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Lysosomal membrane permeabilization causes oxidative stress and ferritin induction in macrophages

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ABSTRACT

Moderate lysosomal membrane permeabilization (LMP) is an important inducer of apoptosis. Macrophages are professional scavengers and are rich in hydrolytic enzymes and iron. In the present study, we found that LMP by lysosomotropic detergent MSDH resulted in early up-regulation of lysosomal cathepsins, oxidative stress and ferritin up-regulation, and cell death. Lysosomotropic base NH_4Cl reduced the ferritin induction and oxidative stress in apoptotic cells induced by MSDH. Cysteine cathepsin inhibitors significantly protected cell death and oxidative stress, but had less effect on ferritin induction. We conclude that oxidative stress induced by lysosomal rupture causes ferritin induction with concomitant mitochondrial damage, which are the potential target for prevention of cellular oxidative stress and cell death induced by typical lysosomotropic substances in different disorders.

1. Introduction

The lysosomal compartment is essential for the degradation of phagocytosed extracellular material as well as of intracellular organelles and long-lived proteins [1,2]. Since many iron containing metallo-proteins are degraded in lysosomal compartment, lysosomes are generally rich in transition metal iron. Iron released inside lysosomes is transported to the cytoplasm and then stored in ferritin for further use in the synthesis of iron-containing biomolecules.

Ferritin is a ubiquitous and highly conserved iron-binding protein. Its heavy-chain also has enzymatic properties, converting Fe(II) to Fe(III) as iron is internalized and sequestered in the ferritin mineral core. Recently, ferritin has been viewed not only as an iron-storage protein, but is also related to apoptosis [3,4]. Ferritin heavy chain up-regulation by NF-κB inhibits TNFα-induced apoptosis by suppressing reactive oxygen species (ROS) [5]. The heavy-ferritin has an anti-apoptotic activity unrelated to its ferroxidase activity or its capacity to modify cellular iron metabolism [6]. However, isoferritins with homology to immunomodulatory ferritin isoforms (p43-PLF, melanoma-derived-H-chain ferritin) are able to stimulate p53 expression to mediate apoptosis via Fas (CD95) signaling [3].

Reactive oxygen species (ROS) are involved in a number of apoptosis models. Excessive production of ROS perturbs the redox balance and lead to oxidative stress [7]. To overcome cellular oxidative stress, cells possess different antioxidant enzyme systems including superoxide dismutase, catalase, and glutathione systems [8,9]. Glutathione depletion has been found as a common feature of apoptotic cell death triggered by a wide variety of stimuli [10]. In addition, lysosomal cathepsins B and D are involved in oxidative stress induced apoptosis [11] and down-regulation of cathepsin D reduced H_2O_2-mediated ROS production and apoptosis [12].

In a previous study, we found that *O*-methyl-serine dodecylamide hydrochloride (MSDH) induced lysosomal membrane permeabilization (LMP) with the release of lysosomal enzymes followed by mitochondrial membrane permeabilization (MMP) and apoptotic or necrotic cell death [13]. Afterward this detergent has been found to induce lysosomal rupture and apoptosis in several different types of cell models [14,15]. It remains unknown whether oxidative stress driven by lysosomal iron is involved in MSDH induced apoptosis.

Since lysosomes not only contain abundant hydrolytic enzymes but also are rich in low mass iron, we hypothesized that LMP by specific lysosomotropic detergent may cause subsequent alterations in cellular iron metabolism and oxidative stress. The aim of this study was therefore to investigate whether lysosomal rupture induced by MSDH causes alterations in lysosomal cathepsins, intracellular ferritin levels and oxidative stress.

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2. Materials and methods

2.1. Chemicals

Ham's F10 and RPMI-1640 culture medium, FBS, penicillin, and streptomycin were from Invitrogen Ltd. (Paisley, UK). MSDH was a kind gift from Dr. Gene Dubowchik, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5100, Wallingford, CT 06492-7660, USA. Ammonium chloride (NH_4Cl) was from Fluka AG (Buchs, Sweitzerland). Benzyloxycarbonyl-Phe-Ala-fluoromethylketone (zFA-fmk) was from Enzyme Systems. Desferrioxamine (DFO) was from Ciba-Geigy (Basel, Switzerland). E64d, ferric ammonium citrate (FeAC), phorbol myristate acetate (PMA) and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise stated.

2.2. Cells, lysosomal rupture using MSDH and detection of apoptosis

J-774 cells, a murine macrophage cell line, were grown in F-10 culture medium with 10% FBS, 2 mM glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere (5% CO_2; 95% air). Cells were sub-cultivated twice a week and used for experiments 24 h later.

THP-1 cells were cultured in RPMI-1640 culture medium with 10% FBS, 2 mM glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere (5% CO_2; 95% air). Cells were sub-cultivated twice a week and subdivided cells were differentiated into macrophages by incubating with 300 nM phorbol myristate acetate (PMA) for 24 h, washed with culture medium, further cultured for 24 h under standard culture condition and then used for experiments.

