

Effect of *N*-acetylcysteine and *L*-NAME on aluminium phosphide induced cardiovascular toxicity in rats

Archana AZAD, Shyam Bala LALL¹, Shivani MITTRA

(Department of Pharmacology All India Institute of Medical Sciences New Delhi-110029, India)

KEY WORDS aluminium compounds; acetylcysteine; *N*^G-nitroarginine methyl ester; malondialdehyde; catalase; glutathione peroxidase

ABSTRACT

AIM: To investigate the protective effects of *N*-acetylcysteine (NAC) and *N*^w-Nitro-*L*-arginine methyl ester (*L*-NAME) on aluminium phosphide (AIP) poisoning induced hemodynamic changes, myocardial oxygen free radical injury and on survival time in rats. **METHODS:** AIP (12.5 mg/kg) was administered intragastrically under urethane anaesthesia. The effect of pre- and post-treatment with NAC and *L*-NAME alone and in combination was studied on haemodynamic parameters [blood pressure (BP), heart rate (HR), and electrocardiogram (ECG)] and biochemical parameters (malonyldialdehyde, catalase, and glutathione peroxidase). **RESULTS:** AIP caused significant hypotension, tachycardia, ECG abnormalities, and finally marked bradycardia. The mean survival time was (90 ± 10) min. There was significant increase in myocardial malonyldialdehyde (MDA), and decrease in catalase and glutathione peroxidase (GSH Px) levels. NAC infusion (6.25 mg·kg⁻¹·min⁻¹, iv for 30 min) caused insignificant hemodynamic and biochemical changes. Pre- and post-treatment of NAC with AIP significantly increased the survival time, stabilized BP, HR, and ECG, decreased MDA and increased GSH Px levels compared to AIP group. *L*-NAME infusion (1 mg·kg⁻¹·min⁻¹, iv for 60 min) as such caused significant rise in BP but precipitated ECG abnormalities. Pre- and post-treatment of *L*-NAME with AIP neither improved the survival time nor the biochemical parameters despite significant rise in BP. Co-admin-

istration of both the drugs with AIP worsened the hemodynamic and biochemical parameters with reduction in the survival time as compared to AIP. **CONCLUSION:** NAC increased the survival time by reducing myocardial oxidative injury whereas *L*-NAME showed no such protective effects in rats exposed to AIP.

INTRODUCTION

Aluminium phosphide (AIP), a solid fumigant pesticide is extensively used for household storage of grains in the Northern India despite its restricted sale in the country. It is available as greyish tablets (3 g each) under the common trade names of Celphos and Quickphos. Retrospective hospital based studies on acute poisoning cases indicated AIP as one of the most common cause of intentional poisoning and mortality^[1-3]. The phosphine gas released from AIP in the stomach, exerts widespread toxic effects. The majority of deaths occur within the first 12-24 h and are due to peripheral vascular collapse, cardiac failure, pulmonary edema, and acute respiratory distress syndrome^[1-4]. A recent *in vivo* study in rats demonstrated plasma cholinesterase inhibition after AIP exposure^[5]. Methemoglobinemia has also been observed in AIP poisoning^[6]. A major limiting factor in the management of these patients is the nonavailability of specific antidote and thus supportive treatment is the mainstay.

The underlying mechanism of cardiotoxicity and peripheral circulatory failure caused by phosphine is not well understood. *In vitro* studies indicate phosphine to be a potent inhibitor of cytochrome-c oxidase in mitochondria^[7-9]. A number of clinical studies have shown myocardial ischemic changes in AIP poisoning^[10]. A study on rat model of AIP poisoning demonstrated dose-dependent myocardial depletion of glycogen, creatine-phosphate and lactate complimented with ischemic changes seen in ECG^[11]. Myocardial cellular injury due to increased lipid peroxidation is also suggested in clinical^[12] and ex-

¹ Correspondence to Dr Shyam Bala LALL. Phn 91-11-685-0691.

Fax 91-11-685-9391. E-mail sball@medinst.ernet.in

Received 2000-08-30

Accepted 2001-01-11

perimental studies^[12]. Further, acute respiratory distress syndrome (ARDS), a cardinal feature of AIP poisoning is thought to be due to an oxidant injury^[1,4].

