Curcumin Acts as a Chemosensitizer for Leiomyosarcoma Cells In Vitro But Fails to Mediate Antioxidant Enzyme Activity in Cisplatin-Induced Experimental Nephrotoxicity in Rats

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Abstract

Background. Cisplatin (cis-diamminedichloroplatinum) is a widely used chemotherapeutic agent for the treatment of various cancers. Although it represents an effective regimen, its application is accompanied by side effects to normal tissues, especially to the kidneys. Cisplatin generates free radicals and impairs the function of antioxidant enzymes. Modulation of cisplatin-induced oxidative stress by specific antioxidant molecules represents an attractive approach to minimize side effects. *Methods.* We studied the ability of curcumin to sensitize leiomyosarcoma (LMS) cells to cisplatin. Assays for cell proliferation, mitochondrial function, induction of apoptosis, and cell cycle arrest were performed using various concentrations of cisplatin and a concentration of curcumin that caused a nonsignificant reduction in cell viability. Moreover, the effect of curcumin was examined against cisplatin-induced experimental nephrotoxicity. Renal injury was assessed by measuring serum creatinine, blood urea nitrogen (BUN), and the kidney's relative weight. Oxidative stress was measured by means of enzymatic activities of superoxide dismutase and glutathione peroxidase in the rats' blood and malondialdehyde levels in rats' urine. *Results.* In our study, we found that curcumin sensitizes LMS cells to cisplatin by enhancing apoptosis and impairing mitochondrial function. In an in vivo model of cisplatin-induced experimental nephrotoxicity, intraperitoneal administration of curcumin failed to preserve blood's antioxidant enzyme activity and decrease lipid peroxidation. Nevertheless, curcumin was able to protect nephrons' histology from cisplatin's toxic effect. *Conclusion.* Our results showed that curcumin can act as chemosensitizer, but its role as an adjunctive cisplatin-induced oxidative stress inhibitor requires further dose-finding studies to maximize the effectiveness of chemotherapy.

Keywords

curcumin, chemotherapy, nephrotoxicity, cytotoxicity, oxidative stress

Introduction

Chemoprevention is defined as "the use of drugs, vitamins, or other agents to try to reduce the risk of, or delay the development or recurrence of, cancer."[1] It constitutes an approach that hopes to identify new substances that can suppress the transformation of precancerous cell into cancerous ones[2] by affecting cell functionalities, including the

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cell cycle, the mechanism of apoptosis, signal transduction, oncogene activation, angiogenesis, and many others.[2,3]

Cisplatin is a first-generation platinum drug and one of the most effective chemotherapeutic agents used in the treatment of ovarian, testicular, and head and neck cancers.[4] The clinical success of cisplatin and its derivatives was a milestone for the development of numerous metal-based anticancer compounds.[5] However, its efficacy is hindered by the dose-related side effects to normal tissues[6] such as nephrotoxicity,[7] hepatotoxicity,[8] and cardiotoxicity.[9,10] The generation of free radicals and the induction of oxidative stress by the platinum complexes impair the antioxidant function of many enzymes and nonenzymatic molecules and contribute to the cisplatin-induced toxicity.[11] Cisplatin also interacts with the genetic material of the cells and forms covalent adducts with purine DNA bases. This interaction is the root cause for the cytotoxic effect of cisplatin.[12]

During the past 2 decades, a significant number of natural compounds has been identified that possess chemopreventive effects, and among them is curcumin (diferuloylmethane), which is the main component of the spice turmeric derived from the rhizome of the plant *Curcuma longa*, a member of the *Zingiberaceae* (ginger) family.[13] Curcumin is used as a traditional medicine in Asia and has been shown to play antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, and hepatoprotective roles.[14] It has been shown that curcumin acts as a chemopreventive agent through the induction of apoptosis and cell cycle arrest in many different cancer cells by affecting molecular targets such as upregulating Cdk inhibitors and p53 and downregulating cyclin D1, Cdk-1, cdc-2, and NF-kB.[3,15] Moreover, several studies focus on the effects of curcumin on oxidative stress. Indicatively, curcumin preserves mitochondrial functions and activates AKt-GSK3β-signaling (a regulator of cell survival and proliferation) in osteoblasts exposed to oxidative stress[16]; regulates intracellular Ca^{2+} levels, VEGF levels, PARP expression levels, and caspase-3 and -9 values in an experimental oxidative stress model in human retinal pigment epithelium (ARPE-19) cells[17]; and attenuates oxidative stress in diabetic rats by activating the Keap1-Nrf2-ARE signaling pathway.[18]

New approaches for improving the outcomes (maximizing the clinical response and minimizing toxicity) in cancer therapy are investigated. Toward this direction, the adjunctive role of natural compounds with chemotherapeutic drugs has attracted considerable interest.[19-21] We have previously shown that pretreatment of leiomyosarcoma (LMS) cells with epigallocatechin gallate (EGCG) sensitizes cells to cisplatin.[22] The aim of the present work was to evaluate the effects of curcumin on cisplatin cytotoxic activity in a series of in vitro and in vivo experiments to discover less toxic but equally effective methods to induce cancer cell death.

Materials and Methods

Cell Lines

LMS cells isolated from tumors histologically identified as LMS in Wistar rats[23] and human cell line MRC-5 (normal fetal lung fibroblasts, ATCC, CCL-171) were used. The cells were grown in Dulbecco's modified Eagle medium in monolayer cultures, supplemented with 10% fetal bovine serum, 0.5% L-glutamine (1% for MRC-5 cells), and 1% of penicillin-streptomycin solution) at 37°C in a 5% CO_2-95% air humidified incubator.

