

Effects of Treatment with Zinc Sulphate on the Oxidative Stress State during Chronic Exposure to Lead in Humans

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الخلاصة

حديثاً تم الإثبات بان الإجهاد التأكسدي من ضمن العوامل المسببة في التسمم بالرصاص ، وتبعاً لذلك فقد برزت الفعالية العلاجية والوقائية لمضادات الأوكسدة. كرسست هذه الدراسة لإظهار الفعالية الوقائية لدى الزنك على الإجهاد التأكسدي المستتار بزيادة التعرض للرصاص. لقد تم إعطاء العاملين المتعرضين للرصاص سلفات الزنك بجرعة 100 ملغم يومياً لمدة شهرين ، وقد تم تقييم الإجهاد التأكسدي وذلك بقياس المالوندايلديهيد (MDA) والكلاتاتايون (GSH) في خلايا الدم الحمراء وفي بلازما الدم إضافة إلى ذلك تم قياس مستوى البروتين الكلي ، الألبومين ، الهيموكلوبين ، PCV. في هذه الدراسة أيضاً تم قياس مستويات بعض الفلزات الزهيدة لمقدار (الرصاص، النحاس، الزنك) في عينات دم العاملين في المسبك. ان التحليل الإحصائي للنتائج باستخدام الزنك اظهر نقصان ملحوظ في معاملات أكسدة الدهون المالوندايلديهيد (MDA) وزيادة ملحوظة في مستوى الكلاتاتايون في خلايا الدم الحمراء والبلازما عند العاملين بسبب التعرض المزمن للرصاص. كما أن العلاج باستخدام الزنك أدى إلى انخفاض ملحوظ في مستوى الرصاص بدم العمال المتعرضين مصحوباً بتحسين بمستوى الزنك والنحاس. مما سبق نستنتج أن استخدام الزنك يومياً للعاملين المتعرضين للرصاص قد يكون ذو نفع كبير في تقليل الإجهاد التأكسدي المصاحب للتعرض المزمن للرصاص.

ABSTRACT

Oxidative stress has been recently implicated in the pathogenesis of lead poisoning. Consequently, the potential protective effects of antioxidant agents had been raised. This study was designed to explore the potential protective effects of zinc against the oxidative stress due to chronic exposure to lead. Lead-exposed workers were treated with 100mg zinc sulphate / day for 2 months, and the extent of oxidative stress was evaluated by measuring of erythrocytes and plasma content of GSH and MDA, in addition the Cu, Zn and Pb blood level were measured also. The results of this study showed that treatment with zinc sulphate significantly lowered MDA production and increased glutathione levels in plasma and erythrocytes. Blood lead levels, which were elevated significantly due to chronic exposure, showed a reproducible reduction after treatment with zinc sulphate, associated with improvement in copper and zinc levels in the plasma of lead workers. In conclusion, daily supplementation with zinc as antioxidant to lead exposed workers seems to be beneficial in the prevention of oxidative stress associated with chronic exposure.

INTRODUCTION :

Lead is the most ubiquitous toxic metal, and is particularly detectable in all phases of the environmental conditions and mostly all biological systems⁽¹⁾. Because of it's toxicity to most living organisms, the major issue regarding lead is the determination of the toxic dose, and the major systems at high risk of toxicity⁽²⁾. Lead alters the integrity of the cellular and mitochondrial membranes, thereby, increasing cellular fragility and facilitates degenerative processes⁽³⁾, also interferes with many enzyme systems in the body, thereby affecting the functions of most organs⁽⁴⁾. Clinical manifestations of lead toxicity include symptoms referable to the central and peripheral nervous systems, hematopoietic, renal and gastrointestinal systems⁽⁵⁾.

