A binuclear complex constituted by diethylthiocarbamate and copper(I) functions as a proteasome activity inhibitor in pancreatic cancer cultures and xenografts

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It is a therapeutic strategy for cancers including pancreatic to inhibit proteasome activity. Disulfiram (DSF) may bind copper (Cu) to form a DSF–Cu complex. DSF–Cu is capable of inducing apoptosis in cancer cells by inhibiting proteasome activity. DSF is rapidly converted to diethylthiocarbamate (DDTC) within bodies. Copper(II) absorbed by bodies is reduced to copper(I) when it enters cells. We found that DDTC and copper(I) could form a binuclear complex which might be entitled DDTC–Cu(I), and it had been synthesized by us in the laboratory. This study is to investigate the anticancer potential of this complex on pancreatic cancer and the possible mechanism. Pancreatic cancer cell lines, SW1990, PANC-1 and BXPC-3 were used for in vitro assays. Female athymic nude mice grown SW1990 xenografts were used as animal models. Cell counting kit-8 (cck-8) assay and flow cytometry were used for analyzing apoptosis in cells. A 20S proteasome assay kit was used in proteasome activity analysis. Western blot (WB) and immunohistochemistry (IHC) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were used in tumor sample analysis. The results suggest that DDTC–Cu(I) inhibit pancreatic cancer cell proliferation and proteasome activity in vitro and in vivo. Accumulation of ubiquitinated proteins, and increased p27 as well as decreased NF-κB expression were detected in tumor tissues of DDTC–Cu(I)-treated group. Our data indicates that DDTC–Cu(I) is an effective proteasome activity inhibitor with the potential to be explored as a drug for pancreatic cancer.

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Introduction

Cancer cells are more sensitive than normal cells to proteasome inhibition, and the proteasome-mediated degradation pathway is considered an important target for cancer treatment (Chen et al., 2006). The proteasome inhibitor bortezomib has been reported to inhibit tumor activity in a variety of cancer models, which was approved by the US Food and Drug Administration (FDA) for clinical treatment in multiple myeloma (MM) (Kane et al., 2006), bringing investigator confidence on inhibition of this pathway. Though copper (Cu) is an essential cofactor for tumor angiogenesis processes, some copper complexes demonstrate the capacity to inhibit proteasome activity, inducing apoptosis in various types of human cancer cells. Disulfiram (DSF) used clinically as an alcohol deterrent in the USA, is capable of binding copper to form a complex named DSF–Cu which acts as a proteasome inhibitor preferable to cancer cells (Chen et al., 2006).

Copper is largely absorbed from the stomach and the small intestine. Absorbed copper is transported to the liver in portal blood bound to albumin and is transmitted to peripheral tissues mainly bound to ceruloplasmin and, to a lesser extent, albumin. During the process of cellular copper uptake, copper enters the cell through various transmembrane transporters and copper(II) is reduced to copper(I) (Knopfell and Solioz, 2002; McKie et al., 2001). In vivo, DSF is rapidly converted to its reduced metabolite, DDTC (Escarabajal and Aragon, 2003; Pike et al., 2001), and if copper ions are available, DDTC–Cu(I) or −Cu(II) complex will be formed (Fig. 1). As other investigators described (Chen et al., 2006; Iljin et al., 2009), the activity of DSF to inhibit the cellular proteasome in vivo might be attributed to DDTC–Cu complexes.
We hypothesized that DDTC–Cu(I) play a critical role as a proteasome activity inhibitor in cancer cells. The present study is focused on whether DDTC–Cu(I) has the potential to induce apoptosis in pancreatic cancer cultures and xenografts by inhibiting proteasome activity. Though DDTC–Cu complex has been reported by other investigators before (Pang et al., 2007), their studies described the copper(II) complex mainly and this article is the first to describe the copper(I) complex especially to the best of our knowledge.

Materials and methods

Cell lines and reagents. The human pancreatic cancer cell lines, SW1990, PANC-1 and BXPC-3, were originally obtained from the ATCC (American Type Culture Collection, Manassas, VA, USA). Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). All cells were maintained at 37 °C and 5% CO2, and used at early passage numbers (passage 3–6). Sodium diethylthiocarbamate trihydrate (C7H14NS2·2Na·3H2O), copper chloride dihydrate (CuCl2·2H2O), and sodium sulfite (Na2SO3) were purchased from Sigma-Aldrich Corp. (MO, USA). The 20S proteasome assay kit was purchased from Enzo Life Sciences, Inc. (NY, USA). The rabbit polyclonal antibodies against human ubiquitin or NF-κB p50, and a mouse monoclonal antibody against human p27 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). RPMI 1640 and fetal bovine serum were obtained from Invitrogen (Carlsbad, CA, USA).

