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Original Research Article

Diethyldithiocarbamate complexes with metals used as food supplements show different effects in cancer cells

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ABSTRACT

Diethyldithiocarbamate (ditiocarb), a metabolite of the old anti-alcoholic drug disulfiram (Antabuse), forms proteasome-inhibiting metal complexes with copper or zinc that suppress cancer cells both *in vitro* and *in vivo*. The drug has been used in a clinical trial (NCT00742911) along with copper gluconate as a dietary supplement in patients with cancer spreading to the liver. In this study, we demonstrate the effect of synthetic complexes of disulfiram with four various metals (Mn, Fe, Cr and Cu) used as food supplements. These complexes may be spontaneously formed in the blood during the use of disulfiram with divalent metals and thus may suppress the growth of cancer *in vivo*. The cytotoxic effect of the compounds and the compounds' ability to inhibit the cellular proteasome were tested in the osteosarcoma cell line U2OS. After 48 h, copper and manganese complexes exhibited cytotoxic effect on the cell line, in sharp contrast to both iron and chromium complexes.

Introduction

Dithiocarbamate compounds have been effective against cancer in animal models and in the clinical studies (Cvek and Dvorak, 2007; Cvek, 2011). Chemically, dithiocarbamates are the reduced form of thiuram disulfides whit strong complex-forming properties and rich coordination chemistry (Thorn and Ludwig, 1962), which facilitates a large array of uses. For instance, thiuram disulfides and dithiocarbamates are used in the rubber vulcanization process (Nieuwenhuizen et al., 1997) or as pesticides, with potential side effects, in agriculture (Environmental Health Criteria, 1988; Chou et al., 2008). Disulfiram, a thiuram disulfide clinically used under the brand name Antabuse, is converted to diethyldithiocarbamate in the body and shows intriguing biological activity and a safe pharmacological profile (Børup et al., 1992; Cvek and Dvorak, 2007). Most importantly, patients on disulfiram exhibit

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inhibited aldehyde dehydrogenase (ALDH) activity (Suh et al., 2006). ALDH is an essential enzyme in ethanol metabolism, converting acetaldehyde to acetate. Consequently, disulfiram's nonspecific interaction with enzyme sulfhydryl groups (Johansson, 1992) has been utilized in the treatment of chronic alcoholism (Bell et al., 2012). Other cellular effects and properties of these compounds are described in several recent publications (Xu et al., 2011; O'Brian et al., 2012). Among these effects and properties, the most discussed is antitumor activity (Cvek, 2011), which was described, for the first time, in the late 1970s by Lewison (1977). Moreover, it was experimentally demonstrated that the administration of disulfiram prevents induced tumorigenesis in laboratory animals (Wattenberg, 1975; Sunderman et al., 1984). These findings gradually led to the establishment of a double-blind, placebo-controlled phase II clinical trial for disulfiram's main metabolite, diethyldithiocarbamate (ditiocarb), in patients with nonmetastatic, high-risk breast cancer. At 5 years, overall survival was 81% in the ditiocarb group compared with 55% in the placebo group (Dufour et al., 1993). The results of this clinical trial confirmed the potent anticancer activity of diethyldithiocarbamate in humans. The antitumor effect of disulfiram has been potentiated by synchronous administration with zinc gluconate, leading to clinical remission in a melanoma patient with a liver metastasis (Brar et al., 2004). It was consistently found that a complex of ditiocarb and metal (e.g., copper that is formed in the body after disulfiram digestion; Johansson, 1992) is responsible for the antitumor activity, rather than disulfiram or ditiocarb alone (Viola-Rhenals et al., 2006). The diethyldithiocarbamate–copper complex most likely targets the cellular proteasome and can inhibit the proteasome (Chen et al., 2006; Cvek et al., 2008). The proteasome is essential for cancer cells (Drexler et al., 2000) and is involved in many important cell signaling pathways and processes, such as nuclear factor-κB signaling, cell cycle progress, proliferation, the immune response and maintenance of cellular protein homeostasis (Finley, 2009; Bedford et al., 2010). Thus, inhibition of the chymotrypsin (CT)-like activity of the proteasome is selectively lethal to cancer cells in an apoptotic manner (Adams, 2004; Cvek and Dvorak, 2011). Moreover, many organic complexes with diverse metals are likely able to inhibit the proteasome and suppress cancer cells in this way (Chen et al., 2005; Verani, 2012). Given the strong metal-binding properties of diethyldithiocarbamate (Hogarth, 2012), we hypothesize that this compound can chelate other biogenic metals in the human body and create various complexes with diverse biological activities *in vivo*. To examine the biological activity of such complexes, we synthesized diethyldithiocarbamate complexes with metals used as dietary supplements, i.e., Cu, Mn, Fe and Cr (Wong, 2012). Although the exact mechanism of action of disulfiram and its metabolites in cancer cells remains unclear, several clinical trials have been performed in the United States. For instance, disulfiram was used in combination with copper for the treatment of refractory solid tumors involving the liver (ClinicalTrials.gov Identifier NCT00742911) or in combination with arsenic trioxide for the treatment of metastatic melanoma (ClinicalTrials.gov Identifier NCT00571116). These trials suggest the great potential of diethyldithiocarbamate–metal complexes to become a cheap and effective cancer cure.

