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H_2O_2-MEDIATED DAMAGE TO LYSOSOMAL MEMBRANES OF J-774 CELLS

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The effects of hydrogen peroxide on cell viability and, in particular, on lysosomal integrity were investigated in a model system of cultured, established, macrophage-like J-774 cells. The cells were found to rapidly degrade added hydrogen peroxide, withstanding concentrations $\leq 250\,\mu M$ without cell death; however, all tested concentrations (100–500 μM) substantially decreased cellular ATP to approximately the same degree. Concentrations of hydrogen peroxide $\geq 500\,\mu M$ resulted in a pronounced and rapid decrease in cell viability preceded by the loss of lysosomal integrity, as judged by the relocalization of acridine orange, a lysosomotropic weak base, in pre-labelled cells. Hydrogen peroxide-induced relocalization of acridine orange and cell death were either enhanced or much prevented, according to if the cells were initially allowed to endocytose ferric iron or the specific iron-chelator deferoxamine, respectively. Depletion of ATP, however, was not associated with the loss of lysosomal integrity and viability regardless of iron or deferoxamine pretreatment. Pre-exposure to E-64, an inhibitor of lysosomal thiol proteases, resulted in the reduction of both lysosomal membrane damage and cell death. The results are interpreted as indicating (i) generation of hydroxyl radicals within the secondary lysosomal compartment due to the occurrence of reactive ferrous iron, leading to (ii) peroxidative alterations of the lysosomal membrane resulting in (iii) loss of lysosomal membrane integrity with dissipation of the proton gradient and leakage of lysosomal contents, including hydrolytic enzymes, into the cell sap. The partial protection by E-64 may result from hydroxyl radical scavening by accumulated non-degraded autophagocytosed lysosomal material, and/or decreased availability of reactive redox-cycling iron due to decreased enzymatic digestion of autophagocytosed iron-containing metalloproteins. Moreover, our results show that the normal lysosomal content of iron, capable of redox cycling, of the cell line under study is enough to induce oxidative damage leading to loss of lysosomal integrity. It is suggested that lysosomal damage may be an important cause of cell degeneration under conditions of increased intra- or extracellular hydrogen peroxide-formation.

KEY WORDS: Hydrogen peroxide, oxygen radicals, lysosomes, iron, cultured cells.

INTRODUCTION

Hydrogen peroxide (H_2O_2), is a product of numerous oxidases residing in different cellular compartments and is thus ubiquitous. It may also derive from non-enzymatic auto-oxidative processes and as a result of electron leakage from the electron transport complexes of the inner mitochondrial membrane and the endoplasmic reticulum to oxygen[1] thus leading to the intermediate formation of superoxide anion radicals ($O_2^{\cdot-}$) with ensuing spontaneous or enzymatic (SOD) dismutation to H_2O_2.[2,3]

Under normal conditions the combined activities of catalase and glutathione peroxidase handle H_2O_2 without upsetting the normal GSH/GSSG ratio, which in

turn is dependent on the activity of glutathione reductase and a sufficient supply of NADPH from the hexose monophosphate shunt.[4] The consequences of increase in cytosolic H_2O_2, which might occur, for instance, during reperfusion conditions following ischemia,[5] in the metabolism of xenobiotics[6] and in inflammatory tissue with high production of $O_2^{\cdot-}$ and H_2O_2 by activated invading leukocytes,[7,8] are still being investigated.

Hydrogen peroxide-induced oxidative stress results in multiple damaging effects on cell metabolism, function and structure. One such effect is disturbed calcium homeostasis mediated by release of Ca^{2+} from intracellular calcium stores and by decreased function of membranous Ca^{2+} pumps.[9] Some enzymes, such as glyceraldehyde-3-phosphate dehydrogenase of the glycolytic pathway, are directly inactivated through disulfide bond formation[10] which, together with an inhibition of ADP phosphorylation, may cause a reduction in cellular ATP levels.[11] Hydrogen peroxide-induced single-strand breaks of DNA have been suggested to lead to activation of poly(ADP-ribose) polymerase resulting in depletion of NAD,[12] an essential cofactor in glycolysis. However, whether these or other metabolic alterations really can account for the acute and irreversible cell damage occurring in several types of mammalian cells in culture by the addition or generation of H_2O_2 in the 10 μM ranges is still a matter of dispute.[3,13,14] Furthermore, it has been shown that H_2O_2 toxicity is dependent on cellular sources of iron[3,15,16,17] thus suggesting that damage is directly inflicted by hydroxyl radicals ($HO^{\cdot}$) generated from H_2O_2 through Fenton type chemistry which is dependent on the availability of transition metals such as iron or copper in reduced form.

