Antagonism of cadmium cytotoxicity by differentiation inducers

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Abstract

Studies on the antagonism of toxicity can provide information about toxic mechanisms and suggest chemotherapeutic strategies. A rapid cell growth assay that measures the effects of test agents on the accumulation of cell protein (Shopsis and Eng, Toxicol. Lett. 1985;26:1) has been applied to studies of the antagonism of the cytotoxicity of cadmium. Exposure of Balb/c mouse 3T3 cells to 15 μmol/L Cd²⁺ for 24 h or 7 μmol/L Cd²⁺ for 48 h caused a 50% decrease in total cell protein. Zn²⁺ and selenite ion, antagonists of Cd toxicity in vivo, antagonized Cd²⁺ cytotoxicity when added in micromolar concentrations at the initiation of exposure to Cd²⁺. A diverse group of chemicals that can induce differentiation in vitro in cultured erythroleukemia and other cells were also found to antagonize the cytotoxic effects of Cd²⁺ to 3T3 cells. Dimethyl sulfoxide (DMSO), hexamethylene bisacetamide, N,N-dimethyl formamide, N-methyl formamide, dimethyl acetamide, hypoxanthine, hemin, ouabain, and sodium butyrate, when added to cultures simultaneously with Cd²⁺, each antagonized Cd²⁺ toxicity. These agents were used at concentrations equal to or lower than the concentrations at which they induce cellular differentiation. Other cytotoxicity assays and morphological studies confirmed these observations. DMSO added as much as 6 h after the initiation of a 24-h exposure to Cd²⁺ still protected cells; conversely, pretreatment of cultures with butyrate or DMSO for 24 h followed by their removal did not confer protection against subsequent Cd²⁺ challenge. Ethanol and methanol (noninducers of differentiation) did not antagonize Cd²⁺ cytotoxicity, and differentiation-inducing agents did not protect the cells from Zn²⁺- or Hg²⁺-induced cytotoxicity. DMSO treatment does not induce an increase in the concentrations of metallothionein or glutathione in these cells.

Abbreviations: AZA, 5-azacytidine; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; DMSO, dimethyl sulfoxide; DTNB, 5,5’-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; GSH, reduced glutathione; GSSG, oxidized glutathione; HMBA, hexamethylene bisacetamide; MELC, murine erythroleukemia cells; MT, metallothionein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SEM, standard error of the mean; Tris, tris(hydroxymethyl)aminomethane
Introduction

Cadmium is a highly toxic trace element with no known role in normal metabolism. It accumulates in mammalian kidneys and liver, binding to the low-molecular-mass cysteine-rich protein metallothionein (Piscator, 1964). Cd\(^{2+}\) has a half-life in humans of 10-30 years (Friberg et al., 1974). It has been implicated in a variety of disease processes, including bone, kidney, liver and cardiovascular damage (reviewed by Bernard and Lauwerys, 1986), and its acute administration causes testicular damage in rodents (reviewed by Samarawickrama, 1979). The mechanisms by which Cd\(^{2+}\) exerts toxic action are unknown.

Studies with agents that antagonize toxicity can provide information about toxic mechanism and may indicate possible prophylactic and chemotherapeutic solutions to toxic hazards. Zn\(^{2+}\) (Parizek et al., 1969; Leber and Miya, 1976) and selenite (Parizek et al., 1971; Gasiewicz and Smith, 1976) antagonize the acute toxicity of cadmium in vivo. Several laboratories have also demonstrated the antagonism of Zn\(^{2+}\) to Cd\(^{2+}\) toxicity in vitro (Huang et al., 1980; Stacey and Klaassen, 1981) and suggested metallothionein induction and competitive inhibition of Cd\(^{2+}\) uptake as mechanisms of antagonism. Dithiothreitol (Stacey, 1986) and 5-azacytidine (Waalkes et al., 1985) have also been shown to protect cells from Cd\(^{2+}\), the latter by potentiating the cells’ capacity to produce metallothionein. Stacey and Klaassen (1981) reported that selenite did not protect primary hepatocytes from the toxic effects of Cd\(^{2+}\). Jones and his collaborators have demonstrated the effectiveness of dithiocarbamates as Cd\(^{2+}\) antagonists that function by chelating the cation (Gale et al., 1988; Zhao et al., 1990).

In 1971 Friend et al. reported that dimethyl sulfoxide (DMSO) can induce differentiation in cultured mouse erythroleukemia cells (MELC), causing these tumor cells to cease proliferating and express differentiated functions, including the synthesis of hemoglobin. Subsequently a number of other chemical and physical agents were identified as differentiation inducers for MELC and other cells (reviewed by Rifkind et al., 1984; Marks et al., 1985). The mechanism underlying this differentiation induction in vitro has not been determined. In addition to DMSO, the inducing chemicals include other polar-planar compounds such as hexamethylene bisacetamide (HMBA) and N-methyl formamide, and other apparently unrelated substances, including hypoxanthine, hemin, and ouabain (reviewed by Marks and Rifkind, 1978), and vitamin D\(_3\) and retinoic acid (Haskovec et al., 1990).

In the course of investigating the effects of solvents on in vitro toxicity determinations (Shopsis and Eng, 1986) it was observed that DMSO protected cultured mouse fibroblasts (Balb/c 3T3 cells) from cadmium toxicity. In this paper we examine further the antagonism of DMSO towards Cd\(^{2+}\)-induced cell damage and explore the effects of other differentiation-inducing agents, Zn\(^{2+}\) and sodium selenite on Cd\(^{2+}\)-induced cytotoxicity.

Materials and methods

Cells and culture conditions

Balb/c 3T3, a mouse fibroblast cell line obtained from the American Type Culture Collection, Rockville, MD, USA, was maintained in a 7% CO\(_2\) atmosphere in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin G, 100 μg/ml streptomycin, and 1.25 μg/ml Fungizone (referred to subsequently as complete medium). Cells were subcultured after treatment with trypsin–EDTA as described previously (Shopsis and Sathe, 1984).