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An electrocardiographic, molecular and biochemical approach to explore the cardioprotective effect of vasopressin and milrinone against phosphide toxicity in rats

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ABSTRACT

The present study was conducted to identify the protective effect of vasopressin (AVP) and milrinone on cardiovascular function, mitochondrial complex activities, cellular ATP reserve, oxidative stress, and apoptosis in rats poisoned by aluminum phosphide (AlP). Rats were divided into five groups (n = 12) including control, AlP (12.5 mg/kg), AlP + AVP (2.0 Units/kg), AlP + milrinone (0.25 mg/kg) and AlP + AVP + milrinone. After treatment, the animals were connected to an electronic cardiovascular monitoring device to monitor electrocardiographic (ECG) parameter. Finally, oxidative stress biomarkers, mitochondrial complex activities, ADP/ATP ratio and apoptosis were evaluated on the heart tissues. Results indicated that AlP administration induced ECG abnormalities along with a decline in blood pressure and heart rate. AVP and milrinone significantly ameliorated these changes in all treated groups. Considerable protective effects on oxidative stress biomarkers, complex IV activity, ADP/ATP ratio and caspase-3 and -9 activities in treated groups were also found. These findings were supported by flow cytometry assay of cardiomyocytes. In conclusion, administration of AVP and milrinone, not only improve cardiovascular functions in AlP poisoned rats in the short time, but after a long time can also restore mitochondrial function and ATP level and reduce the oxidative damage, which prevent cardiomyocytes from entering the apoptotic phase.

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1. Introduction

Aluminum phosphide (AlP), a solid fumigant insecticide and rodenticide, is usually used to protect food products from pest during the storage and transportation processes (Bumbrah et al., 2012; Mostafazadeh, 2012; Proudfoot, 2009). This agent is known as rice tablet in Iran and extensively used by farmers despite restricted sale (Mehrpour et al., 2012; Moghhadamnia, 2012; Mostafalou et al., 2013). This may be due to its special properties such as being highly potent against all stages of insects and cost beneficial, having no effect on seed viability, and leaving little residue on food products (Anand et al., 2011; Bumbrah et al., 2012; Moghhadamnia, 2012). Despite all good properties, it is very dangerous for non-targeted species such as humans so that more than 70% of the acute intoxicated patients die following AlP ingestions (Anand et al., 2011; Mostafalou et al., 2013; Singh et al., 1989). Poisonous effects of AlP tablet are due to fatal phosphine gas released when it comes into contact with water or hydrochloric acid in the stomach (Gurjar et al., 2011; Moghhadamnia, 2012). The exact mechanism of phosphine is still unknown; however, the results of some animal studies showed that oxidative stress, inhibition of cytochrome oxidase and cellular oxygen utilization in mitochondria, denaturation of oxyhemoglobin molecule and interfering with several enzymes or ion channels are plausible mechanisms of AlP toxicity (Anand et al., 2011; Mehrpour et al., 2012; Moghhadamnia, 2012; Mostafazadeh, 2012; Nath et al., 2011). The signs and symptoms of phosphine toxicity are nonspecific and most organs are affected and usually result to multi-organ failure (Bumbrah et al., 2012; Gurjar et al.,

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2011). However, the heart is the predominantly affected organ and most of intoxicated patients die of cardiovascular complications and intractable hypotension (Bogle et al., 2006; Chugh et al., 1991; Moghhadamnia, 2012; Mostafazadeh, 2012). Severe and refractory hypotension usually occurs following many poisonings and overdoses.

Conventional vasopressors, especially those having direct effects on alpha receptors, may not regularly improve hypotension in the setting of acute poisonings. There are several reports indicating that vasopressin (AVP) has had beneficial effects on severe hypotension and shock. Barry et al. have reported about the successful use of intravenous AVP in a case of a patient who had ingested a bottle of amitriptyline and showed hypotension unresponsive to conventional vasopressors and pH manipulation (Barry et al., 2006). In patients with massive calcium channel blockers and caffeine overdoses, AVP was successfully used to treat refractory hypotension which was unresponsive to calcium, glucagon, insulin, and conventional vasopressor therapies (Holstege et al., 2003; Kanagarajan et al., 2007). Also, low-dose AVP leads to significant increases in vascular tone in septic shock and in late vasodilated hemorrhagic shock and improves response to infused catecholamines (such as norepinephrine) (Russell, 2007). This hormone probably causes vasoconstriction and thereby increases systemic vascular resistance and blood pressure by the two main mechanisms. First, activation of V_1 receptors in vascular smooth muscles by AVP increases cytoplasmic Ca^{2+} through the phosphotidyl-inositol-biphosphate (PIP2) cascade. Second, blockage of K_{ATP} channels within the smooth muscle cell membrane by AVP facilitates myocyte depolarization and thus vasoconstriction (Dünser et al., 2001; Russell, 2007).