Cell Viability

LMS and MRC-5 cells were seeded in 96-well plates (at densities of 5×10^3 and 7×10^3 cells/well, respectively) for 24 hours before treatment with cisplatin or curcumin. The IC_{50} value of cisplatin or curcumin was then estimated by means of the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. The maximum concentration of curcumin that caused a nonsignificant reduction in LMS cell viability (after 24 and 48 hours) was further used in the following experiments as a reference value (curcumin reference value [CRV]).

LMS cells were then incubated for 24 hours with the CRV before medium removal and the addition of cisplatin with fresh medium. Cells were harvested after 48 hours; MTT was added, and cell viability was calculated using a microplate spectrophotometer (Multiskan Spectrum, Thermo Fisher Scientific, Waltham, MA). All experiments were performed in triplicate.

Quantification of Apoptosis

Induction of apoptosis was estimated with Annexin V-FITC and propidium iodide staining as previously described.[22] Briefly, LMS cells were seeded into 6-well plates at a density of 6×10^4 cells/well for 24 hours with or without addition of the CRV. The medium was removed, and the cells were then incubated additionally for 48 hours with fresh medium supplemented with the IC_{50} of cisplatin, estimated by means of the MTT assay. Vehicle controls with dimethyl sulfoxide and cisplatin were also used. Cells were analyzed on a fluorescence-activated cell sorting flow cytometer (Partec ML, Partec GmbH, Germany).

Cell Cycle Analysis

LMS cells were stained with 50 ng/mL propidium iodide and analyzed on a BD FacsCalibur Flow Cytometer while the data obtained were manipulated with the CellQuest v.3.1 software.[24]

Mitochondrial Viability Assay

The mitochondrial viability stain kit (Abcam, ab129732) was used to determine the metabolic capacity of live cells. LMS cells were seeded into 96-well plates at a density of 5×10^3 cells/well and incubated for 24 hours with the CRV before the addition of cisplatin. Oligomycin (3 μM) was selected as a positive control for the inhibition of the mitochondrial function. A microplate spectrophotometer (Multiskan Spectrum, Thermo Fisher Scientific, Waltham, MA) was used for the measurement of absorbance at 570 nm). All experiments were performed in triplicate.

Animals and Treatment

Experiments were performed on female Wistar rats (*Rattus norvegicus*), 20 weeks old, weighing approximately 200 ± 10 g. Animals were housed in metabolic cages, 1 animal per cage, at a constant temperature of 25°C, under a photoperiod of 12 hours of light and 12 hours of darkness. Rat chow (Viozois SA, Animal Feed Company of Epirus, Greece) and water were provided ad libitum.

Experiments were handled with human care and in accordance with the European Union directive for the care and use of laboratory animals (EEC Directive 2010/63/EU; University of Ioannina, 20EEP02).

The rats were randomly divided into 8 groups (5 animals per group): (1) the control group (CG), no treatment; (2) cisplatin group (CPG), intraperitoneal administration of cisplatin at a single dose of 5 mg/kg body weight (day 4); (3) curcumin groups (C4G, C8G, and C16G), intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively, with 2 single doses every 2 days (day 0 and day 2); and (4) curcumin prior to cisplatin groups (C4CPG, C8CPG, and C16CPG), intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively, with 2 single doses every 2 days (day 0 and day 2) before the injection of cisplatin (day 4). All animals were killed humanely on the 9th day from the beginning of the experiment (day 0). Autopsy was immediately performed, and the rats' kidneys were dissected and fixated in 10% buffered formalin. A histological evaluation was later performed by an experienced pathologist of the University Hospital of Ioannina.

Rats were left for acclimatization in the metabolic cages for 5 days before the start of the experiment. The amount of food as well as water consumption, urine output, and fecal weight were monitored daily. Blood and urine samples were collected at the end of the experimental period (day 9). Blood samples were centrifuged at 3500 rpm for 30 minutes to separate the plasma from the red blood cells. All samples were stored at −80°C until used.

Estimation of Endogenous Antioxidant Enzyme Activity

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity in the blood plasma of the rats was determined by means of spectrophotometry using the Biovision Superoxide Dismutase Activity Colorimetric Assay kit (K335-100) and the Glutathione Peroxidase Activity Colorimetric Assay kit (K762-100), respectively.

Assay of Lipid Peroxidation

Malondialdehyde (MDA) levels in urine were determined as previously described.[25]

Statistical Analysis

The statistically significant difference between data means was determined by the Student *t*-test, and 1-way analysis of variance (ANOVA) was used for statistical evaluation of differences between groups (SPSS version 20.0, Statistical Package for the Social Sciences software, SPSS, Chicago, IL). *P* values of <.05 were considered as significant.

Results

Effect of Curcumin and Cisplatin on Cell Viability

LMS and MRC-5 cells were incubated for 24 and 48 hours with increasing concentrations (1.25-80 μM) of curcumin or cisplatin (Figures 1A-1D). Both curcumin and cisplatin caused a concentration- and time-dependent inhibition of cell viability. The IC_{50} value of curcumin and cisplatin against LMS cells, after 48 hours of incubation, was calculated at 35.3 ± 5.5 and 8.6 ± 1.1 μM, respectively, whereas for the MRC-5 cells they were 47.2 ± 5.1 and >80 μM. The CRV for the LMS cells was estimated at 7.5 μM, which was the same for both 24- and 48-hour exposure of cells to curcumin.

