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Original Contribution

Role of intracellular labile iron, ferritin, and antioxidant defence in resistance of chronically adapted Jurkat T cells to hydrogen peroxide

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ABSTRACT

To examine the role of intracellular labile iron pool (LIP), ferritin (Ft), and antioxidant defence in cellular resistance to oxidative stress on chronic adaptation, a new H_2O_2-resistant Jurkat T cell line "HJ16" was developed by gradual adaptation of parental "J16" cells to high concentrations of H_2O_2. Compared to J16 cells, HJ16 cells exhibited much higher resistance to H_2O_2-induced oxidative damage and necrotic cell death (up to 3 mM) and had enhanced antioxidant defence in the form of significantly higher intracellular glutathione and mitochondrial ferritin (FtMt) levels as well as higher glutathione-peroxidase (GPx) activity. In contrast, the level of the Ft H-subunit (FtH) in the H_2O_2-adapted cell line was found to be 7-fold lower than in the parental J16 cell line. While H_2O_2 concentrations higher than 0.1 mM fully depleted the glutathione content of J16 cells, in HJ16 cells the same treatments decreased the cellular glutathione content to only half of the original value. In HJ16 cells, H_2O_2 concentrations higher than 0.1 mM increased the level of FtMt up to 4-fold of their control values but had no effect on the FtMt levels in J16 cells. Furthermore, while the basal cytosolic level of LIP was similar in both cell lines, H_2O_2 treatment substantially increased the cytosolic LIP levels in J16 but not in HJ16 cells. H_2O_2 treatment also substantially decreased the FtH levels in J16 cells (up to 70% of the control value). In contrast in HJ16 cells, FtH levels were not affected by H_2O_2 treatment. These results indicate that chronic adaptation of J16 cells to high concentrations of H_2O_2 has provoked a series of novel and specific cellular adaptive responses that contribute to higher resistance of HJ16 cells to oxidative damage and cell death. These include increased cellular antioxidant defence in the form of higher glutathione and FtMt levels, higher GPx activity, and lower FtH levels. Further adaptive responses include the significantly reduced cellular response to oxidant-mediated glutathione depletion, FtH modulation, and labile iron release and a significant increase in FtMt levels following H_2O_2 treatment.

Introduction

The response of cells to either an acute (single high dose) or chronic (repeated low/moderate doses) exposure to oxidising agents is quite different. Depending on the degree of the oxidising insult, acute exposure could trigger a series of intracellular anti-oxidant defence mechanisms that counteract the damage caused but if these are not sufficient, cells will die by apoptosis or necrosis, again depending on the extent of the oxidative insult [1,2]. In chronically exposed cells, it is anticipated that the antioxidant defence mechanism will be altered as repeated exposure of cells to oxidants usually provokes the development of a series of adaptive responses that are distinct from those following acute exposure. Because of such adaptive responses, cells may withstand high toxic doses of the oxidising agent that would otherwise be lethal. Excess production of reactive oxygen species

Abbreviations: Apaf-1, apoptosis protease activating factor-1; BSA, bovine serum albumin; BSO, buthionine-[*S*,*R*]sulfoximine; CA-AM, calcein-acetoxymethyl ester; CA-Fe, CA-bound iron; CM, conditioned media; DFO, desferrioxamine mesylate; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate-buffered saline; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; Ft, ferritin; FtH, ferritin heavy chain; FtL, ferritin light chain; FtMt, mitochondrial ferritin; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; h, hour(s); H_2O_2, hydrogen peroxide; IR, ionizing radiation; IRP, iron regulatory protein; K_d, dissociation constant; LIP, labile iron pool; LL, lower left quadrant; min, minute(s); NADPH, reduced nicotinamide adenine dinucleotide phosphate; NR, neutral red; $^{\cdot}OH$, hydroxyl radical; PI, propidium iodide; PNG, glucose-free EMEM media containing pyruvate; RA, rheumatoid arthritis; ROS, reactive oxygen species; SD, standard deviation; SFM, serum-free media; SIH, salicylaldehyde isonicotinoyl hydrazone; TBHP, *tert*-butyl-hydroperoxide; TfR, transferrin receptor; UVA, ultra-violet A

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(ROS) has been implicated in progression of cardiovascular, neurodegenerative, and chronic inflammatory diseases as well as cancer and aging [2–7]. The study of the mechanisms underlying the adaptive responses of cells to oxidising agents should provide clues to understanding the promotion and progression of such disorders.

The involvement of hydrogen peroxide (H_2O_2) in numerous types of cell and tissue injury is well-documented [8–12]. Although H_2O_2 itself has low reactivity toward cell constituents, it is capable of forming potent ROS in the presence of trace amounts of catalytic labile iron via Fenton chemistry. The potentially toxic labile iron exists in cells as a transit pool of catalytically active iron complexes which is distinct from intracellular iron associated with proteins and is known as the labile iron pool (LIP). Iron belonging to this pool is considered to be in steady-state equilibrium, loosely bound to low-molecular-weight compounds, accessible to permeant chelators, and metabolically and catalytically reactive [13]. Under physiological conditions, cells protect themselves either by the H_2O_2-degrading enzymes catalase and glutathione peroxidase (GPx) [14] or by minimising the intracellular level of potentially harmful redox-active LIP via the cytosolic iron regulatory proteins 1 and 2 (i.e., IRP-1 and IRP-2) which function as posttranscriptional regulators of both iron uptake via transferrin receptor (TfR) and iron sequestration by the iron-storage protein, ferritin (Ft) [15,16]. However under pathological conditions including acute oxidative stress, these conventional cellular defences are often insufficient, because the system is overwhelmed either by an increase in H_2O_2 formation [10,17,18] and/or by an excess presence of labile iron [16,19]. The increase in intracellular LIP in oxidative stress conditions such as short exposure to H_2O_2 and the damaging effect of iron-catalysed oxidative damage has been shown in numerous cellular and animal studies [19–27].

