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Organic cadmium complexes as proteasome inhibitors and apoptosis inducers in human breast cancer cells

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ABSTRACT

Although cadmium (Cd) is a widespread environmental contaminant and human carcinogen, our studies indicate an organic Cd complex to be a potent inhibitor of proteasomal chymotrypsin-like (CT-like) activity, further capable of inducing apoptosis in a cancer cell-specific manner. It has been reported that the ligands indole-3-butyric acid (L1) and indole-3-propionic acid (L2) have cancer-fighting effects when tested in a rat carcinoma model. In addition, 3, 5-diaminobenzoic acid o-vanillin Schiff bases (L3) have high antimicrobial activity and a large number of Schiff base complexes have been reported to have proteasome-inhibitory activity. We therefore hypothesized that synthetic forms of Cd in combination with L1, L2 and L3 may have proteasome-inhibitory and apoptosis-inducing activities, which would be cancer cell-specific. To test this hypothesis, we have synthesized three novel Cd-containing complexes: $[Cd_2(C_{12}H_{12}O_2N)_4(H_2O)_2]\cdot 2H_2O$ (Cd1), $[Cd_2(C_{11}H_{10}O_2N)_4(H_2O)_2]\cdot 2H_2O$ (Cd2) and $[Cd(C_7H_4N_2O_2)(C_8H_6O_2)_2]\cdot 2H_2O$ (Cd3), by using these three ligands. We sought out to characterize and assess the proteasome-inhibitory and anti-proliferative properties of these three Cd complexes in human breast cancer cells. Cd1, Cd2 and Cd3 were found to effectively inhibit the chymotrypsin-like activity of purified 20S proteasome with IC_{50} values of 2.6, 3.0 and 3.3 μM, respectively. Moreover, inhibition of cancer cell proliferation also correlated with this effect. As a result of proteasomal shutdown, the accumulation of ubiquitinated proteins and the proteasome target IκB-α protein as well as induction of apoptosis were observed. To account for the cancer specificity of this effect, immortalized, non-tumorigenic breast MCF10A cells were used under the same experimental conditions. Our results indicate that MCF10A cells are much less sensitive to the Cd1, Cd2 and Cd3 complexes when compared to MDA MB 231 breast cancer cells. Therefore, our study suggests that these Cd organic complexes are capable of inhibiting tumor cellular proteasome activity and consequently induce cancer cell-specific apoptotic death.

1. Introduction

The ubiquitin proteasome system (UP-S) regulates a number of key cellular processes, including cell proliferation and death through degradation of specific proteins involved [1,2]. Selective degradation of proteins is indeed a slippery slope, critical for protein homeostasis in normal cells, but dysregulated in cancer cells. The UP-S has therefore been extensively studied as a novel molecular target for the development of novel drugs in an attempt to restore protein homeostasis as the ultimate therapeutic strategy. The 20S proteasome, the main component of the UP-S, is a high molecular weight protease complex with a proteolytic core containing subunits including β1, β2 and β5, which are responsible for its caspase-like, trypsin-like and chymotrypsin-like (CT-like) activities, respectively [3]. It is well established that inhibition of the β5 proteasomal subunit, and therefore its CT-like activity, is primarily associated with apoptosis induction in tumor cells [4–6]. Furthermore, this shutdown also leads to the accumulation of several target proteins (*i.e.*, IκB-α), followed by an induction of programmed cell death, or apoptosis [7–9].

Metal-based anti-cancer drugs were developed many years ago. Our laboratory has studied a number of the metal-based drugs, including organic copper-, zinc-, and gold-based complexes, all of which are

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capable of inhibiting the tumor cell proteasome and thus proliferation, thereby inducing cancer cell death [10–14]. We have also reported that mixtures of disulfiram (DSF) and cadmium (Cd) can selectively inhibit proteasome activity in human breast cancer cells, over their "normal", immortalized, non-tumorigenic counterparts, and ultimately result in apoptosis [15]. Cd is an environmental hazard whose effects from a long term exposure are substantially controversial. Several incidents have been reported about general population of Cd poisoning due to continual consumption of contaminated food and water [16]. It has recently been proposed that Cd ingestion may increase one's risk of developing breast cancer [17]. This however, is an area of ongoing investigation and Cd's actual carcinogenic effects, in regard to breast cancer incidence, remain to be determined.

