Life Sciences 139 (2015) 30–39

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Molecular and biochemical evidences on the protective effects of triiodothyronine against phosphine-induced cardiac and mitochondrial toxicity

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 12 July 2015
Accepted 26 July 2015
Available online 1 August 2015

Keywords:
Triiodothyronine
Aluminum phosphide
Phosphine gas (PH_3)
Oxidative stress
Mitochondrial toxicity
Apoptosis

ABSTRACT

Aim: Aluminum phosphide (AlP) is a widely used fumigant and rodenticide. While AlP ingestion leads to high mortality, its exact mechanism of action is unclear. There are ample evidences suggesting cardioprotective effects of triiodothyronine (T3). In this study, we aimed to examine the potential of T3 in the protection of a rat model of AlP induced cardiotoxicity.

Main methods: In order to induce AlP intoxication animals were intoxicated with AlP (12 mg/kg; LD50) by gavage. In treatment groups, T3 (1, 2 and 3 μg/kg) was administered intra-peritoneally 30 min after AlP administration. Animals were connected to the electronic cardiovascular monitoring device simultaneously after T3 administration. Then, electrocardiogram (ECG), blood pressure (BP), and heart rate (HR) were monitored for 180 min. Additionally, 24 h after AlP intoxication, rats were deceased and the hearts were dissected out for evaluation of oxidative stress, cardiac mitochondrial function (complexes I, II and IV), ATP/ADP ratio, caspases 3 & 9, and apoptosis by flow cytometry.

Key findings: The results demonstrated that AlP intoxication causes cardiac toxicity presenting with changes in ECG patterns such as decrement of HR, BP and abnormal QRS complexes, QTc and ST height. T3 at a dose of 3 μg/kg significantly improved ECG and also oxidative stress parameters. Furthermore, T3 administration could increase mitochondrial function and ATP levels within the cardiac cells. In addition, administration of T3 showed a reduction in apoptosis through diminishing the caspase activities and improving cell viability.

Significance: Overall, the present data demonstrate the beneficial effects of T3 in cardiotoxicity of AlP.

1. Introduction

Aluminum phosphide (AlP) is a commonly used insecticide, rodenticide and fumigant. Poisoning by deliberate self-ingestion of AlP is a common cause of death and socioeconomic loss worldwide, especially in developing countries [2,13,26]. AlP is sold as pallet, tablet, porous blister pack, sachets, and as dusts [43].

While the exact mechanism of AlP toxicity is still unclear, several studies suggest that phosphine gas (PH_3) is a key player in AlP toxicity. PH_3 is a highly reactive radical, which can freely diffuse into intracellular compartments. PH_3 is released from AlP upon contact with water, moisture or hydrochloric acid of the stomach [43]. There are ample evidences suggesting that PH_3 can initiate a nucleophilic attack and reduce vital enzymes [3].

AlP intoxication is mostly fatal by causing multiorgan damage through denaturation of cell membranes [53,56,57]. While AlP can cause a wide range of clinical manifestations, circulatory failure is the most common cause of mortality and morbidity in AlP ingested patients [5,60]. Ventricular arrhythmias or dysfunction is a primary outcome of

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http://dx.doi.org/10.1016/j.lfs.2015.07.026
0024-3205/

AlP induced cardiac toxicity. Virtually any type of arrhythmia and conduction abnormality such as tachycardia and bradycardia can occur. On the other hand, there are several studies reporting reduced left ventricular ejection following AlP poisoning [8,12].

Evidences for the mechanism of PH_3 demonstrated that it disrupts mitochondrial activity through inhibition of cytochrome c oxidase (complex IV), decrement of complex I and II activity and also impairment in ATP synthesis [59,20]. Consequently, inhibition of the electron transport chain (ETC) could lead to a raise of reactive oxygen species (ROS) production [3]. In addition, PH_3 can exacerbate oxidative stress through inhibition of catalase, peroxidase and increasing the superoxide dismutase (SOD) activity [9].

Most patients with established AlP poisoning die despite intensive care. There are several choices used for the management of AlP poisoning, including gastric lavage with potassium permanganate solution and oral sodium bicarbonate (Bicarb) with activated charcoal. In addition, oral coconut oil and intravenous magnesium have been proposed to be effective [56,49]. Despite many efforts conducted in order to overcome this challenge, the management of AlP poisoning is still a big obstacle in the world. Besides, there is no specific antidote and the major part of management remains supportive only [3].

Thyroid hormones (THs) are inotropic hormones, which could be potentially used to support hemodynamics [21,22,33,47,48]. Subsequently, several clinical studies appeared using triiodothyronine (T3) or thyroxine (T4) to treat heart failure. Short-term intravenous administration of T3 to patients with advanced congestive heart failure was reported to improve cardiac output and decrease arterial vascular resistance [28].

On the basis of the evidence obtained from cells, animals, and even humans, it seems likely that timely treatments targeting the TH signaling may promote endogenous regeneration of the damaged myocardium [39,54,50]. THs are well-known regulators of mitochondrial biogenesis and function [65,27]. THs have been shown to limit cell apoptosis under stress conditions in several models [24,39]. A previous study demonstrated that THs are potent regulators of cardiac muscle contractility [10]. In the present study, we aimed to investigate the potential of T3 in the cardioprotection of a rat model of AlP-poisoning through examining electrocardiographic and biochemical parameters of toxicity.

