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INHIBITION OF PROSTATE CANCER CELLULAR PROTEASOME ACTIVITY BY A PYRROLIDINE DITHIOCARBAMATE-COPPER COMPLEX IS ASSOCIATED WITH SUPPRESSION OF PROLIFERATION AND INDUCTION OF APOPTOSIS

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1. ABSTRACT

Recent research suggests that copper could be used as a novel selective target for cancer therapies. Copper is a co-factor essential for tumor angiogenesis processes and high levels of copper have been found in many types of human cancers, including prostate, breast and brain. We have reported that organic copper-containing compounds, such as 8-hydroxyquinoline-copper(II), are a novel class of proteasome inhibitors and tumor cell apoptosis inducers (Daniel et al., Biochem Pharmacol. 2004;67:1139-51). Most recently, we have found that when complexed with copper, the known antioxidant pyrrolidine dithiocarbamate (PDTC) forms a potent proteasome inhibitor in human breast cancer, but not normal cells (Daniel, Chen, et al., submitted). In the current study, we investigate whether the PDTC-copper complex can play similar roles in inhibiting the proteasomal activity and consequently inducing apoptosis in human prostate cancer cells. We used tetrathiomolybdate (TM), a strong copper chelator currently being tested in clinical trials, as a control. We report here that after binding to copper, PDTC, but not TM, can inhibit the proteasomal chymotrypsin-like activity, suppress proliferation, induce apoptotic cell death, and inhibit uptake of radiopharmaceutical 2-[^{18}F]Fluoro-2-deoxy-D-glucose in cultured human prostate cancer cells. In contrast, PDTC, TM or copper alone or a TM-copper mixture had no such effects. Our study suggests that high copper levels in human prostate cancer *in vivo* can be targeted by a ligand such as PDTC, resulting in formation of an active proteasome inhibitor and apoptosis inducer specifically in prostate tumor, but not normal cells.

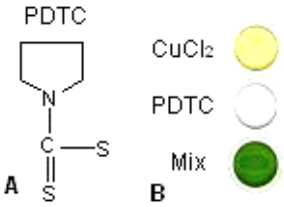

Figure 1. Copper complex formation as indicated by color change. A. Chemical structure of PDTC. B. 50 mM of PDTC was mixed in a 1:1 molar ratio with $CuCl_2$. All solutions were made in DMSO. The appearance of intensified color of mixture indicated formation of copper complex.

2. INTRODUCTION

Copper is an essential trace metal for animals. The amount of copper in an organism is tightly regulated (1, 2). High levels of copper have been found in many types of human cancers including prostate, breast, colon, lung, and brain (3-11). Consistently, angiogenesis, the formation and differentiation of blood vessels, is essential for tumor growth, invasion, and metastasis (12-15). Indeed, molecular processes of angiogenesis require of copper, but not other trace metals, as an essential cofactor (12-21).

The ubiquitin/proteasome system plays an important role in the degradation of cellular proteins. This proteolytic system involves two distinct steps: ubiquitination and degradation (22, 23). The eukaryotic proteasome contains at least three known activities, which are associated with its β subunits. These are the chymotrypsin-like (cleavage after hydrophobic residues, β5 subunit), trypsin-like (cleavage after basic residues, β2 subunit), and caspase-like or peptidyl-glutamyl peptide-hydrolyzing (cleavage after acidic residues, β1 subunit) activities (24, 25). Inhibition of the proteasomal chymotrypsin-like, but not trypsin-like, activity has been found to be associated with induction of apoptosis in tumor cells (26-31).

Dithiocarbamates are a well-defined class of metal-chelating compounds. These compounds have been previously used for treating bacteria, fungi and AIDS (32, 33). Pyrrolidine dithiocarbamate (PDTC; Figure 1A), a member of the dithiocarbamates, is an antioxidant that has been used as a therapeutic agent for treatment of inflammation, atherosclerosis and metal intoxication (34, 35). It has been shown that PDTC is able to inhibit NFκB activation and induce tumor cell apoptosis (36). Although the involved molecular mechanism remains unknown, it has been found that copper uptake is required for PDTC-mediated activities (37) and that PDTC is a copper-binding compound (38). PDTC and other dithiocarbamates have been found to induce apoptosis in conjunction with copper in different types of cancer cells (37, 39).

