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## Protective Effects of Sildenafil and Dipyridamol from Lead-induced Lipid Peroxidation in Perfused Rat Liver

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**Abstract:** This study was designed to explore the protective effects of sildenafil and dipyridamole from toxicity of lead in an established model of rat liver perfusion. The rats were anesthetized with ketamine and chlorpromazine. The portal vein and the inferior vena cava were cannulated and the liver was perfused *in situ* through the portal vein with Krebs-Henseleit buffer. After 25 min of equilibration, lead (0.5 mM) and drugs (sildenafil 50 µM, dipyridamole 50 µM) were perfused for 45 min and samples of perfused fluid were collected for assessment of thiobarbituric reactive substances as index of lipid peroxidation. Lead treatment induced a marked lipid peroxidation. Cotreatment with sildenafil and dipyridamole significantly reduced lead-induced lipid peroxidation but did not reach control values. It is concluded that lead toxicity is mediated through lipid peroxidation in rat liver which is preventable with sildenafil and dipyridamole. This action of sildenafil and dipyridamole backs to their potential to increase intracellular cGMP which seems playing as an antioxidant.

**Key words:** Lead, liver, toxicity, lipid peroxidation, cGMP

### INTRODUCTION

Lead is an environmental pollutant inducing a broad range of physiological, biochemical and behavioural dysfunctions in both laboratory animals and humans with multiple mechanisms involved<sup>[1-13]</sup>. In recent years, one of the most important mechanisms suggested for lead toxicity is a disruption in prooxidant/antioxidant balance and inducing oxidative stress in cells with generation of highly reactive oxygen species like hydroxyl radical, hydrogen peroxide, superoxide anion and lipid peroxides<sup>[4,10,14-21]</sup>.

On the other hand, the phosphodiesterases (PDEs) are a superfamily of enzymes, which catalyse the hydrolysis of the cyclic nucleotides cAMP and cGMP to their corresponding inactive 5-monophosphate counterparts. The cyclic nucleotides play a prominent role in the regulation of important cellular functions and PDE inhibition can therefore elicit a variety of pharmacological effects. Sildenafil and dipyridamole are cGMP PDE inhibitors<sup>[22-24]</sup>. There is evidence that liver is one of organs that response very well to effects of PDEs inhibitors like sildenafil and dipyridamole in increasing hepatic cells cGMP<sup>[25]</sup>. In addition, our recent studies

indicated a protective role for cGMP from induction of oxidative stress inside cells<sup>[21,26]</sup>.

The first aim of this study was to confirm lipid peroxidation as a mechanism of toxicity of lead in liver and the second was to examine how increasing intracellular cGMP by sildenafil and dipyridamole may protect from lead-induced lipid peroxidation in rat liver.

### MATERIALS AND METHODS

**Animals:** Male albino Wistar rats (6-8 weeks) weighing 200-250 g were used in this experiment. They were housed individually in standard rat cages in a room on a 12 h light-dark cycle at 22°C and 50±5% relative humidity, with free access to standard diet and tap water until use. The animals were adapted to the conditions for 7 days before starting of the experiments.

**Chemicals:** Lead chloride, 2-thiobarbituric acid (TBA), 1,1,3,3-tetraethoxy-propan, trichloroacetic acid (TCA), n-butanol, NaCl, KCl, CaCl<sub>2</sub>, KH<sub>2</sub>PO<sub>4</sub>, NAHCO<sub>3</sub>, glucose form (Merck chemical company, Germany), sildenafil citrate and dipyridamole from (local pharmaceutical companies) and ketamine, chlorpromazine, heparin from (local pharmacies) were used in this study.

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**Experimental design:** All ethical manners for use of laboratory animals were considered carefully. The animals were randomized to eight groups with six animals in each group. The liver of animals in each group were perfused by perfusion buffer alone (control) or with lead chloride (0.5 mM), sildenafil (50  $\mu$ M), sildenafil (50  $\mu$ M) plus lead chloride (0.5 mM), dipyridamole (50  $\mu$ M) and lead chloride (0.5 mM) plus dipyridamole (50  $\mu$ M) as treated groups.