N-acetylcysteine (NAC), an important antioxidant and cytoprotective agent is reported to replenish intracellular glutathione, one of the pirote of cellular defense against oxidative stress^[13]. It is hypothesized that NAC may protect the myocardium from ischemic and free radical induced injury in AIP poisoning and further *L*-NAME, a nitric oxide synthase inhibitor may reduce the capillary fluid leakage and consequent intractable circulatory shock seen in AIP poisoning. The present study was therefore, aimed to investigate the effects of NAC and *L*-NAME alone and in combination on the survival time, hemodynamics (BP, HR, ECG) and cardiac biochemical parameters (MDA, catalase, glutathione peroxidase) at various time intervals in rat model of AIP poisoning.

MATERIALS AND METHODS

Male Wistar rats (200 – 250 g), from the Central Animal Facility of All India Institute of Medical Sciences, were acclimatised for one week under controlled temperature of 22 °C ± 2 °C and 12-h light/dark cycle in the animal house of the department with access to food and water *ad libitum*. The animals were categorised into 7 groups of 30 – 35 rats each. Group 1 (sham operated control), Group 2 (AIP exposed), Group 3 (NAC alone), Group 4 (AIP + NAC), Group 5 (*L*-NAME alone), Group 6 (AIP + *L*-NAME), Group 7 (*L*-NAME + AIP + NAC).

Each group was further divided into four subgroups of 6 – 8 rats each. In one group hemodynamic parameters [blood pressure (BP), heart rate (HR), electrocardiogram (ECG)] and survival time was recorded while the other three groups, were sacrificed at 30, 60, and 90 min after AIP treatment for biochemical studies (malonyldialdehyde, catalase, glutathione peroxidase) on the cardiac tissue. The hemodynamic parameters (BP, ECG, HR) were continuously recorded on a videograph (Coulbourn Instruments, USA) in each animal during the study period and the records, computed at 30, 60, and 90 min were compared.

Under urethane anaesthesia (30 mg/kg, ip), right external jugular vein was cannulated for intravenous infusions, trachea was cannulated for suction and to ease breathing, left carotid artery was cannulated and connected to the pressure transducer after callibrating the instrument for recording the BP. ECG electrodes were placed

in the proper position and connected to the instrument for Lead II recordings. After obtaining the baseline records of BP and ECG, the upper abdomen of the animal was exposed by midline incision. The pylorus of the stomach was identified and a gastrotomy hole was made after applying a purse string suture in control group (Group 1). In Group 2, weighed amount of AIP (12.5 mg/kg, United Phosphorus Limited, India) was administered in the stomach and the purse string suture was immediately closed^[12]. In Group 3, *N*-acetylcysteine (6.25 mg · kg⁻¹ · min⁻¹, Samarath Pharmaceuticals, Bombay) was infused intravenously at 15 min pre- and post-treatment of sham operation. In Group 4, same dose of NAC was administered 15 min prior and 15 min after AIP exposure. In Group 5, *L*-NAME (1 mg · kg⁻¹ · min⁻¹, Sigma) was infused at 30 min pre- and post-treatment of sham operation. Group 6 received *L*-NAME alone 30 min before and 30 min after AIP administration and in Group 7 AIP and *L*-NAME were given at 30 min pre- and post-treatment in the same doses, and NAC in the same dose at 30 min post treatment.

The BP, HR, and ECG were continuously recorded in each animal and the survival time was noted. The biochemical studies were carried out in rest of the subgroups after the scheduled treatment and recording of hemodynamic parameters at 30, 60, and 90 min. The animals were sacrificed and the heart was removed and placed in liquid nitrogen for estimation of malonyldialdehyde (MDA), catalase and glutathione peroxidase (GSH Px) by spectrophotometric (Beckman, USA) methods of Okhawa *et al*^[14], Hans^[15], and Khaper and Singal^[16] respectively.

Statistics The hemodynamic and biochemical parameters in Group 2, 3, and 5 were compared with Group 1 (control group). Group 4, 6, and 7 were compared with Group 2 (AIP treated). The comparisons were also made between the basal values and values at different time intervals. The results were expressed as $\bar{x} \pm s$. Two way and one way analysis of variance (ANOVA) and unpaired *t*-test wherever needed were applied to calculate the *P* value. *P* value of at least 0.05 was considered significant.

RESULTS

Hemodynamic studies

Effect of AIP Intra gastric administration of AIP caused significant fall in blood pressure (*P* < 0.05), with initial tachycardia followed by progressive bradycar-

dia (Tab 1). The ECG changes after AIP administration were observed within 30 min and included initial ST elevation followed by QRS broadening, increased PR and QT intervals (Tab 2). The mean survival time in this group was significantly reduced, (90 ± 10) min as compared to (360 ± 26) min in controls ($P < 0.05$).

