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Prooxidative effect of copper–metallothionein in the acute cytotoxicity of hydrogen peroxide in Ehrlich ascites tumour cells

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Abstract

This study was concerned with the role of copper (Cu) and Cu–metallothionein (Cu–MT) in oxidative stress. Hydrogen peroxide (H_2O_2)-induced oxidative injury was examined in Ehrlich ascites tumour cells isolated from host mice pretreated with 0, 1 or 2 mg of $CuSO_4$ (ip) 24 h earlier. Control Ehrlich cells contained low levels of Cu and Cu treatment produced dose-related increases in cellular Cu and Cu–MT levels and corresponding increases in sensitivity to oxidative toxicity of H_2O_2 (LC_{50}, cell blebbing, lipid peroxidation, GSH depletion, and increase in intracellular free $[Ca^{2+}]_i$). Hydrogen peroxide treatment also resulted in the oxidation of MT thiolates, reduction in the binding of Cu to MT resulting in translocation of Cu to other subcellular sites. D-penicillamine, a Cu-chelating agent, obliterated the sensitization effect of Cu-pretreatment and reduced the redistribution of MT-bound Cu, suggesting the participation of Cu ions derived from MT in promoting oxidant stress. Additional experiments with desferoxamine and mannitol have revealed the involvement of a Cu-dependent Fenton reaction in the mediation of the prooxidative effect of Cu–MT. These data suggest that cells with high levels of Cu–MT may be particularly susceptible to oxidative stress.

Keywords: Copper–metallothionein; Prooxidant; Oxidative stress; Ehrlich ascites tumour cells

1. Introduction

Oxidative stress is a condition characterized by elevation in cellular steady-state concentration of reactive oxygen species generated from the incomplete reduction of molecular oxygen (Sies, 1985). There is evidence that oxidative stress is involved in various biological and pathological processes including cancer, inflammation, myocardial ischemic/reperfusion injury, diabetes mellitus, rheumatoid arthritis, and chemically-induced tissue injury (Sies, 1985; Willcox et al., 2004; Djordjevic, 2004; Stohs, 1995). Also, a variety of anticancer drugs are known to exert their therapeutic effects via the generation of reactive oxygen species (Pelicano et al., 2004; Kovacic and Osuna, 2000; Doroshow, 1986).

Because of the presence of cellular defence systems (i.e. glutathione, α-tocopherol, superoxide dismutase), the generation of intracellular reactive oxygen species may not always lead to cellular injury. However, when these defence systems are overwhelmed, such as in the presence of acute oxidative stress, it can lead to changes in the function and structure of cellular components including DNA damage, depletion of intracellular ATP,

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acceleration of lipid peroxidation, alterations in calcium homeostasis, increased formation of intracellular oxidized sulphydryls and membrane blebbing, all of which can contribute to the cause of cell death (Chen et al., 2000; Farber, 1990; Pascoe and Reed, 1989).

Metallothionein(s) (MT) are ubiquitous, low molecular weight cytosolic proteins capable of binding a variety of essential as well as toxic metals. All mammalian MT contain 61 amino acids including 20 cysteines which all participate in the binding of metal ions (Kagi and Schaffer, 1988; Mason et al., 1980). Each molecule of MT can bind 12 g atoms of Cu in a trigonal arrangement or 7 g atom of Zn in a tetrahedral coordination. MTs exist in low levels in tissues of normal adult laboratory animals; however, their synthesis can be induced markedly by certain metal ions such as zinc (Zn) and copper (Cu) (Kagi and Schaffer, 1988; Maret, 2000; Nordberg and Nordberg, 2000). They are recognized for their role in the regulation of essential metal metabolism (Zn and Cu) and in the detoxication of toxic metals (Kagi and Schaffer, 1988; Mason et al., 1980; Maret, 2000; Nordberg and Nordberg, 2000; Theocharis et al., 2003). Also, in vitro studies have shown that the cysteinyl thiolate groups of MT can also act as efficient interceptors of reactive oxygen species (Thomas et al., 1986; Cousins and Coppen, 1987; Maret, 2000) and that Zn ions released from oxidized MT may also contribute to the protective effect of Zn–MT against oxidative stress (Cousins and Coppen, 1987; Maret, 2000). Also, the antioxidant properties of MT have been confirmed in studies with transgenic mice or cells from transgenic mice where targeted disruption of metallothionein I and II genes enhance the sensitivity of such biological systems to oxidants such as *tert*-butylhydroperoxide, the redox cycling toxin paraquat, doxorubicin, and acetaminophen (Lazo et al., 1995; Kang et al., 1997; Liu et al., 1999).

