care of Assiut University approved the protocol for the experiment.

2.2. Chemicals

Melatonin, 2-thiobarbituric acid, 1,1,3,3-tetra-methoxypropane, dimethyl sulfoxide and sodium dodecyl sulfate were purchased from Sigma Co. (St. Louis, MO). Melatonin was dissolved in ethanol before being diluted with saline. The final concentration of ethanol in the melatonin solution was <1%. All other chemicals were of highest quality available.

2.3. Experimental protocol

2.3.1. Experiment I

In this experiment, 20 mice were randomly divided into three groups: The first group (7 mice), designated Hypoxia (Hypox), was subjected to oxygen shortage (hypoxic hypoxia) as described before (3 h). The second group (7 mice), designated Hypoxia+Melatonin (Hypox+M), was injected subcutaneously with melatonin at a dose of 10 mg/kg body weight (Reiter, 1998). The administration of melatonin was 30 min before subjection the animals to hypoxia. The third group (6 mice) served as controls and left in the normal conditions. The administration of melatonin into the non-treated maintained animals did not change basal levels of LPO and morphological changes in different studied organs (El-Sokkary, 2000; Reiter et al., 2000) and we did not repeat this in the present work.

2.3.2. Experiment II

In this experiment, the animals (12 mice) were subjected to hypoxia as those of experiment I. After 3 h, the hypoxic mice were divided into two subgroups: The first subgroup (6 mice), was subjected to normal oxygenation again for another 3 h (the same time of hypoxia) to study the recovery after the oxygen shortage (hypoxia) and designated Reoxygenated (Reoxgn). The second subgroup (6 mice), designated Reoxygenation+Melatonin (Reoxgn+Mel), was given subcutaneous injection of melatonin (10 mg/kg body weight) and subjected to normal oxygenation again to study the role of melatonin in the recovery after the oxygen shortage (hypoxia). At the end of the experiments, the animals were sacrificed by cervical dislocation.

The two experiments were done concomitantly with each other.

2.4. Tissue preparations

The specimens from the liver were rapidly excised, and cut conveniently into small pieces, which were used for histology and histopathology as well as ultrastractural investigation. Other portions of the liver were frozen and stored at −20 °C for lipid peroxidation measurement.

2.5. Measurement of lipid peroxidation

The method was based on that of Ohkawa et al. (1979). A 10% (w/v) tissue homogenate from the liver in phosphate buffer was required for this assay (this homogenate contained 1% v/v

dimethyl sulfoxide to prevent further oxidation). An aliquot (0.2 ml) of tissue homogenate was mixed with 0.2 ml 8.1% w/v sodium dodecyl sulfate solution, 1.5 ml 20% v/v acetic acid solution (pH 3.5) and 1.5 ml 0.8% w/v thiobarbituric acid solution. The mixture was made up to 4.0 ml with distilled water and heated to 95 °C for 1 h. The samples were cooled and centrifuged at 2000 ×*g* for 10 min and absorbance measured at 532 nm. Results of malondialdehyde were expressed as nmol/g tissue.

2.6. Histological and histopathological examinations

For the histological and histopathological examination, pieces of the liver were fixed in 10% neutral buffered formalin (pH 7.2), dehydrated in ascending series of ethyl alcohol (70%–100%), cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 5 μm in thickness were prepared.

2.7. Electron microscopy

For electron microscopy, portions of the liver were fixed in 2.5% glutaraldehyde in cacodylate buffer. The specimens were washed in cacodylate buffer (0.1 M, pH 7.2) for 1–3 h and then post fixed in 1% osmium tetraoxide for 2 h. The specimens were placed in propylene oxide for 60 min, then in pure epon 812 and incubated in a special polymerization incubator (one day at 37 °C, second day at 45 °C and then three days at 60 °C). Semithin sections were obtained and stained with toluidine blue and examined using a light microscope. Representative fields of semithin sections were selected. Ultrathin sections were mounted in copper grids and stained with uranyle acetate, lead citrate and investigated with transmission electron microscope (Bancroft and Stevens, 1982).

2.8. Statistical analysis

Results of the lipid peroxidation were expressed as means ± S.E.M. Differences between means were tested using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls *t*-test. The percent of stimulation (*S*%) or inhibition (*I*%) in the mean values of LPO was calculated.

3. Results

3.1. Experiment I

The animals submitted to hypoxia enhanced the levels of lipoperoxids in liver tissue compared with the values obtained in control mice (Table 1). The hypoxia-induced stimulation in LPO was 88% which statistically was significant ($P<0.01$). When melatonin was given 30 min before subjection the animals to hypoxia, it inhibited (69%) the increase of LPO levels, which was statistically significant ($P<0.01$).

