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ORIGINAL ARTICLE

Sunitinib improves chemotherapeutic efficacy and ameliorates cisplatin-induced nephrotoxicity in experimental animals

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Abstract
Purpose Therapeutic inhibition of angiogenesis has a benefit in the treatment of neoplastic diseases. Cisplatin is a widely used anti-cancer agent; however, it has serious side effects on non-tumor cells and causes nephrotoxicity due to its reactive oxygen species–mediated effect. Thus, a combination between cisplatin and angiogenesis inhibitors may be useful in cancer treatment. In the present study, the effect of sunitinib, a multi-targeted receptor tyrosine kinase inhibitor, on the antitumor activity as well as the nephrotoxic side effect of cisplatin was examined.
Methods The antitumor activity was evaluated both in vitro using cultured Ehrlich ascites carcinoma (EAC) cells and in vivo using a mouse model of solid tumor. In addition, the effect of cisplatin and/or sunitinib on the angiogenic marker, VEGF, was examined. Nephrotoxicity was induced in rats by single i.p. injection of cisplatin (6 mg/kg).
Results Sunitinib significantly potentiated the cytotoxic effect of cisplatin in vitro and in vivo. The nephrotoxicity of cisplatin was evidenced by decrease in the body weight, increase in kidney/body weight ratio and decrease in the percent survival of rats. The toxicity was also confirmed biochemically by measuring some kidney function parameters and oxidative stress markers. Sunitinib significantly decreased cisplatin-induced changes in serum creatinine, blood urea nitrogen, creatinine clearance and micro total protein in urine, renal malondialdehyde levels and reduced glutathione contents. In addition, sunitinib effectively blunted cisplatin-induced proximal and distal tubules necrosis.
Conclusion The potential for sunitinib to ameliorate the cisplatin-evoked toxicity as well as to improve the chemotherapeutic effect could have beneficial implications for patients undergoing chemotherapy with cisplatin.

Keywords Cisplatin · Sunitinib · Angiogenesis · Ehrlich ascites carcinoma (EAC) · Vascular endothelial growth factor (VEGF) · Nephrotoxicity

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Introduction

Angiogenesis, the formation of new blood vessels sprouting from the pre-existing vasculature, is critical for growth of human tumors and is a prerequisite for the formation of metastases [19]. Of the pro-angiogenic growth factors identified, vascular endothelial growth factor (VEGF) is the most well known and thought to be the most important angiogenic cytokine in cancer and other types of pathological angiogenesis. VEGF is commonly expressed in a wide variety of human and animal tumors [33] and has been shown to induce angiogenesis in both in vitro [40] and in vivo systems [30]. After being released, endothelial growth factors interact with one or more of the following receptors: VEGFR1, VEGFR2 and VEGFR3. These receptors are transmembrane tyrosine kinases predominantly found on endothelial cells that stimulate downstream signal transduction cascades.

Sunitinib malate is a multi-targeted, oral small molecule tyrosine kinase inhibitor with selective activity against VEGF receptors. Sunitinib has demonstrated growth-inhibitory activity against a variety of tumors in preclinical models including human colon xenografts and small cell

lung cancer [20]. It has been approved for the treatment of metastatic renal-cell carcinoma and gastrointestinal stromal tumors [31].

Cisplatin is a widely used anti-cancer agent for the treatment of patients with a variety of tumors, particularly with head and neck, testicular, ovarian, bladder and lung cancer [7]. However, cisplatin has serious side effects on non-tumor cells and causes nephrotoxicity. Approximately, 25–35% of patients develop evidence of nephrotoxicity [42]. Previous studies [29, 45] showed that reactive oxygen species (ROS) generated by cisplatin play an important role in the onset of renal injury. Unopposed intracellular accumulation of ROS leads to lipid peroxidation and DNA damage. The present study aimed at studying the impact of sunitinib on both chemotherapeutic efficacy and nephrotoxicity of cisplatin.

Materials and methods

(a) Experimental animals

Female Swiss albino mice weighing 20–30 g were used to study the cytotoxic effect of cisplatin alone and in combination with sunitinib. Sprague-Dawley rats of either sex weighing 110–160 g were used for evaluating nephrotoxicity. Animals were purchased from Urology and Nephrology Center, Mansoura University, Egypt and were kept at constant environmental and nutritional conditions throughout the experimental period at room temperature 25°C ± 2 with a 12-h on/off light schedule. Standard food and water were allowed to animals all over the experiment. The in vivo experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Pharmacy, Mansoura University, Egypt.

