J. Med. Toxicol. (2012) 8:281–284
DOI 10.1007/s13181-012-0219-1

TOXICOLOGY INVESTIGATION

Aluminium Phosphide Poisoning and Oxidative Stress

Serum Biomarker Assessment

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Published online: 10 March 2012

Abstract According to previous animal studies, aluminium phosphides (AlPs) may induce oxidative stress leading to generation of free radicals and alteration in antioxidant defense system. This study was conducted to evaluate the existence and degree of oxidative stress in patients with acute AlP ingestion. A total of 44 acute AlP ingested patients as well as 44 age- and sex-matched controls were included. All patients had acute poisoning symptoms with AlP at the time of presentation and had blood samples analyzed for lipid peroxidation, total antioxidant capacity and total thiol. Our findings showed that there is a significant increase in lipid peroxidation in AlP ingested group along with a reduction in total antioxidant capacity and total thiols groups. These clinical data confirm previous experimental models that showed AlP exposure might significantly augment lipoperoxidative damage with simultaneous alterations in the antioxidant defense system. Hence, our findings might justify use of antioxidants in treatment of acute AlP poisoning which needs to be clarified by additional clinical trials.

Keywords Aluminium phosphide · Oxidative stress · Poisoning · Antioxidant capacity

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Introduction

Phosphides are used throughout the world as pesticides to protect stored grains from rodents and pests. In Iran, aluminium phosphide (AlP) is accessible as 3-g tablets that are a combination of 56% AlP (total dose of 1,680 mg) and 44% ammonium carbonate [1]. In the past 35 years, high mortality rates have been reported following significant exposures to aluminium, zinc or calcium phosphides. Exposure is rarely accidental with the majority of cases involving intentional acts of suicides.

After ingestion, solid phosphide including AlPs produce a toxic phosphine gas following any contact with water, moisture in the air, or hydrochloric acid in the stomach. Although the exact mechanism has not been well defined, it has been demonstrated that phosphine acts at the mitochondrial level, and once systematically absorbed, it will interfere with synthesis of enzymes and proteins [2–4]. In addition to the corrosive action of phosphine, the mechanism of toxicity includes formation of highly reactive hydroxyl radicals [5, 6]. Cellular injury due to lipid peroxidation is also suggested [7]. Previously, a reduction in the level of catalase and rise in the activity of superoxide dismutase in patients of AlP poisoning have been reported [8]. The reduction of glutathione concentration in different tissues in AlP poisoning also explains the cellular injury, as glutathione is a protecting factor against oxidation by catalyzing the reduction of the oxygen peroxide in O_2 and H_2O

[9]. Indicators of oxidative stress (reduced glutathione and malondialdehyde) are showed to reach peak levels within 48 h of exposure to poison [7, 10]. To our knowledge, there is paucity of data regarding measurement of total antioxidant capacity in patients with AlP poisoning although presence of phosphine-induced oxidative damage in animal studies has been well established [11]. Moreover, any decrease in plasma levels of thiol groups, as an important part of antioxidant defense system, might imply the presence of oxidative stress in an AlP-poisoned patient. This might result in generation of free radical and alteration in antioxidant system. Hence, this study was conducted to evaluate the existence and capacity of oxygen free radicals, in patients with acute AlP ingestion.

Materials and Methods

This study was conducted at the emergency department of Imam Hossein hospital, Tehran, Iran during the years 2007–2010. The ethical committee of human research of the Shahid Beheshti University of Medical Sciences approved the study protocol. Eligible patients were men and women older than 12 years with acute AlP poisoning who had not received advanced medical care in another medical center. Patients were excluded if they had concomitant ingestion of other drugs in their history. The diagnosis of acute AlP poisoning was based on history of oral ingestion of known AlP compound.

A total of 44 patients were included in this study, whose mean age was 32 years. Fifty-four percent were male and 46% were female. Sex- and age-matched control group were chosen from healthy hospital staff. A written consent was obtained from the controls. Mortality in the study was 95.6%, and the two survivors were caught during ingestion and prompt management possibly was the only factor in their rescue.

Samples were collected from the patients before commencement of treatment. Lipid peroxidation, total antioxidant capacity, and total thiol groups level were measured in plasma of both patients and control subjects through chromatography. Laboratory technicians measuring study endpoints were blinded to the poisoning or control status of the subjects.

The antioxidant capacity of plasma was determined by measuring of the plasma ability to reduce Fe^{3+} to Fe^{2+}. The complex between Fe^{2+} and 2,4,6-tripyridyl-*s*-triazine as a reagent for iron gives a blue color with absorbance at 593 nm [12].

Total thiol groups of plasma were evaluated spectrophotometrically at 412 nm using 5,5-dithiobis-2-nitro benzoic acid as the reagent [13].

Lipid peroxidation of plasma was determined by the reaction of thiobarbituric acid with malondialdehyde, the end product of lipid peroxidation. This method used the thiobarbituric acid dissolved in sodium sulfate solution and both liberation of lipid peroxide and color reaction have been performed simultaneously by heating serum protein precipitate with this reagent in a weak acid solution [14].

Data were analyzed using SPSS 17 (SPSS Inc, Chicago, IL, USA). Numerical data were expressed as mean ± standard deviation. The Kolmogorov–Smirnov test was used to test the normal distribution of numerical variables. Independent samples *t*-test and Mann–Whitney test were used to compare the variables.