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Generation of reactive oxygen species (ROS) due to exposure to lead, even when not undergo valence changes, may be attributed to not fully understood mechanism, which may involved during the course of lead exposure, with consequent emergence of oxidative stress⁽⁶⁾. Lead was found to have no pro-oxidant catalytic activity with respect to lipid peroxidation, but recently, marked elevation in MDA production was observed as a result of incubation of lead with poly unsaturated fatty acids in vitro and in vivo systems⁽⁷⁾. Therefore induction of ROS by lead and subsequent depletion of antioxidant cell defenses can result in generalized disruption of the prooxidant/antioxidant balance in lead-burdened tissues. This could contribute to tissue injury via oxidative damage to critical biomolecules⁽⁸⁾. Many literatures supported that zinc is an antioxidant that hinders the free radical reactions⁽⁹⁾. It exerts its effects in an indirect manner, by protecting the cell from damaging effect of oxygen radicals⁽¹⁰⁾. Accordingly this study was designed to evaluate the possible protection role of zinc against lead exposure induced oxidative stress in lead markers.

MATERIALS AND METHODS:

This study was carried out on thirty adult male workers age range (35.8±6.9) of Iraqi Smelter Plant in Khan Dhary city, during the period of (January 6, 2002 to April 12, 2002). These workers were selected on the basis that they were in direct exposure to lead and have been employed for at least 1 year before the investigation was carried out. The daily exposure to lead of each worker should be at least 6-8 hrs, and the total period of exposure range from 1-25 years. Additionally twenty healthy subjects were selected to serve as controls with the same age group compared with lead exposed group. All workers included in the study received 100mg single daily dose of zinc sulphate for two months. Blood samples were collected from each subject (10ml) before starting treatment (zero time) and after 30 and 60 days of treatment for the determination of plasma and erythrocytes levels of glutathione (GSH) and malondialdehyde (MDA), plasma levels of zinc and copper. The glutathione content of erythrocytes and plasma were measured by weighing 0.1gm of packed RBCs or 0.5ml of fresh plasma sample and mixed with 0.1ml D.W and 0.65ml of 1m M Na₂ EDTA in 5% TCA. After centrifugation at 3000g for 5-7 min. at 4°C, 0.3ml of supernatant was added in a tube containing 2.6ml of phosphate buffer (pH of 8.0), then we add 0.1ml 5, 5 Dithiobis (2-nitro benzoic acid) Solution (3mM DTNB) in 0.1M phosphate buffer pH of 8.0, the assay mixture was then ready for determination of GSH spectrophotometrically within 2 minutes at 412nm. The concentration of GSH was determined according to a standard curve of GSH, prepared for this purpose, and the results were expressed as μ mole GSH/gm Hb for erythrocytes and as μ mole GSH/L for plasma⁽¹¹⁾. Measurement of erythrocyte and plasma-MDA levels, which are an index of lipid peroxidation, are based on the reaction with thiobarbituric acid (TBA) forming TBA₂-MDA adducts. The method included the preparation of one ml aliquot of 10% suspension of the packed red blood cells in 1.9ml saline azide (2mM sodium azide in 0.9% sodium chloride solution), or 0.25ml of plasma was incubated with 1.75ml of saline azide. After that 1.0ml of 0.1M sodium arsenite in 28% trichloroacetic acid solution was added. The mixture was then centrifuged and aliquot of 2.0ml of the supernatant was mixed with 0.5ml distilled water and 0.5ml TBA solution in 0.05M sodium hydroxide. The mixture was incubated in a boiling water bath for 15min. to achieve color development. After cooling (under tap water) the absorbance of light was determined at 532 and 453nm. The results were expressed as nmol MDA/gm Hb for erythrocytes and μmol MDA/L for plasma⁽¹²⁾. For the measurement of plasma copper levels, the plasma sample is diluted with an equal volume of deionized water and directly aspirated into the atomic absorption instrument, and the amount of copper was calculated through comparison with standard copper sulphate solution treated in the same manner. In case of zinc determination, the plasma sample was diluted 5 folds with deionized water and aspirated into the atomic absorption spectrophotometer. Calculations were done through comparison with standard zinc chloride solution prepared for this purpose⁽¹³⁾.

Blood lead levels were measured by using atomic absorption spectrophotometer, using the slotted quartz tube method .five ml. of blood was mixed with 1ml 10% TCA, after vigorous shaking the mixture was centrifuged at 3000 rpm for 10 min., then supernatant was taken for measurement of lead at 283.2nm.⁽¹⁴⁾. Statistical analysis of data was performed utilizing Student`s t-test and analysis of variance (ANOVA). P values less than 0.05 was considered significant.