(b) Drugs and chemicals

- Cisplatin (Bristol-Myers-Squibb Co.) was obtained as the pharmaceutical drug (5 mg/ml vial) and was diluted with isotonic saline.
- Sunitinib (supplied by Pfizer).
- Roswell Park Memorial Institute (RPMI 1640) medium (supplied by Sigma–Aldrich chemical Co.)
- All other chemicals used in this study are of fine analytical grade.

(c) Ehrlich ascites carcinoma cells

Ehrlich ascites carcinoma cells (EAC) were established in the Netherlands Cancer Institute. The Ehrlich tumor line was maintained in the laboratory of Faculty of Pharmacy, Mansoura University in female Swiss albino mice by serial intraperitoneal passage at 7- to 10-day intervals.

Cytotoxicity and angiogenesis study

In vitro experiment

Cell culture EAC cells suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, containing various concentrations of drugs, were cultured in cell culture sterile tubes at a density of 2×10^5 cells/ml/tube. The tubes were incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h. The trypan blue dye exclusion test was used to determine the rate of cellular growth inhibition. The dye stains the dead cells only [50].

In vitro cytotoxic assay Ascitic fluid from the intraperitoneal cavity of the donor animal was aseptically aspirated, 7–8 days after EAC cells inoculation and washed three times with *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid buffered Hanks' balanced salt solution. EAC cells were counted under the microscope using a haemocytometer and resuspended in normal saline so that each 0.1 ml contained 2×10^5 cells [3]. EAC cells were incubated in RPMI 1640 medium for 24 h in cell culture tubes, each tube contained 0.1 ml cells and 0.9 ml medium (final concentration of the cells = 2×10^5 cells/ml). After 24-h incubation, the tubes were centrifuged at $67 \times g$ and cells were separated. EAC cells were resuspended in RPMI 1640 medium and drugs were added so that the content of each tube was 0.8 ml medium, 0.1 ml cells and 0.1 ml drug. The final drug concentrations of cisplatin were 30, 50, 100, 150 and 200 μM.

The cytotoxicity dose–response curve for cisplatin was constructed in order to determine the concentration that inhibits 50% of cell survival (IC_{50}). EAC cells were incubated with 85 μM cisplatin (IC_{50}), sunitinib, 1 and 2 μM or the combination for 24 h. After incubation of cells with drugs, cells were separated, washed by phosphate buffered saline and resuspended in a drug-free medium. EAC cells were stained with trypan blue dye and the percent survival of cells was determined by trypan blue dye exclusion method. Cytotoxicity was determined 3 times and the mean was recorded.

Control experiments in which EAC cells were incubated in a drug-free medium were also conducted. Percent survival of cells = (T/C) × 100 was calculated, where T and C represent the number of viable cells in a unit volume of the test drug tube and the control tube, respectively.

In vivo experiment

Ascitic fluid was withdrawn under aseptic conditions from tumor-bearing mice by needle aspiration from the peritoneal cavity, 7–8 days after EAC cells inoculation and washed three times with normal saline by centrifugation at $67 \times g$. EAC cells obtained after washing were tested for

viability using trypan blue. The cells were examined microscopically using a haemocytometer, suspended in normal saline so that each 0.1 ml contained 5×10^5 viable EAC cells. The cells were counted under the microscope.

Solid tumors were induced in mice by S.C. inoculation of 0.1 ml containing 5×10^5 viable tumor cells on the left flank anterior to the hind leg [41]. Tumor growth was determined by caliper measurement of the largest diameter and its perpendicular [43].

$$\text{Tumor size } \left(\text{mm}^3\right) = 0.5 \times a \times b^2$$

where a is the largest diameter and b is its perpendicular.

When the primary tumor reached a size of 50–100 mm^3, 40 mice were grouped into 4 groups (10 mice each). Group (1) received normal saline (EAC-bearing control, 5 ml/kg). Group (2) received cisplatin (2 mg/kg from 0.1% solution) I.P. Group (3) received sunitinib (53.5 mg/kg, orally). Group (4) received cisplatin and sunitinib. Treatment continued for 21 consecutive days. Tumor size at day 0 (five days after tumor inoculation) and after treatment (day 21) was measured. Antitumor activity was calculated by the determination of ΔT (change of tumor size in the treatment group) and ΔC (change of tumor size in the control). The degree of tumor growth inhibition can be obtained from $\Delta T/\Delta C \times 100$ [43]. Serum samples were collected for determination of VEGF. An additional group of 10 mice receiving normal saline (5 ml/kg) served as negative control (tumor non-bearing mice). It was used for determining normal VEGF levels.

