Effects of zinc and allopurinol in ameliorating oxidative stress in lead-exposed workers

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Abstract

Background: Oxidative stress has been recently implicated in the pathogenesis of acute and chronic exposure to lead. Consequently, the potential role of using antioxidants of various types to provide protective effects became a major task in this respect.

Objective: This study was designed to explore the potential antioxidant effects of zinc and allopurinol in ameliorating the oxidation stress induced due to chronic exposure to lead.

Methods: Twenty-four male workers, chronically exposed to lead, were enrolled in the study and treated with a single daily dose of ٥٠ mg zinc sulfate and ١٠٠ mg allopurinol for ٢ months. Erythrocyte and plasma MDA, GSH; blood lead, plasma copper and zinc level were measured each month during treatment and one month later after termination of treatment. Only eighteen workers completed the study.

Results: During treatment, zinc and allopurinol significantly reduced excessive MDA production and elevated GSH level in association with decreasing blood lead level and improving the picture of the essential trace elements, copper and zinc in the plasma which were previously altered as a result of lead exposure.

Conclusion: the use of antioxidants like zinc and allopurinol successfully eliminated the oxidative consequences of lead exposure, and the treatment should be continuously maintained as long as there is exposure to lead.

Key words: Lead toxicity, oxidative stress, zinc, allopurinol, trace elements.

الخلاصـــة

الخلفيـة العلميـة: لقد أصبح معلوماً دور ظاهرة فرط الأكسدة في ميكانيكية حدوث الضرر عند التعرض الحاد والمزمن للرصاص وتبعاً لذلك برزت إمكانية استخدام مضادات الأكسدة لأغراض الوقاية والعلاج في مثل هذه الحالات .

الهدف: تم تصميم هذه الدراسة لإظهار فعالية كل من الخارصين والالوبيورينول في الحد من ظاهرة فرط الإجهاد التاكسدي المستثار بالتعرض المزمن للرصاص عند العمال الذين يعملون في تصنيعه .

طرق العمل : تم اختيار أربع وعشرون من العمال المتعرضين للرصاص بشكل مزمن ومعالجتهم بجرع يومية تتألف من ٥٠ ملغم من كبريتات الخارصين و ١٠٠ ملغم من مادة الالوبيورينول لمدة شهرين وتم تقييم الإجهاد التاكسدي من خلال قياس مستوى MDA و GSH في كريات الدم الحمراء والبلازما وكذلك تم قياس مستوى الرصاص في الدم ومستوى العناصر النزره (الخارصين والرصاص) في بلازما الدم . شارك في الدراسة إلى نهايتها ثمانية عشر عاملا فقط .

النتائج: لقد تحقق من خلال العلاج بمضادات الأكسدة المستخدمة في الدراسة خفض ملحوظ لمستوى تكوين MDA وارتفاع في مستوى GSH في البلازما وكريات الدم الحمر وكان متزامناً مع انخفاض كبير في مستوى الرصاص في الدم مع تحسين صورة العناصر النزرة الخارصين والنحاس في بلازما الدم والتي كانت قد تأثرت بالأساس نتيجة للتعرض المزمن لمركبات الرصاص .

الاستنتاجات: إن استخدام مضادات التأكسد مثل الخارصين والالوبيورينول تؤدي بنجاح إلى الحد من ظاهرة فرط الإجهاد التاكسدي وتبعاتها نتيجة للتعرض المزمن للرصاص لدى العاملين في تصنيعه ، وان هذا الاستخدام يجب أن يكون بشكل منظم طالما كانت حالة التعرض لهذا العنصر السام مستمرة .