Chemical synthesis. The DDTC–Cu(I) complex for cell or animal treatment was synthesized at a concentration of 1 mmol/L as per the quantity equivalent to the molar quantity of DDTC or copper (all the concentrations to describe DDTC–Cu(I) were equivalent to the molar quantity of DDTC or copper in this article). First, sodium diethylthiocarbamate trihydrate or copper chloride dihydrate as well as sodium sulfite was dissolved in sterilized water to produce a solution of 200 mmol/L concentration respectively. Second, a total of 50 μL of a sodium diethylthiocarbamate solution, 25 μL of a sodium sulfite and 50 μL of a copper chloride which were prepared in the first step were added to 9 mL of sterilized water orderly and mixed gently. The third, sterilized water was then added to the prepared solution to ensure that the solution had a total volume of 10 mL, and the solution was gently mixed again. Finally, the complex solution was packed and stored at 4 °C.

Cell proliferation assay. Cellular proliferation was assessed by quadruplicate plating at a density of 5000 cells per well in a 96-well plate. The designated concentrations of fresh media containing the drugs or the vehicle were added to cell cultures containing the standard growth media for that cell line on the morning after plating for 12 h once the cells had attached. Viable cells were quantified after the 24-hour treatments using cell counting kit-8 (cck-8) reagent (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer’s protocol. The cck-8 solution (10 μL) was added to each well, followed by incubation for 2 h at 37 °C. The absorbance at 450 nm was determined using an Elx800 Absorbance Microplate Reader (Bio-TEK Instruments Inc., Winooski, VT, USA). Cell viability was expressed as a percentage of that of the control (untreated) cells. For each concentration of the complex, the mean value of the mean absorbance from four wells was calculated. IC50 value was calculated from a sigmoidal dose–response curve fit using Prism GraphPad 5 Demo (GraphPad Software, CA, USA).

Flow cytometric apoptosis assay. An Alexa Fluor® Annexin V/Dead Cell Apoptosis kit (Invitrogen, CA, USA) was used, according to the manufacturer’s instructions, to distinguish and quantitatively determine the percentage of apoptotic cells after treatment by the drugs. Cells were seeded at a density of 4 × 105 cells/mL in 6-well plates. After treatment, the cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), and resuspended in 100 μL of binding buffer. A total of 5 μL of Annexin V and 10 μL of propidium iodide (PI) were added, and the mixture was incubated for 30 min in the dark. Finally, 400 μL of binding buffer was added to the cells. The labeled cells (10,000 per sample at least) were analyzed by measuring the fluorescence intensity using a FC500 MPL cytometer (Beckman Coulter Inc., Brea, CA, USA) in conjunction with CXP analysis software (Beckman Coulter Inc.).

20S proteasome activity assay. A total of 17.5 ng of 20S proteasome (human) was incubated in 100 μL of assay buffer with or without different concentrations of the DDTC–Cu(I) complex, copper chloride, sodium sulfite, DDTC, or their combinations, and 10 μmol/L of the fluorogenic peptide substrate Suc-LLVY-AMC for 2 h at 37 °C. After incubation, the production of hydrolyzed AMC groups was measured with a Synergy H4 Hybrid Multi-Mode Microplate Reader (Bio-TEK Instruments Inc., Winooski, VT, USA) with an excitation filter of 365 nm and an emission filter of 460 nm. Whole-cell extracts (10 μg) of cells treated as indicated were incubated for 1 h at 37 °C in 100 μL of assay buffer with 20 μmol/L fluorogenic substrate (Chen et al., 2006).

Xenograft experiment. Five-week-old female athymic nude mice were purchased from the Shanghai Laboratory Animal Center (Chinese Academy of Science) and housed under specific pathogen-free conditions according to Fudan University animal care guidelines. The experimental animal protocols were reviewed and approved by the animal care committee of the Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences and the Fudan University Shanghai Cancer Center. Mice were maintained in laminar flow rooms under constant temperature and humidity.

