Selected abstracts of the clinical trials linking Melatonin to Glutathione production

1. Melatonin mitigates mitochondrial malfunction.

J Pineal Res. 2005 Jan;38(1):1-9.

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Melatonin, or N-acetyl-5-methoxytryptamine, is a compound derived from tryptophan that is found in all organisms from unicells to vertebrates. This indoleamine may act as a protective agent in disease conditions such as Parkinson's, Alzheimer's, aging, sepsis and other disorders including ischemia/reperfusion. In addition, melatonin has been proposed as a drug for the treatment of cancer. These disorders have in common a dysfunction of the apoptotic program. Thus, while defects which reduce apoptotic processes can exaggerate cancer, neurodegenerative disorders and ischemic conditions are made worse by enhanced apoptosis. The mechanism by which melatonin controls cell death is not entirely known. Recently, mitochondria, which are implicated in the intrinsic pathway of apoptosis, have been identified as a target for melatonin actions. **It is known that melatonin scavenges oxygen and nitrogen-based reactants generated in mitochondria. This limits the loss of the intramitochondrial glutathione and lowers mitochondrial protein damage**, improving electron transport chain (ETC) activity and reducing mtDNA damage. Melatonin also increases the activity of the complex I and complex IV of the ETC, thereby improving mitochondrial respiration and increasing ATP synthesis under normal and stressful conditions. These effects reflect the ability of melatonin to reduce the harmful reduction in the mitochondrial membrane potential that may trigger mitochondrial transition pore (MTP) opening and the apoptotic cascade. In addition, a reported direct action of melatonin in the control of currents through the MTP opens a new perspective in the understanding of the regulation of apoptotic cell death by the indoleamine.

PMID: 15617531 [PubMed - indexed for MEDLINE]

2. Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells.

Free Radic Biol Med. 1999 Oct;27(7-8):838-47.

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In the present study, we show that melatonin induces the expression of gamma-glutamylcysteine synthetase (gamma-GCS), the rate-limiting enzyme of glutathione (GSH) synthesis, in ECV304 human vascular endothelial cells. One micromolar melatonin induced the expression of gamma-GCS mRNA followed by an increase in the concentration of GSH with a peak at 24 h. An electrophoretic mobility shift assay showed that melatonin stimulates the DNA-binding activity of activator protein-1 (AP-1) as well as retinoid Z receptor/retinoid receptor-related orphan receptor alpha (RZR/RORalpha). ECV304 cells transiently transfected with a plasmid containing the gamma-GCS promoter-luciferase construct showed increased luciferase activity when treated with melatonin. The melatonin-dependent luciferase activity was found in the gamma-GCS promoter containing AP-1 site. The luciferase activity mediated by AP-1 was repressed in the promoter containing RZR/RORalpha site. In addition, cell cycle analysis showed that melatonin increases the number of cells in the G0/G1 phase; however, treatment of the cells with buthionine sulfoximine, a specific inhibitor of gamma-GCS, abolished the effect of melatonin on the cell cycle, suggesting induction of cell arrest by melatonin requires GSH. **As conclusion, induction of GSH synthesis by melatonin protects cells against oxidative stress and regulates cell proliferation.**

PMID: 10515588 [PubMed - indexed for MEDLINE]

3. Melatonin protects against mercury(II)-induced oxidative tissue damage in rats.

Pharmacol Toxicol. 2003 Dec;93(6):290-6.

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Mercury exerts a variety of toxic effects in the body. Lipid peroxidation, DNA damage and depletion of reduced glutathione by Hg(II) suggest an oxidative stress-like mechanism for Hg(II) toxicity. Melatonin, the main secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant. N-Acetylcysteine, a precursor of reduced glutathione and an antioxidant, is used in the therapy of acute heavy metal poisoning. In this study the protective effects of melatonin in comparison to that of N-

