Potent anticancer activity of pyrrolidine dithiocarbamate–copper complex against cisplatin-resistant neuroblastoma cells

Haiyuan Zhang[a,b], Jiu-sheng Wu[a] and Fangyu Peng[a,b,c]

Effective drugs are urgently needed for the treatment of advanced neuroblastoma refractory to conventional chemotherapy. Pyrrolidine dithiocarbamate (PDTC) is a copper-binding ligand, which showed cytotoxicity on many human tumor cells after binding with copper ions. In this study, we synthesized a copper–PDTC complex, which was characterized as a $Cu(PDTC)_2$ complex, with elemental analyses (Fourier transform infrared, electrospray ionization mass spectra, and ultraviolet-visible spectroscopy). The $Cu(PDTC)_2$ complex suppressed the proliferation of BE(2)C cells, a human neuroblastoma cell line, with an IC_{50} of 8.0 μmol/l, which was more potent than cisplatin (IC_{50} of 80 μmol/l). Treatment of BE(2)C cells with the $Cu(PDTC)_2$ complex caused the S-phase arrest of cell cycle progression, cellular apoptosis, and necrosis, and increased the expression of p53 protein. The $Cu(PDTC)_2$ complex holds potential as a new drug for the treatment of refractory neuroblastoma in children. ***Anti-Cancer Drugs*** **19:125–132 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Neuroblastoma (NB) is a malignant tumor originating in the peripheral sympathetic nervous system in children [1]. The prognosis for children diagnosed with advanced NB with high risk factors, such as amplification of N-*myc* oncogene and deletion of chromosome 1p36, remains poor [2,3]. Rapid development of acquired resistance to conventional chemotherapeutic drugs is one of the major causes of treatment failure [4,5]. Effective drugs are urgently needed for the treatment of drug-resistant NB.

Metal–dithiocarbamate compounds, such as platinum(II), palladium(II), and gold(III) conjugates, have been known to be effective against tumor cells resistant to cisplatin [6–9]. Pyrrolidine dithiocarbamate (PDTC), a member of the dithiocarbamates, is known to be highly cytotoxic to many human tumor cells in the presence of copper ions [10–15]. The molecular mechanism for the cytotoxicity of the copper–PDTC [$Cu(PDTC)_2$] complex remains to be elucidated. It might be related to apoptosis induction by inhibiting the activity of the transcription factor, nuclear factor-κB [16–18], and by the inhibition of proteosome [10,11].

Although PDTC has been tested for anticancer activity on NB cells [19–21], there is no report about the anticancer activity of the copper complex of this ligand on NB cells. We herein hypothesize that $Cu(PDTC)_2$ complex has more potent anticancer activity on NB cells compared with the PDTC ligand and that the $Cu(PDTC)_2$ complex might be useful as a new drug to treat NBs that are resistant to conventional chemotherapeutic agents such as cisplatin and other platinum drugs. To test this hypothesis, we synthesized and characterized the $Cu(PDTC)_2$ complex, and evaluated its anticancer activity on BE(2)C cells, a human NB cell line resistant to cisplatin and other chemotherapeutic agents.

Materials and methods

Ammonium PDTC, triethylamine and $CuCl_2.2H_2O$ were purchased from Sigma-Aldrich (St Louis, Missouri, USA). Infrared (IR) spectra were recorded from 4000/cm^{-1} to 300/cm^{-1} as KBr pellets on a Tensor 27 FT-IR spectrophotometer (Billerica, Massachusetts, USA). Ultraviolet-visible spectroscopy (UV-Vis) was carried out and spectra were recorded from 190 to 900 nm on a Cary 50 spectrometer (Palo Alto, California, USA). Electrospray ionization mass spectra (ESI-MS) were measured in a triple quadrupole Micromass QuattroLC spectrometer (Micromass, Manchester, UK) with an electroscopy/atmospheric pressure chemical ionization source. Elemental analyses were performed by Midwest Microlab (Indianapolis, Indiana, USA).