Determination of VEGF

VEGF was quantitated using an ELISA assay (Ray Bio® mouse VEGF ELISA kit) according to the protocol of the manufacturer.

Nephrotoxicity

Rats were divided into 4 groups of 8 rats each. Group (1) received normal saline (2.5 ml/kg). Group (2) received single dose of cisplatin (6 mg/kg) I.P. Group (3) received sunitinib (25 mg/kg/day, orally) for 5 consecutive days. Group (4) received cisplatin and sunitinib. On day 4 after cisplatin injection, animals were housed separately in metabolic cages for collection of 24-h urine samples that centrifuged for 15 min at $604 \times g$, and kept frozen until analyzed. Micro total protein and urinary creatinine were measured in these samples. Creatinine clearance was estimated from the collected urine during 24 h. On day 5, all rats were weighed and the percent survival was recorded. All died animals were replaced to complete 8 rats/group. Blood samples were collected for determination of creatinine and blood urea nitrogen, then rats were killed by an overdose of ether. The kidneys were isolated and washed with ice-cold isotonic saline (0.9%). The left kidneys were used for determination of thiobarbituric acid-reactive substances (TBARS) levels, reduced glutathione contents and superoxide dismutase activities. The right kidneys were fixed in 10% neutral buffered formalin for histopathological examinations.

Biochemical measurements

Determination of serum creatinine

Creatinine was measured in rat sera as described by Henry et al. [25]. In brief, creatinine in alkaline solution reacts with picrate (Jaffe reaction) to form a colored complex that was measured spectrophotometrically at 550 nm. A kit from Human Diagnostics Co., Egypt was used.

Determination of blood urea nitrogen (BUN)

Urea was measured enzymatically in rat sera according to the reported procedures [24]. Briefly, urea in the sample was hydrolyzed by urease enzyme to yield ammonia and carbon dioxide. In a modified Berthelot reaction, the ammonium ions react with a mixture of salicylate, sodium nitroprusside and hypochlorite to yield a blue-green chromophore that was measured spectrophotometrically at 580 nm. A kit from Human Diagnostics Co., Egypt was used.

Determination of creatinine clearance

Creatinine clearance can be estimated from the amount of creatinine excreted in the urine during 24 h [28].

Determination of micro total protein in urine (MTP)

MTP in urine was determined quantitatively by colorimetric method as described by Watanabe et al. [49]. Briefly, in acidic medium, protein in the specimen reacts with pyrogallol-red in the presence of molybdate ions to form a purple color complex measured spectrophotometrically at 600 nm. A kit from Fortress Diagnostics Co., UK was used.

Preparation of kidney homogenate

The isolated left kidneys were rinsed in ice-cold isotonic saline (0.9%) and weighed quickly. Homogenization was carried out in ice-cold 0.1 M phosphate buffer (pH 7.4) to yield 10% w/v tissue homogenates [39], and the following biochemical parameters were assessed.

Determination of lipid peroxidation (LP)

The level of LP in the kidney was estimated as TBARS according to Ohkawa et al. [38]. The absorbance was determined at 532 nm spectrophotometrically, and the concentrations were expressed as nmol/g wet tissue.

Determination of reduced glutathione (GSH)

The level of acid-soluble thiols, mainly GSH, in the kidney was assayed colorimetrically, based on its reaction with Ellman's reagent according to the method earlier described by Ellman [18]. The absorbance was measured at 412 nm, and the concentrations were expressed as μmol/g wet tissue.

Determination of superoxide dismutase (SOD)

The enzymatic activity of SOD was assessed according to Marklund [34]. SOD activity was expressed as U/g wet tissue. One unit of SOD activity is defined as the amount of the enzyme causing 50% inhibition of auto-oxidation of pyrogallol.

Histopathological analysis

The right kidneys were rapidly removed and divided vertically into two halves and fixed by immersion in 10% neutral buffered formalin solution (pH 7.4). After fixation, the kidney tissue block was dehydrated in graded alcohol, embedded in paraffin, sectioned at 3 μm and then stained with hematoxylin and eosin (H&E) stain for evaluation by light microscope and photographed by using a Leica Qwin Image analyzer (Cambridge, England). The severity of the injury was judged by two-independent observers blinded to the experimental protocol.

Statistical analysis

Data are expressed as mean ± SEM (Significance was calculated at $P < 0.05$). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test, in addition to linear regression analysis for the best fitting line of all standard points [13]. Also paired Student's t-test was used as a test of significance for comparison between two arithmetic means of the same subject before and after treatment [13]. Chi-square test was used for comparison of two proportions [13]. Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA, USA).