Preincubation of LMS cells with the CRV dramatically reduced the IC_{50} of cisplatin to 2.5 ± 0.8 μM. This corresponded to a more than a 3-fold reduction in the dose required to achieve a similar cytotoxic effect and in the death of almost 80% of the cell population (vs 50% with cisplatin; Figure 1E). Preincubation of the MRC-5 cells with the CRV had no effect against the cytotoxic activity of cisplatin (Figure 1F).

Apoptosis

Cisplatin (IC_{50} value) alone induced apoptosis in LMS cells, whereas CRV itself had no effect. Preincubation of cells
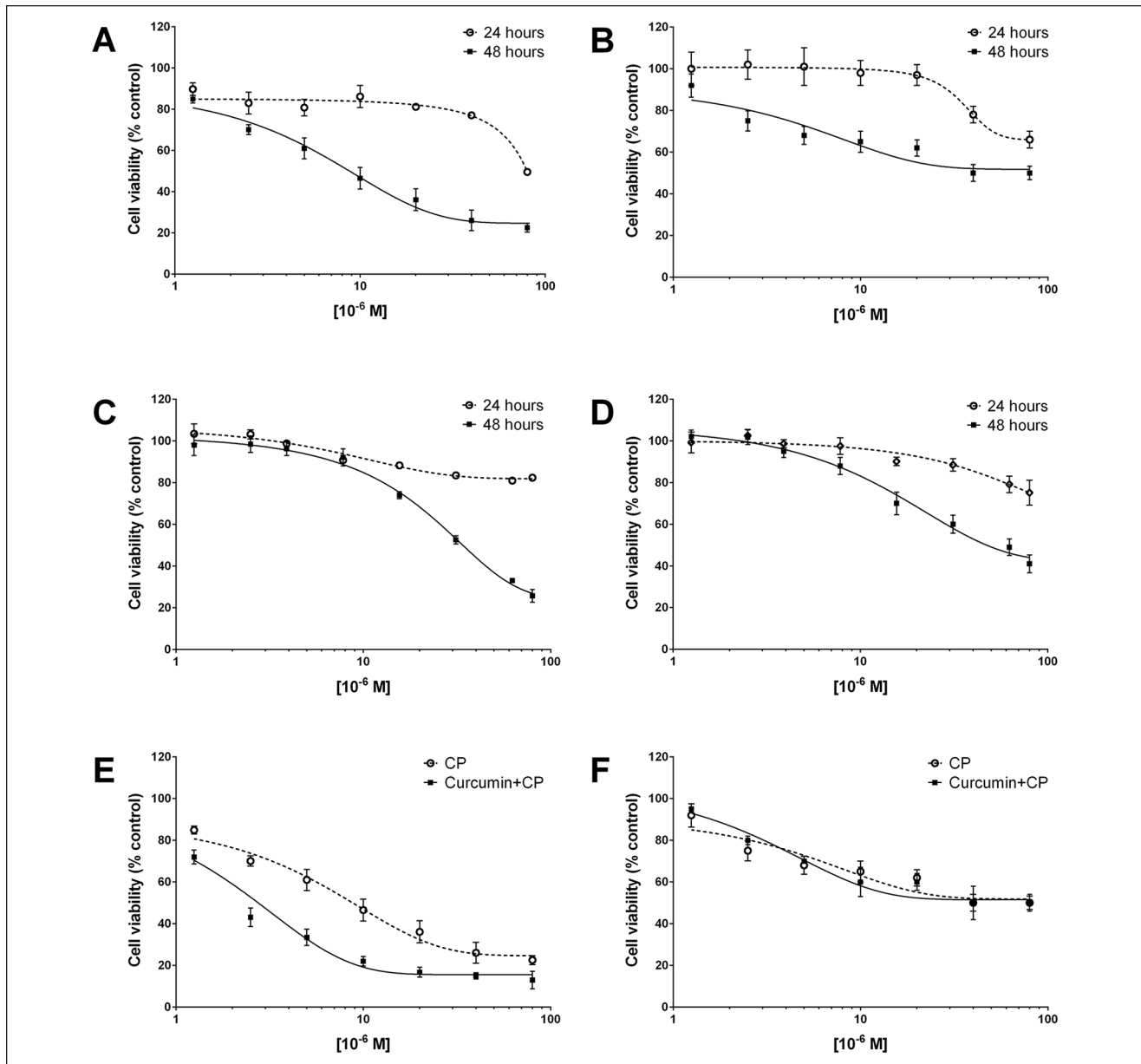
Figure 1. Cell viability of LMS treated with CP (A) or curcumin (C) in various concentrations for 24 and 48 hours: Cell viability of MRC-5 cells treated with CP (B) or curcumin (D) in various concentrations for 24 and 48 hours. Cell viability of LMS cells (E) and MRC-5 cells (F) preincubated with CRV (7.5 μM) for 24 hours and treated with CP for 48 hours. The power curve fit model was used for curve fitting (GraphPad Prism 6 Software).
Abbreviations: LMS, leiomyosarcoma; CP, cisplatin; CRV, curcumin reference value.

with the CRV showed an increase of approximately 16% in apoptotic cells compared with treatment with cisplatin alone (Figure 2A). Representative flow cytometry images for apoptosis are shown in Figure 2C (1-4).