Synthesis and characterization of copper–pyrrolidine dithiocarbamate complexes

$CuCl_2{\cdot}2H_2O$ (1 mmol, 0.133 g), dissolved in 10 ml of water, was added to 40 ml of aqueous solution containing

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various concentrations of PDTC (1 mmol, 0.164 g; 2 mmol, 0.328 g; or 3 mmol, 0.492 g). The resulting solution was stirred overnight at room temperature. Upon completion of the reaction, rotary evaporation of the solution to 20 ml yielded a brown precipitate. The precipitate was collected with filtration and washed with diethyl ether. Recrystallization from boiling acetone yielded brown microcrystals, which were filtered off and dried under vacuum. The products obtained using different reactant ratios were found to be the same chemical form of $Cu(PDTC)_2$. Analysis calculated for $C_{10}H_{16}N_2S_4Cu$ (%): C, 33.65; H, 4.49; N, 7.85; S, 35.90. Found (%): C, 33.68; H, 4.53; N, 7.75; S, 35.65. ESI-MS (methanol, m/z): 356 ($M + H^+$). Selected IR (KBr, υ/cm^{-1}): 1498 s [a bond for amino-dithiocarbamate (N–CSS)], 947 m (C–S), 390 m (Cu–S). UV-Vis [λ(nm)/ε, dimethylformamide]: 268 (13 100), 287 (11 520), 434 (5740).

MTT assay

MTT assay was performed in essentially the same manner as described previously [22], using an MTT assay kit from Chemicon (Temecula, California, USA). BE(2)C cells, a subclone of the SK-N-BE(2) human NB cell line, were purchased from American Type Culture Collection (Manassas, Virginia, USA). The BE(2)C cells were cultured in Eagle's minimum essential medium: F12 (1 : 1) medium (Biosource International, Camarillo, California, USA), supplemented with fetal bovine serum (10%), penicillin (100 units/ml), streptomycin (100 mg/ml), and glutamine (100 mg/ml), at 37°C in an atmosphere of 5% CO_2. Twelve hours after the seeding of the cells in a 96-well plate (1×10^4/0.1 ml/well), the $Cu(PDTC)_2$ complexes were dissolved in medium containing 0.01% dimethylsulfoxide (DMSO) at the noted concentration and were added and incubated for 24 h. The cells in the positive control group were treated with cisplatin, whereas the cells of the negative control group were treated with PDTC ligands or medium containing 0.01% DMSO. At the end of the treatment, MTT solution (1 mg/ml in serum-free medium, 100 µl/well) was added, followed by incubation at 37°C for another 4 h. The blue crystal of the MTT product was dissolved in DMSO and optical density (OD) was measured on a plate reader at 570-nm wavelength, with a reference at 630 nm. The proliferation of the treated cells was recorded as percentages against the cell proliferation of negative controls [(OD of the experimental samples/OD of the negative control) × 100%]. Each experiment was repeated at least three times, and each point was determined in triplicate.

Microscopic examination of cytopathological effects

To evaluate the cytopathological effects on the BE(2)C cells, the $Cu(PDTC)_2$ complex or the PDTC ligand at noted concentrations were added to the cells (4×10^4/well) seeded in a 24-well plate and incubated for 24 h. The cells in the negative control group were treated with culture medium containing 0.01% DMSO. At the end of the treatment, the cells were examined under microscope and recorded for cytopathological effects using a phase contrast microscope equipped with a Spot digital camera (Diagnostic Instruments, Sterling Heights, Michigan, USA).

Cell cycle analysis

The DNA contents and cell cycle progression of the BE(2)C cells were determined by flow cytometry, after the drug treatment. The cells were seeded in six-well plates at a density of 1.6×10^5 cells/well. Twelve hours later, the cells were treated with $Cu(PDTC)_2$ or PDTC ligand for 12 and 24 h, respectively. The cells of the negative control were treated with medium containing 0.01% DMSO for the same period of time. At the end of the treatment, the cells were harvested and washed with ice-cold phosphate-buffered saline, and fixed with 70% ethanol. The cells from the cell pellet were resuspended in 100 µl of RNase A (1 mg/ml) and 400 µl of propidium iodide (PI) (50 µg/ml), and incubated at 37°C for 30 min. Analysis was performed on a FACScan flow cytometer (Beckman Coulter, Miami, Florida, USA). Ten thousand events were analyzed for each analysis.