Results

Cytotoxicity and angiogenesis

Effect of sunitinib on the cytotoxic effect of cisplatin on cultured EAC cells

The IC_{50} of cisplatin was found to be about 85 μM. Sunitinib showed a significant cytotoxic effect when compared to control group but non-significant from cisplatin-treated group. Combination of cisplatin with sunitinib showed a significant cytotoxic effect compared to both control and cisplatin-treated groups (Table 1).

Effect of cisplatin and/or sunitinib on tumor size in mice

Implantation of EAC cells resulted in a solid palpable tumor mass that appeared after 5 days from inoculation (day 0). The size of tumor progressively increased with time and reached about sixfold its initial mass after additional 21 days (day 21) that considered 100% tumor growth.

Treatment with cisplatin significantly decreased the relative tumor size compared to the control group, showing 20% tumor growth (i.e., 80% tumor growth inhibition). Mice treated with sunitinib showed 16% tumor growth (i.e., 84% tumor growth inhibition). Administration of cisplatin with sunitinib showed an increase in tumor growth inhibition (103%) compared to control group (Table 2).

Effect of cisplatin and/or sunitinib on VEGF

As in Fig. 1, EAC-bearing group showed a non-significant change in VEGF concentration at day 21. Similarly,

Table 1 Effect of sunitinib (1, 2 μM) on the cytotoxic effect of cisplatin (85 μM) on cultured EAC cells

Treatment	% Survival of cultured EAC cells
Control	97.0 ± 0.3
Cisplatin	50.0 ± 5.1*
Sunitinib (1 μM)	64.0 ± 6.6*
Sunitinib (2 μM)	52.0 ± 3.6*
Cisplatin/sunitinib (1 μM)	28.0 ± 2.3*#
Cisplatin/sunitinib (2 μM)	20.0 ± 2.0*#

Data are expressed as mean ± SEM, $n = 3$

* Significantly different from control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

Significantly different from cisplatin-treated group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

Table 2 Effect of cisplatin (2 mg/kg) and/or sunitinib (53.5 mg/kg) on tumor size in mice after 21 days treatment

Treatment	Tumor size (mm^3)			
	Day 0	Day 21	$\Delta T/\Delta C$ (%)	% Inhibition
Control	81.3 ± 4.6	488.3 ± 21.1	100	0
Cisplatin	75.4 ± 8.7	155.8 ± 6.1*	20	80
Sunitinib	65.9 ± 7.1	132.6 ± 4.4*	16	84
Cisplatin/ sunitinib	68.2 ± 6.2	58.1 ± 2.6*#	−3	103

Data are expressed as mean ± SEM, $n = 10$

* Significantly different from control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

Significantly different from cisplatin-treated group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

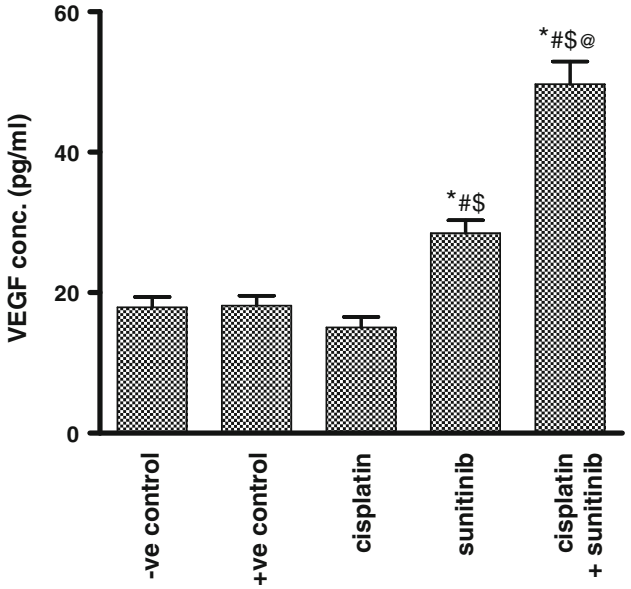

Fig. 1 Mean serum values of angiogenesis marker VEGF (pg/ml). *Significantly different from the corresponding mean value of –ve control group ($P < 0.05$). #Significantly different from the corresponding mean value of EAC-bearing (+ve) control group ($P < 0.05$). $Significantly different from the corresponding mean value of cisplatin-treated group ($P < 0.05$). @Significantly different from the corresponding mean value of sunitinib-treated group ($P < 0.05$). Statistical comparisons were performed using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

cisplatin produced a non-significant change in VEGF values when compared to tumor non-bearing mice (−ve control). Sunitinib produced a significant increase in VEGF when compared to tumor non-bearing, tumor-bearing and cisplatin-treated groups (1.5-fold). Administration of sunitinib with cisplatin significantly increased the VEGF level by nearly threefold.

