

Cholinesterase inhibition by aluminium phosphide poisoning in rats and effects of atropine and pralidoxime chloride¹

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ABSTRACT

AIM: To investigate the cholinesterase inhibition and effect of atropine and pralidoxime (PAM) treatment on the survival time in the rat model of aluminium phosphide (AIP) poisoning. **METHODS:** The rats were treated with AIP (10 mg/kg; $5.55 \times LD_{50}$; ig) and the survival time was noted. The effect of atropine (1 mg/kg, ip) and PAM (5 mg/kg, ip) was noted on the above. Atropine and PAM were administered 5 min after AIP. Plasma cholinesterase levels were measured spectrophotometrically in the control and AIP treated rats 30 min after administration. **RESULTS:** Treatment with atropine and PAM increased the survival time by 2.5 fold (1.4 h \pm 0.3 h vs 3.4 h \pm 2.5 h, $P < 0.01$) in 9 out of 15 animals and resulted in total survival of the 6 remaining animals. Plasma cholinesterase levels were inhibited by 47 %, (438 \pm 74) U/L in AIP treated rats as compared to control (840 \pm 90) U/L ($P < 0.01$). **CONCLUSION:** This preliminary study concludes that AIP poisoning causes cholinesterase inhibition and responds to treatment with atropine and PAM.

INTRODUCTION

Aluminium phosphide (AIP) because of its efficacy against various pests, low cost and ease in handling has been a widely used pesticide in North India. However, during the past decade it has become the commonest chemical for intentional poisoning^[1-4]. Retrospective

five years and ten years studies of acute poisoning cases at the All India Institute of Medical Sciences have revealed a total of 279 and 623 patients of acute poisoning, of which 37 (13.2 %) and 138 (31 %) were of AIP poisoning respectively^[5,6]. The majority of deaths occur within the first 12 - 24 h and these are due to peripheral vascular collapse, cardiac failure, pulmonary edema, and acute respiratory distress syndrome^[1-4,7]. The toxicity is due to instant release of phosphine gas, which exerts widespread toxic effects, upon exposure to moisture. At cellular levels it is suggested to inhibit mitochondrial respiration and electron transport, the critical electrochemical link between respiration and phosphorylation in mitochondria^[8]. Patients are treated symptomatically with oxygen, fluids, bicarbonate, sympathomimetic agents, steroids, magnesium sulfate^[4,9,10], etc. Methylene blue is also suggested to be included in the above regimen^[11]. Search for new approaches to treat AIP poisoning is continuing because of the fatal outcome despite the above suggested treatment modalities. Pazynich *et al*^[12] have reported that phosphine can inhibit cholinesterase in animals and Potter *et al* have shown significant exposure related decrease in cholinesterase activity in pesticide applicers exposed to phosphine^[13].

In this study we report a preliminary observation of cholinesterase inhibition by AIP in a rat model of poisoning and the beneficial effect of atropine and pralidoxime chloride (PAM) treatment.

MATERIALS AND METHODS

Experimental protocol Rats of Wistar strain from the Central Animal Facility ($n = 45$) of either sex, 150 - 200 g, were housed under controlled conditions of temperature and humidity in the departmental animal house with food and water *ad lib* for two weeks before the study. The animals were divided into three groups; group 1 (control, saline treated, $n = 10$), group 2 (AIP exposed, 10 mg/kg, ig, $n = 15$), group 3 AIP (10 mg/kg, ig) + atropine (1 mg/kg, ip) + PAM (5 mg/kg,

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ip) ($n = 20$). In group 3 the rats were treated ip with atropine and PAM at 5 min after the intragastric administration of AIP. Group 1 (control) rats were cannulated for blood pressure recording and were sham operated and saline treated. In group 2, 5 out of 15 rats were cannulated for blood pressure recording and the remaining 10 rats were observed for survival time. In group 3, 5 out of 20 rats were cannulated for blood pressure recording and the remaining 15 rats were observed for survival time. The animals were fasted overnight with free access to water before the experiment. Under pentobarbital anesthesia (40 mg/kg, ip) the carotid was cannulated and connected via the pressure transducer to a videograph (Coulbourn Instruments, USA) in five animals each from both group 2 and 3. In all the animals a 2-cm vertical, midline incision below the xiphisternum was made and the stomach was exposed. A nick with encircling purse string suture was applied near the pylorus and saline or powdered AIP (10 mg/kg, $5.55 \times LD_{50}$) was administered into the stomach and the gastrotomy hole was immediately closed by tightening the suture. The blood pressure and ECG were recorded in the cannulated animals and in the rest the survival time was noted. Plasma cholinesterase levels were estimated in control and AIP only exposed group by drawing the blood samples from the orbitovenous plexus 30 min after the AIP administration. The cholinesterase levels were measured spectrophotometrically (Beckmann, USA) at 405 nm following the Boehringer Mannheim kit instructions.