Tetrathiomolybdate (TM) is a copper chelator that was originally used for patients with Wilson's disease (17, 18). TM has been found to be effective in impairing the growth of mammary tumors in HER2/neu transgenic mice (40) and lung metastatic carcinoma in C557BL6/J mice (41). In a phase I clinical trial with patients suffering from metastatic cancers, TM therapy achieved stable disease in 5 of 6 patients who became copper-deficient (20). However, the disease advanced in some other patients before copper levels were sufficiently lowered (17, 18, 20). These reports support the idea of copper control as an anticancer strategy.

We have previously demonstrated that certain types of copper-binding compounds are potent proteasome inhibitors (42). Most recently, we have also found that PDTC, after interacting with copper, forms a proteasome inhibitor in human breast cancer cells (Daniel KG, Chen D, Orlu S, Cui QC, Miller FR, Dou QP, submitted). In the current study, we investigated the effects of the PDTC-Cu complex in human prostate cancer cells. We report that PDTC is capable of binding copper, spontaneously forming a new complex that has proteasome-inhibitory and apoptosis-inducing activities to human prostate cancer cells. In contrast, copper, PDTC or TM alone, or TM-copper complex had no effects under the same experimental conditions. We propose that targeting highly elevated copper could be tumor-specific and that formation of an active proteasome inhibitory complex between PDTC and copper is a novel strategy that has great potential for prostate cancer therapies.

3. MATERIALS AND METHODS

3.1. Chemicals, reagents, and radiopharmaceuticals

Cupric chloride ($CuCl_2$), dimethyl sulfoxide (DMSO), pyrrolidine dithiocarbamate (PDTC), and ammonium tetrathiomolybdate (TM), 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and bisbenzimide Hoechst #33258 stain were all purchased from Sigma-Aldrich. The substrate for the chymotrypsin-like activity of the proteasome, Suc-Leu-Leu-Val-Tyr-AMC, was purchased from Calbiochem. Polyclonal antibody to poly(ADP-ribose) polymerase (PARP) was obtained from Boehringer, Mannheim. Monoclonal antiubiquitin antibody, secondary antibodies, anti-mouse IgG horseradish peroxidase, and anti-rabbit IgG-horseradish peroxidase were from Santa Cruz Biotechnology Inc. Radiopharmaceutical 2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG), a tracer widely used for positron emission tomography (PET) imaging of cancers, is prepared by the method of Hamacher et al (43) using 1,3,4,6-tetra-O-acetyl-2-O-triflyl-β-D-manno-pyranose as the labeling precursor and ^{18}F radioisotope produced with a cyclotron housed at the PET Center, Children's Hospital of Michigan.

3.2. Preparation of the PDTC-Cu complex

To prepare PDTC-Cu complex, equal molar amounts of PDTC and $CuCl_2$, both dissolved in DMSO at 50 mM, were mixed by drop-wise addition of the $CuCl_2$ solution into the PDTC solution. The mixture was kept at room temperature for 10 minutes to allow formation of the PDTC-Cu complex. The freshly prepared PDTC-Cu complex was used for experiments.

3.3. Cell culture, drug treatment and whole cell extract preparation

LNCaP human prostate cancer cell line was purchased from ATCC. Cell culture medium (RPMI 1640), antibiotics, and trypsin solution were purchased from GIBCO. LNCaP cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum, 100 units/mL of penicillin, 100 mg/mL of streptomycin and 10mM HEPES at 37 ^{o}C in a humidified incubator with an atmosphere of 5% CO_2. Cells were treated with noted molar amounts of compounds, mixtures, or with an equivalent volume of solvent control as indicated in the figure legends. Whole cell extracts were prepared as described previously (44, 45). Briefly, cells were harvested, washed with cold PBS twice, and homogenized in a lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol) for 30 min at 4 ^{o}C. Afterwards, the lysates were centrifuged at 12,000 g for 15 min, and the supernatants were collected as whole cell extract.