In view of the postulated role of the MT-bound Zn ions in mediating the protective effect of MT in oxidative stress, it was hypothesized that Cu–MT would have prooxidant rather than antioxidant properties based on the well recognized toxic effects of Cu ions. Copper being a transition redox metal has the ability to act as catalysts of free radical reactions, stimulate lipid peroxy radical formations and propagation of lipid peroxidation and oxidize membrane sulphydryl groups. (Aust et al., 1985; Hochstein et al., 1980; Gaetke and Chow, 2003). Our hypothesis was supported by the results of our previous study which has attributed the presence of high Cu–MT in the liver of neonatal guinea pig to the heightened sensitivity to ferric nitrilotriacetate-induced hepatotoxicity (Suntres and Lui, 1991).

The present study was concerned with the development and characterization of an in vitro cell model with different levels of Cu–MT, which may be used to examine the influence of Cu on the functional role of MT in oxidative stress. This involved the use of Ehrlich tumour cells (originally derived from a mammary adenocarcinoma) (DCT tumour repository) which can be grown in the peritoneal cavity of host mice and can be obtained in high yields. Hydrogen peroxide was used as the model compound to induce oxidative stress (Stone and Collins, 2002). In addition, as being a commonly used model oxidant, H_2O_2 is an intermediate in the reductive metabolism of O_2 and is actually involved in various oxidant stress-related cell injury.

Results of this study showed that the susceptibility of Ehrlich cells to H_2O_2 toxicity (as measured by cell viability and blebbing, oxidation of GSH, $[Ca^{2+}]_i$, and lipid peroxidation) was directly related to cellular Cu–MT concentrations. Moreover, results from additional studies suggest the involvement of oxidant-induced mobilization of Cu from MT and the participation of Cu ions in a Fenton reaction leading to increases in lipid peroxidation and loss of cell viability.

2. Materials and methods

2.1. Chemicals and animals

Cupric sulphate ($CuSO_4$) was purchased from Fisher Scientific Co. (Toronto, Ont.). Glutathione, 2-thiobarbituric acid (TBA), mannitol, desferoxamine (DEF), D-penicillamine (D-PEN), and 5,5-dithiobis-(2-nitrobenzoic acid) (NbS_2) were purchased from Sigma Chemical Co. (St. Louis, MO). Indo-1 acetoxymethyl ester was a generous gift from Dr. J. Dixon, Department of Physiology, University of Western Ontario, London, Ontario. Sephadex G-75 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). All other chemicals were purchased from BDH (Toronto, Ont.).

Male Swiss albino CD-1 mice (approximately 30 g body weight) obtained from Charles River Breeding Laboratories Inc., Quebec, were housed in groups of six to eight animals in polyethylene cages with free access to pelleted Purina laboratory chow and deionized water. The animals were kept at room temperature (22–24 °C) and were exposed to alternate cycles of 12 h light and darkness.

2.2. Effect of Cu treatments on Ehrlich ascites tumour cells

Ehrlich cells were grown in the peritoneal cavity of animals and transferred weekly to new hosts. Ehrlich cells were harvested 6 days after inoculation, washed four to five times with 0.9% saline and resuspended in Dulbecco's phosphate buffered saline (PBS). Ehrlich cells were counted microscopically using