Examination of liver sections after exposure to hypoxia for 3 h showed different features of morphological changes such as dilatation of central veins, which were congested with blood cells (Fig. 1C) versus those of controls (Fig. 1A). Moreover, a considerable number of hepatocytes showed clear cytoplasmic vacuolation with deeply stained nuclei. Another recorded pathological change was the remarkable collection of inflammatory cells (Fig. 1D).

The most prominent features of the ultrastructure of the hepatocyte are highly vacuolated cytoplasm, indicating damaged mitochondria and other cell organelles. Also, the cytoplasm contains tremendous amounts of lipid droplets when compared with control animals (Fig. 1B). The nucleus is irregular, eccentric with moderately dilated nuclear envelope and condensed heterochromatin (Fig. 1E).

Melatonin administration to mice (10 mg/kg) 30 min before exposure to hypoxia showed that the hepatic lobules retained their normal structure compared with the hypoxic liver. The protective effect of melatonin in avoiding the degenerative alterations caused by the oxidative stress induced by hypoxia could be observed in the examined sections (data not shown). Electron microscopy showed the ovoid form of the nucleus and the nuclear contents retained their normal structure. Also, both of mitochondria and the rough endoplasmic reticulum retained their normal shape and number due to the protective effect of melatonin, although few vacuolation and some lipid droplets were located in certain cellular areas (Fig. 1F).

3.2. Experiment II

Hypoxia significantly stimulated ($P<0.01$) the product of lipid peroxidation levels in the liver homogenates versus those of controls. In the mice, which subjected to oxygen shortage (hypoxia) for 3 h and then left for recovery or reoxygenation without any type of treatment for another 3 h, the levels of the oxidized lipids decreased (42.5%) versus those of hypoxic animals (Table 1). The statistical analysis of these data indicated that this decrease was significant ($P<0.01$). The administration of melatonin reduced significantly the LPO content (66%) in the liver from Reoxgn+Mel-treated mice compared with the values obtained in hypoxic animals ($P<0.01$). Also, melatonin administration significantly reduced the LPO levels in the liver tissue from Reoxgn+Mel animals (40.6%) versus those of Reoxgn mice ($P<0.05$). Thus, using melatonin after the oxygen shortage

Table 1
Mean values of lipid peroxidation ± S.E.M., stimulation (%) and inhibition (%) in the liver homogenates of control and treated mice

Animal groups									
Control	Hypox		Hypox+Mel		Reoxgn			Reoxgn+Mel	
	S% versus Cont.		*I*% versus Hypox		*I*% versus Hypox			*I*% versus Hypox	*I*% versus Reoxgn
1.2±0.27[a]	10.2±0.78[b]	*S*=87.8	3.2±0.25[c]	*I*=68.8	5.9±0.42[d]	*I*=42.5	3.5±0.30[ce]	*I*=65.8	*I*=40.6

S.E.M.: standard error of the mean; *S*: stimulation (%) and *I*: inhibition (%). Values with unlike superscript letters significantly differ.