3.4. Cell proliferation inhibition assay

The effects of each compound and their copper mixtures on LNCaP cells were determined using the MTT dye uptake method. Briefly, LNCaP cells (10^4 /well/ 0.1 ml) were seeded in triplicate in a 96-well plate and incubated at 37 ^{o}C in a humidified incubator with an atmosphere of 5% CO_2 until 70-80% confluence. This was followed by an additional 24-h incubation with fresh medium containing different concentrations of each compound and their copper mixtures. The same amount of DMSO was added as the solvent control. Thereafter, 100 μL MTT solution (1 mg/ml in serum-free medium) replaced the medium in each well. After 4 h at 37 °C incubation, the MTT solution was replaced by 100 μL DMSO. When the blue crystals were dissolved, the optical density (*OD*) was measured in a Wallac Victor3^{TM} multilabel plate reader (Perkin Elmer, USA) at a wavelength of 560 nm. The formula used to evaluate the result is as follows: inhibition of cell proliferation (%) = (*OD* of the experimental samples/*OD* of the control)×100%.

3.5. Cellular and nuclear morphology analysis

LNCaP cells were treated as indicated (see figure legends). Afterwards, the cells were visualized by microscopic imaging with either phase contrast for cellular morphology or Hoescht staining with fluorescence for nuclear morphology. Briefly, the cells were washed with PBS and fixed in 75% ethanol for at least 1 h at 4 °C followed by washing with PBS three times and staining in 50 μM of Hoechst 33258 for 30 min at 4 °C in dark. Subsequently the cells were washed and resuspended in 50 μL PBS and mounted for viewing. Copper mixture-induced apoptosis was monitored by the extent of nuclear fragmentation. Nuclear fragmentation was visualized by Hoechst 33258 staining of apoptotic nuclei using Zeiss Axiovision fluorescence microscope (Carl Zeiss Microscope Inc., Hallbergmoons, Germany).

3.6. *In vitro* proteasome activity assay

The cell-free chymotrypsin-like activity of the proteasome was determined by measuring the release of the AMC groups from a substrate as previously described (26). Briefly, after each treatment, whole cell extract (10 μg) of LNCaP cells was incubated in 100 μl of assay buffer (50 mM Tris–HCl, pH 7.5) and 40 μM fluorogenic peptide substrates, Suc-Leu-Leu-Val-Tyr-AMC (for the chymotrypsin-like activity) for 1 h at 37 °C. After incubation, production of hydrolyzed AMC groups was measured using a Wallac Victor3^{TM} multilabel plate reader with an excitation filter of 380 nm and an emission filter of 460 nm. Changes in fluorescence were calculated against non-treated controls and plotted with statistical analysis using Microsoft Excel^{TM} software.

3.7. Western Blot Assay

To evaluate ubiquitinated protein accumulation and PARP cleavage induced by PDTC-Cu complex treatment, cell lysates (30 μg) from treated LNCaP cells were subject to SDS–PAGE analysis and then transferred to a nitrocellulose membrane. The Western blot analysis was performed using specific antibodies to ubiquitin and PARP as described previously (42, 45), followed by visualization *via* enhanced chemiluminescence (ECL) (Amersham Biosciences).

3.8. Cellular ^{18}F-FDG uptake assay

To evaluate the effects of PDTC-Cu complex on cellular uptake of ^{18}F-FDG, LNCaP cells ($5x10^5$ cells in 1 ml of RPMI1640 /well) were seeded in triplicate on a 12-well cell culture plate 12 hours prior to treatment of cells with PDTC-Cu complex and other control reagents. Following treatment for 12 hours, LNCaP cells were incubated with 5 μCi (185 kBq) of ^{18}F-FDG added in the cell culture medium for 1 hour at 37 ^{o}C, in an atmosphere containing 5% CO_2. At end of incubation, the culture medium containing ^{18}F-FDG was removed and the cells were washed with PBS three times. Subsequently, the cells were digested with 5% trypsin solution and collected in a tube for radioactivity count using a Packard RiaStar multiwell gamma counter (Packard Instrument Co, Meriden CT). Radioactivity of the cells was calculated as percentage of inoculation dose (ID%) normalized to cell number ($5x10^5$/well), in reference to radioactivity of 5 μCi (185 kBq) of ^{18}F-FDG.

3.9. Statistical analysis

All data were expressed as mean ±SD. Statistical analysis was performed with the paired, two-tailed T test using Microsoft Excel software and p values < 0.05 or < 0.01 were considered to represent statistical significance.

4. RESULTS

4.1. PDTC spontaneously reacts with copper to form a new complex.

In order to use endogenous elevated copper in prostate tumor tissues as a targeting mechanism for cancer therapy, it is prerequisite for a ligand to react spontaneously with copper to form a new complex. To test the reactivity of PDTC with copper, 50 mM of PDTC was added to a 50 mM of copper (II) chloride (Figure 1). The reaction of PDTC with copper results in a dramatic color change (Figure 1), indicating formation of a new metal complex.