Cell Cycle

Cell cycle analysis showed an accumulation of LMS cells in S-phase after treatment with cisplatin (IC_{50} value),

indicating that tumor cells had entered S-phase cell cycle arrest. Pretreatment with CRV reduced the number of cells in S-phase from $69.8\% \pm 3.1\%$ to $63.0\% \pm 2.1\%$. Moreover, the percentage of cells in G2/M phase decreased from $18.3\% \pm 0.2\%$ to $11.4\% \pm 1.8\%$. The treatment of cells only with CRV did not show any difference compared with control (Figure 2B). Representative flow cytometry images for cell cycle analysis are shown in Figure 2D (1-4).
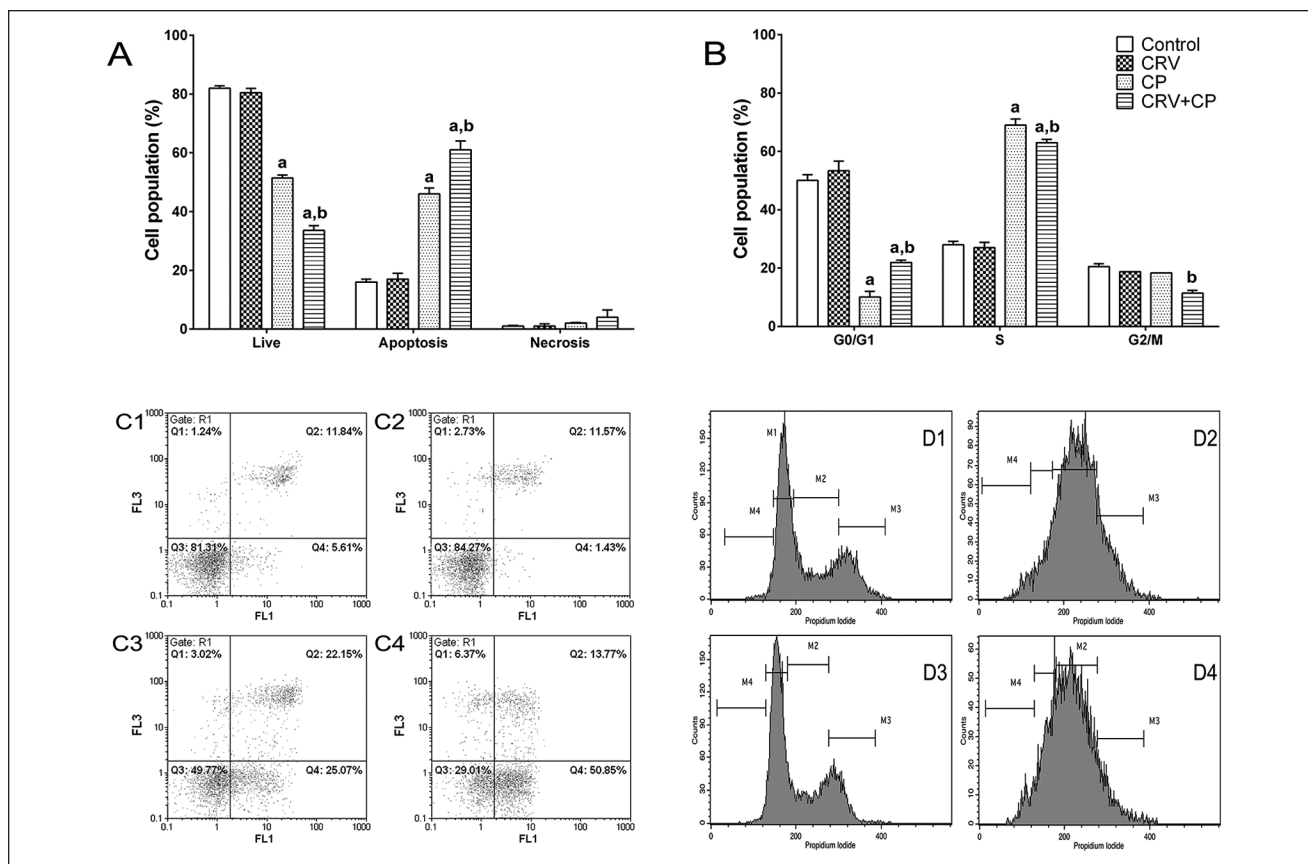
Figure 2. Quantification of the apoptotic effects (A) and cell-cycle analysis (B) of cisplatin (IC$_{50}$), curcumin (CRV, 7.5 μM), and CRV with cisplatin in LMS cells. Representative flow cytometry images for apoptosis (C) and cell cycle analysis (D). C1 and D1, control; C2 and D2, 7.5 μM curcumin-CRV; C3 and D3, cisplatin-IC$_{50}$; C4 and D4, LMS cells preincubated with CRV (7.5 μM) for 24 hours and treated with cisplatin for 48 hours). Data are expressed as mean ± SD.
Abbreviations: LMS, leiomyosarcoma; CP, cisplatin; CRV, curcumin reference value.
[a] Significantly different from control, *P* < .05.
[b] Significantly different from cisplatin, *P* < .05.

Mitochondrial Viability Stain

The results from the mitochondrial viability stain kit showed that treatment of LMS cells with the IC$_{50}$ of cisplatin resulted in a significant damage (more than 50%) in the mitochondria of the cells similar to that caused by oligomycin. Cells treated only with the CRV did not show inhibition of mitochondrial function. The preincubation of cells with CRV increased the damage caused to the mitochondrial function by cisplatin (Figure 3).

In Vivo Results

Effect of the Treatment on Enzymatic Antioxidant Activities. Cisplatin administration impaired the normal activity of the antioxidant enzymes SOD and GPx. Specifically, the dose of 5 mg/mL cisplatin decreased the SOD activity by 30% (Figure 4A) and the GPx activity by 70% (Figure 4B). Pretreatment with curcumin failed to restore the normal

activity of the enzymes, although a slight improvement was recorded.