Another complication of AlP intoxication is cardiac dysfunctions such as lethal heart failure. Phosphodiesterase III inhibitors, especially milrinone, can potentially improve the hemodynamic status of acute heart failure in the setting of acute poisoning because these agents have potent inotropic effects via the increase of intracellular cAMP levels in myocytes (Feneck, 2007; Lescan et al., 2013; Rahimi et al., 2010). These inotropic compounds increase myocardial contractile force with less cardiac oxygen demand than catecholamines (Monrad et al., 1986; Satoh and Endoh, 1990; Tosaka et al., 2007). Unlike catecholamines, phosphodiesterase III inhibitors increase the intracellular cAMP levels by non-adrenergic pathways (Lescan et al., 2013; Monrad et al., 1986; Yano et al., 2000).

It was hypothesized that milrinone, a phosphodiesterase III inhibitor, might improve myocardial contractility accompanied by AVP induced vasoconstriction and correction of hypotension in acute AlP poisonings. The present study, therefore, aimed to investigate the protective effect of AVP and milrinone alone and in combination on hemodynamic (BP, HR, ECG), molecular and biochemical properties (oxidative stress biomarkers, mitochondrial complex activities, ADP/ATP ratio and apoptosis) in a rat model of AlP poisoning.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (GmbH, Munich, Germany) unless otherwise mentioned. AlP from Samiran Pesticide Formulating Co. (Tehran, Iran), Primacor® (Milrinone) from Sepaco Darou Pharmaceutical Center Ssk (Tehran, Iran), and Hypress® (Arginine Vasopressin) from Exir Pharmaceutical Co. (Tehran, Iran) were used in this study. The mitochondria isolation kit was purchased from BioChain Inc. (Newark, New Jersey, USA). Annexin V-FITC/PI was obtained from Beijing Biosea Biotechnology Co, Ltd (Beijing, China). ELISA kits for oxidative stress biomarkers from Cayman Chemical Co. (Michigan, USA) were used in this study.

2.2. Study design and methods

2.2.1. Animals

All experiments were done on animals, according to the ethical guidelines on the use of animals and were approved by the Ethics Committee of Tehran University of Medical Sciences with code number 92-01-33-21796. All male Wistar rats weighing 200–250 g were obtained from animal house of Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran) and housed in controlled environmental conditions of 20 to 25 °C temperature, relative humidity (50–55%), and 12-h light/dark cycle with free access to stock laboratory diet and water.

2.2.2. Determination of AlP LD50

Based on previous studies, AlP LD50 includes a wide range (8.7–12 mg/kg). This may be due to the continuous decomposition of AlP during shelf life. Thus, it was necessary to determine the LD50 of AlP for each experiment. The doses more than 15 mg/kg of AlP usually results in 100% mortality and no mortality is observed in doses less than 8 mg/kg; hence doses ranging between 8 and 15 mg/kg were used and dissolved in almond oil and administered to rats by gavage. Control group received only equivalent amount almond oil. Four rats per group were used at each dose level. Twenty four hours after treatment, mortality was recorded. Finally, AlP LD50 was determined at 12.51 mg/kg by using probit (Baeeri et al., 2013).

