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Curcumin reduces cisplatin-induced neurotoxicity in NGF-differentiated PC12 cells

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ABSTRACT

The potential neuroprotective benefits of curcumin against cisplatin neurotoxicity were investigated. Curcumin is a polyphenol derived from the rhizome of *Curcuma longa* whose pharmacological effects include antioxidant, anti-inflammatory and anti-cancer properties. Cisplatin is a potent chemotherapeutic drug with activity against a wide variety of tumors, although it has notorious side effects. Cisplatin neurotoxicity is clinically evident in patients that have undergone a full course of chemotherapy and develop a peripheral neuropathy that may affect the treatment regimen and the patient's qualify of life. In this study, we examined whether curcumin can protect against cisplatin neurite outgrowth inhibition in PC12 cells, which is an indicator of the protective potential against neuropathy. We also investigated whether curcumin affects cisplatin effectiveness by analyzing the modulation of *p53* gene expression and its effect on cisplatin cytotoxicity in HepG2 tumor cells. Non-cytotoxic concentrations of curcumin reduced *in vitro* neurotoxicity of cisplatin in PC12 cells. The treatment of PC12 cells with cisplatin (10 μg/mL) significantly reduced neurite outgrowth. The tested concentration of curcumin (1.0 and 10 μg/mL) did not result in neurite toxicity but nevertheless diminished cisplatin-induced inhibition of neurite outgrowth by up to 50% ($p < 0.05$). Our results indicate that curcumin does not compromise cisplatin's anticancer activity. Curcumin neither suppressed *p53* mRNA transcription nor protected tumor cells against cisplatin cytotoxicity. These results indicate that curcumin may reduce cisplatin-induced neurotoxicity, and clinical studies should potentially be considered.

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1. Introduction

Curcumin (diferuloylmethane) is a yellow pigment derived from the rhizome of *Curcuma longa*. Turmeric, the dried ground rhizome of *C. longa*, is a spice employed as a flavoring and coloring agent in many food preparations. Additionally, turmeric has been used for thousands of years in Ayurvedic and Chinese medicine. Curcumin is a polyphenol that is recognized as the main biologically active compound in turmeric and is responsible for its medical benefits (Goel et al., 2008; Sharma et al., 2005).

The safety of curcumin has been widely demonstrated, and curcumin has a variety of potential pharmaceutical applications. Curcumin exhibits antioxidant, anti-inflammatory and anticancer properties and has been described as neuroprotective against neurological disorders (Aggarwal and Harikumar, 2009; Maheshwari et al., 2006). Curcumin's antioxidant capacity is similar to other potent antioxidants, such as trolox (a vitamin E analog) (Somparn et al., 2007). Curcumin inhibits lipid peroxidation in different tissues, regulates intracellular levels of antioxidant enzymes (*e.g.*, catalase, glutathione peroxidase and superoxide dismutase) and is an effective intracellular reactive oxygen species (ROS) scavenger (Barzegar and Moosavi-Movahedi, 2011; Soobrattee et al., 2005; Sreejayan and Rao, 1994).

Many of curcumin's pharmacological benefits arise from its antioxidant or anti-inflammatory properties. Curcumin appears to have significant potential for treatment of diseases that result from oxidative stress (Aggarwal and Harikumar, 2009). Reports have shown that curcumin possesses protective effects in different neuronal cell lines and tissues. Curcumin has neuroprotective actions in Alzheimer's disease, multiple sclerosis, Parkinson's disease, epilepsy, and other neurodegenerative disorders that may be related to oxidative stress (Kulkarni and Dhir, 2010; Wang et al., 2010; Xie et al., 2011).

Chemotherapy-induced peripheral neuropathy is a common and incapacitating adverse effect of chemotherapy that may be caused by oxidative stress. Many chemotherapeutic agents,

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including platinum-based anticancer drugs, such as cisplatin, can produce this adverse effect (Jaggi and Singh, 2012). Cisplatin has been widely used in chemotherapy for nearly 40 years; however, its effectiveness may be limited due to adverse effects, such as peripheral neuropathy (Kelland, 2007; Pace et al., 2010). Patients completing a full course of platinum-based anticancer drugs often develop clinically detectable sensory peripheral neuropathies. Peripheral neuropathy results from cisplatin neurotoxicity, which can be severe and significantly affect the patient's quality of life (Brouwers et al., 2009; Siegal and Haim, 1990).