Apoptosis-induction assay

Apoptosis-induction activity of the $Cu(PDTC)_2$ was evaluated with annexin V/PI flow cytometry analysis using an annexin V–PI apoptosis detection kit from R&D Systems (Minneapolis, Minnesota, USA) and with Hoechst 33258 nuclear staining [11,12]. For flow cytometry analysis, the cells (1.6×10^5 cells/well) were seeded in a six-well plate and treated with the complexes at noted concentrations for 12 h. At the end of the treatment, the cells were harvested, stained with annexin V–fluorescein isothiocyanate (FITC)/PI, and subjected to flow cytometry analyses using a FACScan flow cytometer (Beckman Coulter).

For Hoechst 33258 nuclear staining, the cells (1.6×10^5 cells/well) were seeded onto a glass plate in a Petri dish and incubated with $Cu(PDTC)_2$ at noted concentrations and incubated for 12 h. At the end of incubation, the cells attached on the glass plates were washed twice with ice-cold phosphate-buffered saline, and fixed in ethanol and acetic acid (3 : 1) for 10 min. A solution of Hoechst 33258 (20 µg/ml) was added to stain the cells for 10 min in the dark at 37°C. Subsequently, the stained cells were covered with a mounting solution (0.1 mol/l citric acid: 0.2 mol/l disodium phosphate: glycerol, 1 : 1 : 2) and examined for condensed nucleus under a fluorescence microscope, and recorded with a SPOT digital camera (Diagnostic Instruments).

Immunoblot assay

Immunoblot assay was performed in a protocol modified from that described previously [23]. Briefly, the BE(2)C

cells (1.6×10^5 cells/well) were seeded in a Petri dish and were treated with $Cu(PDTC)_2$ or PDTC ligand at noted concentrations for 12 h at 37°C. Subsequently, the cells were harvested and cellular proteins extracted from the cells were quantified with a protein-quantification assay kit from Bio-Rad (Hercules, California, USA). The cellular protein samples were separated by SDS–polyacryamide gel (SDS–PAGE) and transferred onto a polyvinylidine diflouride membrane. After blocking with a Tris-buffered saline (TBS) solution containing 5% nonfat milk and 0.1% Tween-20, the polyvinylidine diflouride blot was reacted with a monoclonal antibody specific for human p53 protein (1 : 3000; R&D Systems), for 1 h at room temperature. After washing with TBS × 3, the immunoreactivity of the p53 protein on the membrane was visualized by incubation with horseradish peroxidase-conjugated rabbit-antimouse secondary antibody from Bio-Rad, followed by chemoluminescence visualization using an enhanced Immun-Star horseradish peroxidase chemoluminescence kit from Bio-Rad. Equal loads of the samples were verified by the immunoreactivity of human β-actin, using anti-human β-actin monoclonal antibody (1 : 1000, Novus Biologicals, Littleton, Colorado, USA).

Statistical analysis

The data was expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for statistical evaluation. Means were compared using Student's *t*-test. A P value < 0.05 was considered significant.