In order to induce LMP, cells were exposed to 7.5 or 25 μM MSDH at otherwise standard culture conditions for 1–24 h. Previously it has been shown that the moderate dose (25 μM) of MSDH causes moderate lysosomal rupture and profound apoptosis, while the lower dose (7.5 μM) causes only negligible rupture without apoptosis [13]. In some experiments, cells were either pre-treated with 10 mM NH_4Cl (for 30 min and then exposed to MSDH for 12–24 h in the presence of NH_4Cl, or pre-treated with zFA-fmk (50 μM) or E64d (10 μM), DFO (500 μM), or Trolox (20 μM) for 1 h and then exposed to MSDH for further 12 to 24 h in the presence of the inhibitors.

Cell morphology examined by phase contrast- and bright field-microscopy after trypan blue and Giemsa staining. Shrunken cells devoid of trypan blue staining but with fragmented or condensed nuclei were counted as apoptotic cells, while trypan blue positive cells were scored as post-apoptotic necrotic or just necrotic. Nuclear condensation and fragmentation were assessed by fluorescence microscopy after Hoechst staining.

Apoptotic and necrotic cells were simultaneously detected by flow cytometry using an Annexin V/PI kit according to the manufacturer's instruction (Roche). In brief, after different treatments cells were collected, stained with Annexin V/PI for 10 min on ice, and analyzed by flow cytometry.

2.3. ROS

Intracellular ROS production was determined by measuring changes in fluorescence resulting from intracellular oxidation of either dichlorodihydrofluorescein diacetate (H_2DCF-DA) or dihydroethidium (DHE) (Molecular Probes, Oregon, USA). Briefly, cells grown on coverslips were washed with culture medium and stained for 15 min at 37 °C with either H_2DCF-DA (5 μmol/l) or DHE (10 μmol/l). After rinsed in culture medium cells were examined using a Nikon microphot-SA fluorescence microscopy, or analyzed by flow cytometry.

2.4. Mitochondrial membrane permeability (MMP)

MMP was assayed by staining with tetramethyl-rhodamine ethyl ester (TMRE). In brief, cells grown on coverslips were washed with culture medium and stained with TMRE (20 nM, 30 min, 37 °C). TMRE-induced red fluorescence was examined and documented using a Nikon microphot-SA fluorescence microscope

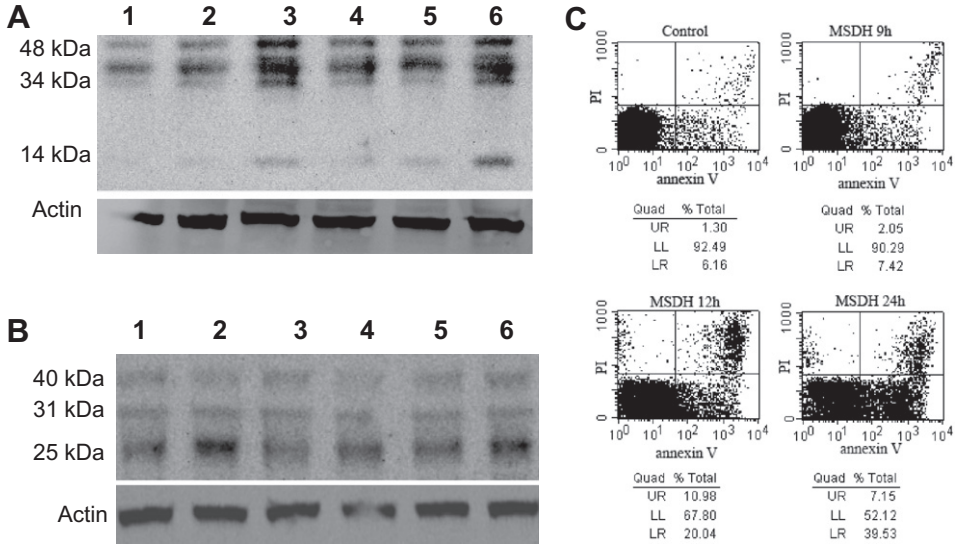

Fig. 1. Up-regulation and activation of lysosomal cathepsins in apoptotic cell death induced by MSDH. THP-1 macrophages were exposed or not to MSDH for 1–4 h and protein levels of cathepsin D (A) and cathepsin B (B) were assayed by Western blot. (A) Cathepsin D expression. Line 1: control 1 h; line 2: MSDH 7.5 μM 1 h; line 3: MSDH 25 μM 1 h; line 4: control 6 h; line 5: MSDH 7.5 μM 6 h; line 6: MSDH 25 μM 6 h. (B) Cathepsin B expression. Line 1: control 1 h; line 2: MSDH 25 μM 1 h; line 3: control 6 h; line 4: MSDH 25 μM 6 h; line 5: control 12 h; line 5: MSDH 25 μM 12 h. (C) Time dependent apoptosis caused by MSDH at 25 μM as assessed by flow cytometry following Annexin V/PI staining. UR: cells in upper right square represent late apoptotic/necrotic cells with Annexin V+ and PI+; LL: cells in lower left square represent viable cells with Annexin V− and PI−; LR: cells in lower right square represent apoptotic cells with only Annexin V+.