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Fig. 1. Disulfiram (DSF) molecule can undergo de-oxidation and transform into diethylthiocarbamate (DDTC) which can react with metals to form coordination complexes.
First, 5 mice were used, and SW1990 cells (5 × 10^6) were injected subcutaneously into one flank of each mouse. After 3 weeks, the mice were sacrificed, and tumors were harvested. Two tumors of medium size were dissected to 1-mm^3 volumes and transplanted into 16 mice. After another 2 weeks, the mice showing tumors were randomly assigned to the control group (n = 8) or the treated group (n = 8). The following managements were administered: (a) control: received an injection of dexamethasone, 0.5 mg/kg, intra-peritoneally (i.p.), every other day (qod); (b) treatment: received 10 nmol/g DDTC–Cu(I) as well as 0.5 mg/kg dexamethasone, i.p., qod. The concentration of DDTC–Cu(I) used was 1 mmol/L. Tumor sizes were measured every 3 days, and tumor volumes (V) were determined by the equation V = (L × W^2) × 0.5, where L was the length and W was the width of tumors (Jimeno et al., 2009). The mice were treated for 25 days and sacrificed on the 25th day. The tumors were then removed and photographed. Tumor tissues were fixed in formalin for hematoxylin and eosin (H&E), immunohistochemical (IHC) staining, and TUNEL assay.

H&E staining, IHC and TUNEL assays. For H&E staining, paraffin-embedded sample slides were deparaffinized, hydrated, and then stained with hematoxylin for 1 min. After rinsing, the slides were stained with eosin for 1 min, rinsed, and sealed with cover slips using Permount. For IHC staining, endogenous peroxidase was blocked with 3% hydrogen peroxide, and the slides were then incubated with the primary anti-human antibodies, anti-p27 (Santa Cruz), anti-NF-κB p50, ubiquitin or GAPDH. Horseradish peroxidase-conjugated secondary antibodies (Roche Applied Science, Mannheim, Germany) and 3,3′-diaminobenzidine-tetrahydrochloridedihydrate (DAB; Dako, Glostrup, Denmark) as a chromogen. The TUNEL assay was performed using an Apotag kit (Roche Applied Science, Mannheim, Germany). Staining was performed by a pathologist in a blinded fashion with coded samples.

Western blotting analysis. Tumor tissues were homogenized on ice in extraction buffer using a micro-pestle. The lysate was then centrifuged at 10,000 × g for 10 min at 4 °C and the supernatants were collected. Equal amounts of protein were separated by SDSPAGE and transferred to a nitrocellulose membrane. Western blot (WB) analysis was performed using specific antibodies (Santa Cruz) against p27, NF-κB p50, ubiquitin or GAPDH. Horseradish peroxidase-conjugated secondary antibodies and the SuperSignal West Pico Chemiluminiscent substrate (Pierce Biotechnology Inc., IL, USA) were used to develop blots.

Determination of copper contents in tissues. To determine copper contents in tumors harbored by mouse as well as organs such as brains, livers and kidneys of the control or the treated group, 0.5–1.0 g of samples were placed in beakers with 5 mL of concentrated nitric acid and dried by heating in a fume cupboard (Kurasaki et al., 1998). The samples were transferred to a muffle furnace and incinerated at 450–500 °C for 4 h. The residues were dissolved with 2 mL aqua regia, and the copper in resolution was determined with an inductively coupled atomic absorption spectrometer (ICP-AAS, Model Z-5000, Hitachi, Tokyo, Japan).

Statistics. The quantitative results are represented as the mean ± standard error of at least three independent experiments. Significant differences were determined with the two-tailed Student’s t test using Office Excel 2003 or GraphPad Prism 5 Demo (GraphPad Software, San Diego, CA, USA). P-values < 0.05 were considered statistically significant.

Results

DDTC–Cu(I) induces apoptosis in pancreatic cancer cells

Based on the cck-8 assay, the 24-hour IC50 values of DDTC–Cu(I) for the three cell lines, SW1990, PANC-1 and BXPC-3, calculated were 0.59, 0.58, 0.61 μmol/L, respectively (Fig. 2). At a concentration of 5 μmol/L, DDTC–Cu(I) could effectively inhibit the proliferation of the three cell lines in vitro, but the precursors of this complex, i.e. DDTC, CuCl2, Na2SO3, or their combinations, i.e. CuCl2 + Na2SO3 (2:1), DDTC + Na2SO3 (2:1) could not (Fig. 3A).