Effect of Cisplatin and Curcumin on MDA Levels. The concentration of MDA in the rats' urine was used as a marker of oxidative stress. No effect on lipid peroxidation was caused by the intraperitoneal injection of curcumin (4, 8, and 16 mg/kg). In contrast, cisplatin produced a significant increase in the MDA levels (1.8-fold increase) compared with the CG, which was further elevated by curcumin pretreatment (Figure 4C).

Effects on Renal Function. Intraperitoneal administration of curcumin alone increased blood urea nitrogen (BUN) in a dose-dependent manner that was significantly higher in C8G and C16G than the CG (*P* < .05). As a result, pretreatment with curcumin failed to reduce the increase of BUN caused by cisplatin (Figure 5A). On the other hand, rats that
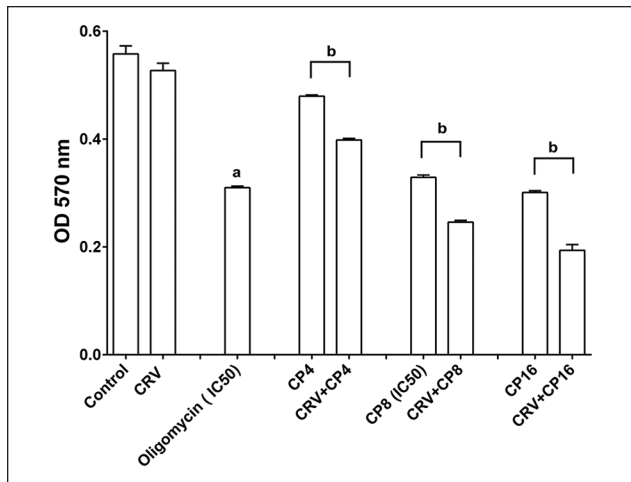
Figure 3. Mitochondrial viability after treatment of LMS cells with CP (IC_{50}), CRV (7.5 μM), and CRV with increasing doses of CP. Data are expressed as mean ± SD.
Abbreviations: LMS, leiomyosarcoma; CP, cisplatin; CRV, curcumin reference value.
[a] Significantly different from control, $P < .05$.
[b] Significantly different from cisplatin, $P < .05$.

were pretreated with curcumin had a significant reduction in creatinine levels compared with those treated with cisplatin ($P < .05$; Figure 5B). Finally, although some changes were observed in the relative kidney weight, no significant differences were recorded (Figure 5C). Nevertheless, these results indicated that cisplatin tends to increase the relative weight of the rat's kidney, and curcumin pretreatment could potentially restore normal kidney weight.

Nutritional and Metabolic Markers. Cisplatin significantly decreased food intake, a reduction that was further increased by the previous intake of curcumin at the highest doses (8 and 16 mg/kg body weight). Curcumin alone had no effect on food intake, but a trend to reduce the fecal weight was seen in all curcumin-supplemented groups (Figure 6A). Measurement of water intake and urine excretion indicated that curcumin could act as a diuretic because the volume of urine was significantly increased for C8G and C16G. Cisplatin alone led to a small increase in water intake (~5.0 mL) but also to a massive increase in urine excretion. Only rats in C4CPG managed to reduce their urine volume, whereas C8CPG and C16CPG groups maintained their high urine excretion (Figure 6B).

Renal Morphology. The histopathological changes in rats' kidneys are presented in Figure 7. No morphological changes were seen in the CG. On the other hand, cisplatin (5 mg/kg body weight) caused marked histological changes and specifically 90% vacuolar degeneration in the proximal tubular epithelium in rats' kidneys. Pretreatment with

curcumin reduced vacuolar degeneration to 40%, whereas curcumin alone had a very mild effect.

Discussion

Cisplatin is an anticancer and chemotherapeutic drug widely used for the treatment of neoplasms.[26] Since its introduction in cancer treatment more than 40 years ago, it has been characterized not only as an effective cancer regimen but also as a toxic agent that can cause damage to healthy tissues and especially to the kidneys.[27]

To overcome this drawback, various measures have been exploited to maximize cisplatin's cytotoxic activity against cancer cells and to minimize the side effects. The use of phytochemical compounds with cytotoxic properties as an adjunctive treatment for cancer represents an area of great scientific interest.[28] In this direction, we have recently shown that pretreatment with EGCG sensitizes LMS cells to cisplatin by enhancing the drug's apoptotic effect without modifying the S-phase cell cycle arrest.[22]

Curcumin is a compound best known for its antioxidant and anti-inflammatory properties,[29] but also possesses a broad spectrum of biological activities, such as high affinity for interaction with biomacromolecules and antivirus, anti-angiogenic, anti-HIV-1, and anticancer properties.[30] Platinum drugs act by causing cell-cycle arrest and apoptosis.[31] As an adjuvant chemotherapy treatment, curcumin has been shown to exert a synergistic activity with cisplatin against hepatic cancer cells[32] and oxaliplatin against colorectal cells.[33] In detail, according to the former study, curcumin with cisplatin exerts an additive effect in the reduction expression of different genes, including c-myc, BCl-X(L), c-IAP-2, NAIP and XIAP,[32] whereas the latter study showed that the combination of oxaliplatin with curcumin induces a 16-fold increase in p53 protein expression in HCT116 cells.[33] In accordance with Howells et al,[33] our results showed that curcumin seems to exert its effects in LMS cells via induction of apoptosis and not by cell-cycle inhibition. In contrast to our results, Montopoli et al[34] demonstrated that combined treatment with curcumin and cisplatin or oxaliplatin in ovarian cancer cells increases cell-cycle arrest and apoptosis.