Effect of *N*-acetylcysteine alone and in combination with AIP Administration of NAC in Group 3 led to significant tachycardia till 60 min, followed by bradycardia ($P < 0.05$). However, the change in BP was not significantly different than controls at all time intervals (Tab 1). Similarly there were no changes in ECG till 30 min, and T wave inversion and ST segment elevation were observed at 60 and 90 min (Tab 2). The mean survival time was (270 ± 11) min as compared to (360 ± 26) min in control group ($P < 0.05$). Pre- and post-treatment of NAC led to significant fall in blood pressure

($P < 0.05$), from the basal level, however, it remained significantly higher at each time interval when compared to Group 2 (Tab 1). The ECG pattern remained normal till 30 min and then the changes similar to those seen in AIP group appeared at 60 and 90 min (Tab 2). The mean survival time in this group was significantly more, (120 ± 17) min as compared to Group 2 ($P < 0.05$).

Effect of *L*-NAME alone and in combination with AIP The intravenous infusion of *L*-NAME (1 mg·kg⁻¹·min⁻¹) produced significant bradycardia at all time intervals ($P < 0.05$), as compared to control group. There was significant rise in BP at 30 min ($P < 0.05$) which was restored to control level after 60 min. However, at 90 min, a significant hypotension was observed (Tab 1). Except significant bradycardia, no other ECG abnormality was noted till 30 min. At 60 min QRS broadening and flat T waves were noted while at 90 min

Tab 1. Effect of AIP, NAC, and *L*-NAME alone and in combination with AIP on heart rate and mean arterial pressure at various time intervals. Values are $\bar{x} \pm s$ of 5 animals in each group. ^b $P < 0.05$ vs Group 1. ^a $P < 0.05$ vs Group 2. ^c $P < 0.05$ vs basal.

Groups	Time since treatment							
	Basal		30 min		60 min		90 min	
	HR (Beats/min)	BP (mmHg)	HR (Beats/min)	BP (mmHg)	HR (Beats/min)	BP (mmHg)	HR (Beats/min)	BP (mmHg)
1	252 ± 18	84 ± 11	264 ± 17	80 ± 6	216 ± 22	75 ± 12	214 ± 13	68 ± 4
2	256 ± 22	90 ± 10	296 ± 16 ^{bh}	54 ± 10 ^{bh}	300 ± 20 ^{bh}	46 ± 4 ^{bh}	190 ± 10 ^{bh}	19 ± 2 ^{bh}
3	260 ± 19	97 ± 4	276 ± 26 ^{bh}	76 ± 5 ^b	266 ± 13 ^b	69 ± 4 ^h	226 ± 16 ^{bh}	64 ± 5 ^b
4	282 ± 15	92 ± 8	306 ± 13 ^{ch}	72 ± 6 ^{ch}	264 ± 16 ^{ch}	61 ± 2 ^{ch}	198 ± 15 ^h	44 ± 4 ^{ch}
5	256 ± 16	82 ± 18	196 ± 16 ^{bh}	119 ± 17 ^{bh}	200 ± 12 ^{bh}	70 ± 8 ^h	140 ± 23 ^{bh}	15 ± 5 ^{bh}
6	256 ± 43	89 ± 11	216 ± 16 ^{ch}	93 ± 4 ^c	150 ± 22 ^{ch}	69 ± 4 ^{ch}	106 ± 13 ^{ch}	33 ± 3 ^{ch}
7	252 ± 10	83 ± 5	226 ± 11 ^{ch}	94 ± 6 ^c	182 ± 13 ^{ch}	63 ± 3 ^{ch}	132 ± 13 ^{ch}	44 ± 4 ^{ch}

Group 1: Control; Group 2: AIP; Group 3: NAC; Group 4: AIP + NAC; Group 5: *L*-NAME; Group 6: AIP + *L*-NAME; Group 7: *L*-NAME + AIP + NAC.

Tab 2. ECG changes after 30 and 60 min of AIP administration in different groups.