Cisplatin's antitumor mode of action is mediated by its direct interaction with DNA; cisplatin forms adducts, which activate several signal transduction pathways, such as those involving the p53 protein, that culminate in the activation of apoptosis in rapidly dividing tumor cells (Ma et al., 2008; Siddik, 2003). However, the exact mechanism of cisplatin neurotoxicity in post-mitotic neurons is not completely understood, although it seems to involve axonal integrity loss in sensory neurons (James et al., 2010; Ozturk et al., 2004). The post-mitotic sensory neurons of the dorsal root ganglia (DRG) are particularly susceptible to cisplatin damage. Studies in DRG neurons have produced evidence that axonal injury involves multiple events that include ROS generation, suggesting that antioxidants might be useful in reducing cisplatin neurotoxicity (Carozzi et al., 2010; McDonald et al., 2005).

It is not known whether the neuroprotective effects of curcumin could be extended to cisplatin-induced neurotoxicity. To investigate this question, the neuroprotective effects of curcumin were evaluated in an *in vitro* model using PC12 cells. This cell line was developed from an adrenal gland pheochromocytoma. PC12 cell is responsive to nerve growth factor (NGF), which causes differentiation into sympathetic-like neurons (Tischler and Greene, 1978). Differentiated PC12 cells are useful for neurotoxicity studies once they have a number of neuronal characteristics. PC12 cell differentiation includes development of synaptic-like vesicles, increased electrical excitability, cessation of mitosis and the development of neurite extensions (Green, 1995).

We evaluated the potential of curcumin to reduce cisplatin neurotoxicity using a neurite outgrowth assay that has been proposed for *in vitro* investigations of chemotherapy-induced peripheral neuropathy; in this assay, cisplatin causes a dose-dependent reduction of NGF-dependent neurite formation (Geldof et al., 1998; Klein et al., 2007; Takeshita et al., 2011; Verstappen et al., 1999, 2004). Cisplatin has been used widely as a chemotherapeutic drug for a variety of malignancies including hepatocellular carcinoma (Zhang et al., 2001). Thus, we also investigated whether curcumin affects cisplatin antitumor effectiveness by analyzing the effect of curcumin on cisplatin cytotoxicity in the human hepatocellular liver carcinoma cell line (HepG2), and the importance of the modulation of *p53* gene expression in the neuroprotective effects of curcumin.

2. Materials and methods

2.1. Chemicals

Curcumin was provided by the Industrial Physics Laboratory of the FCFRP-USP. Cisplatin was purchased from Quiral Química do Brasil (Platinil®, Juiz de fora, Brazil). Nerve growth factor (NGF) and mouse laminin purified protein were purchased from Millipore (Billerica, MA, USA). Dimethylsulfoxide was purchased from Merck (Darmstadt, Germany). RPMI 1640 and DMEM culture medium, fetal bovine serum, horse serum and penicillin/streptomycin antibiotic mixtures were purchased from Gibco™ (Carlsbad, CA, USA). Other reagents were of analytical grade and the purest quality available.

2.2. Cells

Undifferentiated PC12 cells (Cat. No. CRL-1721) and HepG2 cells (Cat. No. HB-8065), obtained from the American Type Culture Collection (Rockville, MD, USA), were cultured in 75-cm^2 flasks in a humidified incubator in a atmosphere of 95% air and 5% CO_2 at 37 °C (Thermo Electron Co.). Undifferentiated PC12 cells were cultivated in RPMI 1640 liquid medium supplemented with 10% horse serum, 5% fetal bovine serum and a penicillin/streptomycin antibiotic mixture. HepG2 cells were cultivated in DMEM with 15% fetal bovine serum and a penicillin/streptomycin antibiotic mixture. For both cell lines, the media were changed every 3–4 days, and cells were passaged when they reached 80–90% confluence. In all experiments, undifferentiated PC12 and HepG2 cells were used between the 3rd and 10th passage.