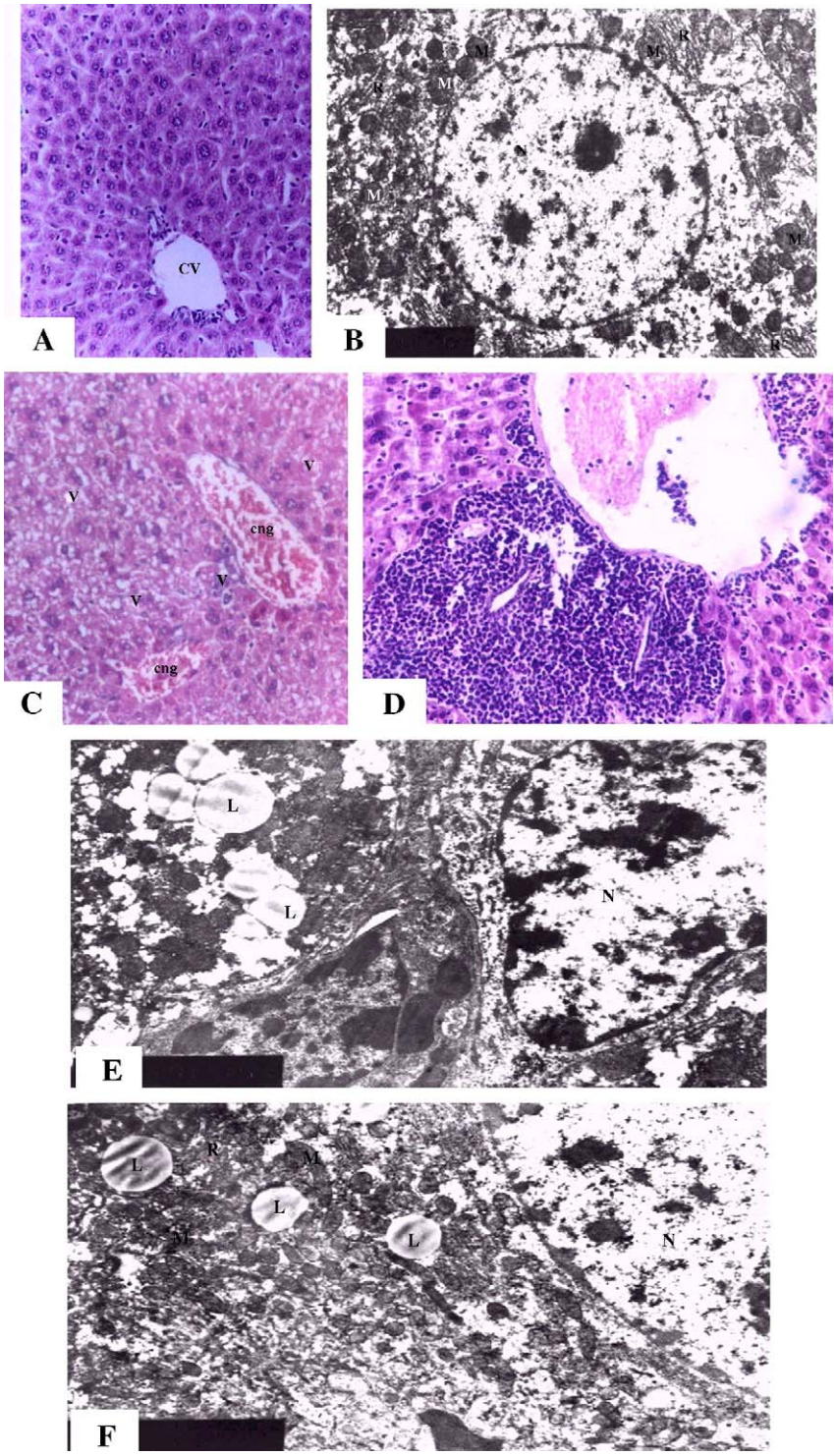

Fig. 1. (A) Liver section of a control mouse displaying the normal architecture of hepatic cords and their radiation from the central vein (CV) (H and E, ×200). (B) Electron micrograph showing a rounded nucleus (N) having well discernable nuclear envelope and chromatin particles. Also, parallel cisternae of rough endoplasmic reticulum (R) and a lot of mitochondria (M) can be observed near the nucleus (×5000). (C) Liver section of hypoxic mouse exhibits marked disturbances of the normal architecture of the hepatic tissues and numerous of vacuolations (V) of the cytoplasm of the liver cells. The central vein exhibits remarkable dilation and distinct features of congestion (cng) (H and E, ×200). (D) Liver section showing a huge collection of inflammatory cells (H and E, ×200). (E) An electron micrograph showing the condensed heterochromatic eccentric nucleus (N) with irregular outlines. The nuclear envelope is moderately dilated. Also, numerous of lipid droplets (L) were clear discerned (×5000). (F) An electron micrograph of the liver of mouse exposed to hypoxia plus melatonin showing open phase nucleus (N), elongated mitochondria (M) and numerous of rough endoplasmic reticulum (R). There are abundance of lipid droplets (L) throughout the cytoplasm (×5000).