The hydroxyl radical reacts site-specifically at diffusion controlled rates with most biomolecules[6] and, thus, the cellular disposition of H_2O_2 and, in particular, of reactive iron is of great importance in oxidative stress-induced cellular pathology. This probably explains why the organism handles iron with the greatest care and almost always keeps it chelated, or in stable metallo-organic compounds, thus preventing an $Fe^{2+} \rightleftharpoons Fe^{3+}$ mediated (redox cycling) electron transfer.[2] During its passage through the acidic vacuolar apparatus iron may exist in loosely bound form,[16] although probably only for short periods of time.[2] This occurs when iron either dissociates from transferrin (requiring low pH) in acidified endosomes, and later is transferred to lysosomes, or directly appears in secondary lysosomes following autophagocytosis and degradation of iron-containing compounds such as ferritin or cytochromes from mitochondria and endoplasmic reticulum. Moreover, it has been shown that both ferritin and hemosiderin promote $HO^{\cdot}$ formation from H_2O_2 at pH 4.5 even in the absence of a reducing agent,[18] i.e. under conditions similar to those inside lysosomes where reducing agents such as ascorbic acid, glutathione and cysteine[19] also occur, thus increasing the probability of $HO^{\cdot}$ formation even further. Secondary lysosomes, therefore, might constitute *loci minora resistentia* since they are likley sites for the formation of $HO^{\cdot}$ by Fenton reactions due to the availability of loosely bound reduced and reactive iron. Lysosomal membrane damage would ensue and this would of course jeopardize cell integrity by the subsequent leakage of lysosomal contents, including hydrolytic enzymes, to the cell sap resulting in uncontrolled autolysis.

The present study is an attempt to elucidate the effects on lysosomal membrane integrity by the exposure of living cells in culture to hydrogen peroxide. This was accomplished by help of the lysosomotropic and metachromatic dye acridine orange, which in living cells gives a concentration-dependent bright red fluorescence to acidic

vacuoles as opposed to a weak green fluorescence of the cytosol and nucleus.[21] The modulating effects of $FeCl_3$, deferoxamine and E-64, an inhibitor of the lysosomal thiol-proteases cathepsin B, H, and L,[22] were investigated as well as the influence of these treatments on celluar ATP levels. The localization of larger cellular concentrations of reactive iron was determined by autometallography and morphological changes were observed with transmission electron microscopy.

MATERIALS AND METHODS

Chemicals

Ham's F-10 medium, fetal calf-serum (FCS) and Hepes buffer were from GIBCO (Paisley, UK). Glutamine, penicillin-G and streptomycin from Flow (Rickmansworth, UK). Acridine orange (Euchrysin 3R) was purchased from Gurr (Poole, UK). Ammonium-sulfide and hydroquinone were from BDH Ltd (Poole, UK), Epon-812 and silver-lactate from Fluka AG (Buchs, Switzerland). Deferoxamine (Desferal^{R}) was purchased from Ciba-Geigy AG (Basel, Switzerland), glutaraldehyde from Bio-Rad (Cambridge, MA, USA) and osmium tetroxide from Johnson Matthey Chemicals (Royston, UK). L-*trans*-epoxysuccinyl-leucyl amino(4-guanido)butane (E-64) was obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide was from Aldrich-Chemie (Steinheim, Germany). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

Cells and Culture Conditions

J-774 cells (established mouse histiocytic lymphoma cells) were routinely grown in 75 ml Costar plastic culture flasks in F-10 medium supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin-G and 100 μg/ml streptomycin in humidified air at 37°C. Cells were detached for experiments by scraping with a rubber policeman and seeded into 35 mm Costar plastic dishes with/without glass coverslips (Ø22 mm) at a density of 1.7×10^5 cells/dish. The cultures were rinsed with F-10 medium 60 min after plating to remove dead and non-adhering cells. Cultures were kept in complete culture medium (see above) for approximately 20 h with 30 μM $FeCl_3$ or for 24 h with 50 μg/ml E-64. Iron-treated cells were transferred to complete medium without $FeCl_3$ for 4 h (thus allowing for the transfer of lately endocytosed iron to secondary lysosomes) and then rinsed in PBS. In experiments involving deferoxamine pretreatment the cells were exposed to medium containing 1 mM deferoxamine for 1 h and then rapidly rinsed in PBS with 100 μM deferoxamine before the start of the experiments.

Exposure to Hydrogen Peroxide

Cells were exposed to H_2O_2 (100–1000 μM) in PBS in the culture dishes at 37°C in air. Deferoxamine was always included at 100 μM in order to prevent extracellular iron-mediated effects due to possible iron contamination of the PBS. This precaution mimics the *in vivo* situation with ubiquitous unsaturated iron-binding transferrin and protects against extrallular $HO^{\cdot}$-generating Fenton-reactions

Estimation of Hydrogen Peroxide Degradation

The degradation of H_2O_2 by J-774 cells was assayed by exposing 1.7×10^5 cells/dish to 2 ml PBS containing 100–500 μM H_2O_2 at 37°C in air. The decline in H_2O_2 concentration was followed for 60 min and fluorimetrically determined (λ_{ex} 315 nm, λ_{em} 410 nm) by p-hydroxyphenylacetic acid dimerization in the presence of horseradish peroxidase.[23,24]

Determination of ATP

ATP levels were determined using the luciferase-linked luminescence method. J-774 cultures with 1.7×10^5 cells/dish, incubated as described above, were extracted with 500 μl of 2.5% trichloroacetic acid. A 20 μl sample was added to 180 μl of an aqueous solution containing 1.2 ng/ml firefly luciferase, 64 nM D-luciferin, 1 mg/ml bovine serum albumin, 10 μM magnesium acetate, 2 nM inorganic pyrophosphate, 2 mM EDTA, and 0.1 M Tris-HCl, pH 7.75. The luminescence was measured using a LKB-Wallac 1250 bioluminometer (Wallac Oy, Turku, Finland). Assay temperature was 25°C and the ATP levels were calculated from a standard curve.