2.3. MTT assay

The MTT assay (Mosmann, 1983) was performed to evaluate cytotoxicity in PC12 and HepG2 cells. PC12 cells were evaluated in undifferentiated (PC12) and NGF-differentiated (dPC12) states. Before performing the MTT assay, PC12 cells were differentiated for 72 h of incubation in differentiation medium. The cell cultures were exposed to serial dilutions of curcumin or cisplatin in preliminary experiments to establish the studied concentrations (data not shown). PC12 cells and dPC12 were plated in 96-well plates (1.0×10^5 cells/mL) with 100 μL of medium, and cisplatin (5.0 or 10 μg/mL), curcumin (0.1, 1.0 or 10 μg/mL) and combinations thereof were added to each well. HepG2 cells were plated in 96-well plates (0.5×10^5 cells/mL) with 100 μL of medium and then treated with cisplatin (10 μg/mL) alone or co-incubated with curcumin (0.1, 1.0 or 10 μg/mL). After 24 h of treatment, the plates were incubated with 10 μL of MTT solution (0.5 mg/mL) for 3 h. The medium was then removed, and the purple formazan crystals were dissolved in 100 μL of dimethylsulfoxide. The absorbance of each well was measured at 570 nm by a microplate reader (Biotek, EL800). Cell viabilities are expressed as the percentage of the mean optical density value from the negative control wells.

2.4. Neurite outgrowth assay

The neurite outgrowth assay was conducted with the Chemicon's NS225 kit according to the manufacturer's instructions (Millipore, Billerica, MA, USA). Prior to the initiation of the neurite outgrowth assay, undifferentiated PC12 cells were incubated in differentiation medium consisting of RPMI 1640 + 1% horse serum + 1% penicilin/streptomycin + 100 ng/mL NGF for 72 h for cell priming. After priming, the cells (100 μL of 1.0×10^6 cell/mL) were transferred to a cell culture insert (previously coated with purified mouse laminin protein) containing permeable membrane pores that passed through the base, which allowed distinction of neurites and cell bodies. The cell culture inserts were placed into culture plate wells containing the differentiation medium with the tested substances. The cells were then treated with cisplatin (10 μg/mL) and curcumin (1.0 or 10 μg/mL) or both curcumin and cisplatin together. For cotreatment treatment, cisplatin was removed after 24 h, and the cultures were then treated with curcumin for an additional 48 h. The optimal treatment concentrations of cisplatin for causing neurite damage have been previously evaluated (Klein et al., 2007). The tested concentrations of curcumin and cisplatin used in the neurite outgrowth assay were evaluated with the MTT assay with differentiated PC12 cells and did not produce considerable cytotoxicity. After the treatment period, cell cultures were washed, fixed, neurite stained, stain extracted and stain solution extracted was quantified at 562 nm, according to the manufacturer's protocol.

2.5. Transcriptional response of the p53 gene

Total RNA was extracted from PC12 cells after treatment for 48 h with non-cytotoxic concentrations of cisplatin (0.1 μg/mL), curcumin (5.0 μg/mL), or curcumin combined with cisplatin. The non-cytotoxic concentrations of cisplatin and curcumin in undifferentiated PC12 cells were obtained from our previously published data (Mendonca et al., 2009). Total RNA extraction was performed using the SV total RNA isolation system (Promega, Madison, WI, USA) according to the manufacturer's instructions. The quality and integrity of the extracted RNA were assessed by both gel electrophoresis in 1.0% agarose and the ratios of the spectrophotometric optical density measurements at 260 nm/280 nm and 260 nm/230 nm. The extracted RNA was converted to cDNA using the kit SuperScript™ III (Invitrogen, Carlsbad, CA, USA). The PCRs were performed using the Biorad Real-Time PCR system with the ABsolute™ QPCR SYBR® Green Mix kit (Invitrogen, Carlsbad, CA, USA) with gene-specific primers for *p53* (*p53*-forward: CATCATCACGCTGGAAGACTC; *p53*-reverse: TTCAGCTCTCGGAACATCTC) and *β-actin* (*β-actin*-forward: TCCTGTGGCATCCATGAACT; *β-actin*-reverse: CCAGGGCAGTAATCTCTTTCTTCTG). A negative control without the RNA template was also included. The relative expression of *p53* mRNA was normalized to the amount of *β-actin* mRNA using the $2^{-\Delta\Delta ct}$ relative quantification method.

2.6. Data analysis

Each experiment was performed three times, and the values are expressed as the means ± the standard deviations. Group comparisons in the neurite outgrowth assay were made using paired *t*-tests, and other results were analyzed using one-way ANOVA followed by Tukey's *post hoc* test. Statistical analysis was performed with the GraphPad Prism 5.0 software. Differences were considered significant at $p < 0.05$.