Results

A total of 44 patients with AlP intoxication and their age- and sex-matched healthy controls were enrolled in the study. The median number of ingested AlP tablets was 1 (range, 0.5–6) — 30 patients had ingested one tablet. A total of 42 deaths occurred in the study population. Most of the deaths occurred within 48 h of admission. The mean time interval from ingestion of tablet to hospital admission was 110.5 ± 52.5 min.

The most frequent presenting symptoms of the patients were dyspnea (n=24, 55%), nausea, vomiting, and abdominal pain (n=20, 45%).

Acute AlP exposure resulted in decreased levels of plasma total antioxidant capacity of patients. Our findings indicate that this plasma level in control subjects is significantly higher than those of patients (3.11±0.27 versus 1.91± 0.18 mmol/ml, P<0.0005; Table 1). The decrease in antioxidant capacity in patients was accompanied by a lower total plasma thiol concentration (0.095±0.01 mmol/l in patients versus 0.15±0.01 mmol/l in controls, P<0.0005; Table 1).

Moreover, measuring the level of thiobarbituric reactive substance (TBRAS) values disclosed that lipid peroxidation in plasma of patients exhibited a significant increase in comparison with healthy controls (9.86±0.75 versus 4.25± 0.37 nmol/ml, P<0.0005; Table 1).

Table 1 Comparison of measured plasma parameters in patients and age and sex-matched healthy controls

	Patients (n=44)	Controls (n=44)	P value
Antioxidant capacity of plasma (mmol/ml)	1.91±0.18	3.11±0.27	<0.0005
Total plasma thiol concentration (mmol/ml)	0.095±0.01	0.15±0.01	<0.0005
TBRAS (nmol/ml)	9.86±0.75	4.25±0.37	<0.0005

Values are expressed as mean ± SD

TBRAS thiobarbituric reactive substance, as an indicator of lipid peroxidation level

As shown in Table 1, these findings may indicate that AlP exerts its toxicity by generating disproportionate oxidative stress through increased reactive oxygen species and compromised antioxidant protection mechanism.

Discussion

There are numerous studies that address the underlying mechanisms of phosphine-induced toxicity. Oxidative stress is one of the major explanations shown to be present in experimental models. The present study confirmed the presence of oxidative stress in acute AlP-poisoned patients since our findings demonstrated a reduction in total antioxidant capacity and total thiol groups as well as a surge in thiobarbituric reactive substances.

Thus far, phosphine-induced oxidative damage to macromolecules has been established in rats [11], nematodes [15], mammalian cell lines [16], and in insects [17]. A detailed analysis of the types of reactive oxygen substances (ROS) induced by phosphine and the roles of specific protectants identified hydrogen peroxide as the most significant ROS and glutathione as the strongest protective antioxidant [16]. The simple and the most significant defense system of human body to counteract free radicals are antioxidant agents. Of these antioxidant systems, the enzymatic system including superoxide dismutase, catalase and thiol-containing enzymes like glutathione, are of great importance [18]. Our findings are in accordance with previous reports that showed interaction of AlP with enzymes such as glutathione peroxides and superoxide dismutase following phosphine exposure in rats [11]. The action of PH3 in rats reported in previous outcomes correlates well with increased H_2O_2 production and lipid peroxidation [16]. Decreased glutathione in rat tissues strongly suggested the involvement of ROS in PH3 toxicity. Depletion of glutathione favors lipid peroxidation and predisposes cells to oxidant damage [19]. However, the intriguing difference presented in our study is the confirmation of the presence of oxidative stress in a patient population. Most previous reports with regard to phosphine-induced oxidative stress were confined to animal studies and experimental models [20]. This difference is of great importance since it is not is not clear that oxidative stress is the cause of phosphine-induced mortality in most previous experimental models. On the other hand, it has been proved that antioxidants, especially melatonin, can stop most of the oxidative damage induced in rat tissues [11, 21], and that melatonin can proprietorially preserve the levels of glutathione [22]. Other therapies such as *N*–acetylcysteine, reloading cellular glutathione, which has been showed to have antioxidant properties, have been proposed [10]. Myocardial suppression and resistant hypotension is a distinguishing feature of AlP poisoning. In AlP-poisoned rats, *N*–acetylcysteine increased survival time and reduced myocardial oxidative injury [23]. These important investigations could be best studied in patients rather than animal models.

In line with our results, a similar observation was reported in the study of Ranjbar et al. [24], where oxygen free radicals and their related interactions like lipid peroxidation were shown to be present in acute organophosphorus insecticides poisoning. These observations imply that imposing oxidative stress might be a shared and general finding in most of poisoned patients, regardless of the poison. Hence, any assumptions for treatment of AlP-poisoned patients based on our results with antioxidant agents requires more human studies, and this warrants studies to show relationship between the level of oxidative damage and phosphine-induced mortality. Moreover, any baseline characteristics of the patients that might impose oxidative stress should be controlled in patient-based studies. Unknown smoking status of the patients and controls, as a confounding variable, could be named as one limitation of the present report and must be addressed in future studies.

In conclusion, our data disclosed that AlP toxicity could be attributed to oxidative pathway involving the disproportionate production of reactive oxygen intermediates in tissues. AlP exerts its toxic effects on multiple organ systems not only by increasing the rate of lipid peroxidation but also by blocking the endogenous antioxidants and thus weakening the antioxidant guard. This clinical data might open avenues for future research to delineate the possible role for exogenous antioxidant agents for treatment of acute AlP poisoning.

Acknowledgement This study was supported by the Emergency Medicine Department of Shahid Beheshti University of Medical Sciences.

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