Determination of Cell Viability

Cell viability was determined using the trypan blue dye exclusion test. In each culture the number of stained (dead) and unstained (living) cells was counted in 10 fields of vision at low magnification using an inverted microscope.

Determination of Lysosomal Stability

Cells cultured on glass coverslips were stained with 2 ml AO-solution (5 μg/ml of F-10 culture medium without FCS) for 15 min at 37°C,[21,25] rinsed rapidly with PBS at 37°C, and then immediately exposed to H_2O_2 in PBS at 37°C in air. The intensities of red and green AO fluorescence from 100 individual cells/coverslip were measured simultaneously with a cytofluorometer system based on a Leitz MPV III (Wetzlar, Germany) photometer microscope using a BG 12 activating filter, a 630 nm barrier filter and a 572 nm interference filter.[21]

Fluorescence micrographs of living AO-stained cells were obtained using the same Leitz MPV III microscope system and Kodak Ektachrome 800 ASA film.

Autometallography for Light Microscopy

Cells were briefly rinsed in PBS (22°C) prior to fixation with 2% glutaraldehyde in 0.1 M Na-cacodylate buffer with 0.1 M sucrose (pH 7.2; effective osmotic pressure 300 mOsm) for 2 h at 22°C (unless otherwise stated). This was followed by short rinses ($\times 5$) in glass-distilled water at 22°C. Cells were then sulfidated at pH ~9 in 1% ammonium sulfide in 70% ethanol for 15 min. Following careful rinsing in running glass-distilled water for 10 min at 22°C, development was performed according to Danscher[26] using a physical, colloid-protected, developer containing Ag-lactate (0.11 g in 15 ml distilled water), hydroquinone (0.85 g in 15 ml distilled water), 60 ml 25% gum arabic, and 10 ml Na-citrate buffer, pH 3.8. The reaction was performed in the dark at 26°C for 15, 20, and 25 min. Following dehydration in a graded series of

ethanol solutions, cells (grown on coverslips) were mounted with Canada balsam, usually without counter-staining. Cells were examined and photographed in an Olympus AHBS photomicroscope (Tokyo, Japan) using Kodak Plus-X pan 125 ASA film.

Electron Microscopy

Cells for TEM were fixed *in situ* in the plastic dishes by addition of pre-warmed (37°C) fixative (2% glutaraldehyde in 0.1 M sucrose/sodium cacodylate-HCl buffer, pH 7.2, total osmolality 510 mOsm, vehicle osmolality 300 mOsm) in a 1:1 ratio to the culture medium for 5 min.[27] Thereafter the fixative was changed to 100% fixative (see above) for 2 h at 22°C. The cell cultures were post-fixed in 1% OsO_4 in 0.15 M Na-cacodylate buffer for 90 min. Dehydration in a graded series of ethanol was performed in the culture dishes. The cultures were stained with 1% uranyl acetate in 50% ethanol overnight during dehydration. The cultures were then embedded in Epon-812 in the plastic dishes as previously described by Abok *et al.*[28] The sections were cut with a diamond knife and stained with lead-citrate.[29] The sections were examined and photographed in a JEOL 2000-EX electron microscope (Tokyo, Japan) at 100 kV.

RESULTS

Effect of a Bolus Dose of Hydrogen Peroxide on Trypan Blue Exclusion

The exposure of the J-774 cells to H_2O_2 at high concentrations (500 or 1000 μM) in PBS with 100 μM deferoxamine reslted in loss of viability for all cells within 120 min, as evaluated with the trypan blue dye exclusion test (this test, however, is rather insensitive and extensive damage might have occurred long before the cells had started to take up trypan blue). Many cells stained blue after only 30 min at these concentrations. The exposure to H_2O_2 at low concentrations (100 or 250 μM), however, had no effecr on cell viability within 120 min, while 350 μM of H_2O_2 caused intermediate damage (Figure 1).

Cellular Degradation of Hydrogen Perxoide and Simultaneous ATP Depletion

When H_2O_2 (100, 250, or 500 μM) in PBS (with 100 μM deferoxamine) was added to J-774 cell cultures there was a rapid decrease in extracellular hydrogen peroxide (Figure 2A). The same concentrations of H_2O_2 also caused rapid depletion of ATP (Figure 2B), although only 500 μM H_2O_2 resulted in loss of viability (Figure 1).

Effects of Deferoxamine and $FeCl_3$ Pretreatments on Cellular Sensitivity to Hydrogen Peroxide

When J-774 cells, pretreated with 1 mM deferoxamine for 60 min in culture, were exposed to 500 μM H_2O_2 in PBS (with 100 μM deferoxamine) there was a marked protection against loss of viability as evaluated with the trypan blue dye exclusion test. However, if pre-exposure to deferoxamine lasted only 10 min, a much less pronounced protective effect was seen (Figure 3). Pretreatment in culture with $FeCl_3$ (30 μM; 20 h) followed by 4 h in fresh medium without iron and subsequent H_2O_2-exposure in PBS with 100 μM deferoxamine resulted in increased loss of viability (Figure 3).