3. Results

3.1. MTT assay

The MTT assay was performed in PC12 and HepG2 cells to evaluate cell viability after different treatments. The viability of the cultures was determined by establishing the relationship between the absorbances obtained in the treated and untreated (negative control) groups. The negative control was considered to have 100% cell viability.

Table 1
MTT assay of undifferentiated and differentiated PC12 cells treated with curcumin, cisplatin or a combination of both compounds for 24 h.

Treatments (μg/mL)	% cell viability	
	Undifferentiated PC12 cells	Differentiated PC12 cells
Negative control	100 ± 0	100 ± 0
CMN 0.1	99 ± 4	83 ± 11
CMN 1.0	88 ± 7	83 ± 9
CMN 10	67 ± 14*	80 ± 5
cDDP 5	74 ± 1	80 ± 12
cDDP 5 + CMN 0.1	79 ± 16	79 ± 8
cDDP 5 + CMN 1.0	74 ± 17	78 ± 14
cDDP 5 + CMN 10	52 ± 6*	81 ± 11
cDDP 10	58 ± 5*	73 ± 3
cDDP 10 + CMN 0.1	63 ± 10*	74 ± 8
cDDP 10 + CMN 1.0	54 ± 14*	86 ± 12
cDDP 10 + CMN 10	45 ± 1*	85 ± 18

CMN, curcumin; cDDP, cisplatin.
The results were obtained from three independent cultures obtained in parallel.
* $p < 0.05$ compared with the negative control, as measured by one-way ANOVA followed by Tukey's *post hoc* test.

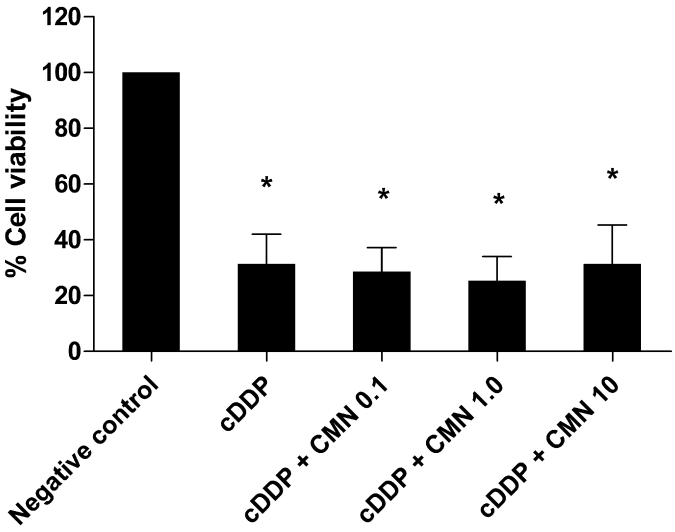

Fig. 1. MTT assay of the human hepatocellular liver carcinoma cell line (HepG2) treated with cisplatin alone or in combination with curcumin. CMN = curcumin and cDDP = cisplatin. Bars indicate the means ± standard deviations obtained from three independent cultures made in parallel. *Significant difference compared with the negative control ($p < 0.05$), as measured by one-way ANOVA followed by Tukey's *post hoc* test.

Undifferentiated and differentiated PC12 cells were treated with curcumin (0.1, 1.0 or 10 μg/mL), cisplatin (5.0 or 10 μg/mL) or combinations of both compounds for 24 h, and the cell viability results are presented in Table 1. We did not observe a cytoprotective effect of curcumin against cisplatin cytotoxicity in undifferentiated or differentiated PC12 cells. Neither curcumin nor cisplatin, alone or combined, significantly affected the cell viability of differentiated PC12 cells.

Undifferentiated PC12 cells were more sensitive to cisplatin and curcumin than differentiated PC12 cells. A significant cellular viability reduction was observed in the undifferentiated PC12 cells treated with cisplatin or curcumin at the concentration of 10 μg/mL ($p < 0.05$). Combination with curcumin (0.1, 1.0 or 10 μg/mL) did not protect these cells from cisplatin cytotoxicity.

We also performed the MTT assay in HepG2 cells to evaluate whether curcumin affects cisplatin cytotoxicity in tumor cells. The HepG2 cells were treated with cisplatin 10 μg/mL alone or co-incubated with curcumin (0.1, 1.0 or 10 μg/mL) for 24 h.

