Toxicology Letters 218 (2013) 273–280

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

Nephroprotective effect of calcium channel blockers against toxicity of lead exposure in mice

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HIGHLIGHTS

- CCBs reduced renal Pb accumulation in mice with Pb-induced kidney damage.
- CCBs alleviated renal pathological damage and nephrocyte apoptosis induced by Pb.
- CCBs inhibited lipid peroxidation induced by Pb.
- CCBs shows the protective effects on Pb-induced nephrotoxicity.

ARTICLE INFO

Article history:
Received 15 December 2012
Received in revised form 6 February 2013
Accepted 9 February 2013
Available online 18 February 2013

Keywords:
Verapamil
Nimodipine
Oxidative stress
Lead
Nephrotoxicity
Calcium channel blockers

ABSTRACT

Exposure to lead (Pb) can induce kidney damage, which is related to induction of oxidative damage and disturbance of intracellular calcium homeostasis. Pb can readily permeate through dihydropyridine-sensitive L-type calcium channels and accumulate within cells. The objective of this study was to investigate protective effects of calcium channel blockers (CCBs) verapamil and nimodipine on nephrotoxicity induced by Pb acetate in mice. One hundred and twenty male mice were randomly divided into 6 groups: control, Pb, low-dose verapamil, high-dose verapamil, low-dose nimodipine and high-dose nimodipine ($n = 20$ per group). Pb acetate was injected intraperitoneally (i.p.) at 40 mg/kg body weight/day for 10 days to establish the Pb toxicity model. While control mice received saline, mice of the treated groups simultaneously received i.p. injections of verapamil or nimodipine daily for 10 days. Both verapamil and nimodipine showed protection against Pb-induced kidney injury, including alleviation of renal pathological damage and decreasing the level of Pb in kidney homogenate and extent of apoptosis in nephrocytes. Moreover, verapamil and nimodipine significantly down-regulated levels of blood urea nitrogen and creatinine in the serum. In addition, verapamil and nimodipine administration decreased malondialdehyde content and increased activities of super oxide dismutase activity and glutathione peroxidase in the kidney homogenate. The findings in the present study implicate the therapeutic potential of CCBs for Pb-induced nephrotoxicity, which were at least partly due to the decrease of Pb uptake and inhibition of lipid peroxidation.

1. Introduction

Lead (Pb) is a ubiquitous heavy metal which is regarded as an occupational toxicant and a major environmental pollutant (Watson et al., 2004). Sources of human exposure to this metal include food, drinking water and air (El-Nekeety et al., 2009). Environmental exposure to low levels of lead (blood lead concentrations as low as 60 $\mu g\,L^{-1}$) has been associated with a wide range of metabolic and cellular deficits (Nriagu, 1988). The kidney is the primary site for the initial accumulation of lead and the critical target organ of following oral or inhalation exposure in humans and animals. Pb may enter the body by ingestion through the intestines, lungs and skin. The urinary tract is the most significant route of Pb excretion (Sabath and Robles-Osorio, 2012). With a biological half-life estimated at 10 years, Pb is excreted extremely slowly from the body and tends to accumulate in the body (Philip and Gerson, 1994). The pathogenesis of Pb toxicity is multifactorial, as it directly

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http://dx.doi.org/10.1016/j.toxlet.2013.02.005

interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis and lowers the level of available sulfhydryl antioxidant reserves in the body (Ercal et al., 2001). The central nervous system, liver, kidneys and cardiovascular system have been established as the main targets of Pb-induced toxicity (Patrick, 2006), which causes serious pathological changes and functional abnormalities in cognition and behavior, as well as gastrointestinal problems, chronic renal failure and cardiovascular disease. Pb has also been implicated in the pathogenesis of saturnine gout and hypertension, which are secondary to the effects of Pb on renal function (Perazella, 1996).

The kidney is a sensitive target organ for Pb exposure. The nephrotoxic effects of Pb may occur at very low levels. Pb bound to low molecular weight proteins (<1% of the total) is filtered freely in the glomerulus and is reabsorbed by proximal convoluted tubule cells by endocytosis. Within the cell, Pb causes mitochondrial damage, formation of free radicals and intracellular depletion of glutathione and apoptosis (Wang et al., 2009). In addition, Pb affects enzymatic reactions in which calcium plays a role, and the calcium-sensing receptor can also be activated by Pb, which suggests that there may be another mechanism for Pb-induced nephrotoxicity (Chiu et al., 2009; Handlogten et al., 2000).

Treatment of Pb intoxication has primarily relied on chelation therapy. Although chelating agents are currently available for the treatment of Pb poisoning, they have been shown to have many side effects such as hepatotoxicity, nephrotoxicity, headache, nausea and increased blood pressure. Moreover, chelating agents are incapable of removing metal from intracellular sites and may cause redistribution of the toxic metal and essential metal loss, resulting in poor clinical recovery (Flora and Pachauri, 2010). Newer therapies may provide effective alternatives for treating the toxic effects of Pb poisoning.

Calcium channels are readily exposed to toxicants and are potentially early targets of the actions of a number of toxicants (Kiss and Osipenko, 1994) due to their portal location within the plasma membrane. Voltage-dependent calcium channels found in a variety of cells are blocked by different metal cations (Gawrisch et al., 1997). Oudit et al. (2003) demonstrated that L-type calcium channels (LTCC) are key transporters of iron into cardiomyocytes under iron-overloaded conditions, and treatments with LTCC blockers such as amlodipine and verapamil can lead to the inhibition of the LTCC current in cardiomyocytes, thereby reducing myocardial iron accumulation, decreasing oxidative stress and improving survival in iron-loaded mice. In recent reports, pre-treatment with LTCC blockers, such as verapamil, demonstrated effects on Cd renal toxicity in rats and altered the distribution of Cd (Xu et al., 2010). Pb is a well-known inhibitor of voltage-dependent calcium channels (Büsselberg et al., 1990; Büsselberg, 1995). It has been shown in bovine adrenal chromaffin cells that Pb not only blocks calcium influx but also can readily permeate through the dihydropyridine-sensitive LTCC and accumulate within the cells (Simons and Pocock, 1987; Tomsig and Suszkiw, 1991). These results suggest that calcium channel blockers (CCBs) may prevent Pb-induced nephrotoxicity by decreasing renal Pb uptake.

