DISTURBED HOMEOSTASIS OF SOME INORGANIC ELEMENTS ASSOCIATED WITH CHRONIC EXPOSURE TO LOW LEVELS OF BENZENE AND POSSIBLE ASSOCIATED HEALTH HAZARDS

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Abstract
Objective: Moderate and long-term exposures to benzene carry the risk of numerous health problems. Involvement of inorganic elements in such hazards has been suggested, but their role in problems resulting from occupational exposure to low-level benzene has not been reported.
Methods: An all male cohort of 60 healthy control subjects and 180 individuals occupationally chronically exposed to low levels of benzene in their daily activity was enrolled in the study. Nineteen elements were determined in plasma and RBCs.
Results: Higher levels of lead, mercury and cadmium were found in the plasma and RBCs of benzene-exposed workers, while the levels of zinc, selenium and copper were lower. Cobalt showed only a small but significant increase. There were no significant differences between other assayed elements in exposed workers and control subjects. Exposure to benzene was found to cause oxidative stress with significant elevation of plasma MDA level and decrease in total antioxidant activity. There was also a tendency for a higher degree of hemolysis in blood samples.
Conclusion: All of the accumulated metals are proposed to contribute to oxidative stress by different mechanisms. They are either redox-active, directly involved in the production of free radicals, or are redox-inert, and contribute to oxidative stress by inhibiting antioxidant defense. The effect of excessive production of free radicals on RBCs membrane may explain the tendency for hemolysis in blood samples from benzene-exposed subjects. Metals with decreased concentrations may cause other metabolic disturbances and accelerate free radical production, probably through decreased participation in antioxidant protection.

Keywords: low level benzene contamination, metal homeostasis, oxidative stress

Introduction:
Moderate and long-term exposure of humans to benzene may cause numerous untoward effects. The most serious health hazards in these cases appear to be of hematologic, immunologic, genetic and malignant nature (Savitz & Andrews, 1997; Rimsky, et al., 1981; Aksoy, 1988; Cooper & Snyder, 1988). Although the involvement of trace metals in such conditions has been suggested (Valco, et al., 2005), disturbances in trace elements homeostasis and their contribution to health hazards resulting from chronic exposure to benzene have not been documented.
Metals and other inorganic elements play important roles in a wide variety of biological processes of living systems. Several essential transition elements, such as zinc, magnesium, iron, copper, cobalt and manganese participate in the control of various metabolic and signaling pathways. However, their rich coordination chemistry and redox properties are such that they are capable of escaping out of the control mechanisms, such as homeostasis, transport, compartmentalization, and binding to the designated tissue and cell constituents. Breakdown of these mechanisms has been involved in a large variety of diseases (Halliwell & Gutteridge, 1990; Halliwell & Gutteridge, 2007; Jomova & Valko, 2011), as it may lead to the metal binding to protein sites other than those designated for that purpose or to displacement of other metals from their natural binding sites (Valco, et al., 2005; Nelson, 4999).

Disruption of metal homeostasis is known to modulate gene expression by interfering with signal transduction pathways. This action may lead to uncontrolled metal-mediated formation of free radicals participating in the modification of DNA bases, enhanced lipid peroxidation and altered sulfhydryl homeostasis (Gutteridge, 1995; Valko, et al., 2007). Metals may interfere with deregulation of cell proliferation by activating various transcription factors controlling cell cycle progression and apoptosis (Évan & Vouisden, 2001), and play important roles in cell growth and development (Valko, et al., 2006). Humans may be exposed to redox-inert elements such as cadmium, and arsenic, which have no known biological function and are even known to be toxic at low concentrations. Exposure to these elements arises from a variety of natural sources, including air, drinking water and food. While redox-active metals undergo redox-cycling reactions, the primary route of toxicity and carcinogenicity for the group of redox-inert elements is depletion of glutathione, bonding to sulfhydryl groups of proteins and other mechanisms of action (Speisky, et al., 2008; Sinicropi, et al., 2010; Peralta-Videa, et al., 2009).

Searching available literature for disturbances in redox-active or redox-inactive metals as a result of chronic exposure to low level benzene was negative. Whether some of the deleterious effects of chronic exposure to benzene in the environment could be attributed, at least in part, to disruption of metal homeostasis has not been reported. The present study was undertaken to investigate the possible disturbance in homeostasis of some elements and their distribution between plasma and erythrocytes as a consequence of chronic exposure to benzene-contaminated work environment. The relation between the changes in metal homeostasis and oxidative stress was also considered.