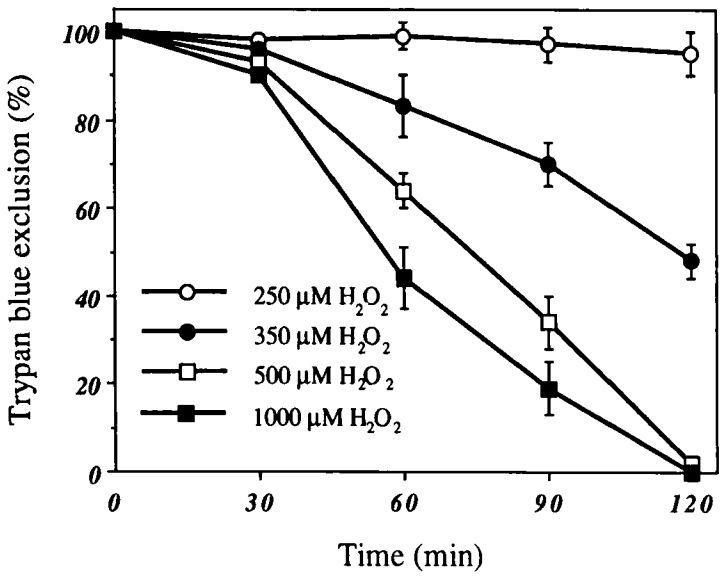


Figure 1 Trypan blue exclusion of J-774 cells 0–120 min after the exposure to a bolus dose of H_2O_2 (0–1000 μM) at 37 C. Values represent mean values of three separate experiments $\pm$SEM.

Effects of Hydrogen Peroxide on Acridine Orange-loaded Cells

When acridine orange-stained J-774 cell cultures were exposed to 500 μM H_2O_2 (in PBS with 100 μM deferoxamine at 37°C) there was a rapid (within minutes) redistribution of the dye (Figure 4) from a red granular lysosomal pattern to a diffuse green nuclear and cytosolic staining. This redistribution was inhibited by pre-treatment of the cell cultures with 1 mM deferoxamine for 60 min but was greatly enhanced by pre-treatment with 30 μM $FeCl_3$ for 20 h (Figure 5A). The increase in green fluorescence (Figure 5B) was recorded because the photomultiplying system is more sesitive to green than red fluorescence and also in order to preclude possible quenching of the red fluorescence effects by the hydrogen peroxide. All cells, irrespective of pretreatment, displayed the same depletion of ATP after exposure to 500 μM hydrogen peroxide (Figure 5C).

In experiments in wich E-64 pretreated cells were exposed to 500 μM H_2O_2 in PBS with 100 μM deferoxamine there was a marked delay in the redistribution of acridine orange-fluorescence from the granular red lysosomal to the diffuse green cytosolic-nuclear pattern (Figure 6). Pretreatment with E-64 had no inhibitory effect on ATP depletion (not shown).

Visualization of Endocytosed Iron

Cytochemical demonstration of heavy metals using autometallography of control J-774 cells (Figure 7A) or of those exposed to 30 μM $FeCl_3$ in complete medium for 20 h and for another 4 h in fresh medium without iron (Figure 7B) showed a lysosomal-type of silver deposition in both control and iron-exposed cells, although