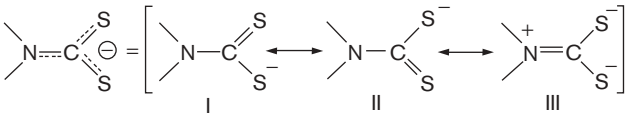
Results

Synthesis and characterization of the copper–pyrrolidine dithiocarbamate complex

The $Cu(PDTC)_2$ complexes were obtained by precipitating the reaction of $CuCl_2$ and PDTC in the molar ratios 1 : 1, 1 : 2, and 1 : 3. After purification, the molecular forms of these precipitates were determined by elemental analysis, which revealed the same complexes of $Cu(PDTC)_2$ from all the reactions of the various molar ratios. Further ESI-MS analysis indicated that the $Cu(PDTC)_2$ complex exhibited a single peak at 356 ($M + H^+$), supporting the mode of $Cu(PDTC)_2$. The $Cu(PDTC)_2$ complex has revealed well-defined molecular vibration in the IR region, which is helpful for determining the mode of coordination and assessing the nature of bonding in the compound. Concerning the dithiocarbamate moiety, three main regions in the IR spectra are of interest: first, the 1400–1580/cm^{-1} region, which is primarily associated with the 'thioureide' band owing to the υ(N–CSS) vibration; second, the 940–1060/cm^{-1} region, which is associated with υ(C–S) vibrations; and last, the 250–420/cm^{-1} region associated with υ(M–S) vibrations [24]. PDTC exhibited a characteristic band at a 1413/cm^{-1} assignable to υ(N–CSS) [25,26]. This band defined a carbon–nitrogen bond order between a single bond (υ = 1250–1350/cm^{-1}) and a double bond (υ = 1640–1690/cm^{-1}) [27]. The appearance of a band in that region indicated that, of three possible resonance structures reported by Chatt *et al.* [28] (Fig. 1), PDTC existed as a considerable contribution of structure β. After chelation with the Cu(II) ion, this band was observed as high energy shifts (from 1413 to 1498/cm^{-1}), indicating a strong chelating effect from PDTC to the copper(II) ion, and also indicating a slight increase of the carbon–nitrogen double-bond character [29,30]. Vibration in the 940–1046/cm^{-1} range has been used effectively for differentiating between the monodentate and bidentate dithiocarbamate ligand (Fig. 2) [31,32]. For the $Cu(PDTC)_2$ complex, the presence of only one band (947/cm^{-1}) in the investigated region, commonly attributed to a υ_a(dithiocarbamate) mode, indicated a completely symmetrical bonding of the PDTC ligand through a bidentate mode (Fig. 2a). Conversely, a split band will indicate an asymmetrically bonded bidentate ligand ($\Delta\upsilon < 20$/cm^{-1}, Fig. 2b) or a monodentate bound ligand ($\Delta\upsilon > 20$/cm^{-1}, Fig. 2c). In the 250–420/cm^{-1} range, a new band of about 390/cm^{-1} was observed that was absent in the spectra for PDTC. It can be assigned to the copper–sulfur stretching modes.

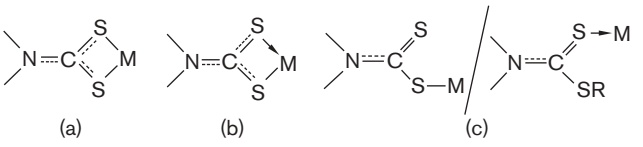
The UV-Vis spectra showed a similar pattern for the $Cu(PDTC)_2$ complex. The bands at 268 and 287 nm exhibited large molar extinction coefficients and corresponded to the intraligand $\pi^* \leftarrow \pi$ transition: the bands were mainly located in the –NCS and –CSS moieties, respectively. The appearance of metal–ligand charge transition (MLCT) at 434 nm strongly supported the binding from Cu(II) to S. To combine the results from the element analysis, ESI-MS, IR, and UV-Vis spectra study, the chemical structure of $Cu(PDTC)_2$ could be determined as presented in Fig. 3.

Fig. 1



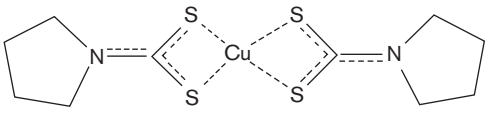
Resonant forms of the dithiocarbamic $-NCSS^-$ moiety.

Fig. 2



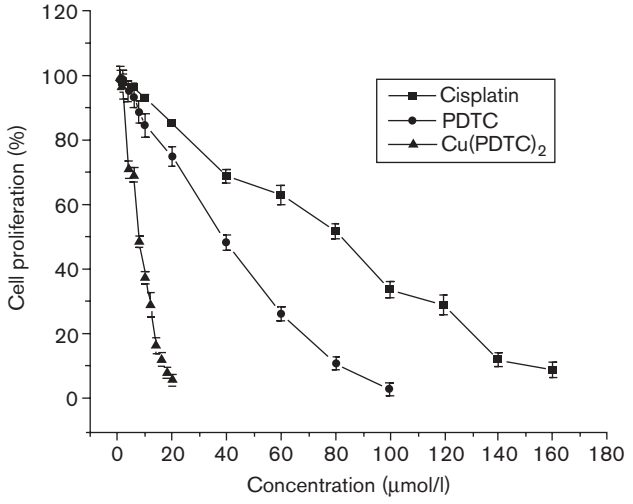
Different ways of metal – sulfur binding in dithiocarbamate complexes: (a) symmetrical bidentate, (b) asymmetrical bidentate, (c) monodentate.