2. Materials and methods

2.1. Ethics

The procedures implemented throughout the study were approved by the Ethics Committee of Tehran University of Medical Sciences in accordance with the Standards for the Care and Use of Laboratory Animals with code number 89-03-33-11232.

2.2. Chemicals

The following compounds were used throughout the study: AlP (>95% purity) was purchased from Samiran Pesticide Formulating Co. (Tehran, Iran). Triiodothyronine (T3) (Sandoz Co.). The mitochondria isolation kit was obtained from Bio-Chain Ins. (Newark, New Jersey, USA). Annexin V-FITC/PI was obtained from Beijing Biosea Biotechnology Co, Ltd. (Beijing, China). Adenosine diphosphate (ADP) sodium salt, adenosine triphosphate disodium salt (ATP), tetrabutylammonium hydroxide (TBAHS), methanol (HPLC grade), column (SUPELCOSIL™ LC-18-T) from Supelco (Antrim, UK), acetic acid, $FeCl_3$–$6H_2O$, sodium sulfate, trichloroacetic acid (TCA), potassium dihydrogen phosphate anhydrous (KH_2PO_4, analytical grade), 2,4,6-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid (TBA), rotenone, 2,6-dichloroindophenol (DCIP), antimycin A, and collagenase. All other chemicals were of the highest purity available and were purchased from Aldrich Chemical Co. Sigma Chemical or Co. (St Louis, Missouri, USA).

2.3. Animals

Sixty male Albino Wistar rats weighing 200–250 g were used in this study. The animals were housed in standard polycarbonate cages in groups of 4–5 and kept in a temperature-controlled room (22 °C) with a 12 h light/12 h dark cycle. Animals were acclimated at least 2 days before experiments with free access to food and water. The experiments were conducted between 09:00 and 13:00. All procedures were carried out in accordance with institutional guidelines for animal care and use. The groups consisted of at least twelve animals and each animal was used only once. Additionally, efforts were made to reduce animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.4. Induction of AlP intoxication

AlP (4–16 mg/kg; dissolved in 2 ml of almond oil) was orally administered (gavage, in almond oil) to different groups of rats. The mortality rate was recorded for 24 h. For this purpose, the oral LD50 of AlP was calculated according to Probit analysis (12 mg/kg) and was used for induction of cardiac toxicity and related events [6,32].

2.5. Experimental design

A total number of 60 animals were assigned randomly to 5 groups, each comprising 12 rats. Rats were treated according to the following schemas:

in the first step of this study, in order to evaluate the electrocardiogram (ECG) parameters, rats were received 12 mg/kg AlP orally (LD50) in all groups except the control group which received almond oil. Then the animals were assigned randomly to 5 groups, including 1) the control group that received almond oil alone; 2) the AlP group that received aluminum phosphide (12 mg/kg); 3) the AlP + T3-1 group that received AlP (12 mg/kg) + T3 (1 μg/kg); 4) the AlP + T3-2 group that received AlP (12 mg/kg) + T3 (2 μg/kg); and 5) the AlP + T3-3 group that received AlP (12 mg/kg) + T3 (3 μg/kg). T3 was administered by intra-peritoneal injection (i.p.) 30 min after AlP administration by gavage in the groups which received T3.

In order to evaluate the biochemical parameters, rats were assigned randomly to 5 groups; all groups except the control group received 0.25 LD50 of AlP including 1) the control group that received almond oil alone; 2) the AlP group that received aluminum phosphide (0.25 LD50); 3) the AlP + T3-1 group that received AlP (0.25 LD50) + T3 (1 μg/kg); 4) the AlP + T3-2 group that received AlP (0.25 LD50) + T3 (2 μg/kg); and 5) the AlP + T3-3 group that received AlP (0.25 LD50) + T3 (3 μg/kg).

The doses of T3, as well as the time interval between drug injection and AlP intoxication (30 min) were chosen according to literature review [16,24,32].

2.6. Determination of electrocardiogram (ECG) parameters

Thirty minutes after AlP gavage, animals were anesthetized by intraperitoneal injection of (30 mg/kg) thiopental sodium and rapidly connected to the PowerLab device (PowerLab 4/35 Data Acquisition Systems, AD Instruments, Australia) for complete monitoring of ECG. For maintaining full general anesthesia, 30 mg/kg thiopental sodium was repeated after 40 min and 2.5 h until the end of the experiment. ECG needle electrodes of the Powerlab™ were inserted under the skin of the right hand and both legs of the anesthetized rat (position II) and continuous ECG data were achieved for 3 h. For each ECG tracing, QRS complexes and the segments of QT, and ST were measured. ECGs were analyzed by PowerLab system software. The heart rate (HR) and systolic blood pressure (BP) were recorded every 3 min by using the tail cuff which was connected to the anesthetized rat tail [6,32].