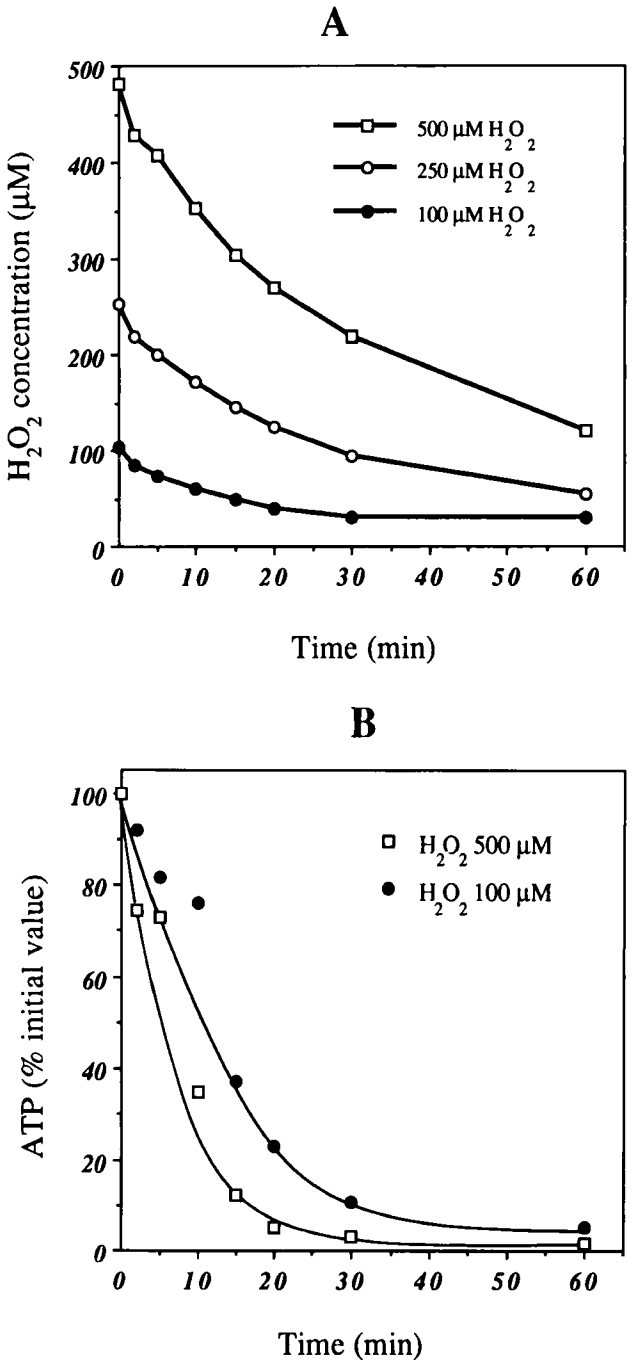


Figure 2 Degradation of H_2O_2 in PBS (2 ml) by J-774 cells in culture after the addition of bolus doses of 100, 250, or 500 μM H_2O_2 (Figure 2A). ATP levels of similarly treated J-774 cell cultures (Figure 2B).

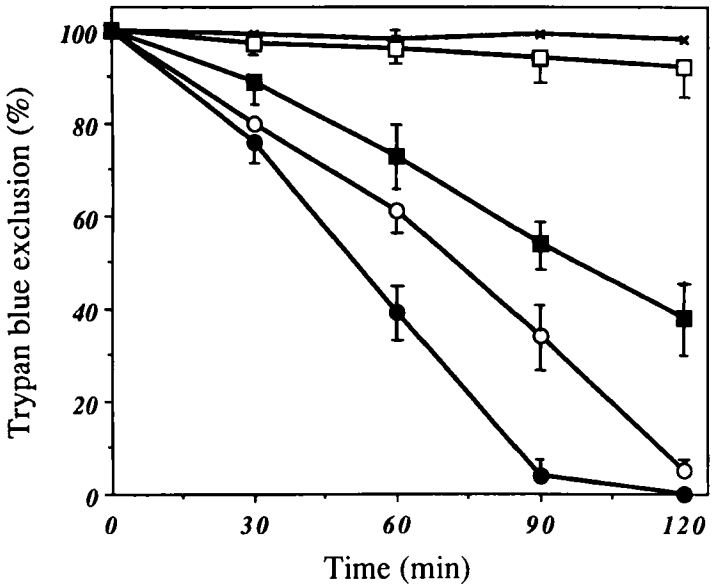


Figure 3 Trypan blue exclusion of J-774 cells pretreated with deferoxamine (1 mM; 10 (-■-) or 60 (-□-) min) or $FeCl_3$ (30 μM; 20 h) (-●-) exposed to 500 μM H_2O_2 in PBS for 0–120 min at 37 C. Controls received 500 μM H_2O_2 (-○-) or PBS only (-x-). Values represent mean values of three separate experiments ±SEM.

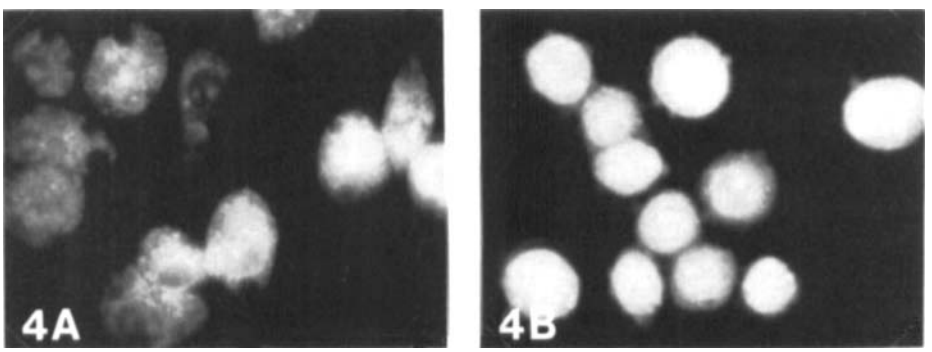


Figure 4 AO-loaded J-774 cells exposed to PBS only (Figure 4A) or to PBS with 500 μM H_2O_2 for 60 min (Figure 4B) demonstrating loss of granular red fluorescence and increase of nuclear and cytosolic fluoescence together with pronounced plasma membrane blebbing.

the reaction was much heavier in Fe-exposed cells. It was also noted that the iron-treatment seems to stimulate cell growth, resulting in increased cell size, which previously has been described.[30]

Electron Microscopy of Hydrogen Peroxide Exposed Cells

J-774 cells exposed to PBS only (Figure 8A) or to 500 μM H_2O_2 in PBS with 100 μM