Drugs AIP (Quick Phos granules, AIP 56%) was from United Phosphorous Ltd, Vapi, Gujarat, India. Atropine sulphate was from Dey's Medical Stores Manufacturing Ltd, India, and pralidoxime chloride from Panacea Biotech Ltd, India.

Statistical analysis The results are expressed as $\bar{x} \pm s$ and non-paired t test was applied to measure the level of significance.

RESULTS AND DISCUSSION

Blood pressure in AIP treated cannulated rats ($n = 5$) of group 2 fell from (85 \pm 11) mmHg to (56 \pm 10) mmHg within the first 15 min which then stabilized at a plateau phase of (42–55) mmHg for 20 min till it fell to (5–10) mmHg ensuing in death. Mean blood pressure of group 1 was (81 \pm 4) mmHg. The blood pressure and ECG changes in rat poisoning model of AIP (10 mg/kg) have been given in details in our previous study^[14]. In the cannulated rats ($n = 5$) of group 3 atropine and

PAM did not prevent the initial AIP induced hypotension, however, the plateau phase was prolonged to more than one hour. The mean survival time in this group was either prolonged or the animals gained consciousness whereupon they were sacrificed. The survival results were computed on the non-cannulated animals of group 2 ($n = 10$) and group 3 ($n = 15$). The survival time in the cannulated and non-cannulated animals varied a little because of the surgical stress. In group 2 the mean death time was (1.4 \pm 0.3) h. In atropine and PAM treated group (group 3), out of 15 animals, 9 had a short term survival and 6 had a long term survival. The short term survival time was (3.4 \pm 2.5) h which was about 2.5 fold of the AIP exposed rats ($P < 0.01$) (Tab 1). The rest of the 6 rats recovered completely and after gaining consciousness from pentobarbital anesthesia the rats urinated frequently, defecated, resumed normal eating and drinking and became active. These rats were observed upto 30 d and were found to be active and healthy.

Tab 1. Survival status of the AIP exposed and atropine and PAM treated rats. $\bar{x} \pm s$. * $P < 0.01$ vs AIP treated rats. Parentheses () include number of animals surviving.

Group	Short term survival in hours/h	Long term survival in days/d
Saline treated ($n = 10$)	– (0/10)	> 30 (10/10)
AIP treated ($n = 10$)	1.4 \pm 0.3 (10/10)	– (0/10)
AIP + atropine + PAM ($n = 15$)	3.4 \pm 2.5* (9/15)	> 30 (6/15)

Mean cholinesterase levels in control (group 1) and AIP only exposed group (group 2) were (840 \pm 90) U/L and (438 \pm 74) U/L respectively. A marked inhibition of 47 % in the cholinesterase activity was observed in group 2 exposed to AIP ($P < 0.01$). Simultaneous observations of pin point pupils, loss of pupillary reflex, lacrimation and irregular rapid breathing accompanied with rales and ronchi were noted in these animals along with bradycardia and severe hypotension. Nasal and airway passages were full of secretions. All these effects support the cholinergic effects of phosphine. However, none of these symptoms were noted in atropine and PAM treated animals. A marked decrease (> 20 %) in red cell (acetylcholinesterase) and plasma (butyryl-

cholinesterase) cholinesterase activity has been reported in a Minnesota population of pesticide applicers in a radiometric assay by Potter *et al*^[13]. An inhibition of >20%, which is taken as a marker for potential pathobiologic effects, has been observed in pesticide applicers who were using both organophosphates and fumigant phosphine. *In vitro* work by Potter *et al* and animal studies by Pazynich *et al* also suggest that phosphine can inhibit cholinesterase^[12, 13]. Lawson *et al* have suggested that phosphine or phosphine derived products may be doing so by formation of organophosphines^[15]. It is of interest to note in this regard that phosphine is a precursor in the synthesis of organophosphates^[16]. Organophosphate poisoning is effectively treated with atropine and PAM.

