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Dimethylthiourea protects against mitochondrial oxidative damage induced by cisplatin in liver of rats

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Abstract

Cisplatin is one of the most effective chemotherapeutic agents. However, at higher doses liver injury may occur. The purpose of this study was to explore whether the hydroxyl radical scavenger dimethylthiourea (DMTU) protects against cisplatin-induced oxidative damage *in vivo* and to define the mitochondrial pathways involved in cytoprotection. Adult male Wistar rats (200–220 g) were divided into four groups of eight animals each. The control group was treated only with an intraperitoneal (i.p.) injection of saline solution (1 ml/100 g body weight). The DMTU group was given only DMTU (500 mg/kg body weight, i.p), followed by 125 mg/kg body weight, i.p. (twice a day) until sacrifice. The cisplatin group was given a single injection of cisplatin (10 mg/kg body weight, i.p.). The DMTU + cisplatin group was given DMTU (500 mg/kg body weight, i.p.), just before the cisplatin injection (10 mg/kg body weight, i.p.), followed by injections of DMTU (125 mg/kg body weight, i.p.) twice a day until sacrifice (72 h after the treatment). DMTU did not present any direct effect on mitochondria and substantially inhibited cisplatin-induced mitochondrial damage in liver, therefore preventing elevation of AST and ALT serum levels. DMTU protected against (a) decreased hepatic ATP levels; (b) lipid peroxidation; (c) cardiolipin oxidation; (d) sulfhydryl protein oxidation; (e) mitochondrial membrane rigidification; (f) GSH oxidation; (g) NADPH oxidation; (h) apoptosis. Results suggest that antioxidants, particularly hydroxyl radical scavengers, protect liver mitochondria against cisplatin-induced oxidative damage. Several mitochondrial changes were delineated and proposed as interesting targets for cytoprotective strategy.

Keywords: Cisplatin; Cytoprotection; Liver; Mitochondria; ROS; DMTU

1. Introduction

Cisplatin [*cis*-$(NH_3)_2PtCl_2$] is one of the most effective drugs to treat testicular, ovarian, bladder and neck cancers. It is also used in the management of endometrial cancer, non-small cell lung cancer, malignant melanoma, penile cancer and adrenocorticol carcinoma. The most common side effects of cisplatin chemotherapy are

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nephrotoxicity and neurotoxicity [1–3]. Cisplatin effectiveness is enhanced by the elevation of the doses. However, at higher doses, less common toxic effects, such as hepatotoxicity, may arise [1,2,4]. Additionally, some pathophysiological conditions such as diabetes and obesity, and some chemicals such as ethanol and nicotine may exacerbate cisplatin-induced liver toxicity, probably due to the enhanced expression of cytochrome P450 2E1 (CYP2E1). This isoenzyme is mainly expressed in the liver and is an effective generator of reactive oxygen species, such as hydrogen peroxide and superoxide anion. Moreover, CYP2E1 is able to potentiate the iron-catalyzed Fenton reaction, which leads to the generation of the potent oxidant hydroxyl radical [5–7]. Therefore, even though hepatotoxicity is not the most frequent toxic effect of cisplatin, it may become a concern under specific conditions. Many new agents, including platinum compounds, have been tested as possible less toxic anticancer agents to substitute cisplatin chemotherapy. Despite that, cisplatin remains one of the most effective antineoplastic drugs used in chemotherapy. For this reason, strategies to protect normal tissues against cisplatin toxicity are of clinical interest and tissue cytoprotective agents are essential to provide protection against the different cisplatin toxicities [1,8–10]. Little is known about cisplatin-induced liver injury and the mechanism of its hepatotoxicity has not been characterized. Cisplatin is transported into cells by the copper transporter Ctr1 and concentrates in the liver, kidneys, large and small intestines. Once inside the cell, its labile chloride ligands are replaced by water molecules, resulting in positively charged and highly reactive electrophilic products (mono-chloro-mono-aquo-diammine-platinum or diaquo-diammine-platinum), which bind DNA, killing dividing tumor cells. These toxic derivatives are also able to cause oxidative damage to macromolecules such as lipids and proteins, probably resulting in normal cells death [2,11–13]. Indeed, some recent studies have suggested that oxidative stress plays an important role in cisplatin-induced liver damage [7,9,14,15]. Moreover, since oxidative stress might interfere with the antineoplastic activity, reducing this oxidative stress by administering antioxidants may enhance the effectiveness of the treatment [16]. The antioxidant activity of certain compounds has been suggested to protect against cisplatin-induced oxidative damage in liver [9,17], but the mitochondrial events involved have not been underlined. Despite the presence of various antioxidant defenses, mitochondria appear to be the main intracellular source of reactive oxidant species and mitochondrial oxidative damage is supposed to be the main event in toxic oxidative stress [18,19]. Mitochondrial ROS are important in the execution of programmed cell death [20]. Involvement of ROS at different phases of the apoptotic pathway leading to activation of intracellular caspases and DNA damage has been clearly established [21]. Considerable evidence supports the role of mitochondria as an early cellular site of cisplatin-induced toxicity [22–24]. Accordingly, understanding the mitochondrial processes involved in cytoprotection could greatly contribute to develop therapeutic strategies to counteract cisplatin-induced toxicity during its early development and, consequently, to enhance the established efficacy of this important anticancer agent. Therefore, in the present study, we investigated the molecular mechanism underlying the protective effect of the known hydroxyl radical scavenger dimethylthiourea (DMTU) against hepatic mitochondrial oxidative damage induced by cisplatin in rats. Its protective effect has been described in different tissues and pathologies [12,25–30]. In a previous study [31], we demonstrated the protective effect of DMTU against the oxidative damage induced by cisplatin in rat's kidney and delineated several mitochondrial events involved in this protection. DMTU efficacy against cisplatin-induced hepatotoxicity has not been previously examined.

2. Materials and methods

2.1. Chemicals

Cis-diammineplatinum(II) dichloride (cisplatin), dimethylthiourea (DMTU) and NADPH were obtained from Sigma-Chemical Co. (St. Louis, MO, USA). Other reagents were of analytical grade. Sodium thiopental was obtained from Cristalia (Itapira, SP, Brazil). All solutions were prepared with ultra-pure water purified by a Milli-Q Gradient system (Millipore, Bedford, USA). Cisplatin solution was prepared in saline.

2.2. Animals

Male Wistar rats (200–220 g) were housed four per cage and maintained in a 12-h light/dark cycle in a temperature-and-humidity-controlled facility. Standard rat chow and water were provided ad libitum. Research protocols were approved by the local ethics committee (Comissão de Ética no Uso de Animais do Campus de Ribeirão Preto-USP, CEUA-USP) and performed in strict accordance with the "ethical principles and guidelines for experiments on animals" of the Swiss Academy of Medical Sciences and Swiss Academy of Sciences.