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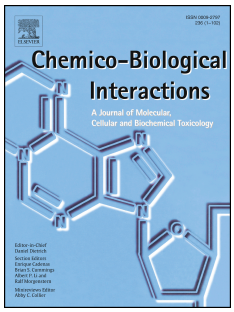

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Carvedilol protects the kidneys of tumor-bearing mice without impairing the biodistribution or the genotoxicity of cisplatin

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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 anti-hypertensive 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.

Keywords: cisplatin; carvedilol; nephroprotection; genotoxicity; biodistribution

Introduction

Cisplatin (Cisp) is an effective drug for chemotherapy; however, the effective dose usually produces significant nephrotoxicity (Launay-Vacher et al., 2008). Minimization of renal toxicity is essential to improve the effectiveness of cisplatin chemotherapy and the quality of life of patients (Hausheer et al., 2010). 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 (Launay-Vacher et al., 2008). 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 (Cvitkovic, 1998). However, amifostine is not always effective against the toxicity induced by cisplatin in healthy tissues (Sastry and Kellie, 2005, Katzenstein et al., 2009). Other limitation factors include high costs, serious side effects and concerns that it might impair the antitumor efficacy (Koyner et al., 2008).

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 obtained, little is known about the impact of these compounds on the antitumor activity of cisplatin (for revision, see dos Santos et al., 2012). We have demonstrated the protective effect of carvedilol (CV) against the nephrotoxicity induced by cisplatin and have

delineated some mechanisms involved in the protection (Rodrigues et al., 2010, 2011, Carvalho Rodrigues et al., 2012, Carvalho Rodrigues et al., 2013).

CV is a β-adrenoceptor blocking agent already used in the treatment of congestive heart failure, mild to moderate hypertension, and myocardial infarction (Feuerstein and Ruffolo, 1995, Watanabe et al., 2000). 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 (Yue et al., 1992, Noguchi et al., 2000, Pauschinger et al., 2005, Stafylas and Sarafidis, 2008). 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 (Rodrigues et al., 2010, 2011, Carvalho Rodrigues et al., 2013).

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 (Cohen and Lippard, 2001). Additionally, we evaluated the potential of carvedilol in relation to two important antioxidant mechanisms, namely, neutralization of free radicals and chelation of ferrous ions (Yue et al., 1992, Noguchi et al., 2000, Dandona et al., 2007).

Methods

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 ultra-pure 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.

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.

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.) 25mg/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 25mg/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 (Carvalho Rodrigues et al., 2013).

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 (Carvalho Rodrigues et al., 2013).

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 (x200).

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 (25ºC). 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) (Batista et al., 2009).

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 (Hayashi et al., 1990). Peripheral blood was collected from the caudal vein, fixed in methanol and stained using acridine orange (125 µg/mL) one minute before the analysis by fluorescence microscopy (Olympus BX 51 – blue light 488nm and yellow filter, 400x) (Lynch et al., 2005). 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 (Krishna and Hayashi, 2000).

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 x 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, x400) (Aparecida Resende et al., 2006).

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-piperidinyloxy). CV (40 µL) was added to DPPH (40µL) or TEMPOL (40µL) solutions, shaken and placed in a capillary tube, then sealed and inserted in a quartz tube measuring 3 mm of diameter in 3 minutes of fixed time (Santos et al., 2009).

Ferrous ion (Fe^{+2}) chelation

Determination of ferrous ion was performed based on a colorimetric reaction between bathophenanthroline disulfonic acid and Fe^{+2}, which generates a red-colored solution with intensity proportional to the ion concentration. We incubated increasing concentrations of CV (0 – 200 µM) with $FeSO_4$ 100 µM for 15 minutes, then we added bathophenanthroline disulfonic acid solution (200 µM), vortexed and incubated for further 15 minutes. Absorbance at 530 and 700 nm was determined. The concentration of remaining ferrous ion was determined based on a calibration curve prepared with different concentrations of $FeSO_4$ (0-50 µM) Hitachi U-3000 spectrophotometer (Rana et al., 2010).