Nephrotoxicity

Effect of cisplatin and/or sunitinib on body weight, kidney/body weight ratio and % survival of rats

Results in Table 3 showed that cisplatin produced a significant decrease in the body weight and percent survival of rats with an increase in kidney/body weight ratio compared to control rats. Administration of sunitinib significantly counter-regulated the induced changes.

Effects of sunitinib on biochemical parameters in cisplatin-treated rats

(a) Effect on kidney functions
Cisplatin caused significant elevation of serum creatinine, BUN and MTP in urine compared to control group with a significant decrease in creatinine clearance. Sunitinib administration significantly ($P < 0.05$) improved kidney function parameters (Table 4).

(b) Effect on antioxidant status
Table 5 points out that after 5 days of treatment, cisplatin significantly increased the levels of MDA and SOD in rat kidney homogenates while decreased reduced GSH level. Sunitinib significantly decreased cisplatin-induced changes in the oxidative stress markers.

Effect of sunitinib on histopathological analysis in cisplatin-treated rats

Cisplatin induced loss of brush border, vacuolation and desquamation of epithelial cells in renal tubular epithelium with cast formation in the lumen compared to control rats. Administration of sunitinib with cisplatin produced a marked reduction in the severity of tubular damage with notable absence of casts in the lumen (Fig. 2).

Discussion

Angiogenesis has been recognized as one of the most important mechanisms of tumor establishment, survival and metastasis [11]. Therefore, it is not surprising that several antiangiogenic agents are currently approved or being investigated in cancer clinical trials and that a combination between commonly known anticancer and antiangiogenic agents may be useful as a new approach to treat cancer.

Table 3 Effect of cisplatin (6 mg/kg) and/or sunitinib (25 mg/kg) on body weight, kidney/body weight ratio and percent survival of rats

Treatment	Body weight (g)		Kidney/body weight ratio $\times 10^{-3}$	% Survival
	Before treatment	After treatment		
Control	104.0 ± 0.4	114.3 ± 0.7	4.9 ± 0.1	100
Cisplatin	132.0 ± 4.0	89.2 ± 6.4°	5.98 ± 0.4*	85.7$^{\$}$
Sunitinib	108.3 ± 1.2	117.3 ± 0.8	4.18 ± 0.2$^{\#}$	100
Cisplatin/sunitinib	143.4 ± 4.7	123.8 ± 5.9	4.76 ± 0.3$^{\#}$	100

Data are expressed as mean ± SEM, $n = 8$

° Significantly different from its corresponding initial value using paired Student's t-test ($P < 0.05$)

* Significantly different from control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

$^{\#}$ Significantly different from cisplatin-treated group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

$^{\$}$ Significantly different from control group using chi-square test ($P < 0.05$)

Table 4 Effect of cisplatin (6 mg/kg) and/or sunitinib (25 mg/kg) on kidney function parameters

Treatment	Serum creatinine (mg/dl)	Blood urea nitrogen (BUN) (mmol/L)	Creatinine clearance (C_{cr}) (ml/min)	Proteinuria (MTP) (mg/day)
Control	1.94 ± 0.17	3.24 ± 0.28	0.12 ± 0.016	2.45 ± 0.16
Cisplatin	11.83 ± 0.7*	21.93 ± 0.57*	0.012 ± 0.001	20.4 ± 2.6
Sunitinib	2.00 ± 0.23$^{\#}$	4.14 ± 0.5$^{\#}$	0.13 ± 0.01$^{\#}$	2.61 ± 0.02$^{\#}$
Cisplatin/sunitinib	6.9 ± 0.3*$^{\#}$	7.24 ± 0.8*$^{\#}$	0.03 ± 0.003*	10.86 ± 1.48*$^{\#}$

Data are expressed as mean ± SEM, $n = 8$

* Significantly different from control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

$^{\#}$ Significantly different from cisplatin-treated group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

Table 5 Effect of cisplatin (6 mg/kg) and/or sunitinib (25 mg/kg) on kidney antioxidant status