RESULTS:

The data presented in (table-1) showed that MDA levels in erythrocytes and plasma of lead workers were elevated significantly compared to controls ($p<0.001$), and treatment with 100mg/day p.o zinc sulphate resulted in significant reduction in MDA levels in both compartments after 1 and 2 months of treatment ($p<0.05$). However MDA production was found to be increased again after 1 month of stopping treatment, reaching values which were still lower than those observed before starting treatment, but still significantly higher than control values. In (table-2), glutathione levels in erythrocytes and plasma of lead workers were found to be significantly lower than those of controls ($p<0.05$); and these levels significantly elevated after 1 month and 2 months of treatment with zinc sulphate. One month of stopping treatment, glutathione levels in both compartments were still significantly higher than before treatment levels and comparable to controls. In (table-3), chronic exposure of workers to lead resulted in significant increase (315%, $p<0.05$) in blood lead levels compared to controls; and treatment with 100mg/day p.o zinc sulphate resulted in a time-dependent decrease in these elevated levels, which were significantly different compared to pre-treatment values ($p<0.05$), but still significantly higher than those observed in controls. Concerning plasma copper levels, (table-3) showed that exposure to lead lead to significantly lower plasma copper values compared to controls; and treatment with zinc sulphate for 2 months produced also significant, time-dependent elevation in plasma copper levels, but still did not match those belongs to controls. The effects of exposure to lead are found to produce significant reduction in plasma zinc levels ($p<0.05$) compared to controls. Treatment with single daily dose p.o of 100mg/day zinc sulphate resulted in significantly, time dependent elevation in plasm zinc levels, which were found to be comparable to those found in controls after 2 months of treatment (table-3).

Table 1 . Effects of treatment with 100 mg zinc sulphate/day on plasma and erythrocytes MDA levels in lead exposed workers.

Subjects groups	n	Malondialdehyde (MDA)	
		Erythrocytes nmol/g Hb	Plasma $\mu\text{mol/L}$
Control	20	7.7+ 0.46	0.96+ 0.15
Before treatment	18	29.66+ 2.65*	3.08+ 0.2 *
After 1 month treatment	18	14.6+ 1.6 *•	1.84+ 0.26 *•
After 2 months treatment	18	8.8+ 1.82 *•#	0.98+ 0.17 •#
After 1 month stopping treatment	18	12.6+2.9 *•	1.6+ 0.3 *•

Each value represent mean + SD

n= Number of subjects

* Significantly different with respect to control ($p<0.05$)

• Significantly different with respect to pretreatment ($p<0.05$)

Significantly different with respect to one month treatment ($p<0.05$)

Table 2 . Effects of treatment with 100 mg zinc sulphate/day on plasma and erythrocytes Glutathione (GSH) levels in lead exposed workers.

Subjects groups	n	Glutathione (GSH)	
		Erythrocytes $\mu\text{mol/g Hb}$	Plasma $\mu\text{mol/L}$
Control	20	12.01+0.75	0.83+ 0.07
Before treatment	18	5.92+ 0.38 *	0.114+ 0.03 *
After 1 month treatment	18	9.21+ 0.53 *•	0.63 + 0.04 *•
After 2 months treatment	18	11.1+ 0.9 •#	0.86+ 0.05 •#
After 1 month stopping treatment	18	10.65+ 0.97 *•	0.82+0.04 •

Each value represent mean + SD

n= Number of subjects

* Significantly different with respect to control (p<0.05)

• Significantly different with respect to pretreatment (p<0.05)

Significantly different with respect to one month treatment (p<0.05)

Table 3 . Effects of treatment with 100mg zinc sulphate/day on blood lead, plasma copper and plasma zinc levels in lead exposed workers.

