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Carvedilol protects the kidneys of tumor-bearing mice without impairing the biodistribution or the genotoxicity of cisplatin

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ABSTRACT

Cisplatin (Cisp) is an effective antitumor drug; however, it causes severe nephrotoxicity. Minimization of renal toxicity is essential, but the interference of nephroprotective agents, particularly antioxidants, with the antitumor activity of cisplatin is a general concern. We have recently demonstrated that the antihypertensive and antioxidant drug carvedilol (CV) protects against the renal damage and increases the survival of tumor–bearing mice without impairing the tumor reduction by cisplatin. So far, reports on the antioxidant mechanism of CV are controversial and there are no data on the impact of CV on the antitumor mechanisms of cisplatin. Therefore, this study addresses the effect of CV on mechanisms underlying the tumor control by cisplatin. CV did not interfere with the biodistribution or the genotoxicity of cisplatin. We also addressed the antioxidant mechanisms of CV and demonstrated that it does not neutralize free radicals, but is an efficient chelator of ferrous ions that are relevant catalyzers in cisplatin nephrotoxicity. The present data suggest that oxidative damage and genotoxicity play different roles in the toxicity of cisplatin on kidneys and tumors and therefore, some antioxidants might be safe as chemoprotectors. Altogether, our studies provide consistent evidence of the beneficial effect of CV on animals treated with cisplatin and might encourage clinical trials.

1. Introduction

Cisplatin (Cisp) is an effective drug for chemotherapy; however, the effective dose usually produces significant nephrotoxicity [22]. Minimization of renal toxicity is essential to improve the effectiveness of cisplatin chemotherapy and the quality of life of patients [16]. The prevention of the renal damage in patients under cisplatin chemotherapy include management of drug dosage, co-administration of other antitumor agents, alternate method of administration, intensive hydration and monitoring of the renal function. Despite that, renal failure still occurs [22]. Another strategy is the administration of the thiophosphate amifostine, a drug approved by FDA as a renal protective agent during cisplatin-based chemotherapy. It is a pro-drug which is converted to an active free thiol that scavenges ROS [7]. However, amifostine is not always effective against the toxicity induced by cisplatin in healthy tissues [18,33]. Other limitation factors include high costs, serious side effects and concerns that it might impair the antitumor efficacy [19].

Several compounds have been tested *in vitro* and in *vivo* for the protection against cisplatin-induced renal damage. The strategies of nephroprotection have mainly targeted on transport and accumulation pathways; oxidative stress (antioxidants); apoptosis; inflammation and hemodynamics. Despite the satisfactory results

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obtained, little is known about the impact of these compounds on the antitumor activity of cisplatin (for revision, see Ref. [11]). We have demonstrated the protective effect of carvedilol (CV) against the nephrotoxicity induced by cisplatin and have delineated some mechanisms involved in the protection [3,4,28,29].

CV is a β-adrenoceptor blocking agent already used in the treatment of congestive heart failure, mild to moderate hypertension, and myocardial infarction [14,39]. Besides its action as β-blocker, CV presents an important antioxidant capacity due a carbazole moiety that is not present in the molecule of other beta-adrenergic antagonists [24,26,36,40]. We have already demonstrated the nephroprotective potential of CV *in vitro*, in tumor-free rats and in tumor-bearing mice treated with cisplatin. Additionally, we have demonstrated that CV does not affect the tumor remission and increases the survival rate of tumor-bearing mice treated with cisplatin [4,28,29].

So far, reports on the antioxidant mechanism of carvedilol are controversial and there are no data on the impact of CV on the antitumor mechanisms of cisplatin. Therefore, in the present study we have addressed the interference of CV with the biodistribution and genotoxicity of cisplatin, events closely related to antitumor activity [6]. Additionally, we evaluated the potential of carvedilol in relation to two important antioxidant mechanisms, namely, neutralization of free radicals and chelation of ferrous ions [8,24,40].

2. Methods

2.1. Chemicals

Cisp (cis-diammineplatinum (II) dichloride) was obtained from Sigma-Chemical Co. (St. Louis, MO, USA). CV was kindly provided by *Baldacci* and *Torrent do Brasil* (São Paulo, SP, Brazil). Heparin (liquemine®) was obtained from Roche (Rio de Janeiro, RJ, Brazil). Sodium thiopental was obtained from Cristalia (Itapira, SP, Brazil). All other chemicals were of the highest purity grade available from Sigma (St. Louis, MO, USA). All solutions were prepared with ultrapure water purified by a Milli-Q Gradient system (Millipore, Bedford, USA). Cisp solution (1 mg/ml) was prepared in saline. CV solution (3 mg/ml) was prepared in 0.5% carboxymethylcellulose. Drug solutions were freshly prepared before animal treatments.

