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Zinc has ambiguous effects on chromium (VI)-induced oxidative stress and apoptosis

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Abstract

Zinc is an important cellular antioxidant. We investigated its role in chromium-induced oxidative stress and apoptosis in human tumor cell line Hep-2. The measured parameters included intracellular labile zinc content (Zinquin-E fluorescence), cell viability (WST-1 assay), oxidative stress (spectrophotometry), mitochondrial potential (flow cytometry), caspase-3 activity, and PARP cleavage (immunofluorescence). We found that Hep-2 cells contain abundant labile zinc stores that may be depleted by the ionophore TPEN or increased by external zinc supplementation. Chromium (VI)-induced cytotoxicity and apoptosis were enhanced in zinc-depleted cells after 24 h, in particular at chromium (VI) concentrations of 50 and 150 μmol/l. On the other hand, elevated levels of labile zinc were able to protect against apoptosis induced by 10 μmol/l chromium (VI) but at higher chromium (VI) concentrations (50 and 150 μmol/l) acted synergistically, significantly enhancing oxidative stress and the course of apoptosis, possibly through oxidative stress and mitochondrial damage.

Keywords: Zinc; Hexavalent chromium; Apoptosis; Oxidative stress; Mitochondria

Introduction

It is now established that hexavalent chromium (Cr (VI)) may induce diverse responses in exposed cells and tissues, involving temporary or terminal growth arrest, malignant transformation or cell death — apoptosis. It has been demonstrated that these different Cr (VI)-induced endpoints might depend on several mutually interfering factors such as employed concentration of this element, the nature and extent of inflicted cellular damage as well as the activity of cellular defense mechanisms [1–3].

Unlike trivalent chromium (Cr (III)), which is relatively nontoxic and safe due to its inability to enter the cell [4], there are several mechanisms whereby Cr (VI) might injure the cells, all of them being dependent on its intracellular reduction. Cr (VI) enters the cell via a nonspecific anion transporter and is subsequently reduced by a series of enzymatically catalyzed reactions to trivalent chromium [5–7]. Cr (III) as well as other intermediates, in particular Cr (V), have been shown to interact directly with DNA and other macromolecules or to induce oxidative stress, thereby causing their damage and leading to apoptosis [8–10].

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Zinc (Zn) is an essential element, which has been shown to protect cells against apoptosis by interfering with several targets along apoptotic pathways such as Bcl-2 and Bax genes, Ca^{2+}/ Mg^{2+}-dependent endonuclease, caspase-3 and microtubular cytoskeleton [11,12]. Furthermore, Zn prevents the formation of reactive oxygen species (ROS), stabilizes plasma membranes and enhances the activity of the copper and Zn dependent superoxide dismutase (CuZnSOD). These antiapoptotic activities are largely due to intracellular labile Zn stores whose increases and decreases have been reported to be crucial in Zn-promoted suppression or increase of apoptosis [13,14].

Several studies have demonstrated that various cellular defense mechanisms are depleted after an exposure to Cr (VI) and, conversely, that the addition of different cytoprotectants to cells prevents or delays Cr (VI)-induced cytotoxicity, oxidative stress and apoptosis [6,15–17]. Nevertheless, so far, none of the studies explored the role of Zn ions in this process. Thus, the aim of this study was to (1) determine intracellular labile Zn stores in Hep-2 cells and (2) investigate the effects of Zn-depletion and Zn-supplementation on the course of cellular injury and apoptosis caused by Cr (VI).

Materials and methods

Chemicals

Potassium chromate (K_2CrO_4); zinc sulfate ($ZnSO_4$); *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN); Zinquin E (ethyl ester), *β*-NADPH; horseradish peroxidase; 4′, 6-diamidino-2-phenylindole (DAPI); Triton-X and cytochrome c were purchased from Sigma-Aldrich (Prague, Czech Republic). 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate (WST-1) was from Boehringer Mannheim-Roche (Basel, Switzerland). JC-9 dye and secondary antibodies for caspase-3 and Poly(ADP-ribose) polymerase (PARP) detection were from Molecular Probes, Inc. (Eugene, USA), EXBIO (Prague, Czech Republic) and N.E. BioLabs GmbH (Frankfurt am Main, Germany). Monospecific antisera for the detection of activated caspase-3 and PARP were obtained from N.E. Biolabs GmbH. All other chemicals used were of highest analytical grade.

Cell culture and treatments

The continuous cell line Hep-2 (ECACC, No. 86030501, Porton Down, United Kingdom) was cultivated in a humidified 5% CO_2 atmosphere at 37 °C in Dulbecco's modified Eagle's medium (DMEM) (Sevapharma, Prague, Czech Republic) with 10% bovine serum (ZVOS Hustopeče, Uherčice, Czech Republic), penicillin G (100 U/ml) and streptomycin (100 μg/ml). For the experiments, the cells were seeded into cultivation flasks (Nunclon, Roskilde, Denmark) or modified cytospin chambers (Hettich, Tuttlingen, Germany). Potassium chromate (1.5, 10, 25, 50 and 150 μmol/l) alone or in a combination with zinc sulfate (10, 50 and 100 μmol/l) was added to the cultures and the observed markers were determined at 12 or 24 h of treatment. Zn depleted cultures were generated from Hep-2 cells previously grown to the confluency of 80% by adding TPEN (25 μmol/l, 2 h, 37 °C) as described elsewhere [18]. After treatment, cultures were rinsed three times in a serum free medium and were kept in this medium for another 24 h. The level of Zn was determined in untreated, TPEN treated and $ZnSO_4$ treated cultures by microfluorometry [14].

Microfluorometry of intracellular labile Zn

Cultures pretreated with 100 μmol/l $ZnSO_4$ (12 h, 37 °C) or 25 μmol/l TPEN (2 h, 37 °C) were washed with phosphate buffered saline (PBS) and incubated with 25 μmol/l Zinquin E in DMEM for 30 min at 37 °C. After 30 min, the slides were washed with PBS and Zinquin fluorescence was examined under a fluorescence microscope Nikon Eclipse E400 (Nikon Corporation, Kanagawa, Japan) using a DAPI-specific set of filters. The images were collected with a digital color matrix camera COOL 1300 (VDS, Vosskűhler, Germany) and LUCIA DI Image Analysis System (Laboratory Imaging Ltd., Prague, Czech Republic). In all experiments, the results were compared with those originating from untreated cultures.

Toxicity of Cr (VI) alone and in combination with Zn

Cytotoxic effects of Cr (VI) on tumor Hep-2 cells were assessed by WST-1 colorimetric assay, which is based on the cleavage of the tetrazolium salt to colored formazan by mitochondrial dehydrogenases in viable cells. Hep-2 cells (6000 cells/well) in 200 μl of DMEM with 10% bovine serum were seeded into 96-well microtiter plates, with the first two columns of wells without cells (blank). The cells were incubated 24 h at 37 °C and in 5% CO_2. After incubation, the medium was replaced with a medium containing the tested concentration of Cr (VI) or Cr (VI) + Zn and cultivated for 24 h at 37 °C and 5% CO_2. After 24 h, the medium was aspirated and 100 μl of WST-1 was added. The cells were further incubated for 2 h under the same conditions. The absorbance of samples was recorded at 490 nm with 650 nm of reference wavelength by a scanning multiwell