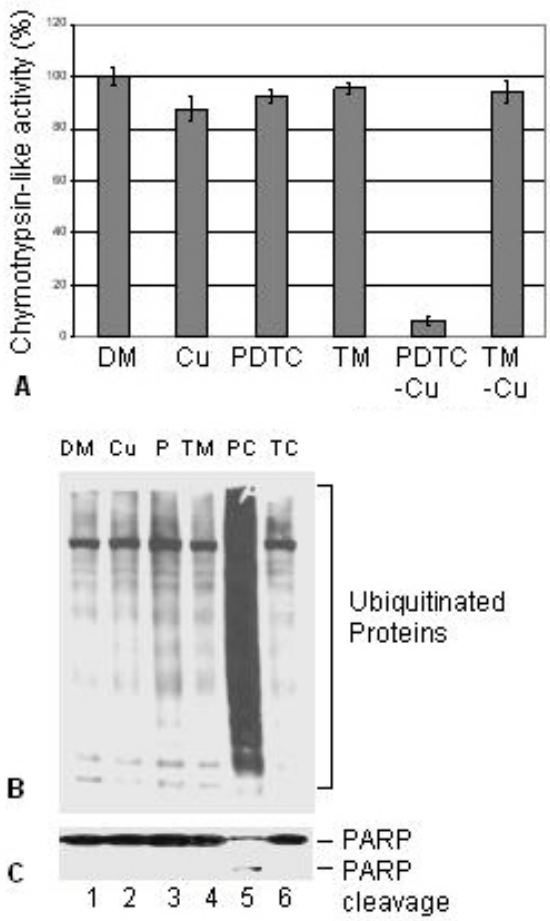

Figure 2. Inhibition of proteasome activity and induction of apoptosis in LNCaP prostate cancer cells by the PDTC-copper mixture. LNCaP prostate cancer cells were treated with 10 μM copper (Cu), PDTC (P), TM, PDTC-copper (PC), or TM-copper (TC), using DMSO (DM) as a control. Cells were collected after 24 h treatment and analyzed for proteasome inhibition and apoptosis. A. Proteasome activity as measured by release of AMCs from the substrate specific for chymotrypsin-like activity as described in Materials and Methods. Values are mean triplicates and error bars denote standard deviations. B. Western analysis was performed using anti-ubiquitin antibody for accumulation of ubiquitinated proteins as an indicator of proteasome inhibition. C. Western analysis for cleavage of PARP as an indication of apoptosis using anti-PARP antibody.

This result is consistent with a previous publication showing that PDTC is strong copper chelator (37). Therefore, PDTC may be capable of combining with endogenous copper in tumor tissues and forming an active proteasome-inhibitory complex.

4.2. The PDTC-Cu complex inhibits the chymotrypsin-like activity of the proteasome in intact LNCaP cells

Previously we reported that organic copper complexes inhibit the cellular proteasomal activity (42; Daniel KG, Chen D, Orlu S, Cui QC, Miller FR, Dou QP, submitted). We then investigated whether the PDTC-copper could inhibit the proteasome activity in human prostate cancer cell. Prostate cancer LNCaP cells were treated for 24 h with copper, PDTC, or PDTC-copper mixture, using TM and TM-copper mixture as controls. After treatment, the cells were collected and protein extracts were prepared for analysis of proteasome inhibition. Inhibition of the proteasome was measured by two assays: the chymotrypsin-like activity assay (Figure 2A), and the accumulation of ubiquitinated proteins by Western blotting (Figure 2B). We found that the prepared PDTC-copper mixture significantly inhibited the proteasome activity in LNCaP cells, as indicated by low levels of the proteasomal chymotrypsin-like activity (Figure 2A) and accumulation of ubiquitinated proteins (Figure 2B, lane 5). Copper or PDTC alone does not inhibit the proteasome activity (Figure 2A, B, lanes 2, 3). We found that neither TM nor the TM-Cu mixture was able to inhibit the proteasome activity in the prostate cancer cells (Figure 2), further supporting that TM acts as a passive copper chelator and eliminator and TM-copper is an inactive complex (42). These data support the hypothesis that a PDTC-Cu complex is capable of proteasome inhibition.