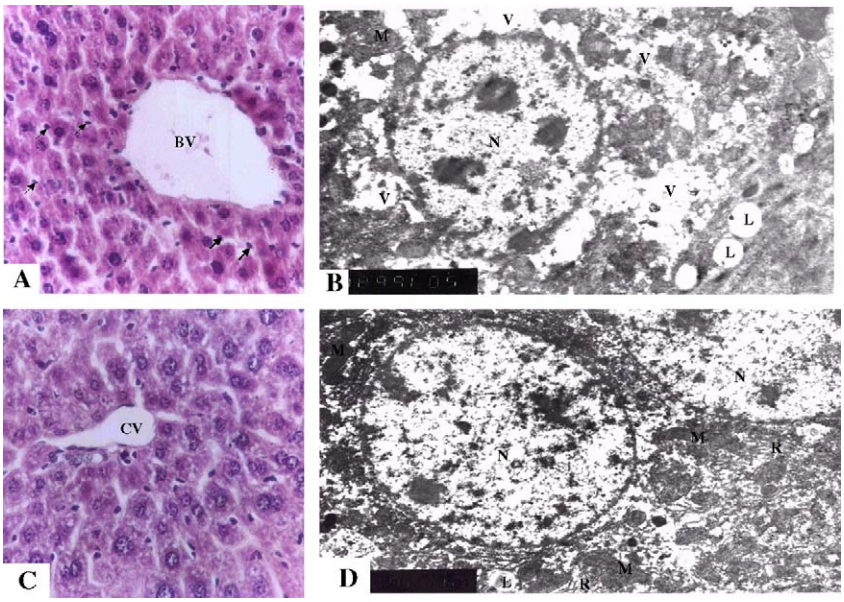

Fig. 2. (A) Section of the liver of reoxygenated mouse showing dilated blood vessels (BV) with blood cells and increased number of darkly stained Kupffer cells (arrows) (H and E, ×400). (B) Electron micrograph of reoxygenated mouse liver showing some destruction in the nucleus (N) and nuclear contents. Few mitochondria (M), vacuoles (V) and lipid droplets are present in the cytoplasm (×5000). (C) Liver section of mouse subjected to reoxygenation+melatonin showing restoration of many degenerations. The hepatocytes appear regular and normal with round central nuclei and central vein (CV) (H and E, ×400). (D) Electron micrograph of mouse subjected to reoxygenation+melatonin showing the restoration of the normality of the nuclear shape (N), mitochondria (M) and rough endoplasmic reticulum (R). Small lipid droplets are present (L) (×5000).

is faster in recovery than if the animal or patient was left without any treatment.

Histological structure of the liver of the reoxygenated group of mice (which subjected to hypoxia for 3 h and then exposed to the air for another 3 h), showed some degenerative alterations in the hepatocytes compared to the hypoxic animals. Fig. 2A displayed dilated blood vessel and an increase in the number of Kupffer cells.

Electron microscopy showed some destruction in the nuclei and the nuclear contents of the hepatocyte. Also, both the mitochondria and the rough endoplasmic reticulum braked down and transformed into noticeable vacuolation. In addition, there was abundance of lipid droplets throughout the cytoplasm (Fig. 2B).

The administration of melatonin immediately after exposure to hypoxia for 3 h exerted a relevant cytoprotection effect that included the recovery of normal cellular morphology (Fig. 2C). The hepatocytes and their nuclei appeared more or less normal in shape and size.

Electron microscopical investigation of the hepatocytes showed the restoration of their normality in the shape of the nuclei and the nuclear contents. Also, both of mitochondria and the rough endoplasmic reticulum restored their normal shape and number although few vacuolations and some lipid droplets located in certain cellular areas (Fig. 2D).

4. Discussion

Oxidative damage is a consequence of the inefficient utilization of molecular oxygen (O_2) by cells. The bulk of the O_2 absorbed by cells is used for mitochondrial generation of energy in the form of ATP (Acuña-Castroviejo et al., 2001).

In this study, our results showed that oxygen shortage (hypoxia) significantly stimulated the levels of the oxidized lipids by 88% in the liver versus control animals. This may be explained by the destructive effect of hypoxia, which leads to an increase of reactive oxygen substances followed by lipid peroxidation. These results are in agreement with those of Joanny et al. (2001) who found that oxidative stress was induced by prolonged hypobaric hypoxia. Also, Magalhaes et al. (2004a,b) reported that hypobaric hypoxia increased the burden of plasma oxidative stress and damage markers all through the hypoxia period. They found significant reduction of the total glutathione content while the levels of lipid peroxidation, oxidized protein and total antioxidant status increased due to hypoxia.

The result of oxygen radical formation is the oxidation of molecules found in tissues, including nucleic acids, membrane lipids, enzymes, and receptors. Membrane-associated polyunsaturated fatty acids are readily oxidized by OH in a process that results in the peroxidation of lipids. Peroxidation of membrane lipids can disrupt membrane fluidity and cell compartmentation, which can result in cell lysis. Thus oxygen radical-initiated lipid peroxidation and protein oxidation may contribute to the impaired cellular function and necrosis associated with reperfusion of ischemic tissues (Cuzzocrea and Reiter, 2001). Oxidative modification of proteins also occurs by reactions with aldehydes produced during lipid peroxidation. Therefore, end products of lipid peroxidation such as malondialdehyde and 4-hydroxy-2-nonenal as well as products from polyunsaturated fatty acids cause protein damage (Refsgaard et al., 2000). Moreover, peroxyl radicals are responsible for oxidation of proteins (Terao and Niki, 1986).