Fig. 3



Schematic representation of the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex.

Fig. 4



Proliferation inhibition of the BE(2)C cells by the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex and the PDTC ligand. The cells were treated with $Cu(PDTC)_2$ at noted concentrations for 24 h. The negative control cells were treated with cell culture medium containing 0.01% dimethylsulfoxide.

Proliferation inhibition activity of the copper–pyrrolidine dithiocarbamate complex

The results of the MTT assay demonstrated the proliferation-inhibition activity of the $Cu(PDTC)_2$ complex on the BE(2)C cells (Fig. 4). After treatment of the cells with $Cu(PDTC)_2$ at various concentrations for 24 h, the proliferation of the BE(2)C cells was inhibited significantly. In contrast, the PDTC ligand showed much less inhibition activity on the proliferation of the BE(2)C cells. The IC_{50} values were determined as 8.0 μmol/l for $Cu(PDTC)_2$ and 40 μmol/l for the PDTC ligand. As expected, the BE(2)C cells were highly resistant to cisplatin, with an IC_{50} of 80 μmol/l. The applied one-way ANOVA indicated significant differences among the IC_{50} values of $Cu(PDTC)_2$, PDTC, and cisplatin ($P < 0.001$).

Morphologically, the BE(2)C cells treated with 8 μmol/l of $Cu(PDTC)_2$ or 80 μmol/l of cisplatin for 24 h displayed marked cytopathological effects, for example, shrinkage, spherical morphology, and detachment of the cells (Fig. 5). In contrast, no obvious cytopathological effects were observed in negative control cells and in the cells treated with 16 μmol/l of PDTC ligands (Fig. 5).

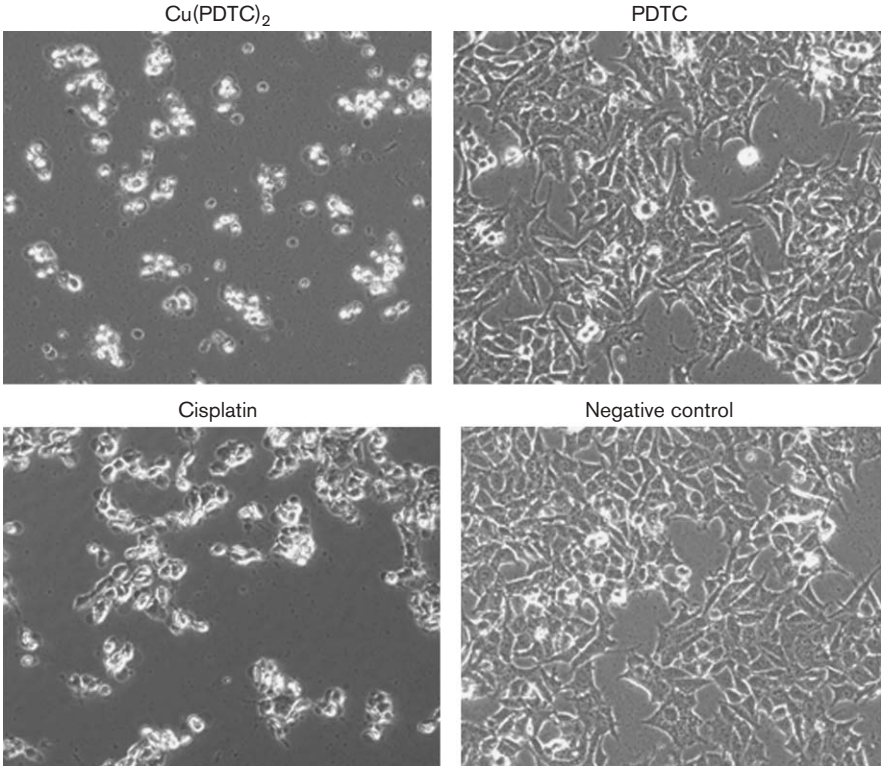
Arrest of cell cycle progression by the copper–pyrrolidine dithiocarbamate complex

