Evaluation of the clinical effect of melatonin on oxidative stress markers in patients with lead poisoning

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ABSTRACT

Lead is a neurotoxic metallic element that can be absorbed by the body, primarily through the lungs and stomach. Generally, lead poisoning occurs slowly, resulting from the gradual accumulation of lead in bone and tissue after repeated exposure. Left untreated, lead poisoning can damage many internal organs, including the kidney and the nervous system. Recent studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species and reducing the antioxidant defense system of cells. This suggests that antioxidants may play an important role in the treatment of lead poisoning as a kind of scavengers of free radicals.

Antioxidant is any substance that reduces oxidative damage such as that caused by free radicals. Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures. Melatonin, a powerful antioxidant, is a hormone produced naturally in the pineal gland at the base of the brain.

This study was designed to evaluate the clinical significance of melatonin in ameliorating the oxidative stress induced due to chronic exposure to lead.

Twenty male patients with chronic lead poisoning and their ٢٠ aged matched normal controls with an age range ٣٥-٤٥ years were included in this study. Treatment included ٣ mg capsule of melatonin antioxidant at night for two months. Heparinized venous blood samples were collected from patients before treatment and at one and two months after treatment as well as from controls to measure erythrocytes malondialdehyde (RMDA), plasma total antioxidant status (TAS), blood lead (Pb) and serum zinc (Zn) levels.

The results of the study showed a significant antioxidant activity of melatonin in eliminating the oxidative consequences of lead exposure revealed by significant reduction in oxidative stress markers (RMDA and Pb) with a significant increase in body antioxidant defense mechanisms(TAS and Zn).

Key words: lead toxicity, oxidative stress, antioxidant, melatonin, zinc

الخلاصة

يعتبر الرصاص من المعادن السامة التي يمكن امتصاصها بالجسم عن طريق الرئة والمعدة. بصورة عامة يحدث التسمم بالرصاص ببطى وينتج عن التراكم التدريجي للرصاص في العظام والخلايا بعد تكرار التعرض له. في حال عدم علاج حالات التسمم بالرصاص بشكل مبكر يحدث تحطيم في الاعضاء الداخلية وتتضمن (الكلى، الجهاز العصبي والدماغ).

أظهرت الدراسات موخرا ان التسمم بالرصاص يحدث اجهاد تاكسدي عن طريق توليد الجذور الحرة وتقليل مانعات الاكسدة في الخلايا .اشارت الدراسات موخرا الى اهمية مانعات الاكسدة في علاج التسمم بالرصاص عن طريق قنص الجذور الحرة.

تعرف مانعات الاكسدة على انها المواد التي تقلل من التحطيم الناتج من الاكسدة مثل الذي تحدثه الجذور الحرة، و تعرف الجذور الحرة على انها جزيئات فعالة جدا احادية الالكترون والتي تتحد مع جزيئات اخرى باخذ الكترون منها وتحور تركيبها الكيميائي.

يعرف الميلاتونين بانه هرمون يفرزمن الغدة النخامية التي توجد بقاعدة الدماغ ويعتبر الميلاتونين مانع اكسدة قوي.

صممت هذه الدراسة السريرية لتقييم دور الميلاتونين في انهاء تاثير الجذور الحرة الناتج من التعرض المستمر للرصاص.

عشرين مريضا شخصوا بالتسمم المزمن بالرصاص شملوا في هذه الدراسة مع عشرين شخصا من الأصحاء، تضمن العلاج ميلاتونين ٣ ملغم ليلا لمدة شهرين. تم اخذ عينات دم من المرضى قبل العلاج وبعد شهر وشهرين من استخدام العلاج كما وتم سحب عينة من الأصحاء. تم قياس كل من المالونداليديهاد في كريات الدم الحمراء، مانع الاكسدة الكلي في البلازما، نسبة الرصاص في الدم، و نسبة الزنك في مصل الدم. أظهرت نتائج الدراسة أهمية الميلاتونين كمضاد للتأكسد في ازالة أو تقليل اثار الاكسدة الناتجة من التعرض للرصاص، حيث ساهم الميلاتونين بتقليل مستويات علامات الاجهاد التأكسدي (المالونداليديهاد، الرصاص) ورفع نسبة مانعات الاكسدة في الجسم (مانع الاكسدة الكلي والزنك).