Introduction

Lead has no pro-oxidant catalytic activity with respect to peroxidation[١] but recently, it was demonstrated that marked enhancement in malondialdehyde (MDA) production was observed as a result of incubation of lead with polyunsaturated fatty acids[٢]. This finding was proved *in vivo* by other investigators, who have pointed to either lipid peroxidation decreased intrinsic antioxidant defenses in various tissues of lead-exposed animals[٣]. Furthermore, direct relationship was observed between the tissue concentrations of lead and the rate of lipid peroxidation in various tissues especially the brain[٤]. Therefore induction of free radicals formation by lead, and subsequent depletion of antioxidant defenses of the cell can result in generalized disruption of the proxidant / antioxidant balance in lead burdened tissues.

Many literatures support that zinc is an antioxidant that hinders free radical reaction[٥], and exerts protective role to the cells from the damaging effects of oxygen radicals[٦]. The acute antioxidant effects of zinc are generally manifested in the presence of demonstrable short term increase in the levels of this metal, and this basically can be conducted through two mechanisms, sulfhydryl stabilization and reduction in the formation of hydroxyl radicals ($OH^{\cdot}$) from superoxide anion and hydrogen peroxide (H_2O_2) through antagonism of redox-active transition metals like iron and copper[٧]. Another available approach is to prevent free radical generation by the enzyme system xanthine oxidase (XO), by the use of the XO inhibitor allopurinol[٨]. This project was designed to evaluate the possible synergetic effect, which may be gained from the use of combination of zinc sulfate and allopurinol when used in the treatment of the toxic consequences of lead exposure especially in lead processing factories.

Subjects and methods

This study was carried out on ٢٤ adult male workers with mean age (٣٤-٩±٧.٤) years, in the Iraqi smelter plant in Khan Dhary sub district-Baghdad; they were selected 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 ١٨ workers completed the study. Additionally twenty healthy subjects were selected to serve as controls. Lead exposed workers received a daily dose of ٥٠-mg zinc sulfate in combination formula with ١٠٠ mg Allopurinol for two months.

Venous blood samples (١٠ ml) were taken by venepuncture from each worker before starting treatment (as baseline sample), after one month and two months during treatment, and one month later after termination of treatment. Blood samples were placed in two heparinized tubes, the first one utilized for lead level analysis, and the second one is used for separation of plasma and erythrocytes fractions for the measurement of other parameters.

Erythrocytes and plasma MDA levels were measured according to the method of Stocks and Dormandy (١٩٧١)[٩] as modified by Gilbert *et al* (١٩٨٤)[١٠]. Erythrocytes and plasma GSH levels were measured according to the method of Godin and Wohaieb method (١٩٨٨)[١١].

Plasma copper levels were measured by atomic absorption spectrometry according to Taylor and Bryant method (١٩٨١)[١٢] , and the same technique was utilized for the measurement of plasma zinc levels according to Taylor and Briggs method (١٩٨٦)[١٣] Blood lead levels were measured by atomic absorption spectrometry according to Brown *et al* (١٩٨٩)[١٤] , and blood hemoglobin content was assayed according to the method of Drapkin and Austin (١٩٣٥)[١٥].

Statistical analysis of data was performed using Student's t-test and the

significance level was considered at p value less than ٠.٠٥.

Table (١): *Effects of treatment with ٥٠ mg zinc sulfate + ١٠٠ mg allopurinol/ day on erythrocytes and plasma MDA and GSH levels in lead-exposed workers.*

Subjects Groups	N	Malondialdehyde (MDA)		Glutathione (GSH)	
		Erythrocyte µmol/g Hb	Plasma µmol /L	Erythrocyte µmol/g Hb	Plasma µmol /L
Control	٢٠	٧.٨٧^{a} ± ٠.٨٦	٠.٩٦ ^{a}± ٠.١٥	١٢.٠٧ ^{a} ±٠.٧٥	٠.٨٣^{a}± ٠.٠٧
Before Treatment	١٨	٢٧.٩٥^{b}± ٢.١٨	٣.١^{b} ± ٠.٢٩	٦.١^{b} ± ٠.٤٦	٠.١١^{b}± ٠.٠٣
After ١ month treatment	١٨	١٢.٦^{c} ±١.٤	١.٤٦^{c} ± ٠.٢٢	٧.٨^{b} ± ٠.٦٧	٠.٥٤^{c}± ٠.٠٥
After ٢ month treatment	١٨	٧.٢^{a} ±٠.٣٧	٠.٧^{a}± ٠.٠٩	١٠.٢^{c}± ٠.٩٦	٠.٧٦^{a}± ٠.٠٧
١month after the end of treatment	١٨	٩.٤^{d} ± ٠.٩	١.٣^{c} ± ٠.٢	٩.٠^{c}± ٠.٩٤	٠.٧^{a}± ٠.٠٥