2.2. Animals

Male Swiss mice (22–28 g), 4–6 weeks-old, were housed four per cage and maintained in a 12-h light/dark cycle in a temperature- and humidity-controlled facility. Standard mouse 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.

2.3. Experimental design

Sarcoma-180 cells were removed from the ascitic liquid of donor mice and implanted subcutaneously in receptor mice. The animals that developed a solid tumor after 8 days were used in the study. Animals were divided in four groups (n = 6) and treated as follows: (i) Controls (C): saline (i.p.) on day 1 and carboxymethylcellulose 0.5% (as gavage), daily on days 1, 2 and 3; (ii) Cisp: only Cisp (i.p.) 25 mg/kg on day 1; (iii) CV: only CV 10 mg/kg (gavage) on days 1,2 and 3; and (iv) CV + Cisp: CV 10 mg/kg (gavage) immediately before Cisp 25 mg/kg (i.p.) on day 1 and then CV 10 mg/kg (gavage) on days 2 and 3. After 3 days, animals were anesthetized with sodium pentobarbitone (50 mg/kg) and euthanized. Blood, kidneys, and tumors were collected for assays [4].

2.4. Renal function

Blood was collected in heparinized tubes and centrifuged. BUN and creatinine were measured by using commercially available diagnostic kits (Labtest®, Lagoa Santa, MG, Brazil) as we previously reported [4].

2.5. Renal morphology

Kidneys were fixed in paraformaldehyde (4%) and embedded in paraffin. Tissue sections (4 μm) were placed on slides, stained with hematoxylin and eosin (HE) and the morphology was analyzed under light microscopy (×200).

2.6. Platinum determination – renal cortex and tumor

Samples from the renal cortex and tumor were lyophilized, weighed (15–85 mg), and placed in sterile conical tubes (15 mL). Tetraethylammonium hydroxide (TMAH) as an aqueous 50% solution (1 mL) was added to each sample and incubated for 48 h (25 °C). The volume was then made up to 15 mL by adding HNO_3 1% and Triton X-100 0.01%. Treated samples were analyzed by monitoring the isotope ^{195}Pt by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS ELAN DRCII). Platinum concentration was based o a standard calibration curve (2; 5; 10 and 20 ppb of platinum) [2].

2.7. Frequency of micronucleated polychromatic erythrocytes (MNPCE) in peripheral blood

The frequency of micronucleated polychromatic erythrocytes (MNPCE) in peripheral blood was determined according to published protocols [17]. Peripheral blood was collected from the caudal vein, fixed in methanol and stained using acridine orange (125 μg/mL) 1 min before the analysis by fluorescence microscopy (Olympus BX 51 – blue light 488 nm and yellow filter, 400×) [23]. The frequency of micronuclei was analyzed in two slides from each animal. For each slide, we counted 1000 PCE. Results are shown as micronucleated polychromatic erythrocytes (MNPCE) in 1000 PCE [20].

2.8. Frequency of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in bone marrow

Bone marrow was collected from animal femurs by using fetal bovine serum (FBS), homogenized and centrifuged (576 × g/5 min). The pellet was used to prepare the slides, which were fixed in methanol. Fixed slides were stained using Giemsa (5 min). Analysis were performed by counting 500 erythrocytes — polychromatic (PCE) plus normochromatic (NCE) — under light microscopy and calculating the PCE/(PCE + NCE) ratio (Olympus BX 51, ×400) [1].

2.9. Electro paramagnetic resonance (EPR) – DPPH and TEMPOL

EPR was performed in the spectrometer Jeol JES-FA200 Band X with cylindrical cavity (TE001) operating with the following parameters: potency 1 mW, central magnetic field 349 mT, modulation frequency 100 kHz, modulation amplitude 0.1 mT. The scavenging activity of CV was evaluated in the presence of two stable free radicals: 500 μM DPPH (1,1-diphenyl-2-picrylhydrazyl) and 100 μM TEMPOL (4-hydroxy-2,2,6,6-tetramethyl-1-