Data concerning the effect of hypoxia on the generation of reactive oxygen species are divergent and depend on experimental conditions (Costa et al., 1993; Nakanishi et al., 1995). A possible explanation for the higher malondialdehyde content in the liver could be the increased MDA influx from other organs for detoxification or the increased decomposition of lipid peroxides by the reduced glutathione system. Another explanation could be a lower production of oxygen radicals by the mitochondria of the liver due to more defective and inefficient respiratory chain (Gonzales-Flecha, 1993; Boffoli et al., 1994) or the direct conversion of oxygen radicals to hydrogen peroxide (H_2O_2) catalyzed by the mitochondrial manganese-dependent superoxide dismutase (Mn-SOD), which was significantly higher expressed in the liver. This increased expression is in agreement with the results of Sanz et al. (1997) and could also be an adaptation to a higher radical release with hypoxia.

Reoxygenation of hypoxic tissue can cause severe cellular damage, known as reoxygenation (or reperfusion) injury in mammals (Halliwell and Gutteridge, 1999). In many cases, reoxygenation can be more harmful than hypoxia or hyperoxia alone. Although these injuries appear to be mediated by ROS, the molecular mechanisms of this process are not well established (Li and Jackson, 2002). Hypoxia and hypoxia/reoxygenation were studied as a model of oxidative stress (Ziegelstein et al., 2004). After reoxygenation, the hepatic MDA content was reduced under basal conditions in rats, possibly due to the higher activity of antioxidative enzymes indicated by the elevated expression of the mRNA of copper/zinc-dependent superoxide dismutase (Cu/Zn-SOD) and GPX (Martin et al., 2002).

In the present study, reoxygenation significantly inhibited the levels of LPO by 42% in the liver compared to the hypoxic mice. Also, the results indicated that the levels of oxidized lipids still high and more than those of control animals. In agreement with our results, Magalhaes et al. (2004a) reported that no additional oxidative stress and damage markers were observed with reoxygenation at the end of the reoxygenation period.

The discovery of melatonin as a direct free radical scavenger (Tan et al., 1993) and as an indirect antioxidant via its stimulatory actions on antioxidative enzymes (Reiter et al., 2000; Rodriquez et al., 2004) has greatly increased interest in the use of this agent in the experimental and clinical setting. Its potential utility in humans is supported by its very low toxicity (Reiter, 1998), its availability in a pure form and the fact that it is inexpensive.

Melatonin actions that have been identified include its ability to directly neutralize a number of toxic reactants and stimulate antioxidative enzymes. Furthermore, several metabolites that are formed when melatonin neutralizes damaging reactants are themselves scavengers suggesting that there is a cascade of reactions that greatly increase the efficacy of melatonin in stymieing oxidative mutilation. Suggested processes which may contribute to melatonin's ability to reduce oxidative stress include stimulation of GSH synthesis (an important antioxidant which is at high concentrations within cells), reducing electron leakage from the mitochondrial electron transport chain (which would reduce free radical generation), limiting cytokine production inflammatory processes (actions that would also lower toxic reactant generation), and synergistic effects with other classical antioxidants e.g. vitamins C, E and glutathione (Reiter et al., 2004).

In the present study, melatonin administration significantly inhibited the levels of oxidized lipids in the liver by 69% in experiment I versus those of hypoxic animals. In experiment II, when melatonin was given for recovery after exposure to hypoxia, it inhibited the levels of oxidized lipids by 66% in the liver versus those of hypoxic mice. Using melatonin for recovery enhanced the recovery profile by 41% in the liver when compared with those that did not receive melatonin. Our results were in agreement with Sener et al. (2003) who found that melatonin treatment abolished the increase in MDA probably in part by scavenging hydroxyl and peroxyl radicals.

The induction of oxidative stress may have a relevant impact in the histology and cellular function in liver. Our results showed severe morphological changes in the liver of hypoxic mice. These changes include necrotic changes with degeneration of vacuolar type and dilated congested blood vessels. The nuclei of the necrotic hepatocytes were pyknotic. There was a remarkable accumulation of inflammatory cells. Most of the previous morphological changes appeared in the reoxygenated group of animals in experiment II of this study. The necrotic and degenerative changes indicate the high susceptibility of mice to oxygen shortage; it also considered as an important indication for the destructive effect of hypoxia due to oxidative stress induction. Our findings regarding the histological changes in the liver of hypoxic animals are in total agreement with Hassanein (1981).

From the ultrastructure point of view, our results showed that the hepatocytes of hypoxic mice were shrunken with eccentric heterochromatic nuclei and irregular outlines. The cytoplasm was highly vacuolated with mitochondrial degeneration and destruction of other living organelles. In the reoxygenated group of animals of experiment II, the same changes in the hepatocytes morphology were detected. Our findings of ultrastructural changes agree with the previous studies that investigated the effect of free radicals generation on the cellular organelles. In agreement with our results, Welt et al. (2004) detected ultrastructural changes in the rat liver exposed to hypoxia. These changes included significant reduction in rough endoplasmic reticulum, decrease in the volume density of mitochondrial cristae and significant decrease in the volume fraction of mitochondria.