4.3. PDTC-Cu mixture effectively inhibits proliferation of human prostate cancer cells

Since PDTC can form a complex with copper, as indicated by color change (Figure 1), and is able to inhibit the proteasomal activity in prostate cancer cells (Figure 2), we then tested whether the complex is able to inhibit proliferation of human prostate cancer cells. Prostate cancer LNCaP cells were treated with copper, PDTC, PDTC-copper mixture, TM or TM-copper mixture for 24 h. We found that the PDTC-copper mixture inhibited LNCaP cell proliferation in a dose-dependent manner (Figure 3). The PDTC-copper mixture showed 50% inhibition at 1 μM and greater than 90% inhibition at 5-10 μM (Figure 3). The IC_{50} value of the PDTC-copper mixture was determined as 1 ±0.07 μM. In contrast, copper alone, PDTC alone, or TM and TM mixed with copper had no significant effect (Figure 3).

4.4. LNCaP cells treated with the PDTC-Cu complex undergo apoptosis

Having demonstrated that the PDTC-copper complex is capable of inhibiting cellular proliferation in prostate cancer cells (Figure 3), we then determined whether this mixture could induce prostate cancer cell apoptosis. Exponentially growing LNCaP cells were treated with copper, PDTC, TM or their mixtures for 24 h, followed by observing cellular and nuclear morphological changes by phase contrast or fluorescence microscope, respectively (Figure 4). The cells treated with the PDTC-copper mixture became spherical and detached (Figure 4A). By staining with Hoescht 33258, the most of the nuclei in the PDTC-copper-treated cells were brighter and denser, compared to those treated with copper, PDTC, TM alone or TM-copper (Figure 4B). These results suggested that the cells treated with the PDTC-copper mixture were undergoing apoptosis. Apoptosis induction was further verified by cleavage of PARP (Figure 2C). Treatment with

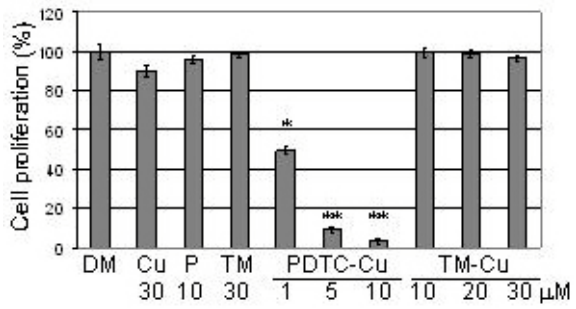

Figure 3. Anti-proliferative effects of the PDTC-copper mixture. LNCaP cells were treated for 24 h with the following: copper (Cu; at 30 μM), PDTC (P; 10 μM), TM (T; 30 μM), PDTC-copper (PDTC-Cu; 1, 5, 10 μM), or TM-copper (TM-Cu; 10, 20, 30 μM). After 24 h the media was removed and cells were treated with MTT solution as described in Materials and Methods. Measurement of MTT conversion by absorbance at 560 nm showed that LNCaP cells responded in a dose-dependent manner to PDTC-copper mixture but not to TM-copper mixture. (* P< 0.05; ** P< 0.01)

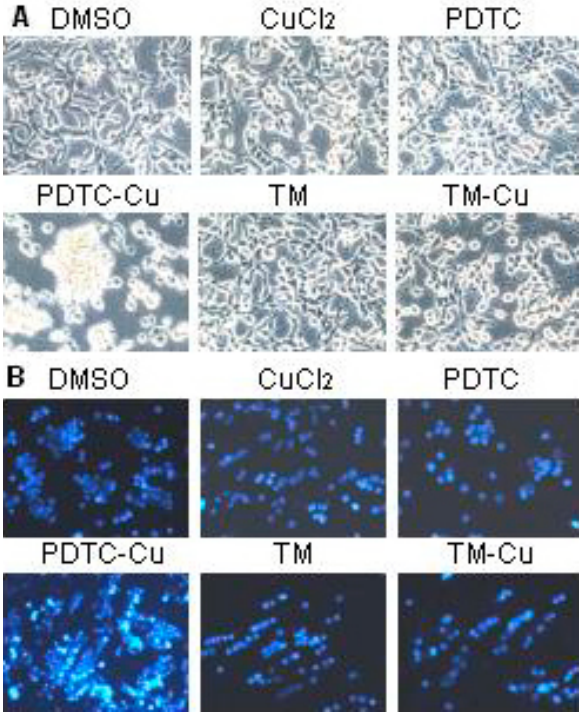

Figure 4. Cellular and nuclear morphological changes indicate induction of apoptosis by PDTC-copper mixture in LNCaP cells.

the PDTC-copper mixture, but not others, induced lost of the intact PARP and appearance of a PARP cleavage fragment (Figure 2C). Taken together, PDTC, when combined with copper, forms an active complex that has the proteasome-inhibitory, proliferation-inhibitory and apoptosis-inducing activities.