Statistical analysis

Data were expressed as mean ± SEM (n=6). Statistical analyses were conducted by one-way ANOVA for multiple comparison with level of significance $p< 0.05$ (GraphPad Software, San Diego, CA, USA).

Results

Carvedilol protected against cisplatin-induced renal damage

The renal damage was assessed by plasma creatinine, BUN and renal tissue morphology. Cisplatin significantly increased BUN and creatinine in plasma (338.7 ± 11.83 mg/dL and 1.94 ± 0.18 mg/dL, respectively) as

compared to controls (49.81 ± 1.71 mg/dL and 0.25 ± 0.01 mg/d, respectively). In the CV + Cisp group, BUN (125.5 ± 21.15 mg/dL) and plasma creatinine (0.50 ± 0.05 mg/dL) were significantly decreased in relation to the Cisp group. The CV group presented BUN (55.17 ± 2.17 mg/dl) and creatinine (0.34 ± 0.005 mg/dL) that were not significantly different from controls (Fig.1A-B).

The kidney morphology of the Cisp group was characterized by tubular dilation and cell vacuolization, which were not observed in the CV+Cisp group (Fig. 1C). These findings confirm the renal damage induced by cisplatin and the nephroprotection of CV as we previously demonstrated (Carvalho Rodrigues et al., 2013).

Carvedilol did not affect the accumulation of Cisp in tumor or kidneys

The accumulation of cisplatin in the kidneys and tumor was assessed by the determination of the platinum concentration in these tissues. No platinum was detected in the kidneys of the C group (< LOD = 0.006 µg/g), while in the Cisp group, it was significantly higher (36.36 ± 12.09 µg/g). No significant difference was observed between the renal concentration of platinum in the Cisp (36.36 ± 12.09 µg/g) and CV+Cisp groups (CV+Cisp: 34.93 ± 14.42 µg/g) (Figure 2A). Accordingly, no platinum was detected in the tumor tissue of controls (< LOD = 0.006 µg/g) and values significantly higher were observed in the Cisp group (17.65 ± 8.20 µg/g). No significant difference was observed between Cisp (17.65 ± 8.20 µg/g) and Cisp+CV groups (20.11 ± 7.10 µg/g). Results for the CV group were higher than the C group for both tissues (kidney, 0.41 ± 0.24 µg/g and tumor, 0.09 ± 0.06 µg/g) but the differences were not significant. The platinum found in the tissues of the CV group might be due to occasional contact with the animals of the CV+Cisp group; nevertheless, this did not

compromise the results, since the CV group presented values extremely lower than Cisp or CV+Cisp groups and not significantly different from controls (Figure 2B).

CV did not affect the genotoxicity induced by Cisp

The frequency of micronuclei in peripheral blood (MNPCE/1000 PCE) was 1.75 ± 1.01 in the CV group, 1.25 ± 0.28 in the C group, 8.03 ± 1.16 in the Cisp group and 7.40 ± 1.11 in the CV + CISP group. Significant difference was only observed between Cisp and C. No significant differences were observed between CV and C or CV+Cisp and Cisp. (Figure 3A-B). The frequency of PCE in bone marrow was significantly lower in the Cisp group (0.02 ± 0.01) as compared to the C group (0.34 ± 0.05). There was no significant difference between the CV+CISP group (0.02 ± 0.00) and the CISP group or between the CV (0.40 ± 0.06) and the C groups (Figure 3C-D).

CV is rather a ferrous ion chelator than a free radical scavenger

The ability of CV to scavenge free radicals was evaluated by using the free radicals DPPH and TEMPOL and monitoring the electron signal by the EPR technique. As shown by Figure 4A-B, CV was not able to scavenge either TEMPOL or DPPH. For TEMPOL, no decreased signal was detected in the presence of CV, even for a concentration 50 times higher (5mM) than the concentration of TEMPOL (100µM) (Figure 4A). For DPPH, a slight decrease was observed in the electron signal, but only for the concentration of CV that was 10 times higher than the concentration of DPPH (500 µM), (Figure 4B). Both findings show the low ability of CV to neutralize free radicals.