Lead is a very toxic element, causing a variety of effects at low dose levels. The accumulation of lead usually is gradual, building up unnoticed until levels become dangerous and cause signs and symptoms.[١] Brain damage, kidney damage, and gastrointestinal distress are seen from acute exposure to high levels of lead in humans.[١]

Chronic exposure to lead in humans results in effects on the blood, central nervous system (CNS), blood pressure, kidneys, and Vitamin D metabolism.[١] Reproductive effects, such as decreased sperm count in men and spontaneous abortions in women, have been associated with high lead exposure.[٢,٣]

In any biological system where reactive oxygen species (ROS) production increases, antioxidant reserves are depleted. In this situation, the negative effects on the human system's ability to deal with increased oxidant stress occur via independent pathways. Once free radicals (FRs) interacts with a tissue, many changes can occur[٤] which can be defined in terms of injury or oxidative stress to denote disturbances in the pro-oxidant antioxidant balance in favor of the former, leading to potential damages.[٥]

An antioxidant is a chemical that prevents the oxidation of other chemicals.[٦] In normal biological systems, free radicals have the tendency to attack healthy molecules around them and turn them into free radicals like themselves, creating a chain reaction which could lead to massive cellular damage.[٧] Antioxidants keep free radicals from turning healthy molecules into free radicals.[٧] The antioxidant breaks off the free radical chain, thus preventing damage in the cells. In addition, antioxidants may also have properties that enable them to repair damages that might have occurred.[٨]

Melatonin is the principal hormone produced by the pineal gland at the base of the brain. It is practically nontoxic and exhibits almost no toxic side effects.[٩] Normally, the production of melatonin by the pineal gland is inhibited by light and permitted by darkness. Many biological effects of melatonin are produced through activation of melatonin receptors,[١٠] whereas others are due to its role as a pervasive and extremely powerful antioxidant[١١] with a particular role in the protection of nuclear and mitochondrial DNA by capturing electrons and thus modifying chemical structures. The antioxidant activity of melatonin may reduce damage caused by some types of Parkinson's disease, may play a role in preventing cardiac arrhythmia and may increase longevity; it has been shown to increase the average life span of mice by ٢٠% in some studies.[١٢-١٤] The therapeutic efficacy of melatonin was studied in a previous study.[١٥] Its ability to restore altered haematopoietic, hepatic and other biochemical variables indicative of tissue oxidative stress in male rats was proved.[١٥] Administration of melatonin provided significant protection against lead-induced disturbed antioxidant defense that may significantly compromise normal cellular function.[١٦] Administration of melatonin also provided a significant protection to thiobarbituric acid reactive substances (TBARS) levels, reduced glutathione (GSH) and oxidized glutathione (GSSG) contents in tissues, suggesting their ability to act as a free radical scavenger and in protecting cells against toxic insult.[١٧,١٨]

Lead-induced oxidative stress has been identified as the primary contributory agent in the pathogenesis of lead poisoning.[١] ROS generated as a result of lead exposure have been identified in lungs, endothelial tissue, testes, sperm, liver, and brain.[٢].Consequently, the potential role of using antioxidants of various types to provide protective effects became a major task in this respect. Therefore the present study was undertaken to examine the antioxidant effect of melatonin in patients suffering from lead poisoning.

Subjects and methods

Study group comprised of forty subjects, ٢٠ patients with chronic lead poisoning and their ٢٠ aged matched normal controls with an age range of ٣٥-٤٥ years, in the Iraqi smelter plant in Khan Dhary sub district – Baghdad; they were select on the basis that they were on direct exposure to lead and have been employed for at least ١ year before this study was carried out. The daily exposure to lead of each worker should be at least ٦-٨ hrs, and the total period of exposure range from ١-٢٥ years, only ٢٠ workers completed the study. Patients involved in this study were under medical supervision. They were non-smokers, non-alcoholics and apparently free from other diseases. Patients were selected on the basis that they were on direct exposure to lead and have been employed for at least one year before this study was carried out. Treatment schedules included ٣ mg capsule of antioxidants (melatonin, American Nutri-ceutical, USA) at night continued for two months. Heparinized venous blood samples were collected from patients before treatment and at one and two months after treatment as well as from controls.