**Subjects, Material and Methods:**

An all-male cohort of 240 individuals, from different districts of Alexandria, was recruited to participate in the present study. This cohort included 60 healthy individuals that served as a control group and 180 individuals occupationally chronically exposed to low-levels of benzene in their daily activity during routine work. A small group worked in printing shops and the rest were involved in work related to the automotive industry like gas station and car wash attendants, car body-shop repair employees, mechanics or drivers. All participants were of the same socio-economic standard with similar living conditions and dietary habits. Exclusion criteria from the cohort included individuals suffering from endocrine diseases like diabetes or thyroid dysfunctions. The Ethics Committee of the Medical Research Institute, Alexandria University; approved the study protocol and all experimental procedures are in accordance with the Helsinki Declaration of 1975, as revised in 1983.

After explaining the objectives of the project and obtaining consent, a 10 ml blood sample was withdrawn over heparin from each individual by qualified personnel. One part of the sample was used fresh for assessment of oxidative stress by estimating total plasma
antioxidant activity and determining the level of malondialdehyde (MDA) as a measure of lipid peroxidation (Satoh, 1978; Ohkawa, et al., 1979). The remaining part of each blood sample was centrifuged and plasma and RBCs were separated. The percentage of hemolysis in the withdrawn samples was also determined (Roper, 2001). Thirteen metals were determined in plasma and RBCs of the collected samples: copper, cobalt, chromium, selenium, cadmium, aluminium, mercury, lead, rubidium, magnesium, manganese, zinc and tin. Six other non-metals were also determined; sodium, potassium, lithium, calcium, phosphorus and boron. All elements were assayed using methods and conditions of metal assays (Elnimr, 1993). All values are presented as the mean ± standard deviation and were analyzed by Statistical Package for Social Science (SPSS) version 10. Paired t-test was used to compare two mean parameter values for the same element and the level of significance was set at P value of 0.05 or less.

**Results:**

Benzene-exposed workers were found to be under oxidative stress. There were significant elevations of plasma MDA level, and decrease in plasma total antioxidant activity as compared to control values. The average MDA level was 45.7% higher in the exposed group as its value was 2.01±1.62 mmol/L as compared to 1.38±1.27 mmol/L for controls, while the mean plasma total antioxidant capacity (0.90±0.64 mmol/L) was 36.6% lower than the corresponding control values (1.42±0.54 mmol/L). A higher degree of hemolysis was observed in the blood samples from benzene-exposed individuals. While the degree of hemolysis ranged between zero and 2% (mean 1.4%) in control samples, it averaged about 5.2% in the exposed individuals, reaching values as high as 9 to 10% in some cases.

In the plasma and RBCs samples from exposed workers, the levels of seven of the assayed elements were found to be statistically different from controls. The levels of lead, mercury, cobalt, and cadmium were higher, and on the other hand, the concentrations of zinc, selenium and copper were lower [Figures 1 and 2]. All other assayed elements in exposed workers had the same mean values in the plasma and RBCs as control subjects. As presented in Figure-1, mercury concentration in the plasma of controls was 0.73±0.12 and 1.12±0.18 µg/L in the exposed group, while it was 2.10±0.55 and 3.10±0.72 µg/L in the RBCs. Such increases were 53.4% and 47.3% respectively. Compared to control values; cadmium was 83.1% and 13.3% higher in the plasma and RBCs of the exposed group respectively, while cobalt showed only a small but statistically significant increase ranging between 15% and 17% in plasma and RBCs.

As shown in [Fig. 2], lead had concentrations in the plasma of exposed workers averaging 88.9±15.6 and 292.2±48.1 µg/L in the RBCs, which were 77.7% and 88.9% above mean control values of 50.04±9.48 µg/L and 161.49±50. µg/L, for plasma and RBCs respectively. On the other hand, lower levels of copper were found in the plasma of the benzene-exposed group, as it decreased by 43.4% from 1246.1±66.52 µg/L to 704.90±70.79 and by 41.2% in the RBCs, which decreased from 1215.22±67.55 µg/L to 714.20±100.78 µg/L. Deficiency in two other metabolically important elements; namely zinc and selenium, was clearly evident in the blood of the benzene exposed group. Zinc, which is almost equally distributed between plasma (1056.84±35.11 µg/L) and RBCs (992.32±13.82 µg/L) in control subjects decreased by 35.7% to 680±67.11 µg/L in the plasma and by 31.9% to 675.73±35.26 µg/L in the RBCs of exposed persons. Deficiency in selenium was also detected as a result of chronic exposure to benzene. Its level in plasma was 150.95±15.49 µg/L, which was lower than that of controls (243.33±7.14 µg/L) by 38.0%. In the RBCs, the level in the exposed group averaged 153.90±11.36 µg/L. This value was 34.8% below the mean of the control group, which was 235.95±5.64 µg/L.
Fig. 1: Concentrations of elements in the RBCs and plasma of control and benzene-exposed workers.