The capacity of CV to chelate ferrous ion was assessed by the bathophenanthroline reaction and spectrophotometry. Figure 5A shows the

calibration curve of ferrous ion, which was used to calculate the remaining concentration of ferrous ion in solution after the addition of different concentrations of CV. The initial concentration of ferrous ion (40 µM) in the solution decreased as the concentrations of CV increased. The half-maximal effective concentration (EC50) of CV was calculated (84.27 µM) based on the non-linear fit obtained by plotting the concentrations of ferrous ions *versus* Log of CV concentrations. Data are presented in Figure 5B.

Discussion

We have previously shown the beneficial effects and the mechanisms of protection of carvedilol (CV) against the renal toxicity induced by cisplatin both *in vitro* and in animal models. First, we demonstrated that CV protected against the mitochondrial toxicity induced by cisplatin in rats (Rodrigues et al., 2010), then we delineated the mechanisms of protection associated with oxidative damage and apoptosis in rats and in renal proximal tubular epithelial cells (Rodrigues et al., 2011, Carvalho Rodrigues et al., 2012). Additionally, we demonstrated that CV protected against the renal damage and increased the survival of sarcoma-180-tumor-bearing mice without affecting the tumor reduction by cisplatin (Carvalho Rodrigues et al., 2013); however, a question as to the antitumor mechanism still remained. Therefore, in the present study, we used the same experimental design to explore the effects of a nephroprotective dose of CV on biodistribution and on the main antitumor mechanism of cisplatin, i.e., genotoxicity. First, we confirmed the nephroprotection by assessing the renal function (plasma urea and creatinine), and the morphological alterations (vacuolization and tubules dilation) of the renal tissue. Results are in line with our previous findings (Carvalho Rodrigues et al., 2013). Then, we assessed the

genotoxicity of cisplatin, the main mechanism of the antitumor action of cisplatin. In fact, two main mechanisms of toxicity have been associated with cisplatin, namely, mitochondrial oxidative stress (Santos et al., 2007) and formation of adducts between cisplatin metabolites and DNA (Eastman, 1999, Hanigan and Devarajan, 2003). The main biochemical lesion induced by cisplatin in cancer cells is inhibition of DNA synthesis; however, the role of cisplatin DNA-binding in renal cytotoxicity is unknown (Launay-Vacher et al., 2008). Differences in the rate of cell division between healthy cells and tumor cells make them more susceptible to one or other mechanism (for revision see dos Santos et al., 2012). Proximal tubular cells are non-dividing and therefore less susceptible to DNA damage (Wainford et al., 2008), while the uncontrolled division rate of tumor cells make them more susceptible to the action of cisplatin on DNA (Hanigan and Devarajan, 2003). The genotoxicity of cisplatin was evaluated by assessing (i) the frequency of micronucleated polychromatic erythrocytes (MNPCE) in peripheral blood and (ii) the frequency of polychromatic erythrocytes (PCE) in bone marrow, both markers of the cytotoxicity of cisplatin in bone marrow. The crosslinks between the aquated metabolites of cisplatin and the purine bases of nuclear DNA impairs the process of replication and transcription leading to cell-cycle arrest (Wang and Lippard, 2005). This effect is detected by (i) decreased nuclear division of cells in bone marrow, i.e., decreased frequency of polychromatic erythrocytes (PCE), which are immature erythrocytes, and among them, (ii) increased frequency of micronuclei. The micronucleus test detects genotoxic damage in interphase cells and higher frequency of MNPCE occurs after treatment with anticancer drugs as compared to non-treated groups (Choudhury et al., 2000, Doherty,

2012). Our results showed increased frequency of micronuclei in both CV and CV+Cisp groups, with no significant difference between them, which shows that CV does not impair this effect of cisplatin. Reduction in the ratio of PCE to normochromatic erythrocytes (NCE) indicates inhibition of erythroblast proliferation or maturation, and destruction of nucleated cells (Krishna and Hayashi, 2000). We observed decreased frequency of PCE in both CV and CV+Cisp groups, with no significant difference between them. This finding is in line with the result of the micronuclei assay and indicates that CV does not interfere with the main antitumor mechanism of Cisp. Both findings support our previous finding that CV does not decrease the tumor remission induced by cisplatin in the same animal model (Carvalho Rodrigues et al., 2013).