The liver is highly sensitive to ischemia/reperfusion injury, which occurs clinically during circulating shock (De La Monte et al., 1984)], disseminated intravascular coagulation (Yoshikawa et al., 1993), liver transplantation and surgery involving this organ (Arthur, 1988). Reoxygenation of the ischemic tissue may promote the generation of various ROS, which are known to have deleterious effects on various cellular functions (Werns and Lucchesi, 1990). The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction and necrosis (Granger and Korthuis, 1995). Ischemia/reperfusion elicits an acute inflammatory response characterized by activation of neutrophils. Activated neutrophils are known to induce tissue injury through the production and

release of ROS and cytotoxic proteins (e.g. proteases, myeloperoxidase, lactoferrin) into extracellular fluid (Kettle and Winterbourn, 1997).

Our study demonstrated the protective effect of melatonin against histological and ultrastructural disturbances in the liver of hypoxic animals. This indicates that such dose of melatonin may be effective in the prevention of destructive effect and oxidative stress induced by oxygen shortage. These results postulated that melatonin scavenges or quench free radicals liberated due to hypoxia in the liver and thus protects the cells from hypoxia and reoxygenation injuries.

References

Abd-Elghaffar, S.Kh., El-Sokkary, G.H., Sharkawy, A.A., 2005. Aluminum-induced neurotoxicity and oxidative damage in rabbits: protective effect of melatonin. Neuroendocrinol. Lett. 26, 609–616.

Acuña-Castroviejo, D., Escames, G., Macias, M., Munoz Hoyos, A., Molina Carballo, A., Arauzo, M., Montes, R., Vives, F., 1995. Cell protective role of melatonin in the brain. J. Pineal Res. 19, 57–63.

Acuña-Castroviejo, D., Martin, M., Macias, M., Escames, G., Leon, J., Khaldy, H., Reiter, R.J., 2001. Melatonin, mitochondria, and cellular bioenergetics. J. Pineal Res. 30, 65–74.

Arthur, M.J.P., 1988. Reactive oxygen intermediates and liver injury. J. Hepatol. 6, 125–131.

Bancroft, J.D., Stevens, A., 1982. Theory and Practice of Histologic Technique, 2nd Ed. Churchill Livingstone, pp. 482–518.

Boffoli, D., Sacco, S.C., Vergari, R., Solarino, G., Santacroce, G., Papa, S., 1994. Decline with age of the respiratory chain activity in human skeletal muscle. Biochim. Biophys. Acta 1226, 73–82.

Burton, G.W., Traber, M.G., 1990. Vitamin E. Antioxidant activity, biokinetics and bioavailability. Annu. Rev. Nutr. 10, 357–382.

Conway, S., Mowat, E.S., Drew, J.E., Barrett, P., Delagrange, P., Morgan, P.J., 2001. Serine residues 110 and 114 are required for agonist binding but not antiagonist binding to the melatonin MT(1) receptor. Biochem. Biophys. Res. Commun. 282, 1229–1236.

Costa, L.E., Llesuy, S., Boveris, A., 1993. Active oxygen species in the liver of rats submitted to chronic hypobaric hypoxia. Am. Physiol. Soc. 264, Cl395–Cl400.

Crompton, M., 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem. J. 341, 233–249.

Cuzzocrea, S., Reiter, R.J., 2001. Pharmacological action of melatonin in shock, inflammation and ischemia/reperfusion injury. Eur. J. Pharmacol. 426, 1–10.

De La Monte, S.M., Arcidi, J.M., Moore, G.W., Hutchins, G.H., 1984. Midzonal necrosis as a pattern of hepatocellular injury after shock. Gastroenterology 86, 627–631.

El-Sokkary, G.H., 2000. Melatonin protects against oxidative stress induced by the kidney arcinogen $KBrO_3$. Neuroendocrinol. Lett. 21, 461–468.

El-Sokkary, G.H., 2002. Inhibition of 2-nitropropane-induced cellular proliferation, DNA synthesis and histopathological changes by melatonin. Neuroendocrinol. Lett. 23, 335–340.

El-Sokkary, G.H., Reiter, R.J., Abdel Ghaffar, S.Kh., 2003. Melatonin supplementation restores cellular proliferation and DNA synthesis in the splenic and thymic lymphocytes of old rats. Neuroendocrinol. Lett. 24, 215–223.

El-Sokkary, G.H., Abdel-Rahman, G.H., Kamel, E.S., 2005. Melatonin protects against lead-induced hepatic and renal toxicity in male rats. Toxicology 213, 25–33.

Goldfarb, A.H., Sen, C.K., 1994. Antioxidant-supplementation and control of oxygen. In: Sen, C.K., Packer, L., Osmo, H. (Eds.), Exercise and oxygen Toxicity, pp. 163–190.

