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Journal of Trace Elements in Medicine and Biology 18 (2005) 269–276
Journal of Trace Elements in Medicine and Biology
www.elsevier.de/jtemb

PATHOBIOCHEMISTRY

Zinc protects HeLa-tat cells against free radical cytotoxicity induced by glucose

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Received 22 September 2004; accepted 29 October 2004

Abstract

In the present study, we investigated the protective effect of zinc on the glucose-induced cytotoxicity in HeLa wild and HeLa-tat cells (30 and 20 mmol/l glucose, respectively). HeLa cells transfected with the protein Tat exhibit a lower antioxidant defense system. Incubation of HeLa wild and HeLa-tat cells with high glucose levels led to a rapid increase in generation of reactive oxygen species (ROS). As expected in the presence of high glucose concentrations, the viability was reduced for both cell lines. The redox status essentially regulated by thiol groups may play an important role in the apoptotic process. Thus, we developed a new method using the *p*-nitrophenyl disulfide to measure cytosolic thiol groups in intact cells. Cellular zinc was measured using inductively coupled plasma mass spectrometry. Intracellular thiol groups and intracellular zinc concentrations were significantly lower in HeLa cells cultured in hyperglycemic conditions, and their concentrations were significantly lower in HeLa-tat cells than in HeLa wild cells. However, the generation of ROS and the induction of apoptosis by a glucose specific mechanism were prevented by zinc (50 μmol/l) and the intracellular thiol groups and zinc concentrations significantly increased in both cell lines to become similar to the initial values. These results suggest that the glucose oxidation and its subsequent effects on the cells can be prevented by a biological antioxidant such as zinc.

Keywords: Zinc; Glucose; Reactive oxygen species; Thiol groups; Cytotoxicity

Introduction

There are strong evidences that glucose, under physiological conditions, is prone to oxidation and consequently generates hydrogen peroxide and reactive intermediates such as hydroxyl-free radicals and α-hydroxyaldehydes [1]. It has been suggested that oxidative stress plays an important role in tissue damage associated with diabetes [2] and that peroxide formation is increased in parallel to elevated glucose oxidation [3]. In different studies, it has been suggested that variations in glucose concentrations are sufficient to induce cell

Abbreviations: DMSO, dimethyl sulfoxide; GSH, reduced glutathione; H_2DCF-DA, 2′, 7′-dichlorodihydrofluoresceine diacetate; HIV, human immunodeficiency virus; ICP-MS, inductively coupled plasma mass spectrometry; MFI, mean fluorescence intensity; Mn-SOD, Mn-dependent superoxide dismutase; MTT, 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; GPx, glutathione peroxidase; PNPD, *p*-nitrophenyl disulfide; ROS, reactive oxygen species; −SH, thiol groups; Tat, transactivator protein of HIV-1; TFA, trifluoroacetylacetone

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doi:10.1016/j.jtemb.2004.10.001

death through a free radical-mediated mechanism [4], delay in various phases of the cell cycle of human endothelial cells [5], inhibition of endothelial cell replication [6] and to induce oxidative stress in smooth muscle cells [7].

Zinc is an essential metal, necessary to the function of many enzymes participating in a wide variety of metabolic processes [8]. In in vitro systems, zinc may function as an antioxidant by protecting sulfhydryl groups from oxidation [9,10], competing with copper and iron to reduce the formation of HO° that are a result of redox cycles and by induction of the antioxidant protein metallothionein, which can scavenge damaging hydroxyls. Regarding these protective effects in different free radical generating systems, it seems of interest to investigate the effect of zinc on cellular thiol group content in the presence of a high glucose concentration.

The Tat protein from human immunodeficiency virus type 1 (HIV-1) is essential for efficient HIV-1 gene expression. Tat acts by binding to a transactivation response element, an RNA stem-loop structure located close to the 5′ end of HIV-1 transcripts [11]. Tat protein can potentially influence cellular gene expression in infected and non-infected cells. In HeLa cells transfected with Tat (HeLa-tat cells) the activity of the manganese dependent-superoxide dismutase (Mn-SOD) is decreased to 50% compared with the level measured in parental cells, accompanied by a reduction of protein expression [12]. A likely consequence of decreased Mn-SOD activity is increased oxidative stress reflected by a decrease in the total amount of glutathione or a decreased ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG). Therefore, HeLa-tat cells are a good model to investigate the implications of free radicals in cell free radical damage.

In the present study, HeLa-tat and parental cells were used to explore the effect of zinc on glucose induced cytotoxicity and free radical production, as zinc has a protective effect against oxidation [13]. This is to our knowledge the first study exploring the role of zinc in glucose oxidation.

Experimental procedures

Materials

Opti-MEM medium, fetal calf serum (FCS), penicillin and streptomycin sulfate were purchased from Gibco-BRL (Grand Island, NY, USA). The solution of 0.05% trypsine, 0.02% EDTA in phosphate buffered saline (PBS) was from Gibco-BRL. MTT kit (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and Complete (proteases inhibitor cocktail) were from Boehringer Mannheim (Mannheim, Germany). Glucose, mannitol, *p*-nitrophenyl disulfide (PNPD), Triton X-100 and dimethyl sulfoxide (DMSO) were from Sigma Chemical Co. (St. Louis, MO, USA). 2′, 7′ dichlorodihydrofluoresceine diacetate (H_2DCF-DA) was purchased from Interchim (Montluçon, France). Stock solutions of 10 mmol/l zinc chloride (Prolabo, Paris, France) were prepared, sterilized by filtration (0.22 μm), and calibrated by atomic absorption spectrometry. Nitric and hydrochloric acids, hydrogen peroxide, pyridine and hexane were purchased from Merck (Darmstadt, Germany) and ammonium acetate from Prolabo and were of high purity grade. Trifluoroacetylacetone (TFA) was purchased from Aldrich (Milwaukee, WI, USA). Immunodetection of carbonylated proteins was performed using Oxyblot kit (Appligene Oncor, Illkirch, France). Hybond-P (PVDF membrane) and ECL kit were purchased from Amersham Pharmacia (Les Ullis, France).

Cell cultures

The HeLa-tat and wild cell lines were obtained from NIH-AIDS Research and Reference Reagent Program (Besthesda, MD, USA).

The control medium comprised the standard culture medium described above containing 4% FCS, 5 mmol/l glucose and 9 μmol/l zinc. The high-glucose medium was identical to the control medium, but supplemented with glucose to increase its concentration up to 30 mmol/l. The zinc supplemented medium comprised the standard or the glucose supplemented medium, but with addition of zinc chloride up to 50 μmol/l zinc.

The mannitol osmotic control medium was similar to the control medium (5 mmol/l glucose) except that it was supplemented with mannitol (15 mmol/l for HeLa-tat and 25 mmol/l for HeLa wild), a nonpermeable hexose, which served as an osmotic control medium for the high-glucose medium.

Cytotoxicity assay

The cytotoxic activity of sugar was determined by a colorimetric assay. Cell viability was determined by a modified MTT assay [14]. HeLa cells were seeded at a density of 3×10^3 cells per well in 96-well flat-bottomed microtiter plates and incubated at 37 °C for 24 h in 0.2 ml culture medium. The supernatant was then removed and replaced by fresh medium containing serial dilutions of glucose supplemented or not with zinc (50 μmol/l). Incubation was continued for 24 h followed by the addition of 10 μl of an MTT solution (5 mg/ml in PBS). After another 2 h incubation, supernatants were removed, followed by the addition of 100 μl DMSO to dissolve the formazan crystals. The absorbance readings