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Reversal of bioenergetics dysfunction by diphenyl diselenide is critical to protection against the acetaminophen-induced acute liver failure

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ABSTRACT

Physiopathological conditions such as acute liver failure (ALF) induced by acetaminophen (APAP) can often impair the mitochondrial bioenergetics. Diphenyl diselenide [$(PhSe)_2$] has been shown protects against APAP-induced ALF. The present study aimed to clarify the signaling mechanism involved in the protection of bioenergetics dysfunction associated with ALF-induced by APAP overdose. Mice received APAP (600 mg/kg) or $(PhSe)_2$ (15.6 mg/kg) alone, or APAP + $(PhSe)_2$, all the solutions were administered by the intraperitoneal (i.p.). Samples of liver, blood and liver mitochondria were collected at 2 and 4 h after APAP administration. APAP-induced ALF was able to induce ALF by means of alteration on liver injury biomarkers, increased Nitrite and Nitrate levels and the impairment of oxidative phosphorylation capacity (OXPHOS). In parallel, APAP overdose promoted activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and Heat shock protein 70 (HSP70) expression. $(PhSe)_2$ was able to abolish the APAP-induced decline of OXPHOS and changes on the Nrf2-ARE pathway. In addition, $(PhSe)_2$ elevated the levels of peroxisome proliferator-activated receptor-γ coactivator (PGC-1α), helping to restore the levels of nuclear respiratory factor 1 (NRF1) associated with mitochondrial biogenesis. In summary, the treatment with $(PhSe)_2$ maintained mitochondrial function, promoted genes related to mitochondrial dynamic and demonstrating to play critical role in the modulation of cellular protective responses during ALF.

1. Introduction

Mitochondrial bioenergetics dysfunction is a physiopathological condition that occurs in many diseases, and results in the inability of cells to maintain energy production [1]. Because the liver, a highly metabolic organ, is affected by acetaminophen (APAP) overdose, the mitochondrial dysfunction that manifests during acute liver failure (ALF) may contribute to alterations in energy metabolism and the presence of hepatocellular necrosis [2]. APAP is widely used due to the analgesic and antipyretic properties [3]. High doses of this drug are shown to induce hepatotoxicity [2,3]. APAP hepatotoxicity is closely related to its highly reactive metabolite generation, called *N*-acetyl-*p*-benzoquinone imine (NAPQI) [3]. Noteworthy, APAP metabolism occurs in liver and the enzyme responsible for this process is cytochrome P450 (mainly CYP2E1) [4]. NAPQI reacts with sulfhydryl groups causing the impairment of redox homeostasis. However, APAP toxicity is a multifactorial process, involving the development of oxidative stress and bioenergetics crisis [2,3,5].

The start of mitochondrial dysfunction triggers injury progression and has demonstrated a close relationship between imbalance of bioenergetics efficiency and energy expenditure, which limits the ability of cells to reestablish the normal homeostasis [2]. Disruption of bioenergetics metabolism and metabolic adaptation after APAP-induced ALF are complex and well-orchestrated processes in which the major limiting factors are glutathione (GSH) levels, the generation of reactive oxygen and nitrogen species (ROS/RNS) and mitochondrial viability [6]. Despite organoselenium compounds have been shown to induce mitochondrial dysfunction at high doses [7]. Diphenyl diselenide [$(PhSe)_2$] is known to antagonize the effects of APAP poisoning [8]. Thiol-disulfite activity (glutathione peroxidase-like and thioredoxin reductase-like activities) of $(PhSe)_2$ appear to be the major mechanism, and these

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activities are manifested through the generation of selenol/selenolate intermediate species [9] . The reactivity of these intermediates make them more powerful nucleophiles compared to known thiol/tiolate groups [10] .

Oxidative phosphorylation (OXPHOS) deficiency and disruptions of survival signaling pathways during ALF are important outcomes during APAP overdose [2,11] . Nonetheless, the adaptive liver response that promotes cell survival involves a complex interaction of transcription factors that are energy-dependent processes, such as heat shock proteins (HSP), nuclear factor erythroid 2-related factor 2 (Nrf2) and antioxidant response elements (ARE) [12]. Thus, the mitochondrial impairment could induce the reduction of the adaptive liver response exacerbating the hepatic damage.

$(PhSe)_2$ being a lipophilic compound that freely crosses the biomembranes could prevent redox imbalance and mitochondrial dysfunction [8,13]. The maintenance of OXPHOS capacity is an obvious strategy to help minimize damage attributable to ALF and could be associated to the induction of adaptive responses to culminate in cell survival [14,15] . $(PhSe)_2$ have been shown to increase the mitochondrial biogenesis and induction of hemeoxygenase type 1, processes that are linked to mitochondrial homeostasis [16] . However, the role of genes associated with this process, such as, nuclear respiratory factor (NRF) 1 and the peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) remains unclear. Additionally, treatment with selenium induces an up-regulation of HSP70 levels [17,18] . HSP70 levels are closely related to the apoptosis inhibition [19] , and the modulation of inflammatory biomarkers has been highlighted in the HSP70 activation [20] .

Given the demonstrated ability of $(PhSe)_2$ to protect against APAP-induced ALF, the current work was aimed to understand the mechanism by which $(PhSe)_2$ protects mitochondrial bioenergetics and the signaling pathway involved in this process.