Annexin V/PI binding was used to distinguish between the inductions of apoptosis versus necrosis. Different staining patterns in this assay enabled us to identify different cell populations: live cells (Annexin V-negative/PI-negative), early apoptotic cells (Annexin V-positive/PI-negative), late apoptotic/necrotic cells (Annexin V-positive/PI-positive), and dead cells (Annexin V-negative/PI-positive). The flow cytometry results showed that DDTC–Cu(I) at concentrations of 1, 3 or 5 μmol/L significantly increased apoptosis in SW1990 cells (Fig. 3B).

As shown in Fig. 2B, around 55% SW1990 cells remained alive after the 24-hour treatment with 5 μM DDTC–Cu(I). However, the cck-8 assay showed that the same treatment was capable of killing approximately 95% of the cells. This discrepancy might result from the different culture conditions involving the different numbers of cells plated or culture plates that 96-well ones were used in the former while 6-well ones in the latter as well as different assay systems. The cck-8 assay results should be more reliable than those from the flow cytometry. That may be verified by a morphological analysis. As shown in Fig. 3C, after the 24-hour treatment, DDTC–Cu(I) induced apparent cell shrinkage and cell rounding at 1 μmol/L and further induced extensive cell rounding and condensed morphology at 3 to 5 μmol/L, few living cells were observed. These data suggest that the DDTC–Cu(I) complex has a significantly apoptosis-inducing effect in pancreatic cancer cells.

DDTC–Cu(I) inhibits proteasome activity

A cell-free proteasome activity assay was done with the following three kinds of chemicals, i.e. DDTC, CuCl2 and Na2SO3, and their different mixtures, i.e. CuCl2 + Na2SO3 (2:1), DDTC + Na2SO3 (2:1)
and DDTC + CuCl₂ + Na₂SO₃ [2:2:1, namely DDTC–Cu(I)]. The chymotrypsin-like (CT-like) activity of the purified human 20S proteasome was inhibited to a similar level by all of the treatments containing copper (Fig. 4A), while it was not by others without copper, indicating that copper is the essential role for the complex as a proteasome activity inhibitor.

To further examine this effect of DDTC–Cu(I), SW1990 cells were treated with the complex at concentrations of 1, 3, 5 μmol/L and the vehicle. After 24 h, the cells were collected, and lysates were prepared to measure proteasome inhibition as shown by decreased levels of the proteasomal CT-like activity. The results showed that proteasomal CT-like activities were decreased by around 8%, 70% and 94% in the cancer cells treated by 1, 3 and 5 μmol/L DDTC–Cu(I), respectively, compared with the vehicle-treated cells (Fig. 4B). Based on morphological analysis, cells treated by 5 μmol/L DDTC–Cu(I) were mostly dead. Taken together, the results indicate that the complex is capable of inducing apoptosis in cancer cells via inhibiting proteasome activity.

**DDTC–Cu(I) arrests the tumorigenesis of pancreatic cancer cells in vivo**

The mice harboring SW1990 cell xenografts were treated with DDTC–Cu(I), and dexamethasone was used to reduce the adverse effects of the treatment. During the 25-day management, only one mouse in the treatment group died (on day 17), and the others survived to the end of the experiment. According to the tumor volumes measured on the 25th day, the tumor growth in the treatment group was inhibited by 62.8% (P < 0.01) compared with the control (Fig. 5A). Meantime, the growth inhibition was reflected by tumor weights (Fig. 5B).

Apoptosis occurred more frequently in tissues derived from xenografts in mice that received DDTC–Cu(I) treatment than that in mice treated by dexamethasone alone as detected by the TUNEL assay (48.7 ± 5.2% versus 3.4 ± 0.7%). Apoptosis induction in tumor samples was also observed in the H&E staining. In contrast, apoptosis was not apparent in the control xenograft tumors (Fig. 5C).

The stabilization of ubiquitinated proteins and p27 as well as the inhibition of NF-κB activation are described as the effects of proteasome inhibitors (McDade et al., 1999; Zavrski et al., 2007). The increased accumulation of ubiquitinated proteins, and up-regulated expression of p27 and or the down-regulated of NF-κB in DDTC-Cu(I)-treated group were observed by WB assay and confirmed by IHC compared to the control (Figs. 5C and D), indicating that DDTC–Cu(I) might arrest the tumorigenesis of pancreatic cancer cells via inhibiting proteasome activity in vivo.