Discussion:

The disturbance in many inorganic elements in the blood of personnel chronically exposed to benzene was quite evident. These elements are essential for a multitude of biological processes and their homeostasis, which is maintained within strict limits, is critical
for life (Valco, et al., 2005). Disruption of such homeostasis may lead to oxidative stress. The generation of free radicals in living systems is closely linked with the participation of redox-active metals which undergo redox cycling reactions and possess the ability to produce reactive radicals in biological systems. Some redox-active metals including cobalt, cadmium, mercury and lead were found in the present work to be higher in both plasma and RBCs of workers chronically exposed to relatively low levels of benzene. The underlying mechanisms of their toxicity involve formation of the superoxide radicals, hydroxyl radicals and other reactive oxygen species (ROS), finally producing mutagenic and carcinogenic malondialdehyde (MDA), 4-hydroxynonenal and other exocyclic DNA adducts. Increased formation of ROS overwhelms body antioxidant protection (Evan & Vousden, 2001), which may be the underlying cause of the decreased antioxidant activity observed in the present study, leading to possible induction of numerous conditions detrimental to health. Besides, depletion of cellular major sulphydryl reserves seems to be an important mechanism of oxidative stress that is induced by redox-inactive metals (Stohs & Bagchi, 1995).

Some heavy metals, including mercury and lead, found to be relatively increased in the present study, cause hemolysis and lipid peroxidation (Ribarov & Benov, 1981). The interaction of heavy metals with oxyhemoglobin has been suggested as an important source of superoxide radical formation in RBCs (Carrell, et al., 1975). It is possible that the observed increased hemolysis of blood samples from benzene exposed population may be due, at least in part, to such interaction.

Relatively elevated levels of lead in the RBCs of control as well as benzene exposed subjects, probably results from pollution from the use of leaded gasoline and lead containing paints. Lead exposure may increase the susceptibility of membranes by altering their integrity via causing deterioration of their components (Gurer & Ercal, 2000) and this was suggested to be the possible mechanism for hemolysis (Levander, et al., 1977. Blood lead level reflects the equilibrium between absorption, deposition in tissues and excretion. Levels associated with chronic exposure may underestimate total body burden because the majority of body lead is stored in teeth and bones. It has also been shown that even without overt toxicity, mildly elevated blood lead levels of 10µg/dL or higher are considered toxic and result in neurological disorders, cognitive impairments and other disorders (Patrick, 2006). Lead inhibitory effects on antioxidant enzymes appear to impair the antioxidant defences of cells and to render them more susceptible to oxidative attacks. Free radical-induced damage by lead is accompanied by two independent, although related mechanisms (Ercal, et al., 2001). The first involves direct formation of ROS and the second mechanism is achieved via depletion of the cellular antioxidant pool (Gurer & Ercal, 2000). Lipid peroxidation in the brains and livers of lead-exposed rats was detected (Shafiq,1984; Shafiq, et al, 199; Sandhir & Gill,1995) and a direct correlation was observed between lead concentration and lipid peroxidation (Shafiq,1984).

Environmental mercury is ubiquitous and consequently it is practically impossible to avoid exposure to some form of mercury (Valco, et al., 2005). The general population is exposed to numerous of its chemical forms including elemental mercury vapor, inorganic and organic compounds (Fitzgerald & Clarkson, 1991]). Inorganic mercury is suggested to increase hydrogen peroxide production by impairing the efficiency of oxidative phosphorylation and electron transport at the ubiquinone –cytochrome b5 step (Lund, et al, 1991; Nath, et al, 1996).

Cobalt is released to the environment from burning coal and oil, from automotive and airplane exhausts and from industrial processes that use the metal or its compounds (Gutteridge, 1995). Other than being an integral part of vitamin B12, cobalt is not known to serve any physiological function (Roth, et al, 1996). Its toxicity is relatively low compared to many other metals (Gal, et al, 2008), but it has been designated as a potent generator of
oxidative stress and free radicals, which in turn induce DNA damage and inhibit DNA repair mechanisms (Galanis, et al, 2009). However, an opposite effect on free radical generation has also been reported, as pretreatment with cobalt was found to attenuate hypoxia-induced oxidative stress (Shukla, et al, 2009). In view of this paradox, and the observed modest increase, it is difficult to evaluate the role of this metal in the production of free radicals and the generation of oxidative stress associated with chronic exposure to benzene.