acetylcysteine against Hg-induced oxidative damage in the kidney, liver, lung and brain tissues were investigated. Wistar albino rats of either sex (200-250 g) were divided into six groups, each consisting of 8 animals. Rats were intraperitoneally injected with 1) 0.9% NaCl, control (C) group; 2) a single dose of 5 mg/kg mercuric chloride (HgCl2), Hg group; 3) melatonin in a dose of 10 mg/kg, 1 hr after HgCl2 injection, Hg-melatonin group; 4) melatonin in a dose of 10 mg/kg one day before and 1 hr after HgCl2 injection, melatonin-Hg-melatonin group; 5) N-acetylcysteine in a dose of 150 mg/kg, 1 hr after HgCl2 injection, Hg-N-acetylcysteine group, and 6) N-acetylcysteine in a dose of 150 mg/kg one day before and 1 hr after HgCl2 injection, N-acetylcysteine-Hg-N-acetylcysteine group. Animals were killed by decapitation 24 hr after the injection of HgCl2. Tissue samples were taken for determination of malondialdehyde, an end-product of lipid peroxidation; glutathione (GSH), a key antioxidant, and myeloperoxidase activity, an index of neutrophil infiltration. **The results revealed that HgCl2 induced oxidative tissue damage, as evidenced by increases in malondialdehyde levels. Myeloperoxidase activity was also increased, and GSH levels were decreased in the liver, kidney and the lungs. All of these effects were reversed by melatonin** or N-acetylcysteine treatment. Since melatonin or N-acetylcysteine administration reversed these responses, it seems likely that melatonin or N-acetylcysteine can protect all these tissues against HgCl2-induced oxidative damage.

PMID: 14675463 [PubMed - indexed for MEDLINE]

4. Melatonin improves oxidative organ damage in a rat model of thermal injury.

Burns. 2002 Aug;28(5):419-25.

Sener G, **Sehirli AO**, **Satiroğlu H**, **Keyer-Uysal M**, **C Yeğen B**.

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Animal models of burn injury indicate oxygen radicals as causative agents in the local wound response, as well as in the development of burn shock and distant organ injury. This study was designed to determine the possible protective effect of melatonin treatment against oxidative damage in the liver, lung and intestine induced by burn injury. Under ether anaesthesia, the shaved dorsum of rats was exposed to a 90 degrees C bath for 10s to induce burn injury. Rats were decapitated either 3 or 24h after burn injury. Melatonin was administered i.p. immediately after burn injury. In the 24h burn group, melatonin injections were repeated for two more occasions. In the sham group the same protocol was applied except that the dorsum was dipped in a 25 degrees C water bath for 10s. Liver, lung and intestine tissues were taken for the determination of malondialdehyde (MDA) and glutathione (GSH) levels, myeloperoxidase (MPO) activity and protein oxidation (PO). Severe skin scald injury (30% of total body surface area) caused a significant decrease in GSH level, significant increases in MDA and PO levels, and MPO activity at postburn 3 and 24h. **Treatment of rats**

with melatonin (10mg/kg) significantly elevated the reduced GSH levels while it decreased MDA and PO levels as well as MPO activity.

PMID: 12163279 [PubMed - indexed for MEDLINE]

5. Melatonin stimulates glutathione peroxidase activity in human chorion.

J Pineal Res. 2001 May;30(4):199-205.

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In preeclampsia, placental production of lipid peroxides is abnormally increased, while placental glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities are decreased. Administration of melatonin, a powerful scavenger of oxygen free radicals, also may protect the placenta from free radical-induced damage by increasing the activity of antioxidant enzymes. To test this hypothesis we administered melatonin to pregnant women before they underwent voluntary interruption of pregnancy between 7 and 9 wk of gestation. Melatonin (6 mg) was administered orally at 12:00 hr, and samples of chorion and maternal blood were obtained at the time of the procedure, 1, 2 or 3 hr later. We measured the melatonin concentration in maternal serum and activities of GSH-Px and SOD and levels of melatonin in chorionic homogenates. Melatonin administration was reflected by markedly increased melatonin concentrations in maternal serum and in chorion, with peak levels achieved 1 hr after melatonin administration (serum, 46.87 +/- 10.87 nM/L; chorionic homogenate, 4.36 +/- 1.56 pmol/mg protein). Between 1 and 3 hr after melatonin administration, GSH-Px activity in chorionic homogenates increased significantly ($P < 0.001$), with peak levels occurring at 3 hr (51.68 +/- 3.22 mU/mg protein per min, 137.3% of GSH-Px activity in untreated control subjects). No significant changes in chorionic SOD activity occurred during the 3-hr post-administration period. **These results indicate that exogenous melatonin increases GSH-Px activity in the chorion and thereby may protect indirectly against free radical injury.** Melatonin could be useful in treating preeclampsia and possibly other clinical states involving excessive free radical production, such as intrauterine fetal growth retardation and fetal hypoxia.