Treatment	TBARS (nmol/g wet tissue)	GSH (μmol/g wet tissue)	SOD (units/g wet tissue)
Control	131.6 ± 5.9	9.7 ± 0.7	34.7 ± 1.3
Cisplatin	464.4 ± 35.7*	2.7 ± 0.3*	55.2 ± 1.6*
Sunitinib	141.6 ± 5.1$^{\#}$	8.73 ± 0.6$^{\#}$	34.7 ± 0.6$^{\#}$
Cisplatin/sunitinib	250.9 ± 11.1*$^{\#}$	8.36 ± 0.9$^{\#}$	47.4 ± 2.3*$^{\#}$

Data are expressed as mean ± SEM, $n = 8$

* Significantly different from control group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

$^{\#}$ Significantly different from cisplatin-treated group using one-way ANOVA followed by Tukey–Kramer multiple comparisons test ($P < 0.05$)

Sunitinib is an oral multi-targeted tyrosine kinase inhibitor with antitumor and antiangiogenic activities through targeting platelet-derived growth factor receptor, VEGF receptor, Fms-like tyrosine kinase receptor 3 (FLT3) and C-KIT (stem-cell factor [SCF] receptors) [31].

Cisplatin, one of the most important conventional cytotoxic agents, is used in treatment of a wide variety of both pediatric and adult malignancies [7]. Dose-dependent and cumulative nephrotoxicity is the major toxic effect of cisplatin that required a reduction in the dose or discontinuation of the treatment [42].

The purpose of the present study was to demonstrate the possible beneficial effect of sunitinib on the anticancer activity and nephrotoxicity of cisplatin.

In the present study, it was found that EAC cells are sensitive to cisplatin in a concentration-dependent manner. This observation is in agreement with the previously reported cytotoxicity of cisplatin on many other cultured experimental tumor cell lines such as cervical cancer cell

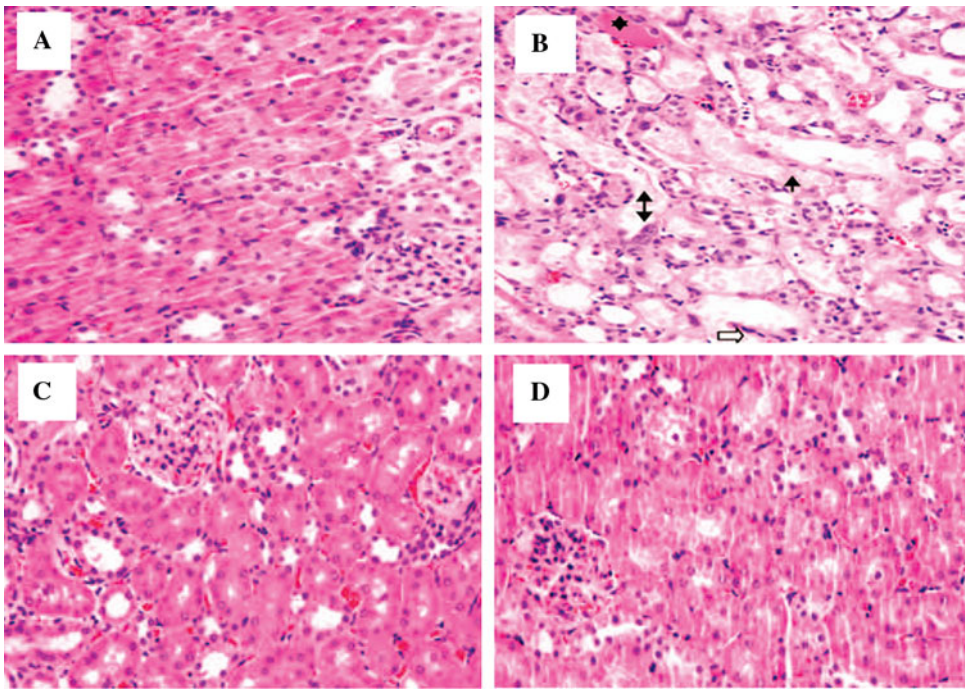

Fig. 2 Light microscopy photomicrographs depicting sections from kidneys of rats receiving **a** saline control; **b** cisplatin given at a single i.p. dose (6 mg/kg); **c** sunitinib (25 mg/kg/day orally for 5 days) and **d** cisplatin+sunitinib, at the above doses. Treatment with either saline or sunitinib alone did not cause renal damage. Kidneys of cisplatin-treated rats caused marked renal injury with marked necrosis (*filled arrows*), loss of epithelial cells (*double arrow*), vacuolization (*open arrow*) and cast formation (*star*). Sunitinib together with cisplatin markedly reduced the severity of the renal damage. H&E stain, magnification ×400

line [26], squamous cell carcinoma of the head and neck [53] mouse EM T6 tumor cells [51] and nasopharyngeal carcinoma [47].