Cisplatin was significantly cytotoxic to HepG2 cells (Fig. 1). Furthermore, cisplatin at 10 μg/mL was more cytotoxic in HepG2 cells than in undifferentiated PC12 cells. The treatment of HepG2 cells with cisplatin (10 μg/mL) reduced cell viability by approximately 70%, and curcumin did not protect these cells from cisplatin cytotoxicity. Co-incubation with curcumin (0.1, 1.0 or 10 μg/mL) also resulted in an approximately 70% reduction in cell viability.

3.2. Neurite outgrowth assay

To determine whether curcumin reduced cisplatin-induced neurite toxicity, two different concentrations of curcumin (1.0 and 10 μg/mL) were added to the cells in combination with cisplatin (10 μg/mL). The results are expressed as the percentage of neurites formed in relation to the negative control group, which was designated as 100%. Curcumin had no effect on the percentage neurites formed at 1.0 μg/mL or 10 μg/mL (Fig. 2A). However, strong neurite toxicity was observed with cisplatin treatment, which decreased the formation of neurites by 62% compared with the control (Fig. 2B).

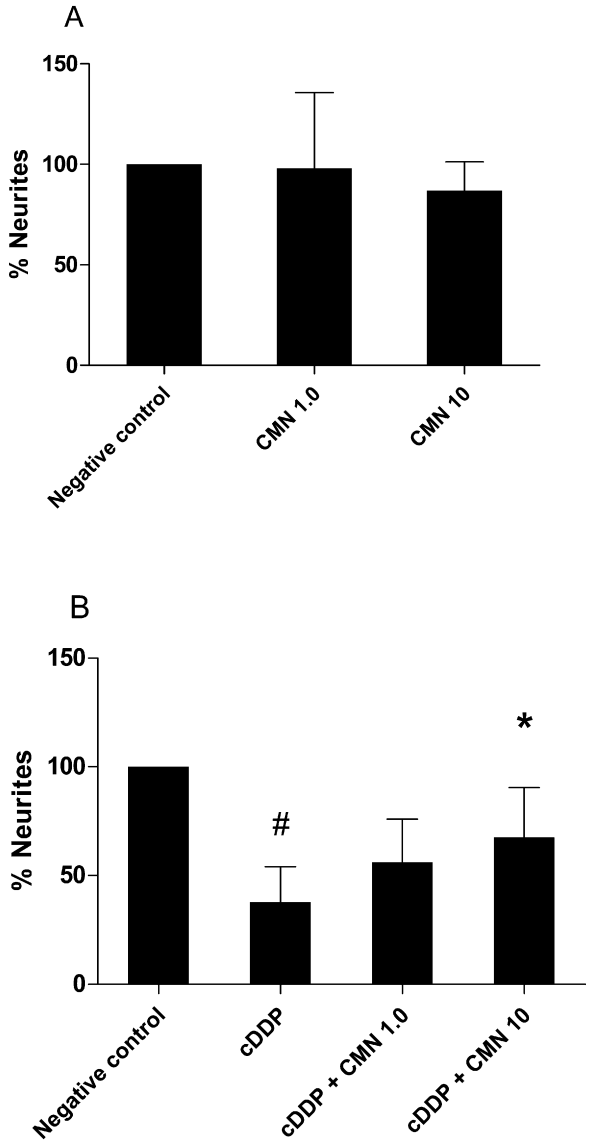

Fig. 2. Percentages of neurites in NGF-differentiated PC12 cells after treatment with (A) curcumin (1.0 or 10 μg/mL) or (B) cisplatin alone (10 μg/mL) or with curcumin (1.0 or 10 μg/mL). CMN = curcumin and cDDP = cisplatin. Bars indicate the means ± standard deviations obtained from three independent cultures made in parallel. #Significant difference compared with the negative control ($p < 0.05$), and *significant difference compared with the cDDP ($p < 0.05$) by paired *t*-tests.

The results presented in Fig. 2B show that curcumin reduced cisplatin-induced neurite toxicity. The lower concentration of curcumin did not result in significant protection; however; curcumin at 10 μg/mL reduced the cisplatin-induced neurite toxicity by approximately 50%, increasing the percentage of neurites from 38% to 68%.

3.3. Transcriptional response of the p53 gene

Undifferentiated PC12 cells were treated with non-cytotoxic concentrations of cisplatin (0.1 μg/mL), curcumin (5.0 μg/mL), or curcumin combined with cisplatin to evaluate the influence of *p53* gene expression on curcumin's protective effects. Undifferentiated PC12 cells were used instead of differentiated PC12 because NGF differentiation seems to influence *p53* transcription, which could complicate data interpretation.