**Perfusion media:** Perfusion fluid was made of krebs-Henseleit buffer. The perfusion medium included the followings in g L<sup>-1</sup>: NaCl 6.896, KCl 0.354, CaCl<sub>2</sub>·2H<sub>2</sub>O 0.373, KH<sub>2</sub>PO<sub>4</sub> 0.162, NaHCO<sub>3</sub> 2.1 and glucose 0.901. The solution was adjusted to pH 7.2-7.4 with 1N NaOH solution<sup>[27]</sup>. The perfusion medium was gassed continuously with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>).

**Perfusion conditions and parameters liver viability:** Temperature, perfusion pressure, flow rate and perfusion fluid pH were closely monitored during the perfusion, particularly during the first 25 min of equilibration<sup>[27]</sup>. These parameters were initially checked every 10 to 15 min and the experiment were not begun until they had reached constant and acceptable values. The temperature in the perfusion system was also set and maintained at 37°C. Perfusion pressure was not raised above 10-15 cm of water with a flow rate of approximately 15 mL/min/g liver weight to provide adequate oxygenation. The perfusion fluid pH was always set between 7.2 and 7.4 by adjusting the CO<sub>2</sub> gases. As soon as perfusion was begun, the liver developed an even, light-brown color was soft and was kept moistened. Blotches or discoloration meant that the liver was not well perfused<sup>[28]</sup>.

**Surgery:** The rats were anesthetized with ketamine and chlorpromazine. Heparin was used to prevent blood clotting prior to anesthesia<sup>[27,29]</sup>. An incision was made along the length of the abdomen to expose the liver.

Sutures were then placed loosely around the inferior vena cava above and below the renal veins. The distal suture around the vena cava was tightened and a 14 g polyethylene catheter was inserted, placed above the renal vein and secured with the proximal suture. The portal vein was immediately cannulated with an 18 g catheter which was secured. The diaphragm was incised and the inferior vena cava ligated suprahepatically. Following attachment of the perfusion tubing to the cannulate, the liver was perfused *in situ* through the portal vein<sup>[27]</sup>. After 25 min of equilibration, drugs and lead were perfused for 45 min and samples of perfused fluid were collected for assessment of any liver injury.

**Lipid peroxidation assay:** Malonedialdehyde levels in perfused fluid were determined using the thiobarbituric

acid test. In a test tube, to 500  $\mu$ L of perfused fluid, 2.5 mL of trichloroacetic acid (20% w/v) was added and centrifuged at 3000 $\times$ g for 10 min. Then 700  $\mu$ L of this supernatant was added to 3 mL f thiobarbituric acid (0.2% w/v). This mixture was incubated for 30 min in a boiling water bath. Then, 4 mL n-butanol was added, the solution centrifuged, cooled and absorption of the supernatant was recorded at 532 nm using a UV-VIS spectrophotometer (160-A Shimadzu). The calibration curve of 1,1,3,3-tetraethoxypropan standard solution was used to determine the concentration of thiobarbituric acid/malonedialdehyde adducts in samples<sup>[30]</sup>.

**Statistical analysis:** Values represent mean $\pm$ SEM. One-way ANOVA followed by Newman-Keuls was used to analyze difference between groups. P values lesser than 0.05 were considered significant.

## RESULTS

Table 1 shows the effects of lead alone or in combination with sildenafil or dipyridamole on perfused rat liver lipid peroxidation. No significant change was observed between treated groups of lead, sildenafil, dipyridamole or combination of sildenafil and dipyridamole with lead in comparison to control group. A significant (p<0.01) increase in lipid peroxidation was observed in lead-treated group when compared to controls. Cotreated groups of lead with sildenafil or dipyridamole decreased lead-induced lipid peroxidation significantly (p<0.01) but did not reach control values.