In view of the importance of Pb intoxication in the pathological process of renal disease and the need for new drugs to treat Pb poisoning, this study investigated the potential of CCBs verapamil and nimodipine to protect against Pb nephrotoxicity in mice and the mechanistic basis of their protective effects.

2. Materials and methods

2.1. Materials

One hundred and twenty male Kunming (KM) mice weighing 20.0 ± 2.0 g were purchased from the Experimental Animal Center, Hebei Medical University and housed in plastic cages with well-ventilated stainless steel grid tops at controlled temperature with a 12 h light–dark cycle. Animals were allowed access to normal chow and drinking water ad libitum. All animal handling procedures were in accordance with the Guidelines of Animal Experiments from the Committee of Medical Ethics, National Health Department of China. Pb acetate was obtained from Shenlong Pharmaceutical Co., Ltd. (Jiangsu, China). Verapamil and nimodipine injection were purchased from Novartis Pharma Stein AG (Basel, Switzerland) and Pharmacosmos A/S (Holbaek, Denmark), respectively. Unless otherwise stated, other chemical reagents were obtained from Sigma (St. Louis, MO, USA).

2.2. Experimental treatments

After one week of acclimation, all mice were randomly divided into 6 groups of 20 animals in each group as follows: control, Pb, low-dose verapamil (L-ver), high-dose verapamil (H-ver), low-dose nimodipine (L-nim) and high-dose nimodipine (H-nim) groups. Mice of the L-ver and H-ver groups were given i.p. injections of verapamil at 1 and 4 mg/kg/day, respectively. Mice of the L-nim and H-nim groups were given i.p. injections of nimodipine at 1 and 4 mg/kg/day, respectively. Meanwhile, mice of the control group and Pb group received isovolumic saline by the same route. Mice of the latter five groups were intraperitoneally (i.p.) injected with Pb acetate 40 mg/kg/day four hours later in the same day and had free access to normal mice chow and drinking water. Mice of the control group received an i.p. injection with isovolumic saline. The entire experimental period lasted for 10 days, and all mice were maintained on normal chow and drinking water ad libitum. Body weight was measured at the beginning and end of the experiment. General characteristics of all mice were observed carefully throughout the experimental period. After recording the final body weight of each surviving overnight fasted animal on the 10th day of the experiment, all mice were anesthetized with sodium pentobarbital (50 mg/kg) to collect blood, and the serum was separated for further analysis. Kidneys were quickly excised and washed in ice-cold phosphate buffer solution (PBS) and immediately frozen in liquid nitrogen.

2.3. Determination of kidney coefficients

Kidneys were removed and weighed using an electronic balance. The kidney coefficients were then calculated as the kidney to body mass ratio × 100.

2.4. Histological examination of kidney tissues

Kidney samples were excised, fixed in 10% formalin, hydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections (5-mm thick) were cut and stained with hematoxylin and eosin (H&E) for histological examination (Drury et al., 1976). Tubulointerstitial damage was graded according to previous study (Shih et al., 1988) on a scale of 0–4 (0 = normal; 0.5 = small focal areas; 1 = involvement of less than 10% of the cortices and outer medullae; 2 = 10–25% involvement of the cortices and outer medullae; 3 = 25–75% involvement of the cortices and outer medullae; 4 = extensive damage involving more than 75% of the cortices and outer medullae). Glomerular damage in the present study was scored by light microscopy according to the scoring system (Katafuchi et al., 1998). Briefly, glomerular lesions (total score: 0–12) included glomerular hypercellularity, glomerular segmental lesions (crescents, adhesions and segmental sclerosis) and global glomerular sclerosis. The glomerular lesion score of each type was determined as follows: 0, no lesion; 1, lesion in <10% of glomeruli; 2, lesion in >10% but <25% of glomeruli; 3, lesion in >25% and <50% of glomeruli; 4, lesion in >50% of glomeruli.

2.5. Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) assay

TUNEL staining was performed using an In Situ Cell Death Detection Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. Briefly, the sections were deparaffinized, hydrated in a successive series of alcohol, washed in distilled water followed by PBS and deproteinized by proteinase K (20 μg/mL) for 30 min at 37 °C. The sections were then rinsed and incubated in the TUNEL reaction mixture. After additional rinses, sections were visualized using converter-POD with 0.02% 3,3′-diaminobenzidine (DAB). The sections were counterstained with hematoxylin. The number of tubular cells undergoing apoptosis was counted in three kidney sections of each animal, and the average number of TUNEL-positive cells/mm^2 was calculated. The results were expressed as the average number of TUNEL-positive cells/mm^2 ± SD for each group. All counting procedures were performed in a blinded fashion.

2.6. Measurements of Pb in blood and kidney homogenate

Pb concentrations in blood and kidney homogenate were analyzed by flame atomic absorption spectroscopy (FAAS). Processing of the samples and analysis of Pb in blood was performed as previously described (Selander and Cramér, 1968). To prepare frozen kidney homogenates for FAAS, the samples were dried at 65 °C for 24 h. Dried samples were then weighed and ashed at 500 °C for 12–16 h in a muffle furnace (Isotemp, Waltham, MA, USA). Ashed samples were digested by