Groups	ECG changes (Lead II) at 30 min					ECG changes (Lead II) at 60 min				
	QRS interval	PR interval	ST wave	QT interval	T wave	QRS interval	PR interval	ST wave	QT interval	T wave
AIP	broadened	increased	elevated	increased	-	broadened	increased	elevated	increased	-
NAC	-	-	-	-	-	-	-	elevated	-	inverted
AIP + NAC	-	-	-	-	-	broadened	increased	elevated	-	inverted
<i>L</i> -NAME	-	-	-	-	-	broadened	-	-	-	flattened
AIP + <i>L</i> -NAME	-	increased	-	-	-	broadened	-	elevated	-	-
<i>L</i> -NAME + AIP + NAC	broadened	increased	elevated	-	-	broadened	increased	elevated	-	flattened

At 90 min, ECG changes in Group 2 to Group 7 remained almost similar to those seen at 60 min with additional abnormalities observed in some animals like inverted P wave rsR' pattern, and right bundle branch block. The reference of comparison for ECG changes at 30, 60, and 90 min was basal ECG pattern in each group. All the above changes were seen in $n = 5$ rats.

occasional rSR' pattern, right bundle branch block (Tab 2) were observed. The mean survival time was (110 ± 14) min as compared to (360 ± 26) min in controls ($P < 0.05$).

Pre- and post-treatment with *L*-NAME resulted in significant bradycardia with increase in BP at 30 min ($P < 0.05$). Though there was fall in BP at 60 and 90 min, yet the values remained significantly higher than Group 2 (Tab 1). The ECG at 30 min showed prolonged P-R interval while at 60 and 90 min and ST elevation with QRS broadening were seen (Tab 2). The survival time was marginally increased to (100 ± 14) min as compared to AIP group ($P > 0.05$).

Effect of *L*-NAME and NAC with AIP Co-administration of both the drugs with AIP led to significant fall in heart rate at all time intervals ($P < 0.05$). The BP remained significantly higher than AIP group throughout (Tab 1). However, ECG showed marked abnormalities including QRS broadening, ST elevation, increased PR interval, and flat T waves at 30 and 60 min and just before the death of animal (Tab 2). The survival time was decreased to (80 ± 6) min as compared to Group 2, (90 ± 10) min.

Biochemical studies

Myocardial malonyldialdehyde The MDA levels increased after 30, 60, and 90 min of AIP administration as compared to the control group. NAC and *L*-NAME alone did not change the MDA levels significantly ($P > 0.05$). Pre- and post-infusion of NAC with AIP resulted in significant decrease in MDA at all time intervals ($P < 0.05$) as compared to AIP alone (Group 2). However, pre- and post-treatment of *L*-NAME with AIP (Group 6) and also co-administration of both the drugs (Group 7) caused insignificant changes in MDA levels when compared to Group 2 (Tab 3).

Catalase Myocardial catalase levels significantly decreased after AIP administration. In groups where NAC and *L*-NAME were administered alone and co-administered with AIP, the catalase levels did not change significantly as compared to control and AIP treated groups respectively (Tab 4).

Glutathione peroxidase The glutathione peroxidase levels in cardiac tissue decreased significantly in AIP exposed Groups ($P < 0.05$). Insignificant changes in GSH Px were noted when NAC and *L*-NAME alone were administered. In Group 4 where NAC was given as pre and post infusion in AIP exposed rats, showed significant increase in GSH Px ($P < 0.05$) at 60 and 90 min,

Tab 3. Effect of AIP, NAC, and *L*-NAME alone and in combination with AIP on myocardial malonyldialdehyde levels at various time intervals. Values are $\bar{x} \pm s$ of 6 animals in each group. ^b $P < 0.05$ vs Group 1. ^a $P < 0.05$ vs Group 2. ^b $P < 0.05$ vs 30 min.

Groups	Malonyldialdehyde/ $\mu\text{mol} \cdot \text{g}^{-1}$		
	After different time intervals in subgroups		
	30 min	60 min	90 min
1	0.060 ± 0.017	0.070 ± 0.020	0.070 ± 0.017
2	0.130 ± 0.020 ^b	0.150 ± 0.036 ^b	0.160 ± 0.039 ^{ab}
3	0.060 ± 0.009	0.060 ± 0.009	0.070 ± 0.014
4	0.060 ± 0.009 ^a	0.080 ± 0.009 ^a	0.110 ± 0.012 ^{ab}
5	0.060 ± 0.017	0.060 ± 0.021	0.060 ± 0.020
6	0.110 ± 0.061	0.130 ± 0.009	0.140 ± 0.029 ^b
7	0.110 ± 0.021	0.140 ± 0.007	0.150 ± 0.036 ^b

Group 1; Control; Group 2; AIP; Group 3; NAC; Group 4; AIP + NAC; Group 5; *L*-NAME; Group 6; AIP + *L*-NAME; Group 7; *L*-NAME + AIP + NAC.