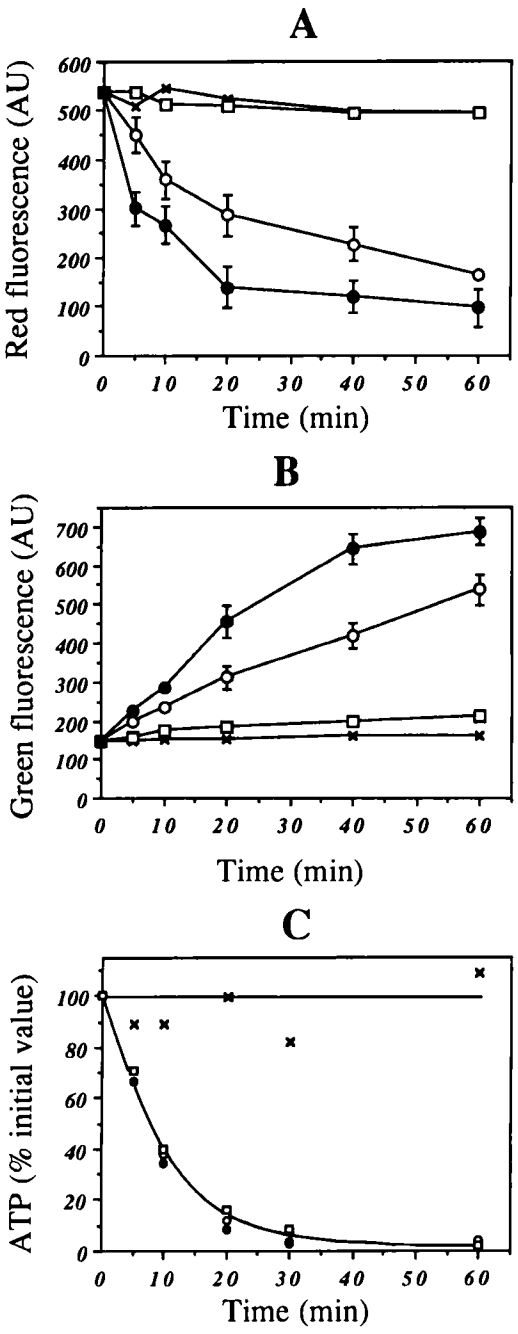


Figure 5 Effects of deferoxamine (1 mM; 60 min) (-□-) or $FeCl_3$ (30 μM; 20 h) (-●-) pre-treatment on red lysosomal (Figure 5A) and green nuclear and cytosolic (Figure 5B) AO-fluorescence of J-774 cells after exposure to 500 μM H_2O_2 for 0–60 min as compared to control cells without deferoxamine pre-treatment (-○-) or unexposed to H_2O_2 (-x-). Values represent mean values of three separate experiments ±SEM. Effects of the above described pretreatment regimes and 500 μM H_2O_2 on cellular ATP content (Figure 5C).

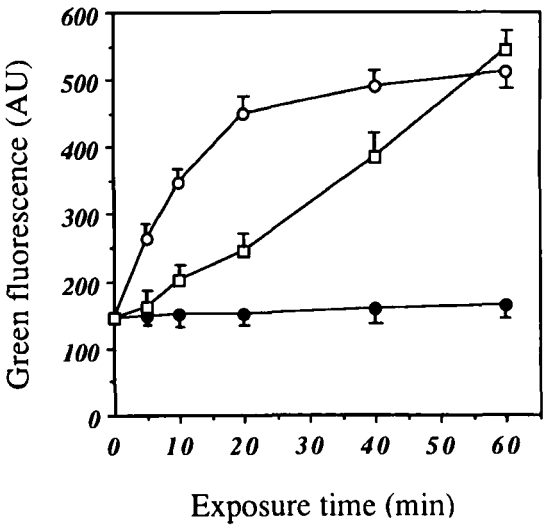


Figure 6 Increase in green nuclear and cytosolic fluorescence of AO-loaded J-774 cells pretreated with E-64 (50 μg/ml; 24 h) (-□-) followed by exposure to 500 μM H_2O_2 in PBS at 37°C for 0–60 min. controls received H_2O_2 (-○-) or PBS only (-●-). Values represent mean values of three separate experiments ± SEM.