4.5. Reduction of F18-FDG uptake by LNCaP cells after treatment with PDTC-Cu complex

To prepare for evaluation of anti-tumor activity of the PDTC-Cu complex *in vivo* with ^{18}F-FDG PET imaging, we tested effects of the PDTC-Cu complex on ^{18}F-FDG uptake by LNCaP cells following treatment with the PDTC-Cu complex *in vitro* (Figure 5). Cellular ^{18}F-FDG uptake was dramatically reduced in LNCaP cells treated with 10 μM of the PDTC-Cu complex (ID% 0.05), in comparison to LNCaP cells treated with 10 μM PDTC (ID% 0.70), 10 μM copper chloride (ID% 0.74), 5% DMSO (ID% 0.94), or no treatment (ID% 1.02) (Figure 5).

5. DISCUSSION

In regards to the current anti-cancer chemotherapies, most drugs used in clinics are toxic to some extent. This is mainly due to their inability to distinguish normal cells from tumor cells. In order to eliminate toxicity, it is necessary to identify some specific properties or features of cancer cells *different from* normal cells. An increased understanding of the tumor-specific differences will facilitate the development of novel anti-cancer drugs that selectively kill cancer cells with limited toxicity or no toxicity to the normal cells.

One such difference is the ubiquitin-proteasome pathway. In cancer cells, dysregulation of this system plays a critical role for tumor progression, drug resistance and altered immune surveillance (46). Previously we reported that proteasome inhibitors induce apoptosis selectively in tumor cells but not in normal cells (47). Bortezomib, the first proteasome inhibitor as a potential anticancer drug, is currently being tested in phase III clinical trials (48, 49). Another attractive tumor-specific feature is high levels of copper found in many types of human cancers including prostate, breast, colon, lung, and brain (3-11). Molecular processes of angiogenesis include: the requirement of copper, but not other trace metals, as an essential cofactor, stimulation of endothelial growth by tumor cytokine production (*i.e.*, vasoendothelial growth factor), degradation of extracellular matrix proteins by metalloproteinases, and migration of endothelial cells mediated by intergrins (12-21).

It has been shown that cancer cells are more sensitive to proteasome inhibition than normal cells (27, 47, 50-52). We hypothesize that an inactive or nontoxic organic ligand could bind with elevated copper found in tumor tissues and result in a complex capable of proteasome inhibition. After the ligand binds with endogenous tumor cellular copper, the formed complex would inhibit the proteasome activity, leading to inhibition of the processes of angiogenesis in tumor tissues and induction of apoptosis in the tumor cells.

In search for new proteasome inhibitors of high potency and low toxicity, we found that several organic copper complexes possessing potent proteasome-inhibitory and apoptosis-inducing activities in tumor but not in normal cells (42). Since PDTC, an antioxidant used to treat inflammation, atherosclerosis and metal intoxication (34,

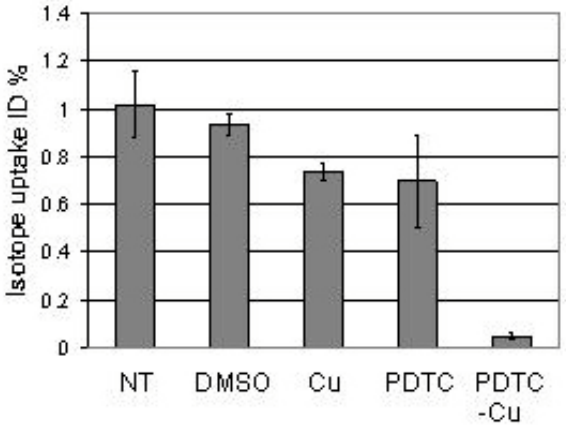

Figure 5. Cellular ^{18}F-FDG uptake assay on LNCaP cells treated with PDTC-Cu compex. Cellular uptake of ^{18}F-FDG was dramatically reduced in LNCaP cells treated with the PDTC-Cu complex in comparison to controls. PDTC, pyrrolidine dithiocarbamate; DMSO, dimethyl sulfoxide; Cu, copper chloride, NT, no treatment, Isotope Uptake ID%, percentage of inoculation dose of ^{18}F-FDG.