Materials and methods

Materials

Sodium diethyldithiocarbamate trihydrate, manganese(III) acetate ($Mn(MeCOO)_3$), iron(III) chloride hexahydrate ($FeCl_3{\bullet}6H_2O$), chromium(III) nitrate nonahydrate ($Cr(NO_3)_3{\bullet}9H_2O$), copper(II) chloride dihydrate ($CuCl_2{\bullet}2H_2O$), fetal bovine serum, dimethylsulfoxide (DMSO) and DMEM were purchased from Sigma–Aldrich. Penicillin/streptomycin (100×) was purchased from PAA. A substrate (Suc-LLVT-AMC) for the determination of CT-like 20S proteasome activity was purchased from Sigma–Aldrich. Complete protease inhibitor cocktail tablets and the phosphatase inhibitor PhosSTOP were purchased from Roche Diagnostics GmbH. A mouse monoclonal antibody against Poly (ADP-ribose) polymerase (PARP-1) was purchased from Cell Signaling Technology. Mouse monoclonal antibodies against ubiquitin (P4D1), a rabbit polyclonal antibody against IκBα (C-15), a goat polyclonal antibody against actin (I-19), secondary antibodies and luminol for Western blotting were purchased from Santa Cruz Biotechnology.

Synthesis of the complexes

Tris-(*N*,*N*-diethyldithiocarbamato)-manganese(III), -iron(III) and -chromium(III) complexes were prepared from aqueous solutions of $Mn(MeCOO)_3$, $FeCl_3{\bullet}6H2O$ and $Cr(NO_3)3{\bullet}9H_2O$, respectively, in the presence of a three-fold molar excess of sodium diethyldithiocarbamate trihydrate, with constant stirring and in air. All complexes precipitated as solid powders, which were collected by filtration; washed, first with water and then with EtOH; and dried in vacuo for 24 h (yields: 80%, 74% and 78%, respectively, for the Mn(III), Fe(III) and Cr(III) complexes). The bis-(*N*,*N*-diethyldithiocarbamato)-copper(II) complex was synthesized and described according to a reported method (Hogarth, 2005; Cvek et al., 2008).

Structural characterization of the complexes

The obtained complexes were characterized by IR spectroscopy, ESI mass-spectrometry and elemental analysis. Infrared spectra (4000–400 cm^{-1}) were recorded on a BIO-RAD FTS 3000 MX instrument in KBr pellets. Far infrared spectra (400–200 cm^{-1}) were recorded on a Vertex 70 spectrophotometer, in CsI pellets. Wavenumbers are in cm^{-1}; abbreviations: *s*, strong; *m*, medium; *w*, weak. ESI^+/ESI^- mass spectra were obtained on a VARIAN 500-MS LC ion trap mass spectrometer (solvent: dimethylsulfoxide; flow: 20 μL/min; needle spray voltage: ±5 kV, capillarity voltage: ±100 V; nebulizer gas (N_2): 35 psi; drying gas (N_2): 10 psi; drying gas temperature (N_2): 350 °C). For the MS spectra description, M denotes the metallic complexes. C, H and N elemental analyses were carried out by the Microanalytical Service of the Instituto Superior Técnico, Lisbon. The obtained spectroscopic features are in agreement (Healy and White, 1972a,b; Almeida et al., 1996; Hogarth, 2012) with the proposed η^2-coordination of *N*,*N*-diethyldithiocarbamate to the metal centers.

Tris-(*N*,*N*-diethyldithiocarbamato)manganese(III), [Mn(η2-S2CNEt2)3]: IR (KBr pellet, cm^{-1}): 1470 s, ν(C–N); 1250 m,