Under physiological conditions, most of the iron that is not metabolised is stored in Ft. Ft is an ubiquitously expressed cytosolic iron-storage protein that forms a hetero-oligomeric protein shell composed of two different subunits, ferritin heavy chain (FtH) with ferroxidase activity and ferritin light chain (FtL) that promotes iron nucleation [28,29]. Ft plays a dual role in LIP homeostasis, acting on the one hand as an iron-sequestering protein and on the other hand as a potential source of labile iron [8,30]. The characterisation of cellular models in which Ft expression is modulated has shown that the ferroxidase catalytic site of the FtH has a central role in regulating iron availability. In turn, this has secondary effects on a number of cellular activities, which include proliferation and resistance to oxidative damage [28,29]. Iron is also liberated from Ft as a consequence of normal turnover in lysosomal compartments, where it is thought to be recycled for synthesis of new iron-containing proteins [31,32]. This source of LIP has been shown to be active in the cell-damaging processes caused by oxidative stress in the form of H_2O_2 or ultraviolet A (UVA, 320–400 nm) radiation, promoting lysosomal rupture and release of potent hydrolytic enzymes to the cytosol [20,33,34] which in turn leads to both proteolytic degradation of cytosolic proteins notably Ft and secondary secondary harm to various cellular constituents notably mitochondrial injury leading to apoptotic or necrotic cell death (depending on the extent of insult) [21,35].

The existence of a storage protein inside mitochondria, called mitochondrial ferritin (FtMt), has been shown to protect the mitochondria of cells from iron-dependent oxidative damage [29,36]. It has been suggested that the primary function of FtMt is the control of ROS formation through the regulation of mitochondrial iron availability, which results in a cytoprotective effect [29]. In HeLa cells, FtMt overexpression has been shown to protect the cells against H_2O_2-induced cytochrome *c* release from mitochondria and reduction of the activity of the mitochondrial Fe/S enzymes [37]. The cytoprotective function of FtMt has also been linked to its iron-sequestering activity capable of reducing the size of cytosolic and mitochondrial LIP, both of which catalyse oxidative damage under oxidative stress conditions [8,37–40].

In this study, we used a cell model composed of two human Jurkat T cell lines (parental, J16; H_2O_2-resistant, HJ16) to assess the mechanisms underlying the increased cellular resistance that occurs after chronic adaptation to oxidative stress. The possible role of LIP, Ft, and FtMt in increasing the resistance of cells to H_2O_2 was also investigated.

Materials and methods

Materials

Cell culture materials were obtained from Gibco (Germany) except for fetal bovine serum (FBS) (PAA Laboratories, Austria) and RPMI-1640 medium (Promocell, Germany). All chemicals were from Sigma-Aldrich Chemical (Poole, UK) except protease inhibitor cocktail tablets, Annexin-V-FLUOS, bovine serum albumin (BSA) that was supplied from Roche (Mannheim, Germany), glutathione reductase (GR), H_2O_2 solution, and Mowiol 4-88 from Calbiochem (CN Biosciences LTD, Nottingham), dimethyl sulfoxide (DMSO) from VWR International Ltd (Leicestershire, England), DPBS (Dulbecco's phosphate-buffered saline with Ca^{2+} and Mg^{2+}) from Cambrex (Belgium), cathepsin B antibody from Santa Cruz Biotechnology, Inc. (Santa Cruz, California), calcein-acetoxymethyl ester (CA-AM) and LysoSensor Green DND-153 from Molecular Probes (Leiden, Netherlands), and an ApoGlow assay kit from Lumitech (UK). Salicylaldehyde isonicotinoyl hydrazone (SIH) was a kind gift from Dr James Dowden (Department of Pharmacy and Pharmacology, Bath University, Bath, UK).

Cell culture

The Jurkat J16 cells are a human T-cell leukemia cell line. The polyclonal H_2O_2-resistant cell line "HJ16" was derived from the J16 cell line after gradual adaptation to 3 mM H_2O_2. For this purpose, the J16 cell culture was diluted in serum-free RPMI at a density of 1×10^6 cells/ml. Cells were then treated with H_2O_2 at a concentration determined by their tolerance (generally a concentration of H_2O_2 causing over 60% cell death), and incubated at 37 °C for 2 h. After this time, cells were harvested by centrifugation (350 *g*, 5 min) and resuspended in an equal volume of 10% FBS RPMI. After 18 h incubation, cell numbers and survival were determined using trypan blue exclusion on a hematocytometer. This protocol was repeated every 2–3 weeks, depending on recovered cell numbers, with increasing concentrations of H_2O_2 over a period of 6 months. An H_2O_2-tolerant cell line (up to a dose of 3 mM) was designated HJ16. Stocks of HJ16 cells were stored in liquid nitrogen and defrosted when needed. Both cell lines were cultured routinely in RPMI-1640 medium supplemented with 10% v/v heat inactivated FBS, 2.7% w/v sodium bicarbonate, 2 mM L-glutamine, and 50 IU/ml of penicillin and streptomycin.

We also attempted to isolate highly resistant clones for comparison. However our attempts failed to identify clones that had higher resistance to a H_2O_2 concentration of 3 mM than the polyclonal HJ16 cell line (data not shown). For this reason and the time-consuming nature of the clonal expansion, in the present study, we concentrated our efforts to perform an in-depth characterization of the polyclonal HJ16 cell line.

Treatments

H_2O_2 treatments (0.05–3 mM) were performed in serum-free media (SFM) for 30 min at 37 °C. Following the treatment, cells were resuspended and incubated in the conditioned media (CM)