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Acute liver damage induced by 2-nitropropane in rats: Effect of diphenyl diselenide on antioxidant defenses

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Abstract

The effect of post-treatment with diphenyl diselenide on liver damage induced by 2-nitropropane (2-NP) was examined in male rats. Rats were pre-treated with a single dose of 2-NP (100 mg/kg body weight dissolved in canola oil). Afterward, the animals were post-treated with a dose of diphenyl diselenide (10, 50 or 100 μmol/kg). The parameters that indicate tissue damage such as liver histopathology, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), urea and creatinine were determined. Since the liver damage induced by 2-NP is related to oxidative damage, lipid peroxidation, superoxide dismutase (SOD), catalase (CAT) and ascorbic acid level were also evaluated. Diphenyl diselenide (50 and 100 μmol/kg) effectively restored the increase of ALT and AST activities and urea level when compared to the 2-NP group. At the higher dose, diphenyl diselenide decreased GGT activity. Treatment with diphenyl diselenide, at all doses, effectively ameliorated the increase of hepatic and renal lipid peroxidation when compared to 2-NP group. 2-NP reduced CAT activity and neither alter SOD activity nor ascorbic acid level. This study points out the involvement of CAT activity in 2-NP-induced acute liver damage and suggests that the post-treatment with diphenyl diselenide was effective in restoring the hepatic damage induced by 2-NP.

Keywords: 2-Nitropropane; Organoselenium; Hepatic damage; Antioxidant; Catalase; SOD; Ascorbic acid

1. Introduction

Selenium is an essential micronutrient for both animals and humans as an integral component of several enzymes with antioxidant properties, including glutathione peroxidase and phospholipid hydrogen glutathione peroxidase [1–3].

The advances in the area of synthesis and reactivity of organoselenium compounds, as well as, the discovery that selenium is an essential trace element in the diet [4] has prompted intense studies on the biological properties of organic selenium compounds [5,6]. The selenium atom participates, as a component, in mammalian thioredoxin system, which has been shown to directly reduce lipid hydroperoxides and play a specific role in peroxynitrite defense [7]. In fact, recent study has reported that diaryl diselenides are potent antioxidant compounds [8], among them diphenyl diselenide presented the highest thiol peroxidase activity and antioxidant potential [9]. The discovery of the pharmacological potential of

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organoselenium compounds [9–11] increases the necessity of explanations about the mechanisms of action on cellular levels, considering the oxidative stress as a focus on the discussion. Indeed, the antioxidant activity could explain some protective effects of diphenyl diselenide on oxidative models of damage [12–14].

In general, reactive oxygen species (ROS) generated by any cellular damage play a pivotal role in accelerating oxidative stress in biological systems [15,16]. Lipid peroxidation, an index of oxidative stress, is known to be stimulated in stressed tissues after single administration with a hepatocarcinogen, which subsequently manifests in serious pathological problems [17].

2-Nitropropane, a nitroalkane, is used as a constituent of paints and inks, in the manufacture of chemicals as industrial solvent and can be found in cigarette smoke [18]. 2-NP has been found to cause hepatotoxicity in occupationally exposed humans [19,20] and in rats and rabbits [21,22]. The mechanism by which 2-NP exerts hepatotoxicity is not clearly understood, but many authors suggested that 2-NP metabolism may increase ROS levels and cause cellular damage [15,23,24]. Thus, liver damage is a therapeutic target of selenorganic compounds, as well as, the various clinical conditions in which hydroperoxides play a role.

The cellular environment has some antioxidative enzymes and non-enzymatic mechanisms (Vitamin C, reduced glutathione) to counteract the damaging effects of reactive oxygen species generated after a single administration of 2-NP [16,25]. Catalase and peroxidases are the primary antioxidant defenses against the increase of free radicals [16]. Similarly, superoxide dismutase (SOD), another enzymatic antioxidant defense, can readily react with damaging superoxide and OH radicals and convert them into less reactive radicals.

Taking these in consideration, the aim of the present study was to investigate the effect of diphenyl diselenide on toxicological parameters and evaluate the role of some antioxidant defenses (enzymatic and non-enzymatic) to counteract the damaging effects of ROS generated after administration of 2-NP in rats.

2. Material and methods

2.1. Chemicals

Diphenyl diselenide (Fig. 1) was synthesized according to literature methods [26] and was dissolved in canola oil. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed that diphenyl diselenide presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of diphenyl diselenide (99.9%) was determined by GC/HPLC. 2-Nitropropane (2-NP) was obtained from Sigma. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

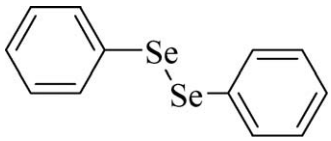

Fig. 1. Chemical structure of diphenyl diselenide.

2.2. Animals

Male adult albino Wistar rats (200–250 g) from our own breeding colony were used. The animals were kept in separate animal rooms, on a 12-h light/12-h dark cycle, at a room temperature of 22 ± 2 °C and with free access to food and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, University Federal of Santa Maria, Brazil.

2.3. Exposure

Six animals per group were usually tested in the experiments. Rats were injected intraperitoneally with a single dose of 2-NP (100 mg/kg body weight dissolved in canola oil) (groups 2, 6, 7 and 8) according to Fiala et al. [23]. Twenty-four hours later, animals were injected with diphenyl diselenide (10, 50 or 100 μmol/kg, i.p.) (groups 3, 4, 5, 6, 7 and 8). The control group received only vehicle (canola oil, 5 mL/kg) (group 1). All groups were sacrificed 48 h after 2-NP injection. Rats were slightly anesthetized with chloroformio for blood collection by heart puncture (hemolyzed serum was discarded). The liver and kidney were dissected and frozen on ice-cold until the time of assay.

The protocol of rat treatments is given below:

Group (1) Canola oil (i.p.) plus canola oil (5 mL/kg, i.p.).
Group (2) 2-NP (100 mg/kg, i.p.) plus canola oil (5 mL/kg, i.p.).
Group (3) Canola oil (5 mL/kg, i.p.) plus diphenyl diselenide (10 μmol/kg, i.p.).
Group (4) Canola oil (5 mL/kg, i.p.) plus diphenyl diselenide (50 μmol/kg, i.p.).
Group (5) Canola oil (5 mL/kg, i.p.) plus diphenyl diselenide (100 μmol/kg, i.p.).
Group (6) 2-NP (100 mg/kg, i.p.) plus diphenyl diselenide (10 μmol/kg, i.p.).