Available online at www.sciencedirect.com

ScienceDirect

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

Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats

Temidayo O. Omobowale[a], *Ademola A. Oyagbemi*[b,*], *Akinleye S. Akinrinde*[b], *Adebowale B. Saba*[b], *Oluwabusola T. Daramola*[a], *Blessing S. Ogunpolu*[a], *James O. Olopade*[c]

[a] *Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*
[b] *Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*
[c] *Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria*

ARTICLE INFO

Article history:
Received 25 December 2013
Received in revised form
3 March 2014
Accepted 6 March 2014
Available online 28 March 2014

Keywords:
Liver
Erythrocyte
Lead acetate
Hepatotoxicity
Oxidative stress
COX-2

ABSTRACT

Lead acetate (PbA) is one of the major environmental contaminants with grave toxicological consequences both in the developing and developed countries. The liver and erythrocyte antioxidant status and markers of oxidative were assessed. Exposure of rats to PbA led to significant decline ($p < 0.05$) in hepatic and erythrocyte glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) content. Similarly, malondialdehyde (MDA) and H_2O_2 concentrations were significantly ($p < 0.05$) elevated. Histopathology and immunohistology of liver of rats exposed to PbA showed focal areas of necrosis and COX-2 expression after 6 weeks of PbA withdrawal. Taken together, hepatic and erythrocytes antioxidant defence system failed to recover after withdrawal of the exposed PbA for the period of the study. In conclusion, experimental animals exposed to PbA did not recover from hepatotoxicity and disruption of erythrocyte antioxidant defence system via free radical generation and oxidative stress.

1. Introduction

The liver is known to be the highest depot of lead in soft tissues followed by the kidney (Mudipalli, 2007). Tissue distribution of lead in the livers, kidneys, heart, lung, spleen, muscles and bones has recently been reported (Kim and Oh, 2013; de Sousa et al., 2013). Documentation has shown that chronic ingestion of lead leads to a significant decrease in liver enzymic and non-enzymic antioxidant including reduced glutathione (GSH) levels, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) with concomitant increase in reactive oxygen species (ROS), Malondialdehyde (MDA) content, generation of superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) content (Qiao et al., 2013; Mohammadi et al., 2013; Wang et al., 2013). Similarly, ROS have been reported to play a critical role in both physiological and pathological conditions with resultant increase in DNA damage and

* Corresponding author. Tel.: +234 8033639776; fax: +234 028103043.
E-mail addresses: ademolaoyagbemi@yahoo.com, ademola.oyagbemi778@gmail.com, aa.oyagbemi@mail.ui.edu.ng (A.A. Oyagbemi).

http://dx.doi.org/10.1016/j.etap.2014.03.002
1382-6689/

apoptosis. Several studies have shown that the source and target of ROS to be the mitochondria (Wang et al., 2007). It has been reported that Pb-exposed individuals had lower values of several haematological parameters such as haemoglobin, haematocrit, red blood cell count, and mean corpuscular volume than controls (Feksa et al., 2012). The levels of hepatic and renal markers such as alanine aminotransferase, aspartate aminotransferase, triglycerides, cholesterol, urea, and uric acid have been reported to increase significantly following administration of lead acetate (Sharma et al., 2013).

Chronic lead toxicity can induce anaemia as a result of death of erythrocytes characterized by excess accumulation of ROS which ultimately downregulate antioxidant system leading to oxidative stress and externalization of phosphatidylserine. Recently, Lakshmi et al. (2013) reported significant increase in the levels of AST, ALT, ALP, BUN and serum creatinine and decreased levels of total protein of rats exposed to lead. They also observed significant decrease in the body weight and organ weights as well as significant decrease in haemoglobin, red blood cell and total leucocyte count in exposed animals (Lakshmi et al., 2013).

The influence of occupational lead-exposure on the gene expression (Sod1) and activity (SOD) of superoxide dismutase, catalase and glutathione peroxidase (GPx, Gpx1) in leukocytes and erythrocytes has been described (Kasperczyk et al., 2012). The gene expression levels of both Sod1 and Gpx1 were significantly increased in the low exposure to lead (LE) group as compared to the control group with an insignificant tendency for increased gene expression of both Sod1 and Gpx1 in the high exposure to lead (HE) group. The SOD and GPx activities in erythrocytes were significantly elevated in both examined subgroups (LE and HE), whereas SOD activity in leukocytes was raised only in the LE group (Kasperczyk et al., 2012).

The aim of this study was to investigate the possibility of recovery or failure of recovery from lead exposed rats following withdrawal.

2. Materials and methods

2.1. Chemicals

Glutathione, 1,2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), trichloroacetic acid, sodium hydroxide, xylenol orange, potassium hydroxide and hydrogen peroxide were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were obtained from British Drug Houses (Poole, Dorset, UK).

2.2. Animal treatment

Twenty four adult male rats weighing approximately 200–290 g obtained from the Experimental Animal Unit of faculty of Veterinary Medicine, University of Ibadan, randomly divided into 4 groups of 14 animals per group. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow ad libitum and liberally supplied with water.

Control group (group I) received normal saline while groups II, III and IV received 0.25, 0.5 and 1.0 mg/ml of lead acetate (PbA) respectively for 6 weeks. One half of the population of the rats was sacrificed at 6 weeks and PbA was withdrawn from the remaining rats for another 6 weeks.

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments (PHS, 1996).

2.3. Blood collection and preparation of erythrocyte

About five millilitres (5 ml) of the blood was drawn from the retro-orbital venous plexus of the animals into vials containing heparin as an anticoagulant before they were sacrificed by cervical dislocation. The erythrocytes were sedimented by centrifugation at 4000 rpm for 10 min after 30 min of collection and the plasma was harvested for plasma biochemistry. The erythrocytes were washed three times with ice-cold phosphate buffer saline at pH 7.4 centrifuged. The erythrocyte membrane was lysed and the pellets were resuspended in PBS at dilutions until the time of use. The pellets thus obtained were washed repeatedly in the same buffer to obtain haemoglobin-free white membranes (Steck and Kant, 1974).

2.4. Preparation of microsomal fraction

Rats were starved overnight and sacrificed by cervical dislocation. Livers were removed, rinsed in 1.15% KCl and homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4) and homogenates were centrifuged at $10{,}000 \times g$ for 20 min to obtain the supernatant fraction.

2.5. Biochemical assays and heamatolgical parameters

The supernatant was collected for the estimation of Catalase (CAT) activity according to the method of Shinha (1972). Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 7.2 at 30 °C as described (Misra and Fridovich, 1972) with modification from our laboratory. Briefly, 50 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. This preparation prevents oxidation of epinephrine and is stable for 4 weeks. 30 μL liver or 10 μL of erythrocyte extract was added to 2.5 ml 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300 μL of adrenaline. The increase in absorbance at 480 nm was monitored every 30 s for 150 s. Glutathione-S-transferase (GST) was estimated by the method of Habig et al. (1974) using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Protein concentration was determined by the method of Lowry (1951). Reduced GSH was determined at 412 nm using the method described by Jollow et al. (1994). Hydrogen peroxide generation was determined as described (Woff, 1994). The MDA level was calculated according to the method of Farombi et al. (2000). Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$. Glutathione peroxidase activity was measured according to Buetler et al. (1963).