Cadmium is another metal that was found to be elevated in the blood benzene-exposed individuals. Increase in lipid peroxidation represented by increased MDA level, has been observed in experimental animals treated with cadmium (Eybl, et al, 2006). While cadmium itself is unable to generate free radicals directly, indirect generation of various radicals has been reported. Such mechanism involves displacement of other redox-active metals from their binding sites thus increasing their free form and enhancing their capability of producing free radicals (Galan, et al, 2001).

There is no mechanism for the excretion of cadmium in humans, and accordingly cadmium accumulates in the tissues. The largest part is deposited in the kidneys, liver, pancreas and lungs. In most studies, the half-life in humans was estimated to be between 15 and 20 years (Jin, et al, 2008) and 20-35 years in kidney cortex (Jomova & Valko, 2011). Therefore, it is possible that the levels of cadmium in the blood of benzene-exposed workers do not reflect its body burden, which could be much higher. The magnitude of the deleterious effects of accumulated cadmium in this case may be more severe than what could be predicted from blood levels.

The decrease in the levels of zinc found in benzene exposed workers amounted to one third the level of controls. A special position among metals is occupied by zinc, which is present in more than 70 different enzymes that function in many aspects of cellular metabolism. In view of the increased levels of some heavy metals in the blood of benzene-exposed workers, it is possible that zinc could be replaced by heavy metals, thereby making the enzymes inactive (Donaldson, 1991).

Mild to moderate zinc deficiency can depress immune function through impairment of macrophage and neutrophil, natural killer cell and complement activity (Wintergerst, et al., 2007). This redox-inert metal is an essential component of numerous proteins involved in defense against oxidative stress, as for example superoxide dismutase (SOD). Besides it possesses neuroprotective properties. Depletion of zinc may enhance DNA damage via impairment of DNA repair mechanisms (Jomova & Valko, 2011; Gutteridge, 1995).

The function of zinc as an antioxidant involves two different mechanisms: (i) the protection of sulphhydroly groups of proteins against free radical attack and (ii) prevention mechanisms causing reduction of free radical formation through antagonism of redox-active transition metals (Bray & Bettgerer, 1990). Zinc deficiency may result in exaggerated toxic action of other metals. An example is the increased susceptibility of testes to cadmium-mediated free radical damage in case of low zinc levels (Oteiza, et al., 1999).

Copper is an essential micronutrient that is incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. It is necessary for the proper growth, development, and maintenance of many body organs. Copper is involved in key redox reactions in essential metabolic processes such as mitochondrial respiration and electron transport (Valco, et al., 2005). Its deficiency alters the role of other cellular constituents involved in antioxidant activities, such as iron, selenium and glutathione, and therefore plays an important role in diseases in which oxidant stress is elevated (Johnson, et al, 1992; Kaegi & Schaffer, 1988). Copper depletion leads to increased cellular susceptibility to oxidative damage and leads to decreased capability to produce superoxide dismutase (SOD), thus increasing their propensity to oxidative damage (Pan & Loo,2000). A marginal (mild) copper deficiency can impair health in subtle ways. Those affected suffer from
lowered resistance to infection, general fatigue, impaired neurological function and elevated risk for coronary heart disease and osteoporosis (Klevay, 1980; Strain, 1994).

Selenium is an essential component of seleno-enzymes and seleno-proteins, and plays a pivotal role in regulating free radical scavenging system (Rotruck et al, 1973; Stadtman, 1980). A low selenium concentration in serum has been associated with increased risk of gastrointestinal and prostatic cancer (Willette, et al, 1983). Selenium deficiency may be associated with myopathy, cardiomyopathy and immune deficiency including oral candidiasis and impaired phagocytic function (Dworkin, 1994).

It is clear from the above discussion that disturbance in trace elements homeostasis can be an underlying cause of some serious hazards resulting from work in professions that necessitate chronic exposure to low levels of benzene. Special attention should be directed to child labor in this case, which is a common practice sometimes. Beside the health hazards, it is possible that the dexterity, cognitive function and manual skills of children and young adolescents working in such professions would be hindered affecting the quality of their lives and the development of their careers.

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References:


Oteiza PI, Adonaylo VN, Keen CL. Cadmium-induced testes oxidative damage in rats can be influenced by dietary zinc intake. Toxicol 1999; 137: 13-22.
Ribarov SR, BenovLC. Relationship between the hemolytic action of heavy metals and lipid peroxidation. Biochim Biophys Acta. 1981; 640: 621-726