Mitochondria are a key target for cisplatin, where its destructive activity leads to the activation of programmed cell death pathways. Cisplatin accumulates in the mitochondria of the cells[35,36] probably because of the affinity of positively charged complexes of cisplatin to the negatively charged mitochondrial matrix.[37] Kruidering et al[38] showed that cisplatin induces mitochondrial dysfunction by inhibiting complexes I to IV of the respiratory chain, which results in the depletion of intracellular ATP. In our study, cisplatin caused mitochondrial damage in LMS cells, and pretreatment with curcumin at all doses magnified the drug's
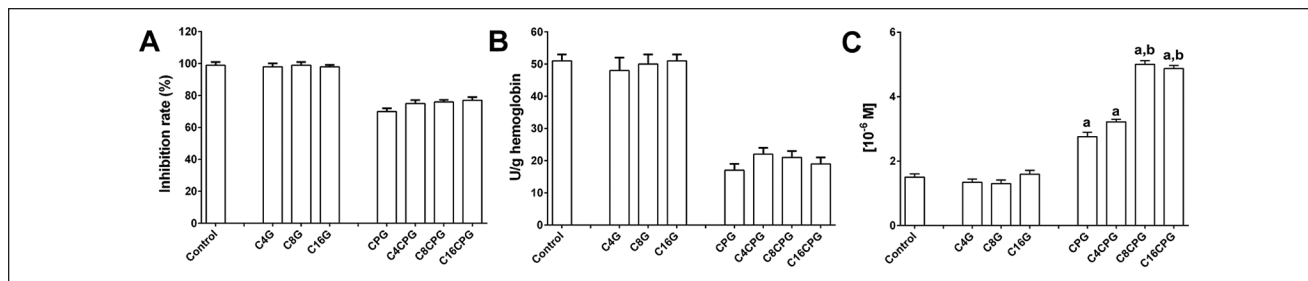
Figure 4. Effects of curcumin (4, 8, and 16 mg/kg body weight) on (A) SOD activity, (B) GPx activity, and (C) malonaldehyde urine concentration in cisplatin (5 mg/kg body weight) treated rats. Enzyme activity for GPx is expressed as units per gram hemoglobin and for SOD as percentage inhibition rate. Data are expressed as mean ± SD. Control group (CG): no treatment; cisplatin group (CPG): intraperitoneal administration of cisplatin at a single dose of 5 mg/kg body weight (day 4); curcumin groups (C4G, C8G, and C16G): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2); curcumin prior to cisplatin groups (C4CPG, C8CPG, and C16CPG): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2) before the injection of cisplatin (day 4).
Abbreviations: GPx, glutathione peroxidase; SOD, superoxide dismutase.
[a] Significantly different from control, $P < .05$.
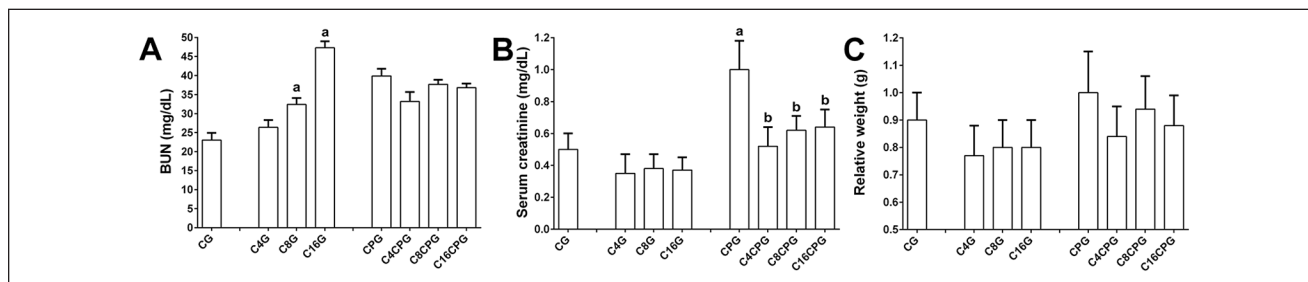[b] Significantly different from cisplatin, $P < .05$.

Figure 5. Effects of curcumin (4, 8, and 16 mg/kg body weight) on (A) BUN, (B) creatinine, and (C) kidney relative weight in cisplatin (5 mg/kg body weight) treated rats. Data are expressed as mean ± SD. Control group (CG): no treatment; cisplatin group (CPG): intraperitoneal administration of cisplatin at a single dose of 5 mg/kg body weight (day 4); curcumin groups (C4G, C8G, and C16G): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2); curcumin prior to cisplatin groups (C4CPG, C8CPG, and C16CPG): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2) before the injection of cisplatin (day 4).
Abbreviation: BUN, blood urea nitrogen.
[a] Significantly different from control, $P < .05$.
[b] Significantly different from cisplatin, $P < .05$.

detrimental activity. According to Kuhar et al,[39] priming Hep-2 cells with curcumin enhances the efficacy of cisplatin by inducing apoptosis (7.1% increase). This adjuvant effect of curcumin was attributed to the increase in Bax and nuclear AIF and a decrease in Bcl-XL, hence through the mitochondrial pathway.

Marullo et al[40] showed that generation of reactive oxygen species (ROS) causes cell death in response to cisplatin-induced mitochondrial damage. Proteins mediating cell death and survival, such as Nf-kB, are regulated by ROS.[41] Moreover, intracellular generation of ROS by cisplatin activates AMPK (AMP-activated protein kinase),[42] which potentially promotes apoptotic cell death.[43] Recently, it was shown that AMPK activation as a result of oxidative stress

induces apoptotic cell death in the neurons of the inner ear.[44] The kidney, ear, and nerves are common targets for the development of cisplatin-related toxicity.[45] Thus, the AMPK-mediated proapoptotic pathway may be responsible for the toxicity seen as a side effect of cisplatin therapy.