* Each value represent mean ± S.D.
* N=Number of subjects.
* Values with non-Identical superscripts (a,b,c,d) are significantly different (p<٠.٠٥).

Table (٢): *Effects of treatment with ٥٠ mg zinc sulfate + ١٠٠ mg Allopurinol/ day on Blood Lead levels, Plasma copper and zinc levels in lead-exposed workers.*

Subjects Groups	N	Blood Lead levels µg /dl	Plasma Copper levels µg /dl	Plasma Zinc levels µg /dl
Control	٢٠	١٢.٨٥^{a} ± ١.٩٣	٩٩.٣^{a} ± ١٠.٨	٩٢.٥٥^{a} ± ١٢.٦
Before Treatment	١٨	٦٠.١^{b} ± ٨.٩	٧٠.٨٦^{b} ± ٣.٣	٧٧.٨٦^{b} ± ٤.٨
After ١ month treatment	١٨	٥٢.٠^{b} ±٧.١٤	٧٨.٥٧^{c} ± ٢.٣	٨١.٤٣^{b} ± ٦.٥
After ٢ month treatment	١٨	٤٤.٧^{c} ± ٩.٤	٩٠.٥٧^{a}± ٥.٦	٩٢.٢٩^{a} ± ٥.٨
١month after the end of treatment	١٨	٤٨.٢٩^{c} ± ١٠.٥	٨٨.٥٧^{a} ± ٣.٩	٨٠.٥٧^{b}± ٤.٤

* Each value represent mean ± S.D.
* N= Number of subjects.
* Values with non-identical superscripts (a,b,c) are Significantly different (P<٠.٠٥).

Results

The results presented in table (١) showed a highly significant elevation in erythrocytes and plasma MDA content in lead-exposed workers (٢٥٥% and ٢٢٣% respectively) compared with controls.

After ١ month of treatment with ٥٠-mg zinc sulfate and ١٠٠ mg allopurinol, significant reduction in the MDA content in erythrocytes (٥٥%) and plasma (٥٤%) was observed compared to pretreatment levels, but even with these levels, the values were still significantly higher than controls. Further reduction in MDA level in both compartments was observed (٧٤% and ٧٧% respectively) comparable with pretreatment levels, and found to be comparable to those in control subjects (Table ١). One month after termination of treatment, MDA levels in both compartments started to increase again reaching values which were significantly different with respect to controls (٢٠% and ٣٥% in both compartments respectively.

Concerning the effects of lead exposure on GSH levels in erythrocytes and plasma of the workers, table (١) demonstrated severe depletion of GSH in both compartments (٤٩% and ٨٦% respectively), which were significantly different compared to controls. After one-month treatment, GSH levels were elevated in plasma only (٣٩٠%) compared to pre-treatment value.

After ٢ months of treatment, GSH levels were increased significantly in both compartments, compared with pre-treatment values (٦٧% and ٥٩٠% respectively) and considered to be comparable to controls in plasma while significantly higher than controls in the erythrocytes. One month after terminations of treatment, no significant changes were observed in GSH content in both compartments, compared to the period of ٢ months of treatment.