2.2.3. Study design

A pilot experiment was designed to determine the optimum dose of AVP and milrinone in rats poisoned with an LD50 dose of AlP. Five doses of AVP (1.0, 2.0, 3.0, 4.0, 8.0 U/kg) and milrinone (0.125, 0.25, 0.5, 0.75, 1.0 mg/kg) were chosen and based on hemodynamic parameters (electrocardiogram [ECG], Blood pressure [BP], heart rate [HR]); the optimum doses were determined. These doses, which decrease cardiovascular complications of AlP with less adverse effects, were used in the next step of the study. After determining LD50 of AlP (12.5 mg/kg) and optimum doses of AVP (2.0 U/kg) and milrinone (0.25 mg/kg), the animals were randomly categorized into five groups of twelve rats each, including group 1 (control), group 2 (AlP), group 3 (AlP + AVP), group 4 (AlP + milrinone), group 5 (AlP + AVP + milrinone). AlP was dissolved in almond oil and administered by gavage. AVP and milrinone were dissolved in saline and administered intraperitoneally. Control animals received only almond oil in appropriate volume. Each group was further divided into two subgroups of six rats each. In one group hemodynamic parameters were recorded while another group was sacrificed at 24 hours after treatment for biochemical studies (oxidative stress analyses, mitochondrial complex activity analyses, ADP/ATP ratio, caspase-3,-9 activity assays and flow cytometry assays). To measure hemodynamic parameters, AlP (LD50 dose) was administered intra-gastrically to all animals except the control. After 30 min, the animals were anesthetized by intraperitoneal injection of ketamine/xylazin (60/6 mg/kg) which was repeated at 30/3 mg/kg post 45 min, 1.5 h and 2.5 h, respectively, to maintain full general anesthesia until the completion of the experiment (3.0 h). After induction of anesthesia, the animal was quickly connected to a PowerLab system (PowerLab 4/35 Data Acquisition Systems, AD Instruments, Australia) to monitor electrocardiogram (ECG), blood pressure (BP), and heart rate (HR), noninvasively. AVP and milrinone were administered by intraperitoneal injection 60 min after AlP administration. But for biochemical studies, 24 hours after treatments, animals were sacrificed and the heart was dissected out and rinsed in ice-cold saline to remove the blood and immediately frozen and stored at −80 °C for various biochemical assays. It should be noted that for biochemical assays, 0.25 median lethal dose [LD50] of AlP was administered by gavage to all animals except the control group, and treatment groups received AVP and milrinone after 60 min. Based on our previous studies, this dose of AlP can induce cardiotoxicity without mortality (Baeeri et al., 2013) and we needed all animals alive after 24 hours to assay the cardioprotective effects of AVP and milrinone in rats poisoned with AlP.

2.2.4. ECG, BP, and HR

After induction of anesthesia, the electrodes were subcutaneously connected to the right hand and both right and left paws of the immobilized rat, the ECG data were obtained for 3 hours. The obtained data were analyzed by PowerLab system software and QRS complexes and the segments of QTc, PR, and ST were measured. In addition to the ECG, the systolic BP and HR was recorded by the tail cuff of PowerLab which was connected to the rat's external tail where the pulse was detected.

2.2.5. Tissue sampling and mitochondrial isolation

As mentioned above, twenty four hours after treatments, the animals were sacrificed and the heart was removed and rinsed in ice-cold saline to remove the blood. The heart tissue was divided into several sections. A small section of tissues (100 mg) was taken for mitochondrial complex assays and the rest of the tissues were stored in −80 °C for other various biochemical studies. For preparation of heart mitochondria, 100 mg of heart tissues was processed according to mitochondria isolation kit protocol.

2.2.6. Determination of NADH dehydrogenase activity

The principle of this assay is based on the consumption of NADH, which passes electrons to complex I, which are then passed to synthetic ubiquinone, as the electron acceptor. NADH dehydrogenase (complex I) activity was assayed in heart homogenate according to the method of Sherwood and Hirst (2006). The mitochondria (100–200 μg of total mitochondrial protein) were added to the reaction mixture containing potassium phosphate buffer (25 mM; pH = 7.4), 25% bovine serum albumin, magnesium chloride (MgCl2; 5 mM), decylubiquinone (2.8 mM), NADH (5.7 mM), antimycin A (3.7 mM), and potassium cyanide (KCN; 2 mM) to start the reaction and the alteration in NADH absorbance was measured at 340 nm for 3 min before the addition of rotenone. After adding rotenone (0.36 mM) to the reaction mixture, the

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