Tab 4. Effect of AIP, NAC, and *L*-NAME alone and in combination with AIP on myocardial catalase levels at various time intervals. Values are $\bar{x} \pm s$ of 6 animals in each group. ^b $P < 0.05$ vs Group 1. ^a $P < 0.05$ vs 30 min.

Groups	Catalase/ $\text{mg} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$		
	After different time intervals in subgroups		
	30 min	60 min	90 min
1	21.9 ± 2.8	19.6 ± 0.8	17.1 ± 3.8
2	16.1 ± 2.6 ^b	15.1 ± 2.0 ^b	12.4 ± 1.6 ^{ab}
3	20.5 ± 2.5	19.7 ± 2.2	19.2 ± 1.0
4	17.2 ± 2.0	15.1 ± 1.2	13.7 ± 2.9 ^b
5	20.0 ± 2.5	18.4 ± 4.5	17.0 ± 2.6
6	18.0 ± 2.5	15.2 ± 1.0	12.5 ± 1.8 ^b
7	17.3 ± 1.8	15.1 ± 2.5	12.4 ± 1.5 ^b

Group 1; Control; Group 2; AIP; Group 3; NAC; Group 4; AIP + NAC; Group 5; *L*-NAME; Group 6; AIP + *L*-NAME; Group 7; *L*-NAME + AIP + NAC.

however, *L*-NAME infusion before and after AIP (Group 6) did not change the glutathione peroxidase levels significantly. Similar results as seen in Group 6 were observed in Group 7 (Tab 5).

DISCUSSION

In the present study AIP produced significant hypotension associated with initial transient rise in heart rate followed by progressive bradycardia. The ECG findings were characteristic of myocardial ischemia and conduction defects. Thus, decreased cardiac output appears to be

Tab 5. Effect of AIP, NAC, and L-NAME alone and in combination with AIP on myocardial glutathione peroxidase levels at various time intervals. Values are $\bar{x} \pm s$ of 6 animals in each group. $^bP < 0.05$ vs Group 1. $^cP < 0.05$ vs Group 2. $^dP < 0.05$ vs 30 min.

Groups	Glutathione peroxidase/mg·min ⁻¹ ·g ⁻¹		
	After different time intervals in subgroups		
	30 min	60 min	90 min
1	0.20 ± 0.014	0.19 ± 0.009	0.18 ± 0.004
2	0.16 ± 0.009 ^b	0.13 ± 0.014 ^b	0.11 ± 0.012 ^{bd}
3	0.19 ± 0.007	0.19 ± 0.009	0.21 ± 0.009
4	0.18 ± 0.007	0.16 ± 0.009 ^c	0.14 ± 0.009 ^{cd}
5	0.18 ± 0.009	0.18 ± 0.009	0.17 ± 0.007
6	0.14 ± 0.017	0.13 ± 0.009	0.11 ± 0.009
7	0.14 ± 0.009	0.13 ± 0.019	0.11 ± 0.019

Group 1: Control; Group 2: AIP; Group 3: NAC; Group 4: AIP + NAC; Group 5: L-NAME; Group 6: AIP + L-NAME; Group 7: L-NAME + AIP + NAC.

the major determinant of the precipitous fall in BP after AIP. Similar hemodynamic and ECG changes complicated with ischemic myocardial biochemical changes have been reported recently in rat model after 10, 20, and 40 mg/kg of AIP administration^[11]. In patients of AIP poisoning persistent hypotension and ECG abnormalities such as ST elevation, T wave inversion, atrial fibrillation, and ventricular tachycardias have been recorded depending on the severity of poisoning^[1,9,10,17,18]. However, ST-T changes are the most common findings, indicating myocarditis and pericarditis^[10]. The above ECG changes are reported to reverse back to normal pattern within 10–14 d in survivors^[19]. The mortality in AIP poisoning is very high and the survival time is highly variable, depending on the severity which in turn depends on the dose of AIP as well as freshness of the tablets consumed^[20]. In our study the survival time was (90 ± 10) min after AIP (12.5 mg/kg) administration.