Erythrocyte MDA levels were measured according to the method of Stocks and Dormandy[١٩] as modified by Gilbert et al.[٢٠] Serum zinc levels were measured by atomic absorption spectrometry according to Taylor and Bryant method.[٢١] Blood lead levels were measured by atomic absorption spectrometry according to Brown et al.[٢٢], and blood

hemoglobin content was assayed according to the method of Drapkin and Austin.[٢٣] Total antioxidant status levels (TAS) were measured using Randox TAS kit (United Kingdom).
Statistical analysis of data was done by using student's- t-test.

Results

The results of this study showed a significant elevation in basal (before treatment) erythrocyte MDA and lead levels with a significant reduction in TAS, hemoglobin and Zinc levels in lead exposed workers compared with their controls, (Table ١).

Two months treatment with melatonin significantly decrease erythrocyte MDA and lead levels with a significant increase in TAS, hemoglobin and Zinc levels (Table ١).

Discussion

People with higher lead levels have a greater risk of long-lasting health problems, and must be followed carefully.[٢٤] A complete recovery from chronic lead poisoning may take months to years. Symptoms resembling chronic intoxication may develop with over a period weeks or months. By removing or avoiding lead sources or with early detection and treatment, we can prevent or limit the harmful effects of lead poisoning.[٢٤]

Total antioxidant status (TAS) is an important marker of disease since ROS circulate freely in the body, and can have a serious repercussions throughout the body .The body utilizes antioxidant reserve to cope with free radicals, and monitoring antioxidants levels may be conductive to the early detection of disease. Therefore, a complete antioxidant profile may ensure accurate detection of variations in antioxidant levels caused by disease onset.[٢٥]

This study showed alterations in oxygen free radical scavenging process in the blood of patients with lead poisoning manifested by decreases body antioxidant defence mechanism (TAS, Hb and Zn) with an increase in oxidative stress markers (RMDA and Pb) (Table١), suggesting the presence of a generalized decrease in antioxidant status in the patients which may be attributed to the utilization of body antioxidant in neutralizing the increased endogenous free radicals which is produced by exposure to lead.

Melatonin is the most potent antioxidant known. It protects cells from free radicals damage, improves and enhances the functioning of the immune system, and delays the aging process. The antioxidant effect of melatonin has been shown to be much more potent than any of the well-known antioxidants (more than twice as effective as vitamin E at scavenging peroxyl radicals).[١١,٢٦] Melatonin has scavenging activity, which in turn decreases both oxidative damage and utilization of glutathione in neutralization phagocytes induced free radicals.[١٨]

Lead toxicity leads to FR damage via two separate, although related, pathways: (١) the generation of ROS, including hydroperoxides, singlet oxygen, and hydrogen peroxide, and (٢) the direct depletion of antioxidant reserves.[٢٧]

Lead perturbs multiple enzyme systems. As in most heavy metals, any ligand with sulfhydryl groups is vulnerable. Perhaps the best-known effect is that on the production of heme. Lead interferes with the critical phases of the dehydration of aminolevulinic acid and the incorporation of iron into the protoporphyrin molecule; the result is a decrease in heme production. Because heme is essential for cellular oxidation, deficiencies have far-reaching effects.[١,٢٨] Prophylactic effect of melatonin on lead-induced inhibition of heme biosynthesis and deterioration of antioxidant systems was stated in male rats.[١٨] The pre-administration of melatonin reduced the inhibitory effect of lead on both enzymatic and non-enzymatic antioxidants.[٢٩] This was accompanied by marked normalization of lipid peroxidation and modulation of copper and zinc levels in liver.

Two months treatment with melatonin (٣ mg capsule at night) corrected body antioxidant status in the patients which was manifested by increased body antioxidant defense mechanism (TAS and Zn) with a significant reduction in oxidative stress markers (RMDA and lead). The action of melatonin on lead-induced changes was attributed to protection of the antioxidant capacity in cells in addition to the ability of melatonin to scavenge free radicals,[٢٦] (Table١).