After treatment with $Cu(PDTC)_2$ (8 μmol/l) or PDTC (16 μmol/l) for 12 h, significant accumulations of cell population were found in the $subG_1$ and S phases, with a corresponding decrease of cell population in the G_0/G_1 phase (Fig. 6). A slight increase of the cell population in the G_2/M phase was also observed. Incubation of the BE(2)C cells with $Cu(PDTC)_2$ for 24 h led to accumulations of more cells in the $subG_1$ and S phases, and reduction of cell numbers in the G_0/G_1 phase, accompanied by a continual increase in the G_2/M cell population (Fig. 6). The data indicated that the $Cu(PDTC)_2$ complex inhibited DNA synthesis and caused S-phase arrest. In contrast, the PDTC ligand exhibited only a small increase of the cell numbers in the S phase and the G_2/M phase at 12 and 24 h, with a very small percentage of the cell population entering the $subG_1$ phase. No significant cell cycle arrest was observed in the negative control group.

Proapoptotic activity of the copper–pyrrolidine dithiocarbamate complex

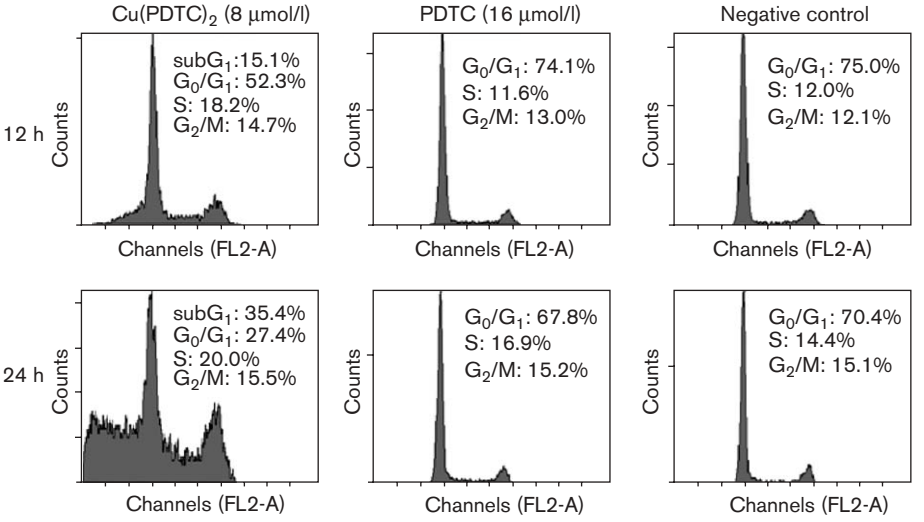
Apoptotic or necrotic cells were detected after incubation of the BE(2)C cells with the $Cu(PDTC)_2$ complex at 8 μmol/l for 12 h, using annexin V–FITC/PI flow cytometry analysis (Fig. 7a). After treatment with the $Cu(PDTC)_2$ complex, $6.2 \pm 0.9\%$ of the cells were stained positive for annexin V–FITC (early apoptosis), and $6.0 \pm 1.3\%$ of the cells were stained positive for both annexin V–FITC and PI (late apoptosis), along with $87.7 \pm 2.1\%$ of the cells staining negative for either annexin V–FITC/PI (viable). In comparison, only $3.6 \pm 0.7\%$ of the BE(2)C cells treated with PDTC at 16 μmol/l for 12 h were stained positive for annexin V–FITC (early apoptosis), along with $0.8 \pm 0.4\%$ of the cells staining positive for both annexin V–FITC and PI (late apoptosis), and $95.6 \pm 0.4\%$ of the cells staining negative for either annexin V–FITC/PI (viable). No apoptotic cells were observed in the group of negative controls. The applied mixed-design ANOVA demonstrated a significant difference between the $Cu(PDTC)_2$ and PDTC ligand with respect to the number of cells, which showed early apoptosis, late apoptosis, or which stayed alive ($P < 0.001$). Proapoptotic activity of the $Cu(PDTC)_2$ complex was confirmed by condensed nuclear morphology and intense Hoechst 33258 unclear staining of the BE(2)C cells treated with the $Cu(PDTC)_2$ complex at the noted concentration (Fig. 7b).