Sunitinib produced a significant cytotoxic effect against EAC cells when compared to control group. A combination between cisplatin and sunitinib significantly produced a synergistic cytotoxic effect on cultured EAC cells.

This study has been extended further to explore if sunitinib could alter the antitumor effect of cisplatin in vivo and thus, modify its therapeutic efficacy. In this serve, a mouse model of solid tumor was used. The antitumor activity of cisplatin was evidenced by the reduction in the relative tumor size compared to untreated tumor-bearing control animals. This is coping with the previous reports of Badary et al. [3].

Sunitinib produced a reduction in relative tumor size by nearly the same percent of inhibition of tumor growth obtained by cisplatin. This observation supports the well-established antitumor effect of sunitinib against a variety of tumors in preclinical models [20]. Combination between cisplatin and sunitinib inhibits the growth of solid tumor in mice confirming the synergistic effect between both drugs.

The growth and metastasis of tumors are dependent on their development of a vascular supply. VEGF is the most pivotal positive regulator of angiogenesis that facilitates tumor growth and metastasis.

In the present study, the effect of combined use of sunitinib with cisplatin on angiogenesis was monitored by measuring serum VEGF level. Cisplatin showed a non-significant effect on serum VEGF level when compared either tumor-bearing or tumor non-bearing control groups.

This result is in agreement with that of [17] in which cisplatin reduced plasma levels of VEGF on day 7 but not on day 14 or 21 postinoculation suggesting that VEGF is essential for initial but not continued in vivo growth of EAC cells. However, sunitinib significantly increased the VEGF level when compared to tumor non-bearing, tumor-bearing and cisplatin groups.

This observation can be explained on the basis of Ebos et al. study [16], who stated that cancer patients treated with sunitinib malate showed increased levels of plasma VEGF and decreased levels of soluble VEGF-receptor 2, thus implicating these overall changes as a possible class effect of such drug and raising the possibility of their exploitation as surrogate biomarkers for pharmacodynamic drug activity exposure and patient benefit. They also reported an identical pattern of change in normal tumor non-bearing mice treated with SU11248/sunitinib. These results suggested that observed sunitinib-induced molecular plasma changes represent a systemic tumor-independent response to therapy and may correlate with the most efficacious antitumor doses but not predictive markers of tumor response of clinical benefit.

Norden-Zfoni et al. [37] also reported that VEGF increased by 2.2-fold during the first 2 weeks of treatment with SU11248 supporting the utility of VEGF as a marker of SU11248 activity.

The significant elevated VEGF levels observed in this study in cisplatin/sunitinib group may reflect the effectiveness of such combination against tumor growth.

This study further extended to address whether or not sunitinib would have effects on cisplatin-induced nephrotoxicity. In the present study, a loss of body weight and an increase in kidney/body weight ratio of rats were observed after cisplatin. These observations are consistent with many reported data [9, 44, 52]. Sunitinib significantly protected rats against reduction in body weight and the increase in kidney/body ratio. Sunitinib also protected rats against cisplatin-induced mortality.

In the present study, treatment with cisplatin induced marked renal injury characterized by a significant increase in serum creatinine and BUN levels that reached to the maximum level at day 5 after cisplatin administration.

Yoshida et al. [52], used the same dose of cisplatin, reported that BUN and creatinine levels increased twofold at day 3 and about fourfold at day 5 after cisplatin injection. Chirino et al. [9] observed that such levels were increased 4.9 and 5.5 times, respectively, in rats administered single dose of cisplatin (7.5 mg/kg i.p.). Also, Shimeda et al. [44] have shown that 5 mg/kg of cisplatin produced a significant increase in serum creatinine (797%) and BUN levels (556%) after 5 days of cisplatin injection.

In the current study, cisplatin-induced nephrotoxicity was also evidenced by a remarkable decrease in creatinine clearance (Ccr) at day 5, a result consistent with Badary et al. [2]. Moreover, cisplatin nephrotoxicity in this study showed a significant increase in urine MTP value. In agreement with this result, Gulec et al. [22] observed significant increases in plasma BUN and creatinine levels as well as MTP values as markers of renal injury at day 7 in rats administered cisplatin as a single i.p. dose (7 mg/kg).

In the present study, the decrease in glomerular function in cisplatin-treated rats could be due to the obstruction and the back leak of glomerular filtrate. Such decrease could not be attributed to structural damage since glomeruli structure was normal in cisplatin-treated rats. These histopathological observations are consistent with the results obtained by Chirnio et al. [9]. The decrease in glomerular function may be secondary to reactive oxygen species (ROS) that induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient [15].