Another concern is that chemoprotectants might interfere with the biodistribution of cisplatin. Sulfur-containing antioxidants, for instance, protects against the renal damage by forming inactive complexes with cisplatin that are eliminated impeding the accumulation of cisplatin in the target tissues and therefore, the antitumor action (Kröning et al., 2000). In fact, decreased accumulation of cisplatin in kidneys is a mechanism of nephroprotection but also implicates in decreased accumulation in tumors. We had previously demonstrated that CV does not alter the platinum concentration in renal mitochondria of tumor-free rats treated with cisplatin, which suggested that the mechanism of protection of CV is not related to decreased renal accumulation of cisplatin (Rodrigues et al., 2010). Here we evaluated the concentration of platinum in the tumor of Sarcoma-180-bearing mice treated with cisplatin. Platinum distribution was not affected by CV, confirming our previous results that showed that CV does not impair the remission of tumor induced by cisplatin

(Carvalho Rodrigues et al., 2013). We also evaluated the concentration of platinum in the kidneys of tumor-bearing mice and it was twice that observed in the tumor tissue, which shows the preferential accumulation and the extensive excretion of cisplatin by kidneys as reported before (Launay-Vacher et al., 2008). CV did not prevent cisplatin to accumulate in the kidneys but even so was able to protect against the renal damage. Altogether, our previous and present findings show that the mechanism of nephroprotection of CV certainly does not involve impaired biodistribution of cisplatin and does not involve decreased genotoxicity of cisplatin. These findings corroborate the importance of the antioxidant effect of CV in the nephroprotection and they are in line with the hypothesis that the main mechanism underlying the nephrotoxicity of cisplatin is oxidative stress while its main antitumor mechanism is genotoxicity. Data on the antioxidant mechanism of CV are controversial. Two different antioxidant mechanisms have been suggested: (i) scavenging of free radicals and/or (ii) chelation of free iron (Yue et al., 1992, Noguchi et al., 2000, Dandona et al., 2007). We assessed both mechanisms and observed that CV is not able to neutralize the stable free radicals DPPH and TEMPOL (Electron Paramagnetic Resonance, EPR). On the other hand, CV efficiently chelated free iron. The chelation of free iron inhibits the Fenton reaction and consequently the formation of hydroxyl radicals, a highly reactive oxygen species that cannot be detoxified by enzymatic reactions (Simůnek et al., 2009). Hydroxyl radicals play a key role in the nephrotoxicity of cisplatin (dos Santos et al., 2007, Santos et al., 2008); therefore, this mechanism supports the effectiveness of CV against the nephrotoxicity of cisplatin. Accordingly, a study showed that CV decreased the intensity of DMPO-OH signal, a spin-trapping

system to evaluate the neutralization of hydroxyl radical (Yue et al., 1992). Carvedilol is extensively metabolized by liver enzymes (Oldham and Clarke, 1997) and the metabolites formed by hydroxylation at some specific positions on the carbazole moiety have higher antioxidant activity than the parent drug (Yue et al., 1992, Schaefer et al., 1998). Nevertheless, our findings suggest that the unaltered drug might contribute to the antioxidant activity of carvedilol by its ability to chelate free iron, which confirms a previous study (Noguchi et al., 2000). Our findings also suggest that the antioxidant activity of CV metabolites might involve iron chelation, which should be further investigated.