Fig. 5



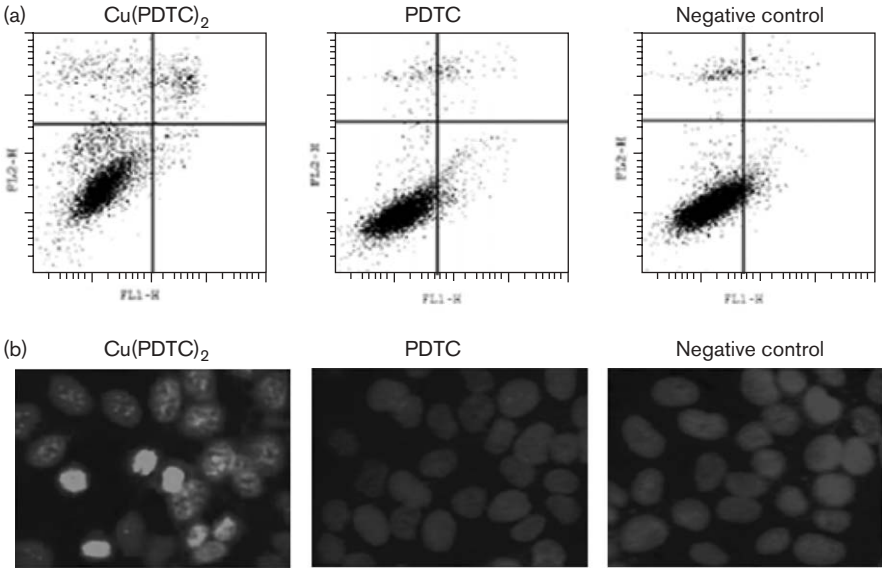
Cytotoxicity of the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex on the BE(2)C neuroblastoma cells. Marked cytopathological effects (shrinkage, roundup, and detachment) were observed on the cells treated with the $Cu(PDTC)_2$ complex (8 μmol/l) for 24 h, or cisplatin at a concentration of 80 μmol/l for 24 h. Little change was observed on the cells treated with PDTC (16 μmol/l) or culture medium containing 0.01% dimethylsulfoxide for 24 h. × 100 magnification.

Fig. 6



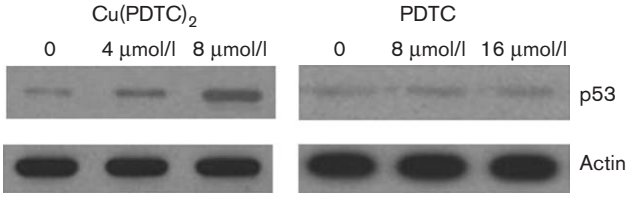
Effects of the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex and the PDTC ligand on the cell cycle progression of BE(2)C cells. BE(2)C cells treated with the $Cu(PDTC)_2$ complex (8 μmol/l) or the PDTC ligand (16 μmol/l) for 12 and 24 h were stained with propidium iodide and analyzed by flow cytometry. Percentages of cells in $subG_1$, G_0/G_1, S, and G_2/M phases are indicated. The cells of negative control were treated with medium containing 0.01% dimethylsulfoxide.

Fig. 7



Apoptosis of the BE(2)C cells treated with the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex. The cells were treated with $Cu(PDTC)_2$ (8 μmol/l) or PDTC (16 μmol/l) for 12 h. The cells of the control groups were treated with cisplatin (80 μmol/l) or culture medium containing 0.01% dimethylsulfoxide, for 12 h. (a) Apoptosis or necrosis of the BE(2)C cells was analyzed with flow cytometry using annexin V–FITC/propidium iodide staining. (b) Intense Hoechst 33258 nuclear staining of apoptotic BE(2)C cells treated with $Cu(PDTC)_2$ was carried out.

Fig. 8



Elevated expression of p53 protein molecules in the BE(2)C cells treated with the copper–pyrrolidine dithiocarbamate [$Cu(PDTC)_2$] complex. The cells were treated with the $Cu(PDTC)_2$ complex or the PDTC ligand at the noted concentration for 12 h. The cellular protein extracts from the treated cells were subjected to immunoblot using p53-specific monoclonal antibody. Marked increase of p53 protein expression was evident in the cells treated with the $Cu(PDTC)_2$ complex, compared with the cells treated with the PDTC ligand.