Chronic exposure of workers to lead resulted in an increase in blood lead levels (٣٦٧%) which was highly significant ($P<٠.٠٠١$) compared to controls (Table ٢). Treatment with a daily dose of ٥٠-mg zinc sulfate and ١٠٠ mg allopurinol resulted in a significant reduction ($P<٠.٠٥$) in blood lead level only after ٢ months of treatment (٢٦%) compared to pre-treatment value. Termination of treatment resulted in a reversible increase in blood lead, but still significantly lower than that of pre-treatment period (Table ٢).

Elevated blood lead was associated with significant decrease in both plasma copper and zinc levels (٢٩% and ١٦%) compared to controls. Meanwhile, treatment with zinc sulfate and allopurinol resulted in a significant increase in plasma copper level after ١ month (١١%) and in plasma zinc after ٢ months (١٨%) compared to pre-treatment values (Table ٢). Termination of treatment resulted in a reversible decrease in plasma zinc only (١٣%) compared to controls, which is previously normalized as a result of treatment, while plasma copper remain unchanged (Table ٢).

Discussion

Generation of highly reactive oxygen species, such as hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide ($H_٢O_٢$), superoxide anion ($O_٢^{\cdot-}$) and lipid peroxides after chronic exposure to lead, may result in systemic mobilization and depletion of the intrinsic antioxidant defenses of the cells, which consequently predispose to a state of oxidative stress (٢). The condition that the data presented in table (١) clearly shown in lead exposed workers, manifested by increased erythrocytes and plasma MDA levels, which are compatible with the observations of others(١٦-١٨); and depletion of GSH in both compartments which other researchers (١٩-٢٠) also observe.

The mechanism by which lead causes it's deleterious effects in this respect has yet to be elucidated; however, part of it's effect may be attributed either to direct effects on cell

membrane structures and functions [٢١], where red blood cell membrane is found to be highly vulnerable to the oxidative damage of lead[٢٢]; or to the blocking of the enzyme glutathione reductase which is responsible for reversible recycling of the oxidized form of glutathione (GSSG) into the reduced form (GSH)[٢٣], this effect may result in decreased GSH:GSSG ratio that will render cells more susceptible to oxidative damage.

The effects of daily doses of zinc sulfate (٥٠ mg) and allopurinol (١٠٠ mg) on the oxidative stress parameters (MDA and GSH) presented in table (١), demonstrated a significant reduction in MDA contents and elevation of GSH levels, which are previously impaired as a result of chronic exposure to lead. These effects clearly explain the antioxidant properties of zinc and allopurinol, which are previously reported in other condition [٢٤]. Zinc has never been shown to interact directly with an oxidant species, but rather prefer to exert it's effect in an indirect manner [٢٥], while allopurinol perform this effect through both directly and indirectly blocking xanthine oxidase [٢٦].

Chronic exposure to lead resulted in a significant increase in it's blood levels (Table ٢), an observation found by others too [٢٧,٢٨]. The toxic effect of lead may be mediated or enhanced by the interactions or deficiencies of nutritionally essential metals like Zn and Cu. Lead and zinc interaction are not well defined as those well defined between lead and calcium or iron. It has been shown experimentally that lead increases zinc excretion and that zinc deficiency enhances lead absorption [٢٩], the effect which is found to be reversed as result of treatment with zinc sulfate and allopurinol (Table ٢) .

Mylorie *et al* (١٩٨٦)[٣٠] have suggested an indirect inhibitory effect produced by elevated blood lead levels on the activity of erythrocytes Cu-Zn-SOD *in vivo*, and found to be due to lead – induced copper deficiency. Inhibition of SOD activity by lead was also observed in an *in vitro* study; and this effect was attributed to decreased scavenging of reactive oxygen species (ROS) which consequently predispose to oxidative damage [٣١]. The observed improvement zinc and copper plasma levels (Table ٢) as a result of treatment with zinc sulfate and allopurinol very well correlates the role of those compounds in the attenuation of the oxidative stress observed in Table ١. In conclusion, the lead-induced oxidative stress after chronic exposure can be successfully interfered with antioxidants like zinc and allopurinol especially why they are regularly administered in fixed daily doses.

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