In conclusion, this study demonstrates, for the first time, that CV protects against the renal damage induced by cisplatin in Sarcoma-180-bearing mice without affecting cisplatin accumulation in the tumor tissue or its genotoxic effect, both events associated with its antitumor mechanism. The antioxidant mechanism of CV is not associated with lower accumulation of cisplatin in kidneys; it might involve chelation of free iron and inhibition of the Fenton reaction that generates hydroxyl radicals, known to play a key role in the nephrotoxicity of cisplatin. Altogether, our studies provide consistent evidence of the beneficial effect of CV on animals treated with cisplatin and might encourage clinical trials.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Figures Legends

Figure 1. Effect of cisplatin and carvedilol on (A,B) renal function and (C) renal tissue morphology: (A) plasma creatinine, (B) BUN, blood urea nitrogen, (C) Representative haematoxylin and eosin (H&E) stained sections (×200 magnification) of tumor-bearing mice kidney. Black arrows indicate damaged proximal tubules and **G** indicates glomerulus**.** Data are expressed as mean ± SEM (n=6). *****Significant in relation to controls; # Significant in relation to the Cisp group. No significant difference was observed between CV and the control group for plasma urea and creatinine. CV, carvedilol; Cisp, cisplatin

Figure 2. Effect of carvedilol on the platinum concentration as a marker of the biodistribution of cisplatin in the (A) kidneys and (B) tumor. Data are expressed as mean ± SEM (n=6). *Significant in relation to controls; No significant difference (NS) was observed between CV and the control group or between Cisp and CV+Cisp groups. CV, carvedilol; Cisp, cisplatin

Figure 3. Effect of carvedilol on the genotoxicity of cisplatin. (A) and (B) Micronucleous Assay in peripheral blood: (A) photomicrographs of polychromatic erythrocytes (yellow arrows) and micronucleated polychromatic erythrocytes (yellow asterisk), stained with acridine-orange and under fluorescence microscopy (x1000); (B) Quantitative analysis of micronucleated polychromatic erythrocytes (MNPCE) per 1,000 polychromatic erythrocytes (PCE). **(C) and (D) Frequency of PCE in bone marrow:** (C) Polychromatic erythrocytes (blue arrows) and normochromatic erythrocytes (red arrows) evaluated in bone marrow smear stained with Giemsa, under optical microscopy (x1000); (D) Quantitative analysis of the frequency of PCE to total erythrocytes (PCE+NCE). Data are expressed as mean ± SEM (n=6). *Significantly different from the control group ($p < 0.05$); NS, non-significant.

Figure 4. Electro Paramagnetic Resonance (EPR) for (A) TEMPOL and (B) DPPH free radicals in the presence of vehicle (DMSO) or carvedilol (CV). CV was not able to neutralize TEMPOL (100 µM) even at a high concentration (5mM). For DPPH (500 µM), a slight decrease was observed in the electron signal only for 5mM CV. Data are representative of the mean of three independent assays, each assay performed in triplicates.

Figure 5. Effect of carvedilol on the concentration of ferrous ion. (A) Calibration curve of ferrous ion and (B) Chelation of ferrous ion by carvedilol. The concentration of ferrous ion in the solution decreases as it is chelated by increasing concentrations of carvedilol. No ferrous ion remained in solution after the addition of 200 µM carvedilol. The concentrations of CV are presented as the corresponding logarithms. EC50 was calculated (84.27 µM) using the equation: Y = 100 / (1 +10 ^ (LogEC50-X) * hillslope); were hillslope is -2,647. Data are representative of the mean of three independent assays, each assay was performed in triplicates.