MDA levels in urine are a common biomarker of lipid peroxidation because of oxidative damage of the cell's membranes. According to the literature, cisplatin causes kidney lipid peroxidation through the production of ROS.[46] Indeed, cisplatin (5 mg/kg) significantly increased MDA levels in rats' urine and, thus, lipid peroxidation. Even though administration of curcumin alone had no effect, pretreatment at higher doses steeply increased lipid peroxidation. Deterioration of lipids in cell membranes through the
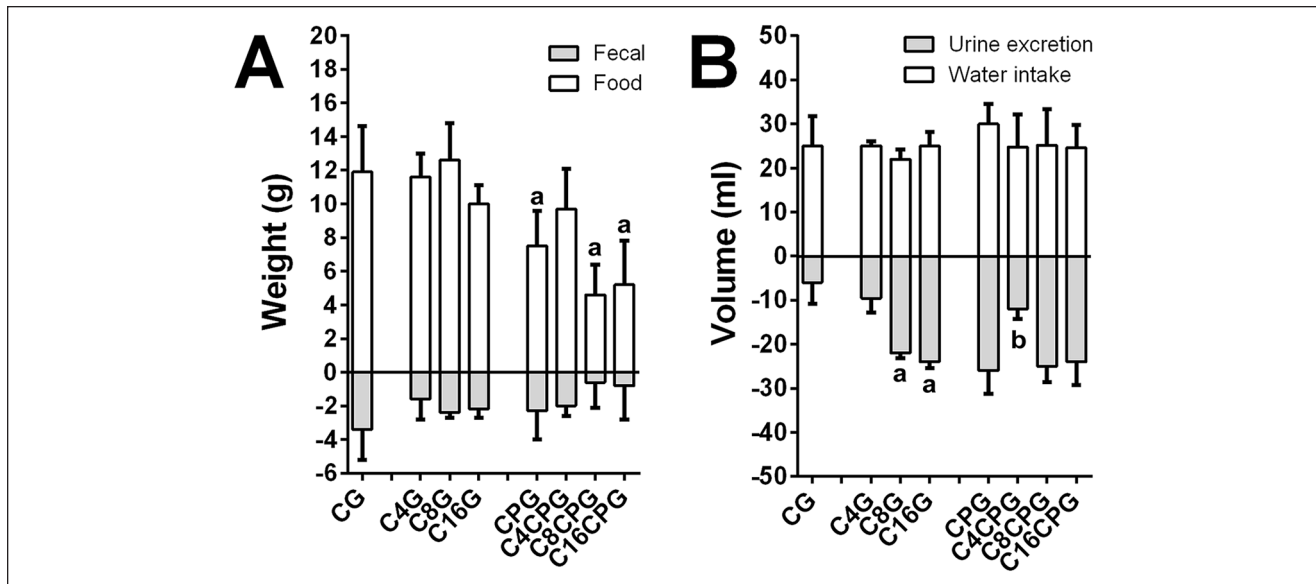
Figure 6. Effects of curcumin (4, 8, and 16 mg/kg body weight) on (A) food consumption and fecal weight and (B) water intake and urine output in cisplatin (5 mg/kg body weight) treated rats. Data are expressed as mean ± SD. Control group (CG): no treatment; cisplatin group (CPG): intraperitoneal administration of cisplatin at a single dose of 5 mg/kg body weight (day 4); curcumin groups (C4G, C8G, and C16G): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2); curcumin prior to cisplatin groups (C4CPG, C8CPG, and C16CPG): intraperitoneal administration of curcumin of 4, 8, and 16 mg/kg body weight, respectively—2 single doses every 2 days (day 0 and day 2) before the injection of cisplatin (day 4).

[a] Significantly different from control, $P < .05$.
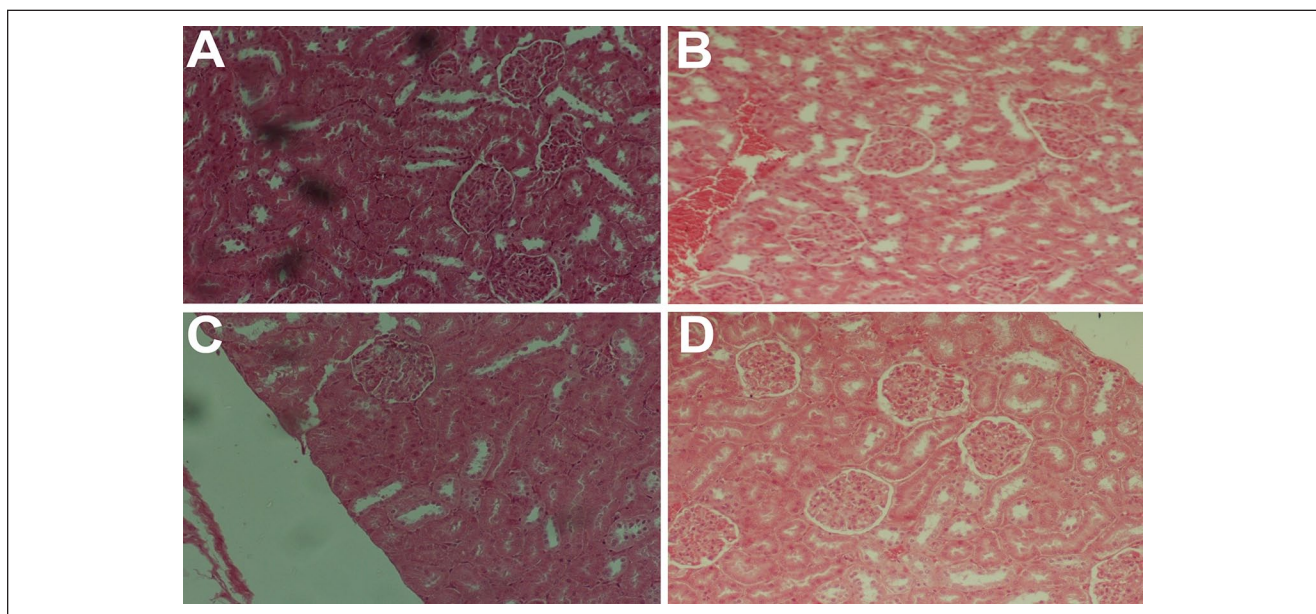[b] Significantly different from cisplatin, $P < .05$.