According to the results obtained by this study which suggested the potential clinical significant of antioxidants (melatonin) in improving oxidative stress markers in patients with lead poisoning and because of that when antioxidants were combined with chelating agents showed a synergism that improved chelating ability resulted in significantly higher urinary lead excretion.[١٨] So we recommend the use of melatonin in combination with a chelating agent by patients with lead poisoning.

Table ١: Case-control comparison of oxidative stress markers at base line level and after one and two month's treatment with ٣ mg melatonin antioxidant.

Variable	Control group N=٢٠	Treated group N=٢٠		
		Baseline	After ١ Month	After ٢ Months
Erythrocyte MDA (OD/g Hb)	٠.٢٠٠ ± ٠.٠٣٨	٠.٩٢٨ ± ٠.٠٥٥ *a	٠.٣٣٠ ± ٠.٠٥٥ *b	٠.١٦٧ ± ٠.٠٣٤ *c
Serum Zinc(µg/dl)	٩٤.٣٥ ± ٧.٢٩	٦٤.٢٥ ± ٤.٥٦ *a	٧٨.٣٥ ± ٥.٩٦ *b	٨٩.٥٠ ± ١.٨٧ *c
Plasma TAS(mMol/L)	١.٢٧ ± ٠.٣٤	٠.٦٠ ± ٠.٠٥ *a	٠.٨٣ ± ٠.٠٩ *b	١.٣٢ ± ٠.١٢ c
Serum Lead (µg/dl)	١٢.٠٤ ± ٠.٧٠	٥٨.٥٠ ± ١.٦٠ *a	٥٢.٣٤ ± ١.٤٩ *b	٤٣.٢٤ ± ٢.١٧ *c
Hemoglobin level (g)	٣٠.١٨ ± ٢.٣٥	٢٦.٠٠ ± ٢.٠٧ *a	٢٧.٢٢ ± ١.٨٨ *b	٢٩.٨٢ ± ١.٣١ c

- Data are presented as mean ± SD
- N= number of patients
- *P<٠.٠٥ with respect to control group.
- Non-identical superscripts (a, b, c) among the treated group considered significantly different, P<٠.٠١.

Refernces

١. Patrick L .Lead toxicity Part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Alternative Medicine Review. ٢٠٠٦.

٢. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology .٢٠٠٢; ١٨٠:٣٣-٤٤.

٣. Levin SM, Goldberg M. Clinical evaluation and management of lead-exposed construction workers. Am J Ind ٢٠٠٠;٣٧:٢٣-٤٣. Med

٤. Sies H. Oxidative stress: introductory remarks. In .Sies-H., ed. oxidative stress New York : Academic Press. ١٩٨٥,١-٨.

٥. Sies H. Oxidative stress: Introduction .In :oxidative stress and antioxidants, Sies-H. Academic Press, San Diego. ١٩٩١; p XV.

٦. Feri B, England L, Ames BN. Ascorbate in an outstanding antioxidant in human blood plasma.Proc.NaH.Acad.Sci.USA.١٩٨٩;٨٦: ٦٣٧٧-٦٣٨١.

٧. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Second edition. Oxford: Clarendon Press, ١٩٨٩.

٨. Andersen HR, et al. Antioxidative enzyme activities in human erythrocytes. Clinical chemistry.١٩٩٧; ٤٣(٤): ٥٦٢-٥٦٨.

٩. Reiter RJ, Tan DX, Manchester LC. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence .Cell Biochem Biophys ٢٠٠١;٣٤:٢٣٧-٥٦.

١٠. Maestroni GJ. The immunotherapeutic potential for melatonin. expert Opin Investig Drugs.٢٠٠١;١٠(٣):٤٦٧-٧٦.

١١. Reiter Mekhiorri D, Sewerynck E, Poeggeler BA. review of the evidence supporting melatonins role as William Fowkes . Smart Drugs II: The Next antioxidant.j Pineal Res.١٩٩٥;١٨(١):١-١١.