**Copper level keeps stable relatively within treated mice bodies**

The tissue copper content analysis results showed that copper contents in the tumors harbored by mice administered DDTC–Cu(I) increased, meantime, copper contents in the brains, the livers, as well as the kidneys of the treated group mice increased also compared with the control group (Fig. 6). Among the tissues, the copper level of tumors is higher than in the three organs, suggesting that copper was enriched in tumor tissues and the amount might be even more than that in the liver which is the organ that stores copper within mammal bodies. Furthermore, we could find that the copper levels in either the treated or the control group were not increased as dramatically as that in the cases of Wilson disease (Suzuki, 1995; Shim and Harris, 2003), indicating that

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**Fig. 3.** The effects of DDTC–Cu(I) on pancreatic cancer cell lines. A. Cck-8 assay. SW1990, PANC-1 and BXPC-3 cell lines were treated with 5 μmol/L of DDTC–Cu (I), CuCl₂ + Na₂SO₃, DDTC + Na₂SO₃, CuCl₂, DDTC or Na₂SO₃ for 24 h, followed by the cck-8 assay as described in Materials and methods. Columns, the mean cell viability of the three cell lines; bars, SD. *P < 0.01 versus the control. B. Annexin V/PI double staining was measured after 0, 1, 3, 5 μM DDTC–Cu(I) treatment in SW1990 cells for 24 h. In all panels, cells in the lower left quadrant are alive, cells in the lower right quadrant are in early apoptosis, cells in the upper right quadrant are in late apoptosis, and cells in the upper left quadrant are dead. C. Cell morphology changes (spherical and detached) were photographed under a microscope after 24 h of the indicated treatments. Scale bars, 50 μm.
the copper element injected into mice still could be regulated by their metabolisms.

**Discussion**

Despite the treatment modalities available for pancreatic cancer (e.g., chemoradiation, surgery, or a combination of these), it has the worst prognosis of all the major malignancies, with less than 5% of patients alive 5 years after diagnosis. Therefore, new treatment modalities for pancreatic cancer patients are urgently needed. A promising approach is the targeted therapy that is to block ubiquitin/proteasome pathways. The complex constituted by copper and DSF attracts worldwide interest recently for its anticancer effect as a proteasome activity inhibitor (Chen et al., 2006; Daniel et al., 2005; Li et al., 2008; Liu et al., 2012). Investigators discovered that DSF–Cu complex could inhibit proteasome activity in vitro, moreover, their studies revealed that if

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**Fig. 4.** Proteasome-inhibitory effects of DDTC–Cu(I). A. Cell-free proteasomal CT-like activity assay for the indicated chemicals or their combinations. The CT-like activity was inhibited by DDTC–Cu(I), CuCl (DDTC + Na2SO3) or CuCl2, but was not inhibited by the other chemicals alone or their combinations, indicating that copper(I) or copper(II) is the central factor for proteasome activity inhibition. Concentration: 10 μmol/L. *P < 0.01 versus the control. B. Proteasomal CT-like activity assay with SW1990 cells on the indicated treatments. *P < 0.01 versus the control.

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**Fig. 5.** Effects of DDTC-Cu(I) on the tumorigenesis of SW1990 cells in vivo. A. Tumor growth chart showing the effect of the treatments in vivo. Points indicate the mean tumor volume in each experimental group; bars, SD. *P < 0.01. B. Comparison of final tumor weights after in vivo treatments. Lower panel, representative tumor pictures of the treatment group or control group. Upper panel, final weights after treatments. Columns, mean; bars, SD. C. IHC assessment of ubiquitin, p27, NF-κB and H&E as well as TUNEL assays. Tumors were collected after 25-day treatment and the prepared tissue slides were used for immunostaining with NF-κB, p27 and ubiquitin antibodies. The TUNEL assay shows apoptotic cells (brown) in tumor tissue. Scale bars for all panels, 20 μm. D. Western blot analysis of tumor tissue extracts with ubiquitin, p27, NF-κB, or GAPDH antibodies. The accumulation of ubiquitinated proteins, p27, and reduced NF-κB expression were detected in the tissue extracts of tumors treated with DDTC–Cu(I).
Fig. 6. Copper contents of tumors, livers, brains or kidneys of mice administered with vehicle or DDTC–Cu(I) for 25 d, n = 8. Error lines are SD.

DSF was injected to animals, DSF might bind copper to form this complex in vivo. However, several kinds of complexes including DDTC–Cu(I) and DDTC–Cu(II) might be formed when DSF is administrated to human or animals, because DSF is metabolized to DDTC in vivo and copper(I) as well as copper(II) exists.