Figure 1

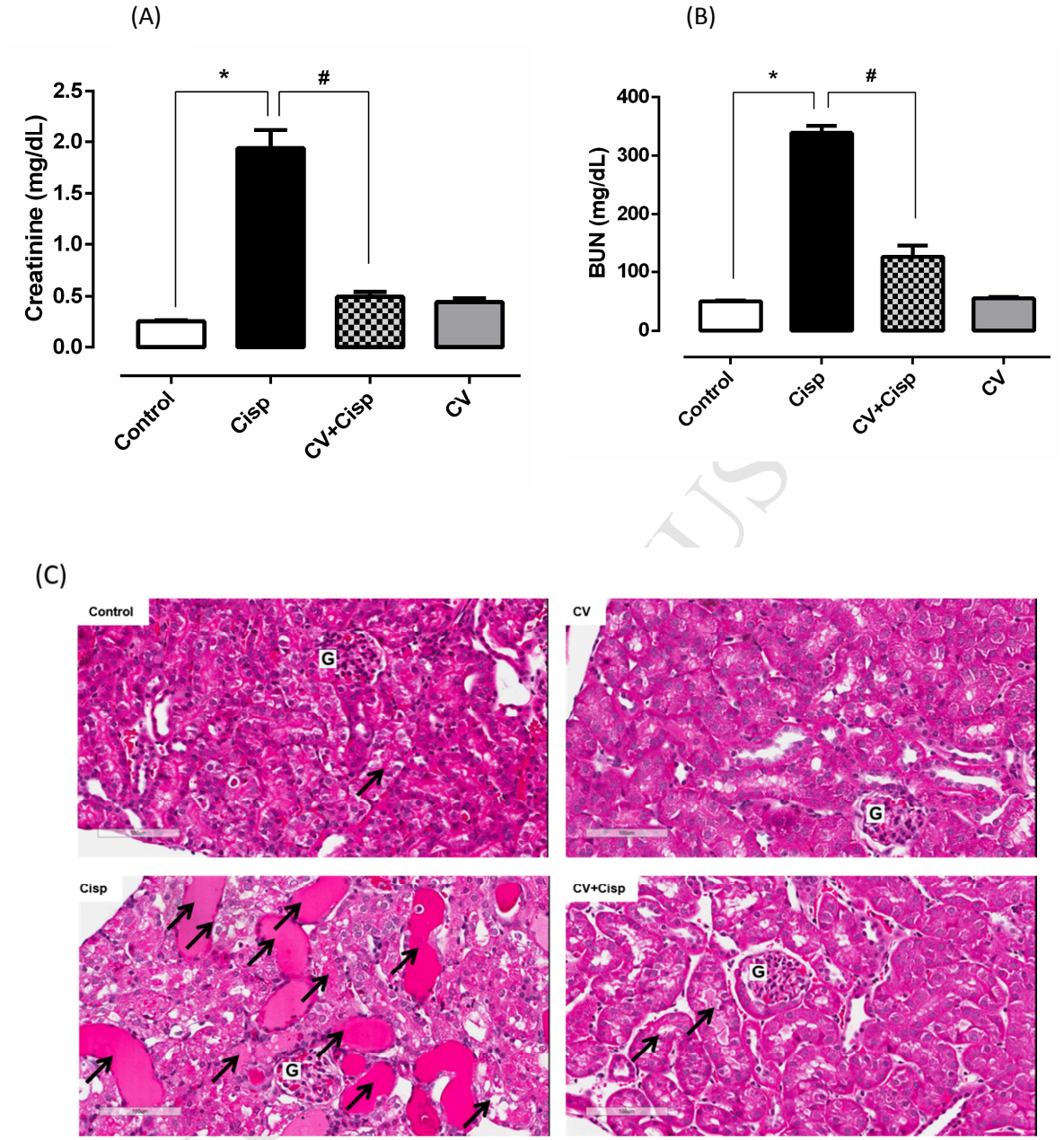

(A)
(B)
Creatinine (mg/dL)
2.5
2.0
1.5
1.0
0.5
0.0
*
#
Control
Cisp
CV+Cisp
CV
BUN (mg/dL)
400
300
200
100
0
(C)
Control
CV
Cisp
CV+Cisp
G