Subjects groups	n	Blood Lead levels $\mu\text{gm/dI}$	Plasma Copper levels $\mu\text{gm/dI}$	Plasma Zinc levels $\mu\text{gm/dI}$	Cu:Zn
Control	20	12.60 \pm 1.5	99.3 \pm 10.86	92.55 \pm 12.68	1.07
Before treatment	18	54.9 \pm 13.6 *	69.67 \pm 8.73 *	78.06 \pm 7.45 *	0.89
After 1 month treatment	18	44.9 \pm 11.6 *•	74.33 \pm 6.68 *•	87.56 \pm 6.93 •	0.85
After 2 months treatment	18	36.78 \pm 9.95 *•#	85 \pm 6.65 *•#	96.83 \pm 6.72 •#	0.88
After 1 month stopping treatment	18	44.1 \pm 10.3 *•	81.17 \pm 6.84 *•	87.28 \pm 5.58 •	0.93

Each value represent mean + SD

n= Number of subjects

* Significantly different with respect to control (p<0.05)

• Significantly different with respect to pretreatment (p<0.05)

Significantly different with respect to one month treatment (p<0.05)

DISCUSSION:

Generation of highly reactive oxygen species (ROS), such as hydroxyl radical, hydrogen peroxide, superoxide anion and lipid peroxide after exposure to lead, might result in systemic solubilization and depletion of the intrinsic antioxidant defenses of the cell. The reported data in this study indicated that a state of oxidative stress was initiated in lead exposed workers, manifested by the increased erythrocytes and plasma MDA levels and depletion of GSH in both compartments (table 1 and 2); and these results are compatible with those observed by others^(15,16). The mechanism through which lead causes its deleterious effects has yet to be elucidated; however, part of these effects may be due to direct effects on cell membrane structures and functions⁽¹⁷⁾. Erythrocytes membrane show a high sensitivity and more vulnerable to the oxidative damage of lead⁽¹⁸⁾. Besides the involvement in the generation of ROS, lead can induce oxidative stress by increasing vulnerability of membranes to the attack by ROS. Lead is shown to inhibit several enzymes having functional thiol groups, including glutathione reductase which is responsible for regeneration of reduced glutathione(GSH) from oxidized form⁽¹⁹⁾; this inhibition results in decreased GSH: GSSG ratio that might render cells more susceptible to oxidative damage. Zinc has never been shown to interact directly with an oxidative species, but rather prefer to exert its effects through an indirect manner⁽²⁰⁾. This has been shown to antagonize the catalytic properties of the redox active transition metals, iron and copper⁽²¹⁾.

Consequently, the effects of daily treatment with single dose zinc sulphate (100mg p.o) in lead-exposed workers lead to significant, time dependent improvement of oxidant stress, revealed by decreasing MDA production and elevation of GSH levels in plasma and erythrocytes (tables 1 and 2). These results are compatible with those observed by Flora and coworkers⁽²²⁾. There is substantial evidence that metal catalyzed formation of hydroxyl radicals predispose to most of the destructive processes during oxidative stress⁽²³⁾. Chronic exposure to lead resulted in significant increase in its concentration in blood (table-3), a result which is found compatible with those reported by others⁽²⁴⁾. An increase in the absorption of lead in lead exposed workers has been recorded to blood levels of more than 40µg/100ml⁽²⁵⁾. Lead workers included in this study have high blood lead levels which may be mostly due to exposure to lead in the work environment through direct contact and aspiration of fumes resulting from melting lead at high temperature. The data presented in (table-3) also demonstrated decrease in plasma levels of Cu and Zn in lead exposed workers, and this finding is compatible with those reported by others^(26,15). Lead and zinc interaction are not as well defined as those between lead and iron. It has been shown experimentally that lead increases zinc excretion and that zinc deficiency enhances lead absorption⁽²⁷⁾; and oral administration of zinc sulphate after chelation therapy has been found to significantly improve the activities of zinc- dependent enzymes, including δ-aminolevulinic acid dehydratase and superoxide dismutase, required for maintenance of RBC integrity and functions^(28,29). Meanwhile, inhibition of Cu-Zn superoxide dismutase by lead can lead to decrease scavenging of ROS and result in oxidative damage⁽³⁰⁾. Daily supplementation with 100mg/day zinc sulphate p.o during the study produces significant increase in plasma zinc and copper levels (table-3). Although zinc competes with copper at the absorption site, plasma copper levels increase could be attributed to the high antioxidant effects of zinc. In conclusion, Zinc sulphate as an adjuvant therapy can be successfully improve the antioxidant status and indirectly ameliorate blood lead levels in lead exposed- workers.

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