The myocardial biochemical changes showed progressive increase in MDA levels indicating continuous lipid peroxidation. Simultaneous decrease in catalase and glutathione peroxidase levels further support the above suggestion. These findings reconfirm our previous observations^[11]. Phosphine gas liberated from AIP is highly toxic and is claimed to act by inhibiting cytochrome-c oxidase thereby impairing mitochondrial metabolism^[8]. Inhibition of electron transfer to the respiratory chain at this site causes cellular hypoxia and leads to generation of superoxide radicals (H₂O₂, O₂⁻). In the blood samples of AIP exposed patients, superoxide dismutase, an en-

zyme that dismutates the free oxygen radicals to H₂O₂ is reported to be raised^[21,22]. In addition, similar to our observations, the level of scavenging enzymes (catalase and GSH Px) were reduced that might have led to further accumulation of free radicals^[21,22]. The excessive H₂O₂ load, is reported to cause protein denaturation and lipid peroxidation in the cell membranes leading to raised MDA levels^[11,12,21,22]. Lipid peroxidation once initiated, further generates oxygen free radicals thus setting up a vicious cycle^[23], a state well supported by our observations. However, in AIP poisoning survivors, restoration of MDA, catalase and superoxide dismutase to normal levels has been seen^[21,22].

The effect of NAC alone on BP in anaesthetized rats was a significant fall associated with reflex tachycardia during the infusion period followed by a lower but stable baseline of both the parameters. Transient initial hypotension may be because of enhanced formation of extracellular nitrosothiol molecules, that have vascular smooth muscle relaxant action^[25] while the maintenance of stable blood pressure afterwards may be due to improvement in myocardial contractility by increasing coronary blood flow and cellular thiol pool of myocardium by NAC^[24]. The ECG remained normal till 30 min of infusion, however, slight inversion of T wave and elevation of ST segment occurred at 60 and 90 min in some of the animals. There is only one case report suggestive of burning chest pain and ECG pattern displaying nonspecific ST elevation and T wave inversion after a loading dose of 150 mg/kg iv of NAC^[25]. The sequential myocardial biochemical studies with NAC revealed significant increase in GSH Px levels and insignificant changes in MDA and catalase levels.

The AIP induced hypotension was significantly less after pre- and post-treatment with NAC. The stabilization of blood pressure at a significantly higher baseline after the infusion could be due to improved myocardial contractility as stated above. The ECG findings remained normal during the period of infusion indicating protection against anoxic myocardial injury. Similar protective effect has been shown in the endotoxemic dogs after the intravenous administration of NAC^[24]. In our study, though the beneficial effect on hemodynamics and ECG was short lived yet it had a significant effect on prolonging the survival time, (120 ± 8) min. Further progressive decrease in MDA and increase in GSH Px at 30, 60, and 90 min of AIP exposure, strongly suggest continued suppression of lipid peroxidation. NAC is a low molecu-

lar weight precursor to glutathione, which can cross the cell membrane and thereby replenish intracellular glutathione stores^[25]. In our study we did not measure the intracellular glutathione levels but determined the amount of GSH Px which is tightly coupled to the cellular concentrations of glutathione. GSH Px reduces H₂O₂ as well as organic hydroperoxides, through the oxidation of reduced GSH thus exerting antioxidant activity^[27]. These observations clearly indicate the usefulness of NAC in AIP poisoning. Increase in survival time by NAC treatment may prove to be of clinical value as this delay would allow phosphine to dissociate from the cellular enzymes^[28]. However, further studies with different dose regimens of NAC are required to prove its beneficial role. Infusion of *L*-NAME alone caused a rise in BP and bradycardia, however after the infusion was stopped there was significant hypotension, tachycardia, and ischemic changes in ECG. Studies on the acute effects of *L*-NAME on hemodynamics in normal rats showed a dose-dependent increase in arterial pressure with bradycardia, without significant effect on cardiac index and ECG recording^[29]. Hypertensive effect is suggested to be due to increased total peripheral resistance. The decline of BP observed after stopping the infusion could be because of myocardial ischemic changes resulting in decreased cardiac index and stroke volume^[30]. The hypotensive effect, probably remained masked by the intense vasoconstriction caused by *L*-NAME during infusion period. In the present study myocardial enzymes (free oxygen radical scavenging enzymes) and MDA levels remained unchanged after *L*-NAME infusion.