2. Materials and methods

$(PhSe)_2$ (98%) and nucleotides were obtained from Sigma Chemical Co. (St. Louis, MO). The antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Other reagents were obtained from standard commercial suppliers.

2.1. Animals

Seven-week-old male adult Swiss albino mice (30–40 g) from our own breeding colony were used. The animals were kept on a separate animal room, on at 12 h light/dark cycle, at temperature of 22 ± 2 °C, with free access to food and water. Mice were acclimated for 7 days before initiation of any procedures. This study was approved by the Ethics and Animal Welfare Committee of Federal University of Santa Maria, Brazil (Permit Number: 095/2010).

2.2. Experimental

Briefly, the animals were randomly divided into four groups: control (vehicle); diphenyl diselenide [$(PhSe)_2$]; acute liver failure induced by APAP (APAP) and acute liver failure treated with diphenyl diselenide [APAP + $(PhSe)_2$]. All the solutions were administered by the intraperitoneal (i.p.) route. Mice in the control and $(PhSe)_2$ groups received saline 0.9% (20 ml/kg), and mice in the APAP and APAP + $(PhSe)_2$ groups received 600 mg/kg APAP (20 ml/kg in saline 0.9%). One hour after saline and APAP treatment, mice received 15.6 mg/kg $(PhSe)_2$ (2.5 ml/kg in canola oil) in the $(PhSe)_2$ and APAP + $(PhSe)_2$ groups. APAP and $(PhSe)_2$ doses have been used in previous studies, APAP (600 mg/kg) demonstrated a significant liver damage, while the $(PhSe)_2$ (15.6 mg/kg) have been demonstrated to be hepatoprotective whiteout induce toxicity in mice [8,21,22] . Food was available *ad libitum* and animals were not fasted prior to dosing.

At 2 and 4 h following APAP administration, animals were killed by cervical dislocation and blood was collected by cardiac puncture using heparin-rinsed 1-ml syringes (20-gauge needles) and centrifuged. The plasma was used for determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities using a commercial kit (Labtest®, Diagnostica S.A., Minas Gerais, Brazil). Excised liver of mice was washed in buffered normal saline and weighed to obtain the absolute liver weights. Relative weights were calculated with the formula: Relative organ weight = (Absolute organ weight/Body weight at sacrifice) × 100%, in according to previous work [23] .

2.3. Histopathology

The caudal portion of the left lobe from liver tissues of mice ($n = 5$) were used for histology sections section and stained with hematoxylin and eosin (H&E) to analysis qualitative of alterations histopathological were taken and analyzed by a pathologist [24].

2.4. Isolation of mice liver mitochondria

Mice liver mitochondria were isolated at 4 °C as previously described [25], with few modifications. The livers of animals sacrificed were removed and immersed in cold medium containing 320 mM Sucrose, 1 mM EDTA, 1 EGTA and 10 mM Tris-HCl, pH 7.4. The tissue was minced using surgical scissors, extensively washed and homogenized in a power-driven, tight-fitting Potter Elvehjem homogenizer with Teflon pestle. The resulting suspension was centrifuged for 10 min at 2500 rpm in a Hitachi CR21E centrifuge. After centrifugation, the supernatant was recentrifuged for 10 min at 10,000 rpm. The pellet was resuspended in a cold medium containing 350 mM sucrose, 1 mM EGTA, 1 mM EDTA, 1 mM ADP, 1 mg/ml bovine serum albumin free fatty acid (BSA) and Tris-HCl, pH 7.4, and recentrifuged at 10,000 rpm for 10 min. The supernatant was decanted, and the final pellet was gently washed and resuspended in 250 mM sucrose and 10 mM Tris-HCl, pH 7.4.

2.5. Mitochondrial respiration rates

Oxygen consumption was monitored polarographically with a Clark-type electrode (Hansatech, UK) at 30 °C. Liver mitochondria were incubated in 1 ml of the respiratory medium consisted of 10 mM Tris-HCl (pH 7.4), 320 mM mannitol, 8 mM K_2HPO_4, and 4 mM $MgCl_2$, 0.08 mM EDTA, 1 mM EGTA, 0.2 mg/ml BSA [26] . Respiration of mitochondria (1 mg of protein/ml) was initiated with substrates at 5 mM malate and glutamate. To induce the phosphorylating (state III) respiration, 200 nmol ADP was used. Next, the Oligomycin (1 μg/ml) was used to induce the non-phosphorylating (state IV) respiration. Respiration rates are given in nmol oxygen/min/mg protein. Respiratory control ratio (RCR) was determined by the State III/State IV ratio.

2.6. Measurement of liver nitrite and nitrate levels

The liver samples were prepared according to a previous [27]. Liver samples were homogenized in 500 μl of 200 mM $ZnSO_4$ and 500 μl of acetonitrile. Next, sample were centrifuged at 16,000 × *g* at 4 °C for 30 min and the supernatants were used for the colorimetric reaction with 400 μl of 2% VCl_3 (in 5% HCl), 200 μl of 0.1% N-(1-naphthyl) ethylene-diamine dihydrochloride, 200 μl of 2% sulfanilamide (in 5% HCl). After incubating at 37 °C for 60 min, Nitrite and nitrate levels were measured at 540 nm using a standard curve. The results were expressed as nmol Nitrite + Nitrate/mg of protein.

2.7. Western blot analysis

Western blotting analysis performed according to a previous method with minor changes [28,29] . The liver samples were homogenized