Alterations in the transcriptional responses of the *p53* gene were analyzed by RT-qPCR using *β-actin* as the housekeeping gene.

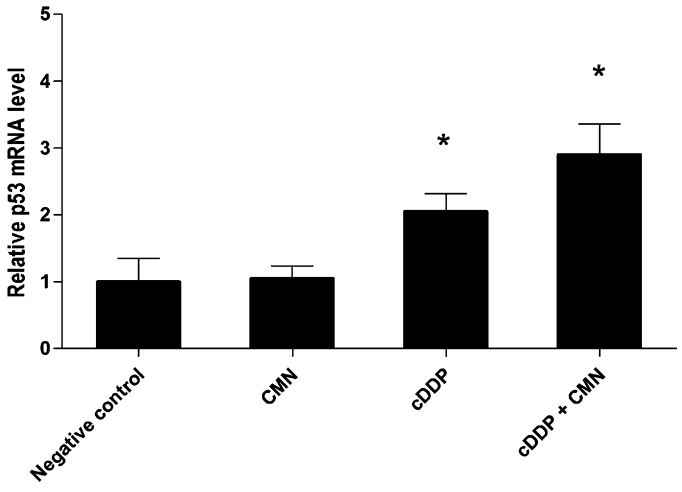

Fig. 3. RT-qPCR analysis of *p53* mRNA transcription of undifferentiated PC12 cells. Cell cultures were treated with cisplatin (0.1 μg/mL), curcumin (5.0 μg/mL) or a combination of both compounds for 48 h. CMN = curcumin and cDDP = cisplatin. Bars indicate the means ± standard deviation of the relative expression level using *β-actin* as a positive control obtained from three independent cultures made in parallel. *Significant difference compared with the negative control ($p < 0.05$), as measured by one-way ANOVA followed by Tukey's *post hoc* test.

The relative expressions of *p53* were calculated with the $2^{-\Delta\Delta ct}$ method by normalizing to the gene expression level of *β-actin*.

Curcumin did not modulate the transcription of *p53* mRNA. The gene expression results in Fig. 3 show that cisplatin increased the expression of *p53* mRNA approximately twofold. The transcriptional levels of *p53* mRNA remained unchanged after treatment of the cells with curcumin when compared with the control. Similar transcriptional levels of *p53* mRNA were observed after treatment with cisplatin or cisplatin in combination with curcumin.

4. Discussion

In this study, we investigated the possible protective effects of curcumin in cisplatin-induced neurotoxicity. The most common cisplatin-induced neurotoxicity is peripheral neuropathy, which can be extremely painful, can result in patient suffering, and may require dose reduction or discontinuation of cisplatin treatment. Curcumin has been shown to protect normal organs, such as the liver, kidneys, oral mucosa and heart from chemotherapy- and radiotherapy-induced toxicity (Goel and Aggarwal, 2010). Although curcumin has been extensively used as a neuroprotective agent to reduce oxidative damage in neurodegenerative disorders, its potential to prevent or reduce peripheral neuropathy had not yet been well evaluated (Huang et al., 2011; Kulkarni and Dhir, 2010). We demonstrated that curcumin attenuates neurite toxicity induced by cisplatin in the *in vitro* model of neurotoxicity with the PC12 cells. Moreover, curcumin did not interfere with the antitumoral activity of cisplatin as assessed *in vitro* in HepG2 cells.

Curcumin is generally considered safe and did have showing considerable toxicity in humans at dosage up to 8 g. The peak plasma concentration of curcumin in clinical trials of patients receiving 8 g of curcumin was 0.7 μg/mL; however, healthy human taking a dose of only 210 mg curcumin in a nanoparticle-based drug delivery system, reached a maximum plasma concentration ranging from 0.2 to 0.6 μg/mL (Cheng et al., 2001; Kanai et al., 2012; Sharma et al., 2005). The concentrations of curcumin used in the PC12 cells were based on our previously published cell survival curve (Mendonca et al., 2009). Additionally, the tested concentrations of curcumin, ranging from 0.1 to 10 μg/mL, cover to most probable *in vivo* pharmacological active concentrations of curcumin (Esatbeyoglu et al., 2012; Gupta et al., 2012).