Pre- and post-infusion of *L*-NAME not only prevented AIP induced hypotension, but also caused rise in BP during the infusion period of 60 min. The progressive fall in BP after 60 min was associated with severe ECG abnormalities. These findings suggest that NO possibly has some role in AIP induced peripheral circulatory shock, however, it itself is an arrhythmogenic. In patients, *L*-NAME was observed to improve hypotension, but it worsened the QRS prolongation induced by desipramine^[31]. Further in the present study there was no beneficial effect on MDA, catalase, and GSH Px levels after pre- and post-infusion of *L*-NAME with AIP, thus it did not protect the myocardium from oxygen free radical injury. The biochemical findings are well supported by the observation of insignificant improvement in survival time in these animals. In some recent experimental^[32,33] as well as clinical studies^[29] use of *L*-NAME in septic shock is shown to increase the mortality. In Group 7,

simultaneous administration of *L*-NAME, AIP, and NAC led to severe hypotension, bradycardia, conduction defects and biochemical changes indicative of complete depletion of catalase and GSH Px, and increased formation of lipid peroxidation end product, MDA^[12]. Thus NAC and *L*-NAME together did not protect the myocardium from anoxic and/or toxic injury and did not prolong the survival time, thereby further strengthening the conclusion that NO may not have a role in AIP induced cardiovascular toxicity. However, before categorically denying its role, it will be interesting to conduct future studies with higher doses of *L*-NAME and with prolonged infusion of selective NOS inhibitors. To conclude, NAC increased the survival time by reducing the myocardial oxidative injury whereas *L*-NAME showed no such protective effects in rats exposed to AIP.

ACKNOWLEDGEMENTS The financial support from United Phosphorus Limited (India) and technical assistance of Ms Meenu are gratefully acknowledged.

REFERENCES

- 1 Bajaj R, Wasir H S. Epidemic AIP poisoning in northern India. *Lancet* 1988; 1: 820-1.
- 2 Siwach SB, Yadav DR, Arora BB, Dalal S, Jagdish. Acute aluminium phosphide poisoning — an epidemiological, clinical and histo-pathological study. *J Assoc Physicians India* 1988; 36: 594-6.
- 3 Lall SB, Peshin SS, Seth SD. Acute poisoning: A ten years retrospective hospital based studies. *Ann Natl Acad Med Sci (India)* 1994; 30: 35-9.
- 4 Anger F, Paysant F, Brousses F, Le Normand I, Develay P, Gaillary, *et al.* Fatal aluminium phosphide poisoning. *J Anal Toxicol* 2000; 24: 90-2.
- 5 Mitra S, Lall SB, Peshin SS. Cholinesterase inhibition by aluminium phosphide poisoning in rats and effect of atropine and pralidoxime. *Acta Pharmacol Sin* 2001; 22: 30-2.
- 6 Lall SB, Peshin SS, Mitra S. Methemoglobinemia in aluminium phosphide poisoning in rats. *Indian J Exp Biol* 2000; 38: 95-7.
- 7 Chefurka W, Kashi KP. The effect of phosphine on the absorption and circuldichronine spectra of cytochrome-C and cytochrome oxidase. *Pesticide Biochem Physiol* 1976; 6: 350-62.
- 8 Bolter CJ, Chefurka W. Extramitochondrial release of H₂O₂ from insect and mouse liver mitochondria using the respiratory inhibitory phosphine, myoxathiazole and antimycin and spectral analysis of inhibited cytochromes. *Arch Biochem Biophys* 1989; 278: 73-80.
- 9 Koley TK. Aluminium phosphide poisoning-part-I. *Ind J Clin Pract* 1998; 9: 14-24.
- 10 Gupta MS, Malik A, Sharma VK. Cardiovascular manifesta-