Copper has a long history of medical application. Its potential anticancer properties have been explored in the last few decades after the discovery of cisplatin (Radulovic et al., 2002). A few copper complexes have been found to be anticancer active and there is a huge scope in the design of more potent but less toxic copper-based anticancer drugs (Iakovidis et al., 2011; Wang and Guo, 2006). Though many copper-based anticancer agents have proved cytotoxic in vitro, their further utility is limited by the poor water solubility and relatively high toxicity in vivo. Our studies focused on the function of complexes constituted by DSF and various metals primarily. We found that reducing agents such as sodium sulfite may decompose DSF to DDTC and form new complexes with copper(I) or copper(II). We also found that a copper complex with a ligand of either DSF or DDTC, has anticancer activity, however, only the complex constituted by DDTC and copper(I) at ratio of 1:1 is resolved in water. To make certain that the structure of DDTC–copper(I) was synthesized in our study, we did a series of assays and the structure was worked out as a binuclear complex according to the mass spectra mainly (seen in Supplementary material 1).

As described above, in the studies on DSF–Cu complex, the proteasome activity inhibition was examined in vitro, however, this effect in vivo was examined by the protocol that DSF was injected into animals, which is based on the possible mechanism that DSF binds copper in vivo. Due to the various copper amounts in different tumors and the intricate internal environment of bodies, whether DSF–copper complex can be formed is unknown for an individual. As cisplatin or other metal complexes used clinically had been prepared manually outside bodies (Lai et al., 2011), an aqueous solution of DDTC–Cu(I) can be produced by the protocol described in this article. Unlike DSF–Cu which needs dimethyl sulfoxide (DMSO) to facilitate dissolution (Chen et al., 2006; Zhang et al., 2010), DDTC–Cu(I) is dissolved in water directly.

In a previous study on DSF–Cu, the investigators described that DSF carries copper ions into tumor cells thereby preventing copper from interacting with many nonspecific proteins. They also theorized that the copper is responsible for inhibiting the proteasome (Chen et al., 2006). The cell-free proteasome activity assay in this study showed that DDTC–Cu(I) was not more potent in inhibiting proteasome activity than CuCl2 or CuCl (CuCl2 + Na2SO3), perhaps because the coordination of DDTC interferes with the interaction between copper ions and the proteasome (Fig. 4A). The data support the hypothesis that it is copper to inhibit proteasome activity.

We carried out a preliminary experiment actually before the reported in vivo experiment. In the preliminary experiment, DDTC–Cu(I) was used without dexamethasone to mice harboring tumors. Though the tumor growth was retarded by DDTC–Cu(I) significantly after 21-day management, some adverse effects such as hemafecia, anorexia, and maranasis were observed (seen in Supplementary material 2), suggesting to us the use of dexamethasone as an adjuvant drug in the formal experiment. No obvious adverse effects were observed when dexamethasone was used along with DDTC–Cu(I), which indicates that DDTC–Cu(I) could demonstrate anticancer activity at a tolerable dosage.

The copper homeostatic mechanisms could play an important role in the prevention of copper toxicity, while exposure to excessive levels of copper can result in a number of adverse health effects including liver and kidney damage, anemia, immunotoxicity, and developmental toxicity (Bonham et al., 2002). Our tissue copper content analysis results showed that DDTC–Cu(I) treatment on mice could increase the copper levels in tumors and other tissues such as livers, brains as well as kidneys. Among the tissues, the copper contents of tumors are higher than in the three organs, indicating that copper was enriched in the pancreatic cancer SW1990 xenograft tumors. However, these increases were not as dramatic as those induced by copper metabolism disorders such as Wilson disease (Suzuki, 1995; Shim and Harris, 2003). Besides, we could find that the copper contents in either the treated or the control group was not beyond the normal range of the tissues (Li et al., 1991), these might be attributed to the regulation of metabolism and/or the reason that DDTC could act as an anti-copper drug.

As an essential trace element for most organisms, copper might act as a “double-edged sword” by promoting physiological and malignant angiogenesis. A few copper complexes exhibit anticancer potential as proteasome inhibitors, inducing apoptosis in various types of human cancer cells. In this study, a new bincular complex, DDTC–Cu(I), was synthesized with DDTC which is the monomer of DSF, copper chloride, as well as sodium sulfite, and the data presented here support that it has the potential to inhibit proliferation of pancreatic cancer cells in vitro and tumor growth significantly in vivo via inhibition of proteasome activity.

Conflict of interest statement

The authors declare no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.taap.2013.09.009.

References