The usual dose of cisplatin for treatment of the majority of tumor types ranges from 50 to 100 mg/m^2 of body surface at every cycle of chemotherapy. Although, the first symptoms of peripheral neuropathy are usually observed after administration of a cisplatin's cumulative dose of 250–350 mg/m^2 (Argyriou et al., 2012). Specenier et al. (2009) observed that after an intravenous administration of cisplatin at a dose of 75 mg/m^2, cisplatin reached a maximum plasma concentration of 4.6 μg/mL. Klein et al. (2007) showed the, in the PC12 cells, concentrations of cisplatin that statistically reduced the neurite formation starts from 1.0 μg/mL and that achieve the greatest effect in reducing neurite outgrowth at the concentration of 10 μg/mL. In our experiments, cisplatin at a concentration of 10 μg/mL reduced in 62% the formation of neurites, and curcumin reduced up to 50% the effect of cisplatin in the formation of neurites.

The mechanism of neurotoxicity appears to involve damage to sensory nerve cells of DRG. Morphological changes have been documented in dorsal root ganglia after platinum drug treatment, but exactly how the damage occurs is unclear. Analysis of neural tissues obtained at the time of autopsy from patients treated with cisplatin showed the DRG as the neural tissue with the greatest degree of platinum accumulation. Tissue platinum concentrations in DRG increased with the cumulative dose of cisplatin, and showed a mean of 1.68 μg/g of tissue. Patients with clinical evidence of neurotoxicity showed the highest histopathological neurotoxicity changes and platinum accumulation (Gregg et al., 1992). Studies in Sprague–Dawley rats treated with 1 mg/kg of cisplatin for 10 days demonstrated that platinum content was elevated in sensory DRG isolated from animals compared with other tissues like heart, muscle, spinal cord and brain. It was also possible to observe that platinum content in DRG was closely related with the amount of approximated 0.0075 ng Pt/μg DNA reported in PC12 cells treated with 2 μg/mL cisplatin for 24 h (McDonald et al., 2005).

Elucidation of the mechanisms involved in cisplatin peripheral neuropathy is useful for the identification of targets for development of effective therapeutic agents for management of such neurotoxicity. Research has explored and recognized mechanisms, including mitochondrial dysfunction and ROS generation, which often lead to axonal damage. In addition to the adduct-forming direct DNA interaction, cisplatin appears to induce ROS generation that leads to oxidative stress in several types of cells including neurons (Jaggi and Singh, 2012). Cisplatin generates superoxide anions, hydrogen peroxide and hydroxyl radicals that may play a part in cellular damage, suggesting that antioxidant therapy could be useful against cisplatin toxicity (Masuda et al., 1994; Santos et al., 2007).

The use of antioxidants during chemotherapy and radiotherapy has been a continuing area of interest and discussion. Phytochemical antioxidants, nutritional agents, and synthetic compounds have been proposed as cytoprotectant agents that may protect normal tissue from the effects of chemotherapy and radiation (Block and Gyllenhaal, 2005). Antioxidants, such as acetylcysteine, amifostine, glutathione and vitamin E, have been proposed as neuroprotective agents for use in the prevention or limitation of cisplatin neurotoxicity; however, the clinical data are currently insufficient to conclude that any of these potential neuroprotectants should be recommended for the prevention of cisplatin-induced peripheral neuropathy (Albers et al., 2011).

Antioxidant strategies have shown promising neuroprotective results in pre-clinical studies. Reduced glutathione prevents cisplatin-induced neuropathy, as measured by sensory nerve conduction velocity (SNCV) in young adult Wistar rats (Hamers et al., 1993; Tredici et al., 1994). In primary DRG cultures, alpha-lipoic acid protects sensory neurons through its antioxidant and mitochondrial regulatory functions (Melli et al., 2008). The antioxidant and free radical scavenger amifostine ameliorates cisplatin-induced neurotoxicity in an NGF-differentiated PC12 cell *in vitro* model (Block and Gyllenhaal, 2005; Verstappen et al., 1999, 2004). However, clinical data on these drugs remain controversial, and their efficacies remain a matter of uncertainty. Although the lack of efficacy of these compounds has not been elucidated in clinical trials, this may be the result of pharmacotechnical or pharmacokinetic thresholds (Argyriou et al., 2006; Klein et al., 2007).