Figure 7. Representative images from the CG, healthy kidney (A); CP, intraperitoneal administration of cisplatin at a single dose of 5 mg/kg body weight (B); intraperitoneal administration of curcumin of 16 mg/kg body weight—2 single doses every 2 days (C); and intraperitoneal administration of curcumin of 16 mg/kg body weight—2 single doses every 2 days (day 0 and day 2) before the injection of cisplatin (day 4) (D). Tissue sections were stained with hematoxylin-eosin and magnified by 200.

production of ROS by cisplatin can also be justified by the severely affected activity of the first-line defense antioxidants, SOD and GPx. Pretreatment with curcumin failed to restore the normal activity of both these enzymes. A rather small statistically significant increase was noted, but this upregulation had no physiological impact. Previous studies that administered curcumin before (2 days) and after (3 days) cisplatin treatment (5 mg/kg) at higher doses (15 to 60 mg/kg vs 4 to 16 mg/kg in our study) concluded that curcumin treatment enhances levels of SOD and reduces lipid peroxidation and, thus, acts protectively on cisplatin-induced experimental nephrotoxicity.[47] A closer comparison of Kuhad et al[47] and our results revealed striking differences in the toxicity induced by cisplatin. In brief, BUN levels increased 2-fold in our study after cisplatin administration versus 7-fold reported by Kuhad et al; there was a 2-fold increase in serum creatinine versus 17-fold increase, and a 1.5-fold increase in MDA levels versus 3-fold increase. We can speculate that restoration of renal function in the Kuhad et al study was achieved as a result of the effects of postcisplatin administration of curcumin. Pretreatment with combined curcumin (200 mg/kg body weight) and a-tocopherol (250 mg/kg body weight) exerts a hepatoprotective effect against cisplatin (20 mg/kg body weight) induced oxidative stress by eliciting an increase in SOD and catalase activity of liver cells.[48] It was reported that at the tissue level, cisplatin (6 mg/kg body weight) causes elevation (brain and liver) of GPx and SOD levels.[49] Here, we report that cisplatin at a single dose diminished GPx activity in rats' plasma and elevated MDA levels. This is in accordance with the results of Ilbey et al.[50] According to the latter, oral intake of curcumin (200 mg/kg) for 10 days after intraperitoneal administration of cisplatin restores GPx activity and abolishes MDA levels.

Our team has previously reported that intraperitoneal administration of catechins and EGCG mediates the activity of SOD and GPx in Wistar rats.[25,51] Specifically, these substances when administered intraperitoneally to Wistar rats reduce the activity of both antioxidant enzymes. One possible explanation for this observation is the shielding of glutathione, which is the GPx substrate, against free radicals by crude catechin extract.[52] In the present study, curcumin dosage regimen failed to regulate SOD and GPx activity. Thus, we hypothesize that curcumin exerts its biological activity in different components of the antioxidant system. A candidate target for curcumin could be heme-oxygenase-1 (HO-1), which is expressed in various cells (spleen, liver, kidneys). Induction of HO-1 represents an efficient antioxidant system and operates as a fundamental defensive mechanism against oxidative stress.[53] Curcumin induces HO-1 expression in various cell types, including astrocytes,[54] endothelial cells,[55] hepatocytes,[56] and others, possibly through the generation of ROS, activation of Nrf2 and p38, and inhibition of phosphatase activity.56

Regarding the kidney damage, the biochemical indices, including BUN and creatinine concentration as well as relative weight of the kidneys, demonstrate that cisplatin severely impaired renal function. Curcumin alone had a dose-dependent effect on BUN increase. Pretreatment with curcumin, however, successfully reduced creatinine levels. Rats injected with the highest amounts of curcumin (8 and 16 mg/kg body weight) excreted significantly larger volumes of urine, possibly an indication that curcumin acts as a diuretic. Higher doses of curcumin (16 mg/kg body weight) offered some protection against cisplatin nephrotoxicity as seen in the histopathology.

Cisplatin-induced oxidative stress is the critical component for cisplatin cytotoxicity that not only affects cancer cells but also impairs the functionality of the healthy tissues. Nonetheless, cisplatin remains a valuable chemotherapeutic agent used to treat a broad spectrum of malignancies; therefore, any new approach that can maximize treatment effects and minimize side effects should be welcomed. Curcumin could be a potential chemosensitizer, but according to our results, further in-depth research is required to fine-tune its application as an adjunctive cisplatin-induced oxidative stress inhibitor and maximize effectiveness of chemotherapy.

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