Gonzales-Flecha, B., 1993. Oxidative stress produced by suprahepatic occlusion and reperfusion. Hepatology 18, 881–889.

Granger, D.N., Korthuis, R.J., 1995. Physiological mechanisms of postischemic tissue injury. Annu. Rev. Physiol. 57, 311–332.

Guerrero, J.M., Reiter, R.J., Ortiz, G.G., Pablos, M.I., Sewerynek, E., Chuang, J.I., 1997. Melatonin prevents increases in neural nitric oxide and cyclic GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (*Meriones unguiculatus*). J. Pineal Res. 23, 24–31.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine. Oxford Sci. Public, Oxford, p. 936.

Hassanein, A.M.M., 1981. Effect of hypoxia on cells and tissues of the albino rat. Ph.D. Thesis, Faculty of Science, Assiut University.

Jackson, M.J., Mc Andle, A., Mc Andle, F., 1998. Antioxidant micronutrients and gene expression. Proc. Nutr. Soc. 57, 301–305.

Joanny, P., Steinberg, J., Robach, P., Richalet, J.P., Gortan, C., Gardette, B., Jamm, Y., 2001. Operation Everest III (Comex'97): the effect of simulated severe hypobaric hypoxia on lipid peroxidation and antioxidant defence systems in human blood at rest and after maximal exercise. Resuscitation 49, 307–314.

Kettle, A.J., Winterbourn, C.C., 1997. Myeloperoxidase: a key regulator of neutrophil oxidant production. Redox Rep. 3, 3–15.

Lapin, I.P., Mirzaev, S.M., Ryzov, I.V., Oxenkrug, G.F., 1998. Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and Pentylenetetrazole in mice. J. Pineal Res. 24, 215–218.

Leeuwenburgh, C., Ji, L.L., 1995. Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. Arch. Biochem. Biophys. 316, 941–949.

Li, C., Jackson, R.M., 2002. Reactive species mechanisms of cellular hypoxia–reoxygenation injury. Am. J. Physiol., Cell Physiol. 282, 227–241.

Li, G., Li, L., Yin, D., 2005. A novel observation: melatonin's interaction with malondialdehyde. Neuroendocrinol. Lett. 26, 61–66.

Magalhaes, J., Ascensao, A., Viscor, G., Soares, J., Oliveira, J., Marques, F., Duart, J., 2004a. Oxidative stress in Humans during and after 4 hours of hypoxia at simulated altitude of 5500 m. Aviat. Space Environ. Med. 75, 16–22.

Magalhaes, J., Ascensao, A., Soares, J.M., Neuparth, M.J., Ferreira, R., Oliveira, J., Amado, F., Duarte, J.A., 2004b. Acute and severe hypobaric hypoxia-induced muscle oxidative stress in mice: the role of glutathione against oxidative damage. Eur. J. Appl. Physiol. 91, 185–191.

Marotto, M.E., Thurman, R.G., Lemasters, J.J., 1988. Early midzonal cell death during low-flow hypoxia in the isolated perfused rat liver. Protection by allopurinol. Hepatology 8, 585–590.

Martin, M., Macias, M., Escames, G., Reiter, R.J., Agapito, M.T., Ortiz, G.G., Acuña-Castroviejo, D., 2000. Melatonin induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. J. Pineal Res. 28, 242–248.

Martin, R., Fitzl, G., Mozet, C., Martin, H., Welt, K., Wieland, E., 2002. Effect of age and hypoxia/reoxygenation on mRNA expression of antioxidative enzymes in rat liver and kidneys. Exp. Gerontol. 37, 1479–1485.

Menendez-Pelaez, A., Poggerler, B., Reiter, R.J., Barlow-Walden, L.R., Pablos, M.I., Tan, D.X., 1993. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J. Cell. Biochem. 53, 373–382.

Motoyama, S., Saito, S., Saito, R., Minamiya, Y., Nakamura, M., Okuyama, M., Imano, H., Ogawa, J., 2003. Hydrogen peroxide-dependent declines in Bcl-2 induces apoptosis in hypoxic liver. J. Surg. Res. 110, 211–216.

Nakanishi, K., Tajima, F., Nakamura, A., Yagura, S., Ookawara, T., Yamashita, H., Suzuki, K., Taniguchi, N., Ohno, H., 1995. Effects of hypobaric hypoxia on antioxidant enzymes in rats. J. Physiol. 489, 869–876.

Ohkawa, H., Ohishi, N., Nagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Ohkuwa, T., Itoh, H., Yamamoto, T., Minami, C., Yamazaki, Y., Kimoto, S., Yoshida, R., 2004. Effects of hypoxia and hypoxic training on 8-hydroxydeoxyguanosine and glutathione levels in the liver. Metabolism 53, 716–719.