Increased expression of the p53 protein in the BE(2)C cells treated with the copper–pyrrolidine dithiocarbamate complex

The results of the immunoblot assay demonstrated increased expression of the p53 protein in the BE(2)C cells treated with the $Cu(PDTC)_2$ complex (Fig. 8). After treatment of the BE(2)C cells with the $Cu(PDTC)_2$ complex at a concentration of 4 or 8 μmol/l for 12 h, larger numbers of the p53 protein molecules were detected; the detection was based on the visual examination of immunoreactivity. In comparison, no increase of p53 protein expression was detected in the cells treated with the PDTC ligand at the noted concentrations of 8 or 16 μmol/l for 12 h (Fig. 8).

Discussion

We attempted to synthesize Cu–PDTC complexes using various reaction ratios of Cu : PDTC at 1 : 1, 1 : 2, or 1 : 3. A single mode of $Cu(PDTC)_2$ was found to exist in the final products after purification and characterization with elemental analyses, ESI-MS, FT-IR, and UV-Vis spectra. In this $Cu(PDTC)_2$ mode, the copper(II) ion coordinated four S atoms to form a symmetric square planar structure. The copper(II) ion was situated on a center of symmetry. This coordination mode is similar to or consistent with the previously reported crystal structure of Cu–dithiocarbamate compounds [33,34].

The $Cu(PDTC)_2$ complex suppressed the proliferation of the BE(2)C cells with an IC_{50} value of 8.0 μmol/l, which was more potent than the activity of cisplatin (IC_{50} value of 80 μmol/l). This suggests that the $Cu(PDTC)_2$ complex holds potential as an effective drug for the treatment of cisplatin-resistant NB cells. The PDTC ligand itself is less potent (IC_{50} value of 40 μmol/l), which indicated that copper ion chelation enhanced the anticancer activity of the $Cu(PDTC)_2$.

The molecular mechanism of the anti-NB activity of the $Cu(PDTC)_2$ complex remains to be elucidated. The data from this study demonstrated that the $Cu(PDTC)_2$

complex caused S-phase cell cycle arrest, which is likely related to the inhibition of DNA synthesis. The treatment of the BE(2)C cells with the $Cu(PDTC)_2$ complex resulted in the significant increase of p53 protein expression, which is consistent with the results reported by others [12]. It is not clear whether the elevated expression of p53 protein is directly related to (or caused) the apoptosis of the BE(2)C cells treated with the $Cu(PDTC)_2$ complex. Mutation of the p53 gene in some of the NB cells was reported previously [35]. It will be interesting to determine whether the p53 gene is mutated in the BE(2)C cells. If the p53 gene in the BE(2)C cells is indeed mutated, the $Cu(PDTC)_2$ complex might induce apoptosis by acting on a p53-independent target of signal-transduction pathway related to the regulation of cell apoptosis.

We have also evaluated the cytotoxicity of the $Cu(PDTC)_2$ complex on nonmalignant human cells, using primary fibroblasts established from human prostate and mammary gland tissues. Minimum cytopathological effects were observed in cells treated with low concentrations of the $Cu(PDTC)_2$ complex (up to 7.5 µmol/l). Significant cytotoxicity was, however, observed when the cells were treated with the $Cu(PDTC)_2$ complex at a concentration higher than 10 µmol/l (images not shown). Additional investigation is being undertaken to study the targeted delivery of Cu(PDTC)2 to NB tissues, in an effort to improve the tumor specificity of this highly potent anticancer agent.

Conclusion

The $Cu(PDTC)_2$ complex was synthesized in various reaction ratios (1 : 1, 1 : 2, and 1 : 3; ratio of Cu : PDTC) and the products from all these reactions were found to be in the $Cu(PDTC)_2$ mode. The $Cu(PDTC)_2$ complex inhibited proliferation, caused S-phase cell cycle arrest, and induced necrosis and apoptosis of the cisplatin-resistant BE(2)C NB cells. Elevated expression of p53 protein molecules was observed in the BE(2)C cells treated with the $Cu(PDTC)_2$ complex. The data from this study indicated that the $Cu(PDTC)_2$ complex merits further investigation as a new drug for the treatment of NB that is refractory to conventional chemotherapeutic agents.

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