Nevertheless, the literature does point to cases where Cd has been shown to affect cell proliferation, differentiation, as well as apoptosis. Studies have shown that Cd has an effect on p38/MAPK isoforms [18] and plays an important role in the promotion of breast cancer cell growth by potentiating the interaction between ERα and c-Jun [19]. Other reports link Cd exposure to genomic instability, aberrant gene expression, and inhibition of DNA damage repair and apoptosis through complex and multifactorial mechanisms [20,21]. Conversely though, Cd has also been shown to induce p53-dependent apoptosis [22] and down-regulation of the x-linked inhibitor of apoptosis protein (XIAP) in human prostate cancer cells [23]. Interestingly, synthetically made meclofenamic acid–Cd complexes have anti-proliferative activity in the breast cancer cell line MCF7, bladder cancer cell line T24, and the non-small cell lung carcinoma line A549 [24]. So the question becomes: is Cd just a causal factor in these studies, or does it really possess the potential to inhibit cancer cell proliferation? The answer and involved mechanism(s) remain unknown. It is likely that Cd may exert a paradoxical effect in breast cancer [25] perhaps dependent on the form it exists in: free Cd, protein-bound Cd, and Cd complexed with novel ligands such as those described in this study, namely, indole-3-butyric acid (L1) or indole-3-propionic acid (L2), may exert either favorable or unfavorable effects in a breast cancer system. We propose that Cd, at least when it is complexed to the above ligands, exerts a very favorable anti-tumor effect in breast cancer cells. Many years ago, L1 and L2 were shown to possess cancer-preventive effects in a rat carcinoma model [26]. L2 also potently and *via* iron-inducing mechanisms, caused oxidative damage to cell membranes and, potentially, prevented carcinogenesis [27]. We have previously reported that the L-glutamine Schiff base copper complex [11], taurine Schiff base copper complex [12] and quinoline-2-carboxaldehyde Schiff base copper complexes [28] could act as potent proteasome inhibitors and induced apoptosis. Similarly, 3, 5-diaminobenzoic acid o-vanillin Schiff base (L3) has also been used in the synthesis of such compounds [29].

We hypothesized that synthetic forms of Cd with L1, L2 and L3 may have cancer-specific proteasome-inhibitory and apoptosis-inducing activities. To test this hypothesis, we have synthesized three novel Cd-containing complexes: $[Cd_2(C_{12}H_{12}O_2N)_4(H_2O)_2]\cdot 2H_2O$ (Cd1), $[Cd_2(C_{11}H_{10}O_2N)_4(H_2O)_2]\cdot 2H_2O$ (Cd2) and $[Cd(C_7H_4N_2O_2)(C_8H_6O_2)_2]\cdot 2H_2O$ (Cd3) (Fig. 1) by using indole-3-butyric acid (L1), indole-3-propionic acid (L2) and 3, 5-diaminobenzoic acid o-vanillin Schiff base (L3), respectively, as ligands. We report here that these Cd complexes are potent inhibitors of the proteasome and inducers of apoptosis, effects which appear to be specific to tumor cells. We have first characterized and assessed these newly synthesized Cd complexes. We then compared the ability of the similar metal complexes containing copper (Cu), zinc (Zn) or Cd to inhibit breast cancer cell proliferation using the estrogen receptor (ER)-positive MCF7 and ER-negative MDA MB 231 breast cancer cell lines. Of the compounds tested, the Cd-containing versions appear to be the most potent inhibitors of cellular proteasome CT-like activity and effective inducers of apoptosis in breast cancer cells, but not in non-tumorigenic breast epithelial MCF10A cells. Additionally, these newly synthesized Cd compounds are superior in potency and cancer selectivity to the DSF-Cd mixture.