- tions in aluminium phosphide poisoning with special reference to ECG changes. *J Assoc Physicians India* 1995; 43: 773-80.
- 11 Lall SB, Sinha K, Mitra S, Seth SD. An experimental study on cardiotoxicity of aluminium phosphide poisoning. *Indian J Exp Biol* 1997; 35: 1060-4.
 - 12 Chugh SN, Mittal A, Seth S, Chugh K. Lipid peroxidation in acute AIP poisoning. *J Assoc Physicians India* 1995; 40: 265-6.
 - 13 Howland MA, Smilksteine M, Weisman RS. Antidotes in depth, *N*-acetyl cysteine. In: Lewis R Goldfrank, editor. *Goldfrank's toxicologic emergencies*. Paramount publishing business and professional group; 1994. 5th ed. p 498-500.
 - 14 Okhawa H, Ohishi N, Yogi K. Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-8.
 - 15 Hans L Catalase. *Methods of enzymatic analysis*. v 2. In: Hans-Ulrich Berneyer, editor. Florida: Verlag Ghemich Int; 1963. p 885-8.
 - 16 Khaper N, Singal PK. Effects of afterload-reducing drugs on pathogenesis of antioxidant changes and congestive heart failure in rats. *J Am Coll Cardiol* 1997; 29: 856-61.
 - 17 Jain SM, Bharani A, Sepaha GC, Sanghavi VC, Raman PC. ECG changes in AIP poisoning. *J Assoc Physicians India* 1985; 33: 406-9.
 - 18 Katira R, Enhence GP, Malhotra KC. A study of AIP poisoning with special reference to ECG changes. *J Assoc Physicians India* 1990; 38: 471-3.
 - 19 Singh S, Dilwari JB, Vashisht R, Malhotra HS, Sharma BK. Aluminium phosphide ingestion. *Br Med J* 1985; 290: 1110-1.
 - 20 Chugh SN, Dushyant, Sant R, Arora B, Malhotra KC. Incidence and outcome of AIP poisoning in hospital study. *Ind J Med Res* 1991; 94: 232-5.
 - 21 Chugh SN, Pal R, Singh V, Seth S. Serial blood phosphine level in acute AIP poisoning. *J Assoc Physicians India* 1996; 44: 184-5.
 - 22 Chugh SN, Arora V, Sharma A, Chugh K. Free radical scavenger and lipid peroxidation in acute AIP poisoning. *Ind J Med Res* 1996; 104: 190-3.
 - 23 Halliwell B, Gutteridge JMC. Free radicals and ischemic tissue injury. *Arch Biochem Biophys* 1985; 246: 501-40.
 - 24 Zhang H, Spapen H, Nguyen DN, Benlabed M, Vincent J. Protective effect *N*-acetyl-*L*-cysteine in endotoxemia. *Am J Physiol* 1994; 266: H1746-H1754.
 - 25 Mark FB, Sheldon MT, Darrell TH, Bradley RM. Anaphylactoid reaction to intravenous acetyl cysteine associated with ECG abnormalities. *Ann Pharmacother* 1992; 26: 22-5.
 - 26 Kharazmi A, Nielsen H, Schiotz PO. *N*-acetylcysteine inhibits human neutrophil and monocyte chemotaxis and oxidative metabolism. *Int J Immunopharmacol* 1988; 10: 39-46.
 - 27 Maiorino M, Gregolin C, Ursini F. Phospholipid hydroperoxide glutathione peroxidase. *Methods Enzymology* 1990; 186: 448-56.
 - 28 Petre J, Meire (abstract). Role of *N*-AC in other types of poisoning. Abstract book of Swiss toxicology information centre and division of clinical pharmacology and toxicology. 1997; 34.
 - 29 Hu CT, Chang KC, Wu CY, Chen H. Acute effects of nitric oxide blockade with *L*-NAME on arterial hemodynamics in the rats. *Br J Pharmacol* 1997; 122: 1237-43.
 - 30 Jurgen AM, Rudolf P, Steven LC, Karan JK, Hajo A. Effect of *L*-NAME, an inhibitor of nitric oxide synthase, on cardiopulmonary function in human septic shock. *Chest* 1998; 113: 1640-6.
 - 31 Pentel PR, Wanankul W, Scarlett W, Keyler DE. Nitric oxide contributes to desipramine induced hypotension in rats. *Hum Exp Toxicol* 1996; 15: 320-8.
 - 32 Minnard EA, Shou J, Naama H. Inhibition of nitric oxide synthesis is detrimental during endotoxemia. *Arch Surg* 1994; 129: 142-7.
 - 33 Wu C, Ruetien H, Thiermermann C. Comparison of the effect of aminoguanidine and *N*^G-nitro-*L*-arginine methyl ester on the multiple organ dysfunction caused by endotoxemia in the rat. *Eur J Pharmacol* 1996; 300: 99-104.
- N*-乙酰半胱氨酸和 *L*-NAME 对磷化铝诱导的大鼠心血管毒性的作用**
- Archana AZAD, Shyam Bala LALL¹, Shivani MITTRA
(*Department of Pharmacology All India Institute of Medical Sciences New Delhi-110029, India*)
- 关键词** 铝化合物; 乙酰半胱氨酸; *N*^G-硝基精氨酸甲酯; 丙二醛; 过氧化氢酶; 谷胱甘肽过氧化酶
- (责任编辑 吕静)