Increased urinary excretion of MTP may be a sensitive indicator of tubular damage, loss to tubular protein reabsorption capability or loss of glomerular barrier to protein filtration [36].

In the present study, sunitinib improved kidney function parameters as evidenced by decreased serum creatinine, BUN levels and urine MTP values, while increasing Ccr value at day 5. Sunitinib is one of protein tyrosine kinase inhibitors, which proved to be effective in the treatment of acute kidney injury (AKI). AG490 and AG126 tyrphostins have recently been shown to protect from AKI in experimental animal models of ischemia–reperfusion and sepsis-induced injury. Tyrphostins are a family of compounds originally designed as protein tyrosine kinase inhibitors. AG490 protects from cyclosporine-induced AKI and AG1714 protects from cisplatin nephrotoxicity.

AG490 induced nephroprotection by inhibiting oxidative stress-related Janus-activated kinase-2 (JAK2) activation [21]. This suggests a relation between protein kinases and oxidative stress which could explain the beneficial effect of sunitinib against nephrotoxicity induced by cisplatin in this study.

Sung et al. [46] reported that genistein, a tyrosine kinase inhibitor, had protective effects on cisplatin-induced renal injury in mice. Genistein was found to significantly decrease ROS production confirming the relation between tyrosine kinases and ROS production.

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids. Malondialdehyde (MDA) is the most abundant aldehyde resulting from lipid peroxide breakdown in biological systems and used as an indirect index of lipid peroxidation [14].

In the present study, MDA production as reflected by TBARS was increased after cisplatin administration such result is in agreement with many reports [1, 9].

ROS such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$) or hydroxyl radical ($OH^{\bullet}$) are normally generated in renal cells and immediately detoxified by endogenous antioxidants such as GSH, catalase or SOD [32]. Cisplatin is able to generate ROS such as superoxide anion, which could form peroxynitrite, during interaction with DNA [35], hydroxyl radicals [4], H_2O_2 [48] and stimulates lipid peroxidation [27].

Conklin [10] reported that the balance normally present in cells between free radicals formation and protection against them is disturbed in cisplatin-induced toxicity. In addition, unopposed intracellular accumulation of ROS can affect cell function by direct effect on cell components, including lipids, proteins, DNA and destroying their structure [23].

Depletion of tissue GSH stores in this study is one of the major factors that allowed lipid peroxidation and subsequent tissue damage. Blokhina et al. [5] found that conjugation of glutathione to lipid peroxidation products could lead to the depletion of the glutathione pool. Renal GSH concentration was significantly decreased in cisplatin-treated rats, an observation that agreed with that of Shimeda et al. [44].

Under oxidative stress, GSH is consumed by GSH-PX (GSH peroxidases) to inactivate H_2O_2 and detoxify peroxides resulted from increased lipid peroxidation [8].

Therefore, it was assumed that cisplatin-induced GSH depletion may be a secondary event following the cisplatin-induced increase in free radical generation, lipid peroxidation and/or decrease in lipid peroxidation protecting enzymes.

In the current study, there was a significant increase in SOD activity in cisplatin-treated rats. This observation is contradictory to others in which the SOD activity was significantly decreased in cisplatin-treated rats [1].

Such controversy could be explained in virtue of the postulate that the cisplatin-induced increase in renal SOD activity is rather a compensatory event that occurred indirectly due to the oxidant injurious effect of cisplatin and not due to a direct effect of the drug.

In this study, sunitinib significantly decreased cisplatin-induced changes in the oxidative stress markers. The significant decrease of oxidative stress markers by sunitinib may further support the postulate that there is a relation between tyrosine kinase receptor inhibition and the observed effects on lipid peroxidation, GSH and SOD activity and thus the nephroprotective effect of sunitinib.

In the present study, there were extensive epithelial cell vacuolization, swelling, desquamation and necrosis with cast formation occurring predominantly in proximal and distal tubules after cisplatin treatment.

Formation of stable protein-S-Pt adducts resulted in dysfunction of membrane associated and cytoplasmic protein [12] and decreasing the activity of important antioxidant enzyme systems [6]. The ability of sunitinib to reverse the depleted GSH and the elevated MDA may be partly responsible for decreasing the extent of tubular damage at day 5 after cisplatin treatment.

In conclusion, the results of the present study indicated that sunitinib ameliorates cisplatin-induced nephrotoxicity and potentiates its antitumor effect in experimental animals.

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