١٢. Ward Dean, John Morgenthaler, Steven Generation: New Drugs and Nutrients to Improve Your Memory and Increase Your Intelligence (Smart Drug Series, V. ٢). Smart Publications.١٩٩١; ISBN ٠-٩٦٢٧٤١٨-٧-٦.

١٣. Anisimov V, Alimova I, Baturin D, et.al . "Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice.". Exp Gerontol .٢٠٠٣; ٣٨ (٤):٤٤٩-٦١.

١٤. Oaknin-Bendahan S, Anis Y, Nir I, Zisapel N . "Effects of long-term administration of melatonin and a putative antagonist on the ageing rat.". Neuroreport .١٧٩٩٥;٦:٧٨٥-٨.

١٥. The ability of melatonin to influence oxidative stress and lower the dose of prednisolone in patients with alopecia areata.VOL ٣, NO ١ ٢٠٠٦; p٢٢. AJPS.

١٦. Gamal H. El-Sokkary, Gamal H.Abdel-Rahman and Esam S.Kamel. Melatonin protects against lead induced hepatic and renal toxicity in male rats. Toxicology .٢٠٠٥; volume ٢١٣, issues ١-٢, , pages ٢٥-٣٣.

١٧. Al-Gaff AN, Humadi S, Wohaieb SA. Effect of melatonin on oxidative stress markers in patients with alopecia areata .Iraqi Journal

Of Pharmacy;College of Pharmacy, Mosul. ٢٠٠٥; Vol ٥,No ١.

١٨. Flora SJ, Pande M, Kannan GM, Mehta A. Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. Cell Mol Biol(Noisy-le-grand) ٢٠٠٤;٥٠ :p٥٤٣-p٥٥١.

١٩. Stocks J, Dormandy TL. the auto-oxidation of human red blood cell lipids induced hydrogen peroxide.Brit-J-Hematol.١٩٧١; ٢٠:٩٥-١١١.

٢٠. Gilbert HS, Stump DD, Roth EF. A method to correct for errors caused by generation of interfering compounds during erythrocyte lipid peroxidation. anal-Biochem.١٩٨٤; ١٣٧: ٢٨٢-٢٨٦.

٢١. Burtis AC, Ashwood RE ,Eds; Tietz'' Text Book Of Clinical Chemistry''٣ ed edition W,B Sannders Company; London. ١٩٩٩;P٤٨٢-٤٨٤.

٢٢. Ismaiel DK. Effect of zinc and allpurinol in ameliorating oxidative stress in lead –exposed workers. Iraqi Journal of Pharmacy, College of Pharmacy, Mosul. ٢٠٠٥; Vol ٥,No ١.

٢٣. Drabken DL, Austen-JH. Spectrophotometer studies II preparation from washed blood cells : nitric oxide hemoglobin and sulfhemoglobin. J- Biol-Chem. ١٩٣٥; ١١٢:٥١-٦٥.

٢٤. Bleecker ML, et al. Differential effects of lead exposure on components of verbal memory. Occup Environ Med. ٢٠٠٥ Mar; ٦٢(٣):١٨١-٧.

٢٥. Ashwaq N.A. Evaluation of oxidative stress indicators in lymphocytes of patients with alopecia areata. Ph.D thesis, ٢٠٠٥.

٢٦. Riter RJ, etal. Melatonin as free radical scavenger: implications for aging and age related disease. Ann NY Acad Sci. ١٩٩٤;٣١,٧١٩:١-١٢.

٢٧. Ercal N, Gurer-Orhan H, Aykin Burns N. Toxic metals and oxidative stress. Part ١. Mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. ٢٠٠١;١:٥٢٩-٥٣٩.

٢٨. Geetu Saxena SJS, Flora. Lead –induced oxidative stress and hematological alterations and their response to combined adminsteration of calcium disodium EDTA with a thiol chelator in rats.Journal of Biochemical and Molecular Toxicology. Volume ١٨, issue ٤, pages ٢٢١-٢٣٣.

٢٩. Mohammed A El-missiry. Prophylactic effect of melatonin on lead –induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rates. Journal of Biochemical and Molecular Toxicology.١٩٩٩; volume ١٤, issue ١,pages ٥٧-٦٢.