Figure 2

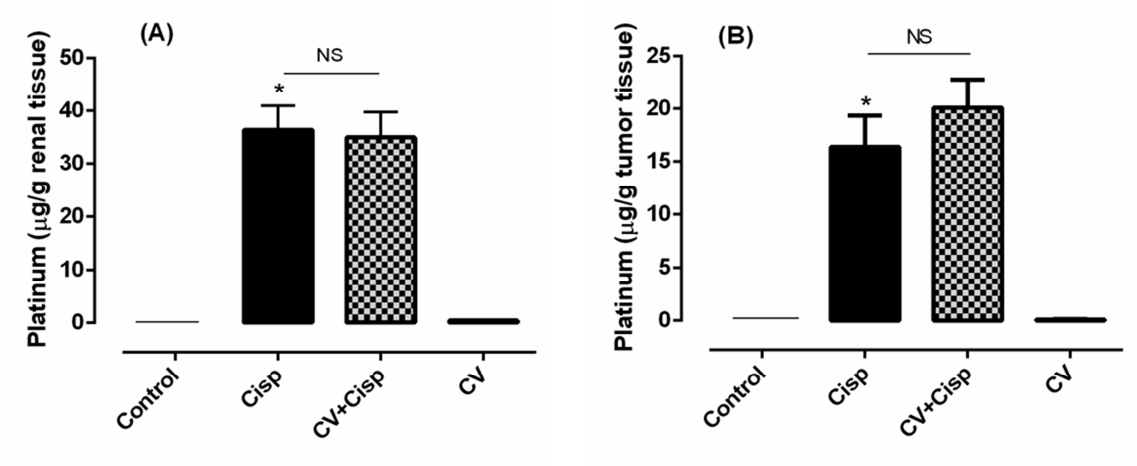


Figure 3

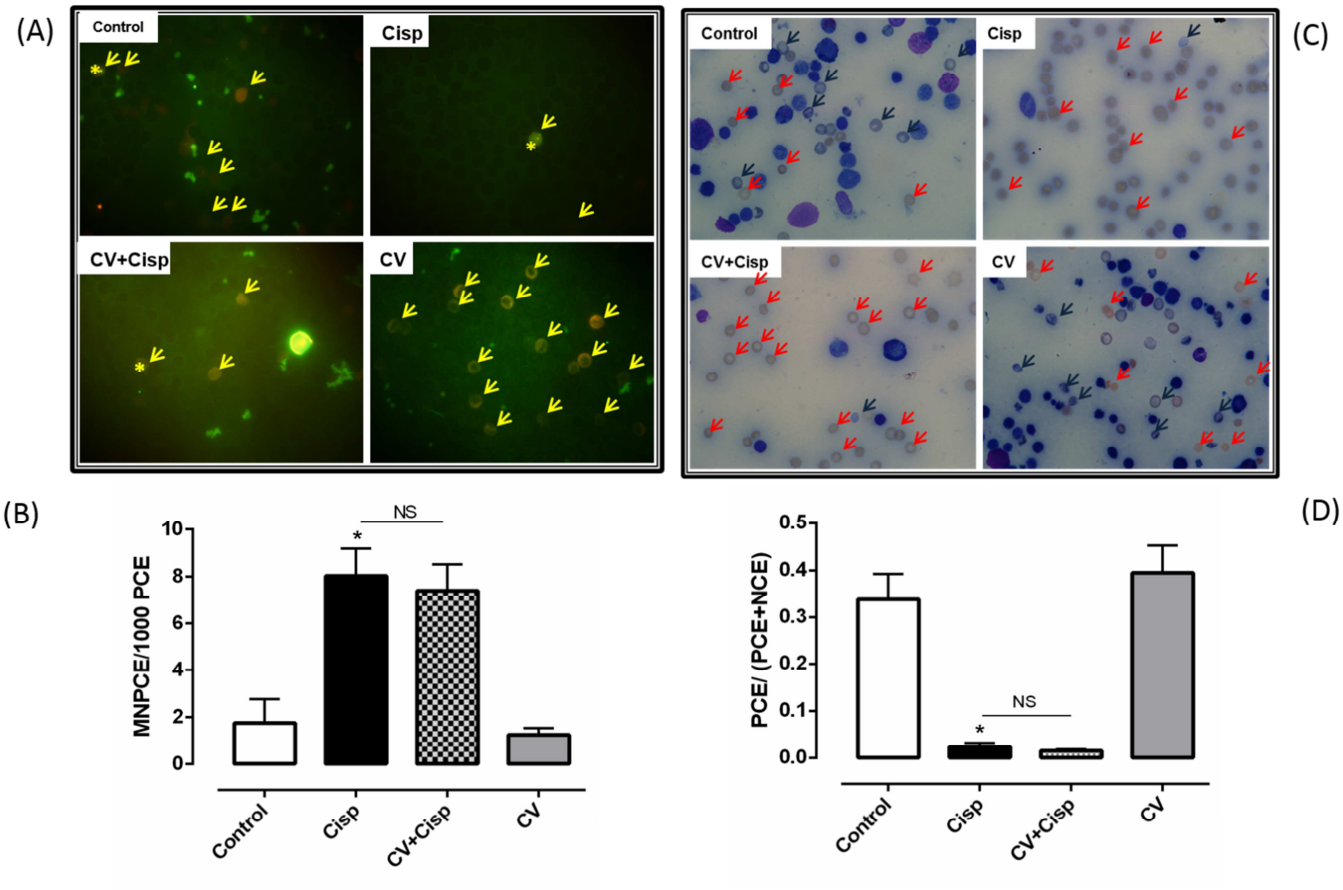


Figure 4

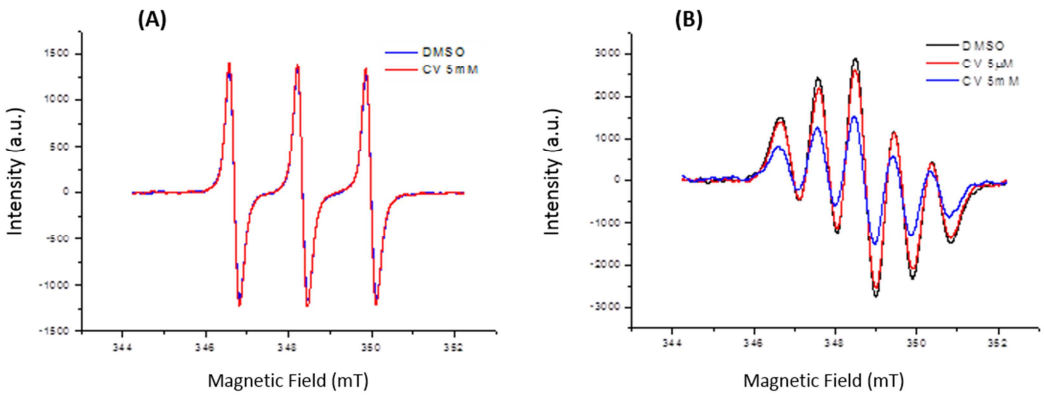
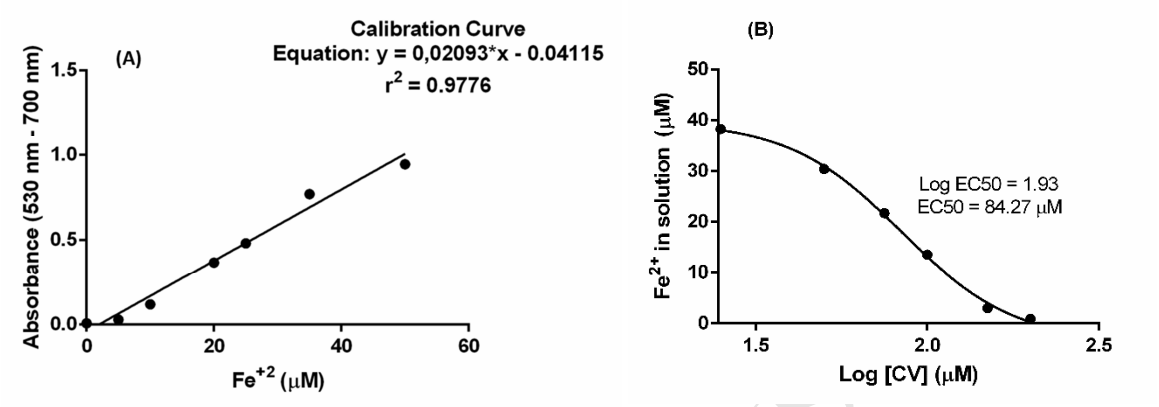


Figure 5



HIGHLIGHTS-R

- Carvedilol protects against the nephrotoxicity of cisplatin in tumor-bearing mice;
- Carvedilol does not affect the genotoxicity or biodistribution of cisplatin;
- Carvedilol is not an efficient scavenger of free radicals;
- Carvedilol efficiently chelates ferrous ion;
- The antioxidant mechanism might involve inhibition of the Fenton reaction.