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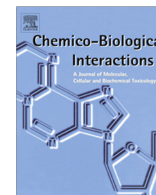

Effect of diabetes on biodistribution, nephrotoxicity and antitumor activity of cisplatin in mice

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ABSTRACT

Both types of diabetes are associated with higher incidence of some types of cancer. Treating cancer in diabetic patients without aggravating diabetes-related complications is a challenge for clinicians. Additionally, little is known about how diabetes affects the treatment of cancer. One of the most effective chemotherapeutic drugs is cisplatin, which is nephrotoxic. Studies suggest that diabetes acts as a protective factor against the nephrotoxicity of cisplatin, but the mechanisms involved have not been elucidated yet. This renal protection has been attributed to decreased accumulation of cisplatin in the kidneys, which could be associated with deficient active transport of proximal tubular cells or to pharmacokinetic alterations caused by diabetes. However, it is uncertain if diabetes also compromises the antitumor activity of cisplatin. To address this issue, we developed a mouse model bearing cisplatin-induced nephrotoxicity, Sarcoma 180 and streptozotocin-induced diabetes. Four groups of treatment were defined: (i) control, (ii) diabetic, (iii) cisplatin and (iv) diabetic treated with cisplatin. The following parameters were evaluated: renal function, oxidative stress, apoptosis, renal histopathology, tumor remission, survival rate, genotoxicity and platinum concentration in tumor and several organs. Results indicate that diabetes protects against the renal damage induced by cisplatin, while also compromises its antitumor effectiveness. This is the first study to demonstrate this effect.

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1. Introduction

Both types of diabetes mellitus (DM) have been associated with increased risk of cancer. Type 2 diabetes is mainly associated with increased risk of breast, colon, pancreatic and liver cancer, while type 1 diabetes is associated with increase in cervical, stomach, pancreatic, and endometrial cancer. The increased risk of cancer in diabetes has been associated mainly with hyperglycemia and hyperinsulinemia [50]. Hyperglycemia supplies the high demand for glucose of tumor cells [78], while insulin is a hormonal stimulator for cellular proliferation [48,80]. In addition, there is a clear relationship between blood glucose reduction and remission of malignant tumors [40]. A study in a model of breast cancer in mice showed that higher values of glycaemia were associated with higher mortality of those animals [58]. It is known that the kidneys of diabetic rats are protected against the nephrotoxicity induced by structurally different compounds, including cisplatin; however, the events underlying this protection are not clear [74,66,67,18]. Cisplatin (cis-diamminedichloroplatinum II) is one of the most effective chemotherapeutic agents, but the exploitation of its full therapeutic potential is limited predominantly due to its nephrotoxicity [16,21,38,42]. The kidney is the main organ of excretion of cisplatin and it is also the major site of cisplatin accumulation [64]. The concentration of cisplatin in proximal tubular epithelial cells is five times higher than in the serum [41]. The resistance to nephrotoxicity is proportional to the duration and severity of the diabetic state; low resistance is observed in rats after a week of experimentally induced diabetes, and total resistance is found after six weeks of uncontrolled diabetes experimentally induced by streptozotocin. The resistance against the nephrotoxicity induced by cisplatin has been found in both insulin-dependent and insulin-independent diabetes [66,10,11]. The treatment of diabetic rats with insulin for 21 days abolishes the protection against cisplatin-induced nephrotoxicity and increases the susceptibility of animals to this toxicity [63]. The attenuation of cisplatin-induced nephrotoxicity in diabetic rats has been associated with

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decreased platinum concentration in plasma [49]. It has also been associated with decreased accumulation of platinum in the kidneys, which has been attributed to deficiencies in the active transport of the drug in tubular epithelial cells [10,62]. However, it is uncertain whether the mechanisms involved in the diabetic renal protection interfere with the antitumor activity of cisplatin and this has been addressed in the present study. For this purpose, we developed an experimental mouse model bearing cisplatin-induced nephrotoxicity, Sarcoma-180-tumor and streptozotocin-induced diabetes, which, to our knowledge, has not been previously studied.

2. Materials and methods

2.1. Chemicals

High purity reagents were used (analytical grade minimum). Reagents were obtained from Sigma–Aldrich®, unless specified differently. Type I ultra-pure water was obtained in the purification system by reverse osmosis (Rios DI-3), followed by purification in Milli-Q Gradient system (Millipore, Bedford, USA). Cisplatin solution (1 mg/ml) was prepared in saline (0.9% NaCl) and streptozotocin solution (15 mg/ml) was prepared in 50 mM sodium citrate buffer, pH 4.5. Both were prepared immediately before use.