## DISCUSSION

In the present study the activities of liver lipid peroxidation in perfusion fluid was assessed. Results showed that lead treatment induces a marked lipid peroxidation. Interestingly, lead-induced lipid peroxidation was inhibited by sildenafil and dipyridamole cotreatment in a similar extent. Increased lipid peroxidation by lead administration in the present study is not a new finding

Table 1: Effects of lead, sildenafil and dipyridamole on lipid peroxidation in perfused rat liver

Treatments	TBARS (mM)
Control	30 $\pm$ 4
Lead	420 $\pm$ 25 <sup>aa</sup>
Sildenafil	40 $\pm$ 5
Lead + Sildenafil	120 $\pm$ 8 <sup>aa</sup> <sup>bb</sup>
Dipyridamole	45 $\pm$ 7
Lead + Dipyridamole	70 $\pm$ 6 <sup>aa</sup> <sup>bb</sup>

Data are mean $\pm$ SEM of 6 experiments. Sildenafil (50  $\mu$ M) and dipyridamole (50  $\mu$ M) were added to perfusion fluid alone or in combination with lead (0.5 mM). Determination of TBARS was performed 45 min after perfusion. <sup>aa</sup> represents that difference between treated and control groups is significant at p<0.01. <sup>bb</sup> represents that difference between lead and other treated groups is significant at p<0.01.

and is supported by many previous reports<sup>[14,31-34]</sup>. Lead is also found to inhibit antioxidant enzymes involved in the prevention of lipid peroxidation such as superoxide dismutase or catalase<sup>[14,35]</sup>. Although lead toxicity is associated with oxidative damage, the mechanisms underlying the ability of this metal to induce and promote oxidative stress and its relation with organ damage are unclear. Added to oxidative stress, disrupting of calcium homeostasis and inhibition of thiol-containing enzymes has been also reported as mechanism of toxic actions of lead<sup>[2,3,5,15]</sup>. Studies indicate that the nitric oxide synthase in the non-neural cells is probably activated by muscarinic receptors leads to an increase in intracellular free calcium that activates nitric oxide synthase leading to the generation of cGMP that opens ion channels to initiate the cell actions<sup>[36]</sup>. It is clearly shown that the inhibitory effect of lead is potentiated in the presence of nitric oxide synthase inhibitor and diminished by nitric oxide precursor<sup>[3,9]</sup>. The inhibitory influence of lead may be exerted on the constitutive nitric oxide synthase catalytic site(s) either by direct binding or by interfering with the electron transfer during catalysis. It is well established that nitric oxide activates guanylate cyclase resulting in an increased conversion of GTP to cGMP and cGMP mediates further actions within the cell<sup>[37]</sup>. Therefore beneficiary effects of sildenafil and dipyridamole may back to their increasing capacity of cGMP which is the active mediator of nitric oxide. Supporting this hypothesis there is evidence that dipyridamole stimulates leukocyte NO production through a calcium-dependent pathway. Also antioxidant effect of dipyridamole in reducing the formation of free radicals in neutrophils has been reported<sup>[38]</sup>. The antioxidant effects of dipyridamole against hydroperoxide-induced lipid peroxidation in erythrocytes has been also reported<sup>[39]</sup>. The pretreatment of lead-exposed rats with L-arginine, a nitric oxide precursor, is found to protect against lead-induced toxicity in rat submandibular gland<sup>[3,9]</sup>. There is also evidence that endogenous NO plays a hepatoprotective role in chronic CCL<sub>4</sub> intoxication in rat with interaction with superoxide anion and other radicals that produce toxic species<sup>[40]</sup>.

Taking collectively, results of this study support the previous findings on the protective properties of cyclic nucleotides against oxidative stress<sup>[21,26]</sup>. It is important to note that liver is an important organ of the body with special functions that cannot be compensated by other organs. Induction of lipid peroxidation by lead in liver cells may disturb proper action of liver which is significance for health. Protective properties of dipyridamole and sildenafil may suggest a place for them in maintaining liver health. Exact mechanism of protective action of cGMP-phosphodiesterase remains to be elucidated by further studies.

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