35), could form a copper complex (37), we hypothesized that PDTC-Cu complex may also possess proteasome-inhibitory activity. Indeed, this hypothesis was supported by the most recent study using human breast cancer cells (Daniel KG, Chen D, Orlu S, Cui QC, Miller FR, Dou QP, submitted). In the current study, we have also demonstrated that PDTC can form a new complex with copper, and the complex can inhibit proteasomal activity and cellular proliferation and induce apoptosis in prostate cancer LNCaP cells.

Once we verified that PDTC could spontaneously bind with copper and form a new complex (Figure 1), we then tested the complex in LNCaP cells to determine whether or not the complex was a proteasome inhibitor. We examined both cellular proteasome activity (Figure 2A) and accumulation of ubiquitinated proteins (Figure 2B). We found that the cells treated with PDTC-copper mixture had significantly reduced chymotrypsin-like activity (Figure 2A) and accumulation of ubiquitinated proteins (Figure 2B), indicating that proteasome inhibition had occurred. In contrast, copper, PDTC alone, TM alone, or TM mixed with copper was incapable of inhibiting the proteasome (Figure 2A and B).

We then tested whether or not the complex was capable of inhibiting proliferation of human prostate cancer cells. The results showed that only PDTC-copper mixture could inhibit proliferation of LNCaP cells in dose-dependent manner, but not copper, PDTC alone, TM alone, or TM-copper mixture (Figure 3).

Afterward we verified the association between PTDC-copper induced inhibition of proteasomal activity and proliferation, we then determined apoptosis-inducing activities of each compound in prostate cancer cells. Our result demonstrated that LNCaP cells treated with PDTC-copper mixture underwent apoptosis, as showed by PARP cleavage (Figure 2C) and cellular (Figure 4A) and nuclear (Figure 4B) morphological changes. However, LNCaP cells treated with copper, PDTC alone, TM alone, or TM-copper mixture failed to undergo apoptosis (Figs. 2C, 4A and B).

Positron-emission tomography (PET) is a sensitive, quantitative, non-invasive molecular imaging technology, which can be used to evaluate response of tumors to anticancer drugs. We tested effects of PDTC-Cu complex on ^{18}F-FDG uptake by LNCaP cells. As expected, ^{18}F-FDG uptake was dramatically reduced in LNCaP cells treated with PDTC-Cu complex (Figure 5), which suggests that the therapeutic effects of PDTC-Cu complex against prostate cancer may be monitored *in vivo* with ^{18}F-FDG-PET imaging. Slight inhibition of ^{18}F-FDG uptake was observed in the cells treated with PDTC alone, which is probably related to anti-tumor effects of small amount of PDTC-Cu complex formed with trace amount of intracellular copper. Unexpectedly, slight reduction of ^{18}F-FDG was also observed in LNCaP cells treated with 10 μM copper chloride (Cu), which may be related to inhibition of glucose transporter activity in LNCaP cells or other unknown mechanisms. These results demonstrated that it would be feasible to evaluate anti-tumor activity of PDTC-Cu complex in vivo non-invasively with ^{18}F-FDG-PET imaging.

The data presented here supports the novel concept of using accumulated copper in prostate cancer cells and tissues as a selection method for chemotherapy. Nontoxic organic compounds such as PDTC can spontaneously bind with tumor cellular copper and form a proteasome inhibitor and an apoptosis inducer. PDTC has been previously explored for use in other diseases and we believe our data support further investigation of this and other similar compounds in an anticopper/anticancer strategy.

6. ACKNOWLEDGEMENTS

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Abbreviations: PDTC, pyrrolidine dithiocarbamate; TM, tetrathiomolybdate; PARP, poly(ADP-ribose) polymerase; AMC, 7-amido-4-methyl-coumarin; MTT, 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; DMSO, dimethyl sulfoxide; [^{18}F]FDG, radiopharmaceutical 2-[^{18}F]Fluoro-2-deoxy-D-glucose; PET, positron emission tomography.

Key Words: Copper; Anti-copper drugs; Chelator; Proteasome inhibitors; Drug discovery; PET

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