Palozza, P., Lubertoc, S., Calviellog, S., Ricc, P., Barfoli, G.M., 1997. Antioxidant and prooxidant role of B-carotene in murine normal and tumor thymocytes: effects of oxygen partial pressure. Free Radic. Biol. Med. 22, 1065–1073.

Refsgaard, H.H., Tsai, L., Stadtman, E.R., 2000. Modifications of proteins by polyunsaturated fatty acid peroxidation products. Proc. Natl. Acad. Sci. U. S. A. 97, 611–616.

Reiter, R.J., 1998. Oxidative damage in the central nervous system: protection by melatonin. Prog. Neurobiol. 56, 359–384.

Reiter, R.J., Tan, D.X., Qi, W., Manchester, L.C., Karbownik, M., Calvo, J.R., 2000. Pharmacology and physiology of melatonin in reduction of oxidative stress in vivo. Biol. Signals Recept. 9, 160–171.

Reiter, R.J., Tan, D.X., Gitto, E., Sainz, R.M., Mayo, J.C., Leon, J., Manchester, L.C., Kilic, E., Kilic, U., 2004. Pharmacological utility of melatonin in reducing oxidative cellular and molecular damage. Pol. J. Pharmacol. 56, 159–170.

Rodriquez, C., Mayo, J.C., Sainz, R.J., Antolin, I., Herrera, F., Martin, V., Reiter, R.J., 2004. Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal Res. 36, 1–9.

Sanz, N., Diez-Fernandez, C., Alvarez, A., Cascales, M., 1997. Age-dependent modifications in rat hepatocyte antioxidant defense system. J. Hepatol. 27, 525–534.

Sarada, S.K.S., Dipti, P., Anju, B., Pauline, T., Kain, A.K., Sairam, M., Sharma, S.K., Ilavazhgan, G., Devendra Kumar, Selvamurthy, W., 2002. Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats. J. Ethnopharmacol. 79, 149–153.

Sener, G., Tosun, O., [a]ehirli, Ö.A., Kacmaz, A., Arbak, S., Ersoy, Y., Ayanoðlu-Dlger, G., 2003. Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion. Life Sci. 72, 2707–2718.

Shan, X., Aw, T.Y., Smith, E.R., Ingelman-Sundberg, M., Mannervik, B., Iyanagi, T., Jones, D.P., 1992. Effect of chronic hypoxia on detoxication enzymes in rat liver. Biochem. Pharmacol. 43, 2421–2426.

Simon-Schnass, I.M., 1992. Nutrition at high altitude. J. Nutr. 122, 778–781.

Suematsu, M., Suzuki, H., Ishii, H., Kato, S., Hamamatsu, H., Miura, S., Tsuchiya, M., 1992a. Topographic dissociation between mitochondrial dysfunction and cell death during low-flow hypoxia in perfused rat liver. Lab. Invest. 67, 434–442.

Suematsu, M., Suzuki, H., Ishii, H., Kato, S., Yanagisawa, T., Asako, H., Suzuki, M., Tsuchiya, M., 1992b. Early midzonal oxidative stress preceding cell death in hypoperfused rat liver. Gastroenterology 103, 994–1001.

Tan, D.X., Chen, L.D., Poeggeler, B., Manchester, L.C., Reiter, R.J., 1993. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J. 1, 57–60.

Terao, K., Niki, E., 1986. Damage to biological tissue induced by radical inhibitor 2,2-azobis(2-aminopropane) dihydrochloride and its inhibition by chain-breaking antioxidants. J. Free Radic. Biol. Med. 2, 193–201.

Welt, K., Weiss, J., Martin, R., Dettmer, D., Hermsdorf, T., Asayama, K., Meister, S., Fitzl, G., 2004. Ultrastructural, immunohistochemical and biochemical investigations of the rat liver exposed to experimental diabetes and acute hypoxia with and without application of Ginkgo extract. Exp. Toxicol. Pathol. 55, 331–345.

Werns, S.W., Lucchesi, B.R., 1990. Free radical and ischemic tissue injury. Trends Pharmacol. Sci. 11, 161–166.

Yoshikawa, T., Murakami, M., Yoshida, N., Seto, O., Kondo, M., 1993. Effect on superoxide dismutase and catalase on disseminated intravascular coagulation in rats. Thromb. Haemost. 50, 869–872.

Ziegelstein, R.C., He, C., Hu, Q., 2004. Hypoxia/reoxygenation stimulates Ca(2+)-dependent ICAM-1mRNA expression in human aortic endothelial cells. Biochem. Biophys. Res. Commun. 322, 68–73.