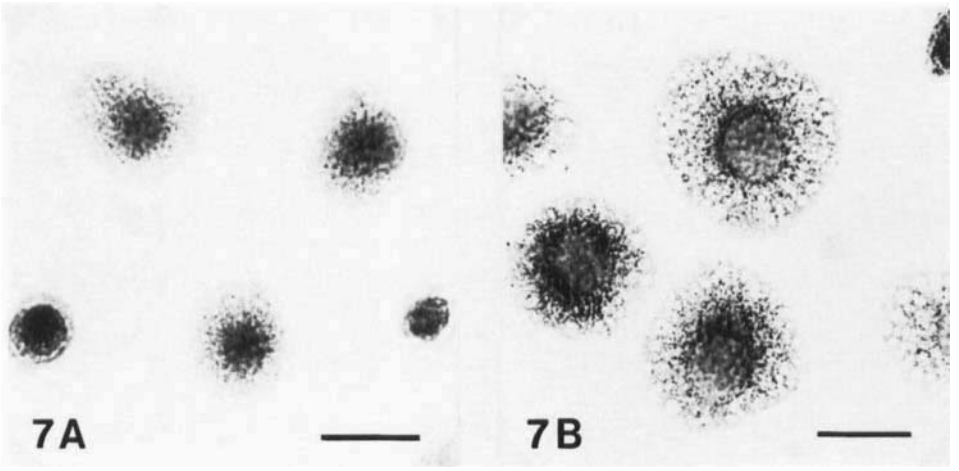
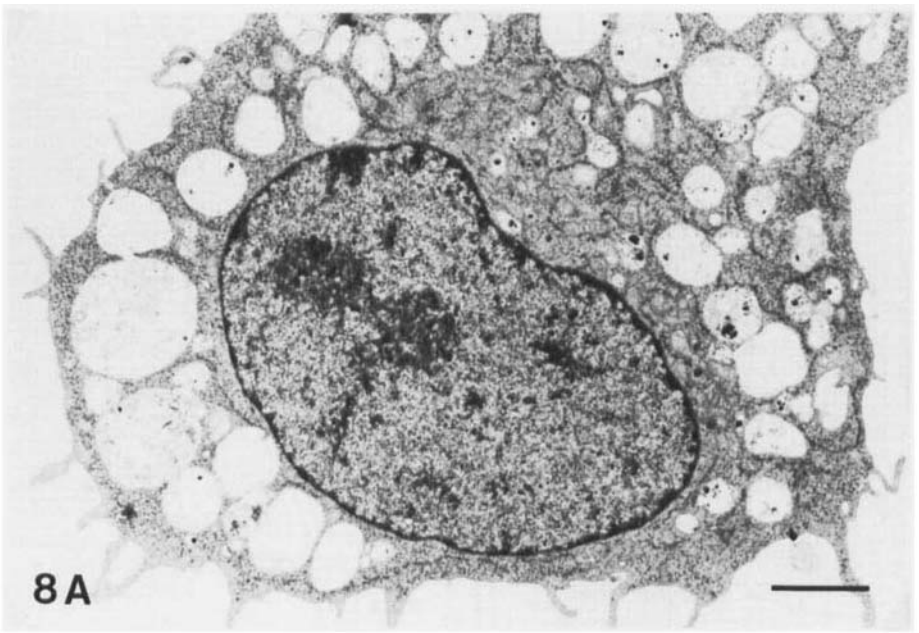


Figure 7 Cytochemical demonstration (autometallography) of reactive iron in control (Figure 7A) and iron-exposed (30 μM; 20 h) (Figure 7B) J-774 cells showing a lysosomal pattern of silver enhanced iron. Bars = 20 μM.

deferoxamine for 10 min (Figure 8B) revealed pronounced plasma membrane blebbing and disorganization of the cytoplasm when exposed to H_2O_2. Definite lethal damage in the form of condensation and pyknosis of the nuclei was also seen.



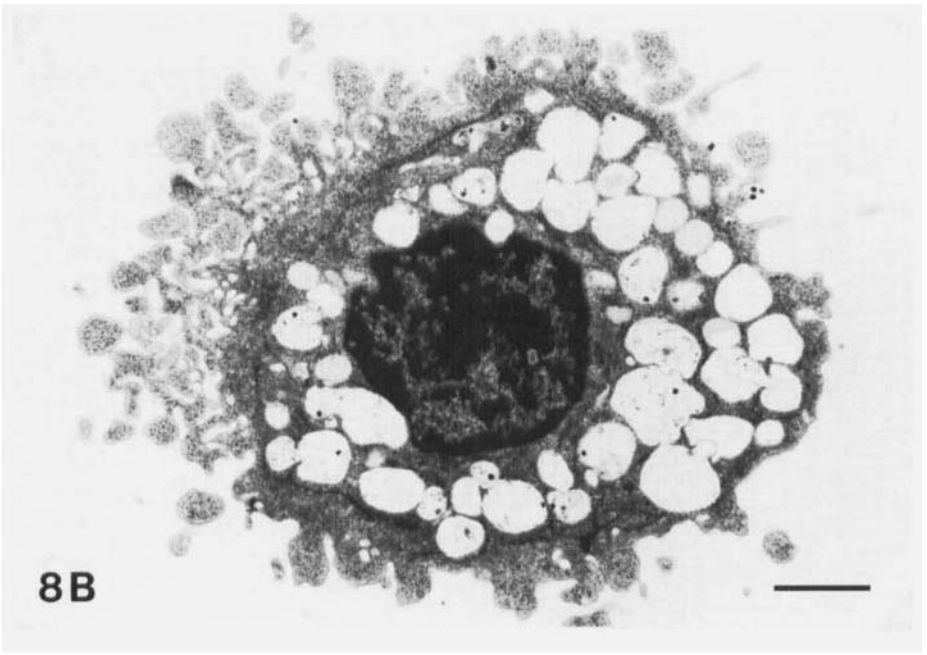


Figure 8 Electron micrographs of control (Figure 8A) and H_2O_2-exposed (500 μM, 10 min) (Figure 8B) J-774 cells displaying pronounced distortion of cellular morphology with bleb-formation and condensation and pyknosis of the nucleus. Bars = 2 μM.

DISCUSSION

This study indicates that cellular oxidative stress, due to abnormal endogenous or exogenous production of H_2O_2, may damage cells through a primary interaction with lysosomal ferrous iron resulting in the formation of hydroxyl radicals (intralysosomal Fenton reaction) which leads to lysosomal membrane damage and leakage of lysosomal contents such as hydrolytic lysosomal enzymes—see Figure 9. In the present study cultured J-774 cells are shown to efficiently degrade hydrogen peroxide when exposed to this compound in rather high bolus doses. Cultures survive 250 μM H_2O_2 without cell death, but increasing numbers of cells were killed during shorter time periods by higher H_2O_2-concentrations. Intracellular ATP-stores were depleted by hydrogen peroxide exposure ranging from 100 to 500 μM, although there was no loss of viability when exposed to $\leqslant$250 μM, thus providing a dissociation between loss of ATP and cell death.