PMID: 11339508 [PubMed - indexed for MEDLINE]

6. Melatonin increases activities of glutathione peroxidase and superoxide dismutase in fetal rat brain.

J Pineal Res. 2000 Mar;28(2):89-96.

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Melatonin is a powerful scavenger of oxygen free radicals. In humans, melatonin is rapidly transferred from the maternal to the fetal circulation. To investigate whether or not maternal melatonin administration can protect the fetal rat brain from radical-induced damage by increasing the activities of antioxidant enzymes, we administered melatonin to pregnant rats on day 20 of gestation. Melatonin (10 mg/kg) was injected intraperitoneally at daytime (14:00 hr) and, to remove the fetuses, a laparotomy was performed at 1, 2, or 3 hr after its administration. We measured the melatonin concentration in the maternal serum and in fetal brain homogenates and determined the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in fetal brain homogenates. Melatonin administration markedly increased melatonin concentrations in the maternal serum and fetal brain homogenates, with peak levels achieved 1 hr after melatonin administration (serum: 538.2+/-160.7 pM/mL; brain homogenates: 13.8+/-2.8 pM/mg protein). **Between 1 and 3 hr after melatonin administration, GSH-Px activity in fetal brain homogenates increased significantly ($P<0.01$).** Similarly, SOD activity increased significantly between 1 and 2 hr after melatonin administration ($P<0.01$). These results indicate that melatonin administration to the mother increases antioxidant enzyme activities in the fetal brain and may thereby provide indirect protection against free radical injury. Thus, melatonin may potentially be useful in the treatment of neurodegenerative conditions that may involve excessive free radical production, such as fetal hypoxia and preeclampsia.

PMID: 10709970 [PubMed - indexed for MEDLINE]

7. Oxidative stress in diabetic rats induced by streptozotocin: protective effects of melatonin.

J Pineal Res. 1998 Sep;25(2):94-100.

Montilla PL, **Vargas JF**, **Túnez IF**, **Muñoz de Agueda MC**, **Valdelvira ME**, **Cabrera ES**.

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We have studied the effect of the administration of two doses of melatonin (melatonin 100 and melatonin 200 microg/kg bw) on diabetes and oxidative stress experimentally induced by the injection of streptozotocin (STZ) in female Wistar rats. STZ was injected as a single dose (60 mg/kg i.p. in buffered citrate solution, pH 4.0) and melatonin (melatonin 100, 100 microg/kg/day i.p.; melatonin 200, 200 microg/kg/day i.p.) beginning 3 days before diabetes

induction and continuing until the end of the study (8 weeks). The parameters analysed to evaluate oxidative stress and the diabetic state were a) for oxidative stress, changes of lipoperoxides (i.e., malondialdehyde, MDA) in plasma and erythrocytes and the changes in reduced glutathione (GSH) in erythrocytes and b) for diabetes, changes in glycemia, lipids (triglycerides: TG; total cholesterol: TC; HDL-cholesterol, HDL-c), percentage of glycosylated hemoglobin (Hb%), and plasma fructosamine. The injection of STZ caused significant increases in the levels of glycemia, percentage of glycosylated hemoglobin, fructosamine, cholesterol, triglycerides, and lipoperoxides in plasma and erythrocytes, whereas it decreased the levels of HDL-c and the GSH content in erythrocytes. The melatonin 100 dose reduced significantly all these increases, except the percentage of glycosylated hemoglobin. With regard to the decreases of plasma HDL-c and GSH content in erythrocytes, this melatonin dose returned them to normal levels. The melatonin 200 dose produced similar changes, though the effects were especially noticeable in the decrease of glycemia (55% vs. diabetes), percentage of hemoglobin ($P < 0.001$ vs diabetes), and fructosamine (31% vs. diabetes). **This dose also reversed the decreases of HDL-c and GSH in erythrocytes.** Both doses of melatonin caused significant reduction of the percentage of glycosylated hemoglobin in those groups that were non-diabetic. These illustrate the protective effect of melatonin against oxidative stress and the severity of diabetes induced by STZ. In particular, this study confirms two facts: 1) the powerful antioxidant action of this pineal indole and 2) the importance of the severity of oxidative stress to maintain hyperglycemia and protein glycosylation, two pathogenetic cornerstones indicative of diabetic complications. Melatonin reduces remarkably the degree of lipoperoxidation, hyperglycemia, and protein glycosylation, which gives hope to a promising perspective of this product, together with other biological antioxidants, in the treatment of diabetic complications where oxidative stress, either in a high or in a low degree, is present.