2.2. Tumor cells (Sarcoma 180, S-180)

Tumor cells were cultured in the ascites of mice for 8 days prior to inoculation in other mice for development of solid tumor. To preserve the tumor cell line, cells were grown (at 37 °C in a humidified 5% CO_2-containing atmosphere) in DMEM culture medium (Dulbeccos Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) and 0.1% of antibiotic solution (PNS, 5 mg/ml of penicillin, 5 mg/ml neomycin and 10 mg/ml streptomycin). For preservation Sarcoma 180 cells were frozen in the same culture medium previously described supplemented with 20% FBS and 10% DMSO (dimethylsulfoxide) [72,22].

2.3. Animal model

Swiss male adult mice (25–30 g) were housed four per cage and were maintained in a 12-h light/dark cycle in a temperature (22–24 °C) and humidity (45–55%) controlled facility. Standard chow and water were provided ad libitum. Animals were separated into 4 groups of treatment ($n = 8$ in each group) as follows:

(a) Control (C): Sarcoma180-tumor bearing mice without treatment.
(b) CIS: Sarcoma180-tumor bearing mice treated with cisplatin.
(c) DB: Sarcoma180-tumor bearing diabetic mice without treatment.
(d) DB + CIS: Sarcoma180-tumor-bearing, diabetic mice treated with cisplatin.

Diabetes was induced by an injection (150 mg/kg, injection volume: 300 μL) of streptozotocin (15 mg/ml). After 10 days 80% of animals presented levels of blood glucose above 250 mg/dl and were considered diabetic. Sarcoma-180 cells from the ascites of mice were injected (100 μL) subcutaneously in the ventral region of other mice, just above the femoral muscle. A solid tumor developed after 8 days of inoculation [70]. Then, an intraperitoneal injection (600 μL) of cisplatin (1 mg/ml) was administered in a single dose (20 mg/kg). The survival rate assays were terminated 170 days after cisplatin treatment. In all other assays the animals were euthanized after 72 h of cisplatin treatment. Blood samples were collected for tests of renal function. The tumor and organs (kidneys, brain, testes, liver, skeletal muscle, heart and lung) were removed immediately for the determination of platinum levels. Research protocols were 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, and were approved by the Ethics Committee on Animal Use (CEUA) of Ribeirão Preto Campus – USP.

2.4. Assessment of the renal function

Urea and creatinine levels were measured in plasma by using commercially available diagnostic kits (Labtest®, MG, Brazil).

2.5. Preparation of kidney homogenate and protein determination

After euthanasia, the kidneys were immediately removed, immersed in cold phosphate buffered saline (PBS) and kept in ice bath. The cortex was carefully separated from the medullae, weighed and homogenized in PBS (1:10) by using a Potter–Elvehjem type homogenizer (three 15-s cycles, 1-min intervals). The homogenate was centrifuged at 350×*g* for 10 min (4 °C) and the supernatant was removed for protein determination and oxidative stress assays. Homogenate protein concentration was determined by a spectrophotometric (Biuret, 540 nm) method [12].

2.6. Reduced glutathione (GSH)

The method previously described by Hissin and Hilf [31] was used. Reduced glutathione was determined in kidney homogenate by fluorimetry (excitation 350 nm, emission 420 nm). A calibration curve containing 7.5, 15, 30, 60, 150 and 300 mM of reduced glutathione was used to calculate the concentrations of GSH in the samples.

2.7. Lipid peroxidation

TBARS (thiobarbituric-acid-reactive substances) were determined in kidney homogenate by using the method previously described by Buege and Aust [9]. The absorbance at 535 nm was monitored and calculations were performed based on a calibration curve containing 0.5, 1, 3, 10, 12.5, 15, 20 and 50 μM of 1,1,3,3-tetramethoxypropane (TMOP).

2.8. NADPH oxidation

The method previously described by Lund et al. [46] was used. NADPH oxidation was monitored by fluorimetry (emission: 450 nm, excitation: 340 nm).

2.9. Histopathology

Kidneys were fixed in paraformaldehyde (4%), dehydrated through a series of graded ethanol baths (70%, 95% and 100%) and embedded in paraffin. Tissue sections (4 μm) were placed on slides, stained with H&E and the morphology was analyzed under light microscopy (×200). The percentage of damaged renal tubules was calculated by counting ten different fields in the outer stripe of the outer medulla and adjacent cortex per slide. The analysis of longitudinal sections of renal tissue was performed by optical microscopy (400×). The degree of renal damage was analyzed based on a scale from 0 to 4, defined as follows: 0 = normal; 0.5 = small focal areas of cellular infiltration and tubular injury; 1 = lesion involving less than 10% of the cortex; 2 = injury involving 25% of the cortex; 3 = injury involving 25–50% of the cortex; and 4 = extensive damage involving more than 75% of cortex [69].

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