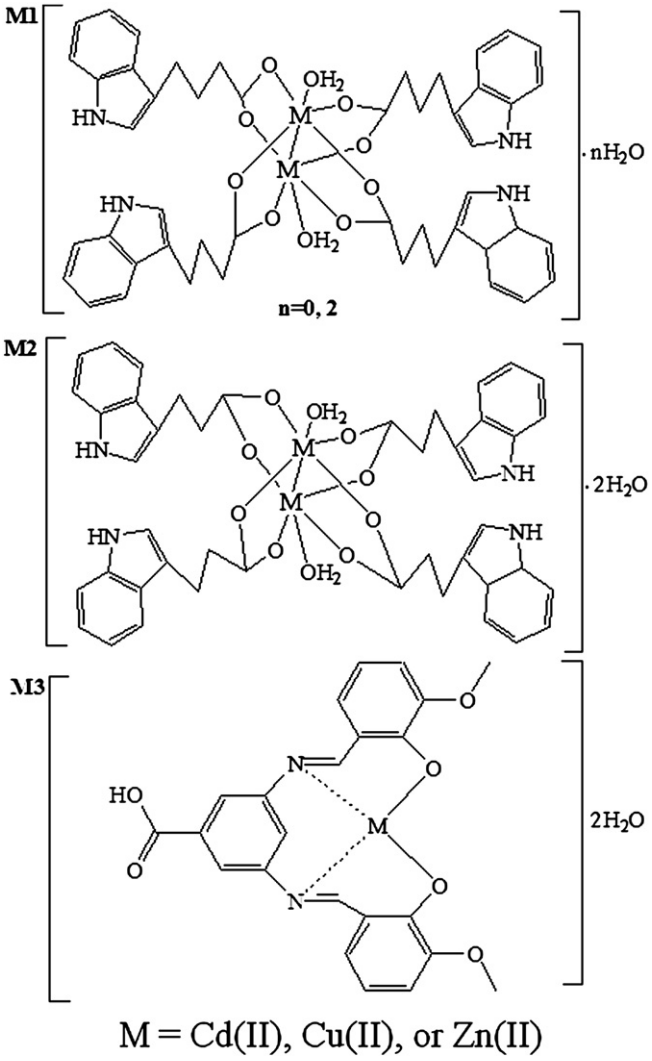

Fig. 1. Chemical structures of M1, M2 and M3 (M = Cd(II), Cu(II), or Zn(II)).

2. Experimental

2.1. Materials

Indole-3-butyric acid, indole-3-propionic acid, 3, 5-diaminobenzoic acid and cadmium acetate were all purchased from Aladdin (Los Angeles, CA). The chemical agents, DMSO and 3-[4, 5-dimethyltiazol-2-yl]-2.5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). All compounds were made as 50 mM stocks in DMSO and stored at 4 °C. DMEM/F12 (1:1), RPMI-1640 and penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Aleken Biologicals (Nash, TX, USA). The fluorogenic peptide substrate Suc-LLVY-AMC (for the CT-activity assay) was purchased from Calbiochem (San Diego, CA). Rabbit polyclonal antibody against human poly (ADP-ribose) polymerase (PARP) (H-250) was purchased from BD Bioscience Pharmingen (San Diego, CA). Mouse monoclonal antibodies against ubiquitin (P4D1) and IκB-α (H-4), goat polyclonal antibody against β-actin (C-11) and all secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA).

2.2. Metal complex syntheses

Cd1, Cd2, Cu1, Cu2, Zn1, Zn2: Synthesis of these complexes followed the previously described procedure [30]. The ligand $C_{12}H_{13}O_2N$ or $C_{11}H_{11}O_2N$ (1 mM) was dissolved in 5 ml of H_2O. M $(CH_3COO)_2\cdot 2H_2O$