Curcumin has demonstrated neuroprotective benefits that extend beyond neuronal diseases. Curcumin protects against arsenic-induced neurotoxic cholinergic dysfunction in rats and attenuates peroxynitrite-induced damage in rat spiral ganglion neurons through its anti-oxidative activity. Curcumin also protects mitochondria from oxidative stress (Liu et al., 2011; Yadav et al., 2011). Our previous study demonstrated that curcumin significantly reduces the DNA damage induced by cisplatin in PC12 cells (Mendonca et al., 2009); the current time is the first demonstration that curcumin reduces the toxic effect of cisplatin on PC12 neurite outgrowth.

Studies have showing that curcumin has neuroprotective benefits on the DRG. Curcumin reduced the histological changes of the DRG and sciatic nerve in Wistar rats subjected to sciatic nerve crush. Curcumin ameliorates the decrease of total number, diameter, and area of the myelinated nerve fibers after sciatic nerve injury (Noorafshan et al., 2011). Recently a study in Wistar rats showed that curcumin reduced the histological changes induced by cisplatin in the sciatic nerve. Histopathological examination of sciatic nerve of animals treated with cisplatin clearly demonstrated degeneration of nerve fiber, with reduction in nerve fiber caliber and areas of demyelization. Animal treated simultaneously with cisplatin and curcumin showed a significant reduction in the sciatic nerve alterations (Al Moundhri et al., 2012).

Our results showed that curcumin protected neurite outgrowth in non-cytotoxic conditions. Frequently, neurotoxicants present a significant hazard for humans despite low associated cytotoxicity (Gartlon et al., 2006). Although high levels of Pt–DNA binding is associated with apoptosis in DRGs *in vitro*, in conditions with reduced sensory nerve conduction velocity in the tails of Swiss albino mice, no DRG neurons are lost after cisplatin treatment. However, the length of outgrowing axons in DRG explants decreased (Ozturk et al., 2004; Ta et al., 2006). Thus, sensory neuron damage may occur even without apoptosis, or cisplatin damage culminates in neuron apoptosis in only in late stages, suggesting that anti-apoptotic strategies may be not effective in reducing peripheral neuropathy.

The use of cytoprotectants has been a subject of controversy due to their potential to protect malignant tissue. Nevertheless, studies in cell culture and in rodents have revealed that, in addition to the protective effects in normal tissue, curcumin can sensitize tumors to different chemotherapeutic agents including cisplatin (Goel and Aggarwal, 2010). Thus, we purpose that curcumin may reduce cisplatin's neurotoxicity without affecting its antitumor activity.

Our results indicate that curcumin does not compromise cisplatin's antitumor activity. In the MTT assay, curcumin did not reduce cisplatin's cytotoxicity in a cancer cell line. HepG2 cells were chosen for this evaluation because cisplatin is a conventional drug used for liver cancers. In addition, the combination of curcumin with cisplatin resulted in synergistic antitumor activity in the hepatic cancer HA22T/VGH cell line rather than implicating curcumin in antitumoral failure (Notarbartolo et al., 2005).

We also investigate the impact of curcumin on *p53* mRNA modulation. The *p53* tumor suppressor gene is of fundamental importance in tumor suppression. This gene serves as a cellular stress sentinel that restricts cellular expansion and eliminates cells

that have encountered acute genotoxic stress (Brady et al., 2011). The functions of *p53* include induction of cell-cycle arrest or apoptosis in response to acute DNA-damage signals (Vousden and Lane, 2007; Vousden and Prives, 2009). DNA is the primary target of cisplatin, which triggers cellular events that culminate in therapeutically beneficial apoptosis. Loss of *p53* function may attenuate DNA damage-mediated apoptotic signal responses, which has implications for chemotherapy resistance (Siddik, 2003). Therefore, strategies that diminish cisplatin's side effects without compromising *p53* function would be broadly valuable for cancer therapy. Curcumin did not change *p53* gene expression; specifically, curcumin did not suppress transcription of *p53* mRNA.

5. Conclusion

This *in vitro* study indicates that curcumin has protective effects against cisplatin-induced neurotoxicity that do not disturb cisplatin's therapeutic effect. Curcumin neither suppresses *p53* mRNA transcription nor protects tumor cells against cisplatin cytotoxicity in HepG2 cells. Effective neuroprotective agents could increase cisplatin therapy effectiveness by reducing adverse effects and enabling increases the required dosages. Thus, clinical studies using curcumin to reduce peripheral neuropathy in patients receiving cisplatin chemotherapy should be considered.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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