Survival of 40% animals after acute AIP exposure (10 mg/kg, $5.55 \times LD_{50}$) is reported for the first time. Inhibition of cholinesterase enzyme after AIP exposure also strengthens the hypothesis that some animals die of respiratory failure before they proceed to a circulatory shock. In the 6 animals who survived completely atropine and PAM must have been able to reverse or break the vicious cycle of respiratory depression, hypotension and ensuing myocardial ischemia and peripheral vascular failure as has been implicated in organophosphate poisoning^[17]. Detailed studies on the above are underway. It may be thus concluded that one of the causes of mortality in AIP poisoning may be due to cholinesterase inhibition leading to respiratory arrest, hence atropine and PAM may also be included in the supportive treatment regimen.

REFERENCES

- 1 Singh S, Dilawari JB, Vashisht R, Malhotra HS, Sharma BK. Aluminium phosphide ingestion. *Br Med J* 1985; 290: 1110-1.
- 2 Khosla SN, Nand N, Khosla P. Aluminium phosphide poisoning. *J Trop Med Hyg* 1988; 91: 196-8.
- 3 Bajaj R, Wasir HS. Epidemic aluminium phosphide poisoning in Northern India. *Lancet* 1988; 1: 820-1.
- 4 Chugh SN, Dushyant, Sant Ram, Arora B, Malhotra KC. Incidence and outcome of all poisoning in a hospital study. *Ind J Med Res* 1991; 94: 232-5.
- 5 Lall SB, Peshin SS, Seth SD. Retrospective five year study of acute poisoning cases at the All India Institute of Medical Sciences. *J Forensic Med Toxicol* 1989; 6: 1-8.
- 6 Lall SB, Peshin SS, Seth SD. Acute poisonings: A ten years retrospective hospital based study. *Ann Natl Acad Med Sci*

(India) 1994; 30: 35-44.

- 7 Siwach SB, Yadav DR, Arora B, Dalal S, Jagdish. Acute aluminium phosphide poisoning — An epidemiological, clinical and histopathological study. *J Assoc Phys India* 1988; 36: 594-6.
- 8 Cherfuka W, Kashi KP, Bond EJ. The effect of phosphine on electron transport in mitochondria. *Pest Biochem Physiol* 1976; 6: 65-84.
- 9 Koley TK. Aluminium phosphide poisoning — Part I. *Indian J Clin Practice* 1998; 9: 14-24.
- 10 Singh S, Sharma BK, Wahi PL, Anand BS, Chugh KS. Spectrum of acute poisoning in adults (10 year experience). *J Assoc Phys India* 1984; 32: 561-3.
- 11 Lall SB, Peshin SS, Mitra S. Methemoglobinemia in aluminium phosphide poisoning in rats. *Indian J Exp Biol* 2000; 38: 95-7.
- 12 Pazynich VM, Mazur IA, Podloznyi AV. Experimental substantiation and prediction of maximum atmospheric phosphine concentrations differentiated by time. *Gig Sanit* 1984; 1: 13-5.
- 13 Potter WT, Garry VF, Kelly JT, Tarone R, Griffith J, Nelson RL. Radiometric assay of red cell and plasma cholinesterase in pesticide applicers from Minnesota. *Toxicol Appl Pharmacol* 1993; 119: 150-5.
- 14 Lall SB, Sinha K, Mitra S, Seth SD. An experimental study on cardiotoxicity of aluminium phosphide. *Indian J Exp Biol* 1997; 35: 13-5.
- 15 Lawson MA, Lieske CN, Fox-Talbot MK, Meyer HG. Spontaneous reactivation of phosphinylated human erythrocyte acetylcholinesterase and human serum butyrylcholinesterase. *Life Sci* 1985; 36: 1715-20.
- 16 Jackson JR. Phosphine and selected metal phosphides. In: *Environmental Health Criteria* 73. Geneva: World Health Organization; 1998. p 12.
- 17 Saadeh AM, Farsakh NA, al-Ali MK. Cardiac manifestations of acute carbamate and organophosphate poisoning. *Heart* 1997; 77: 461-4.

磷化铝中毒抑制大鼠胆碱酯酶及阿托品和氯解磷啶的作用

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关键词 中毒; 磷化铝; 胆碱酯酶类; 血压; 阿托品; 氯解磷啶

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