PMID: 9755030 [PubMed - indexed for MEDLINE]

8. Melatonin stimulates the activity of the detoxifying enzyme glutathione peroxidase in several tissues of chicks.

J Pineal Res. 1995 Oct;19(3):111-5.

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The pineal hormone melatonin has been shown to directly scavenge free radicals and to stimulate, in the mammalian brain, at least one enzyme, glutathione peroxidase, which reduces free radical generation. In the present studies, we examined the effect of melatonin on glutathione peroxidase activity in several tissues of an avian species. Melatonin (500 micrograms/kg), when injected into chicks, increased glutathione peroxidase activity within

90 min in every tissue examined. Tissue melatonin levels, measured by radioimmunoassay, also increased following its peripheral administration. Depending on the tissue, the measured increases in melatonin varied from 75% to 1,300% over the control values. **The melatonin-induced increases in glutathione peroxidase activity varied with the tissue and were between 22% and 134%. These percentage increases in glutathione peroxidase activity were directly correlated with tissue melatonin content. These results suggest that melatonin induces the activity of the detoxifying enzyme, glutathione peroxidase, in several tissues in the chick.** The findings also suggest that melatonin would reduce the generation of highly toxic hydroxyl radicals by metabolizing its precursor, hydrogen peroxide. Because of this ability to stimulate glutathione peroxidase activity, melatonin should be considered as a component of the antioxidative defense system in this avian species.

PMID: 8750343 [PubMed - indexed for MEDLINE]

9. Paraquat toxicity and oxidative damage. Reduction by melatonin.

Biochem Pharmacol. 1996 Apr 26;51(8):1095-9.

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The ability of melatonin to protect against paraquat-induced oxidative damage in rat lung, liver, and serum was examined. Changes in the levels of malondialdehyde (MDA) plus 4-hydroxyalkenals (4-HDA) and reduced and oxidized glutathione concentrations were measured. Paraquat (50 mg/kg) was injected i.p. into either Sprague-Dawley or Wistar rats with or without the co-administration of 5 mg/kg melatonin. Paraquat alone increased MDA + 4-HDA levels in serum and lungs of both rat strains, with these increases being abolished by melatonin co-treatment. **Paraquat also decreased reduced glutathione levels and increased oxidized glutathione concentrations in lung and liver; these changes were negated by melatonin.** The effect of melatonin on paraquat-induced mortality was also studied. Paraquat at a dose of 79 mg/kg was lethal for 50% of animals within 24 hr; when administered together with melatonin, the LD50 for paraquat increased to 251 mg/kg.

PMID: 8866832 [PubMed - indexed for MEDLINE]

10. The effect of melatonin on bleomycin-induced pulmonary fibrosis in rats.

J Pineal Res. 2002 Jan;32(1):21-5.

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The present investigation was designed to determine the protective effects of melatonin against bleomycin (BLM)-induced oxidant lung toxicity. Wistar-albino rats were divided into four groups: saline (SA, 0.4 mL/animal), 1% ethanol-saline (ALC, 0.4 mL/animal), bleomycin sulphate (BLM, 10 mg/kg), or bleomycin sulphate + melatonin (BLM, 10 mg/kg + MLT, 10 mg/kg). All injections were given intraperitoneally (i.p.), twice weekly for a period of 3 wk (a total of seven injections for each group). Twenty-five days after BLM treatment, pulmonary fibrosis was assessed as hydroxyproline content in lung homogenates. Findings show that BLM-induced pulmonary injury resulted in increases in bronchoalveolar lavage fluid (BALF) biomarkers including total protein, lactate dehydrogenase (LDH), glutathione-peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT). Additionally, the levels of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation (LPO), were also increased in BALF. **Conversely, the level of glutathione (GSH) was reduced in BALF of BLM-treated rats. Melatonin provided protection against BLM-induced pulmonary fibrosis by suppressing oxidative stress. It abolished BLM-stimulated LPO and reversed the imbalance between oxidants and antioxidants in the BALFs.** Results thus indicate that melatonin inhibits BLM-induced lung toxicity associated with oxidative damage.

PMID: 11841596 [PubMed - indexed for MEDLINE]