As judged by the relocalization of acridine orange from a lysosomal to a cytosolic

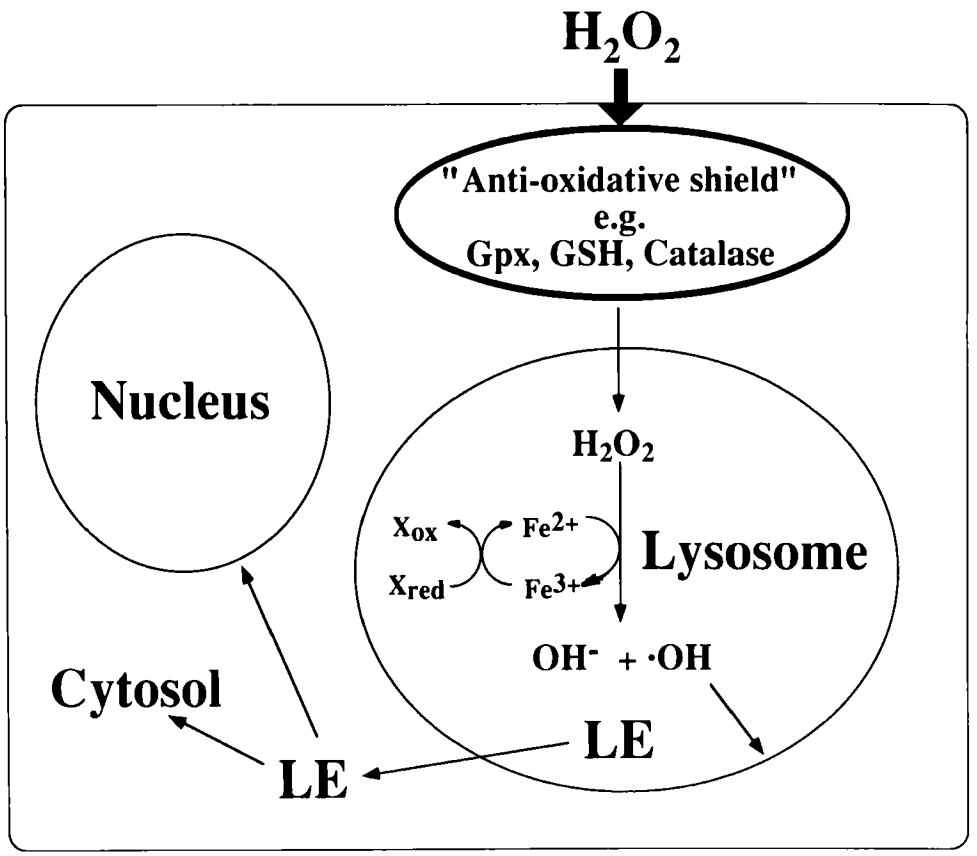


Figure 9 The proposed mode of action of H_2O_2-mediated damage to the lysosomal compartment. Lysosomal enzymes are indicated with LE.

distribution (shift from red granular to diffuse green fluorescence) it was evident that the H_2O_2-exposure resulted in the disappearance, or reduction, of the proton gradient over the lysosomal membrane. It was also obvious that this was a rapid event (within minutes) when cells were exposed to 500 μM H_2O_2 but to some degree also took place after exposure to considerably lower concentrations of hydrogen peroxide. Thus the alteration of lysosomal membrane permeability is an early and dose-responsive result of H_2O_2 exposure. Both acridine orange relocalization and cell death were prevented by pretreatment with deferoxamine. This chelator, however, did not prevent ATP-depletion, underscoring the dissociation between loss of lysosomal integrity and loss of intracellular energy substrates. We have previously shown that acridine orange relocalization is a sign of damage to the lysosomal membrane which may be accompanied by leakage of contents including hydrolytic lysosomal enzymes from the acidic vacuolar apparatus, resulting in early cellular degeneration in the form of blebbing, later by cytoplasmic vacuolization due to increased autophagocytosis, and nuclear pyknosis.[31]

The lysosomal compartment seems to be the most likely location of the "free non-protein-bound pool" of iron. Treatment of cells with the thiol protease inhibitor E-64 leads to inhibition of lysosomal cathepsins and thus to the accumulation of undergraded material in the lysosomal compartment.[31,32] It has previously been found that an induced reduction or increase of autophagocytosis respectively decreased or increased the restoration of sensitivity to oxidative stress of deferoxamine-treated hepatocytes.[33] Protease inhibition by E-64 will lead to less degradation and thus to less iron release from intralysosomally located ferritin and other metallo-proteins. The suppression or preservation, respectively, of both lysosomal stability and viability by endocytosed iron or deferoxamine and E-64 is well in agreement with the hypothesis than H_2O_2 is cleaved (Fenton reaction) within the secondary lysosomes due to the availability of reactive iron, thus resulting in $HO^{\cdot}$-mediated attack on lysosomal contents and membranes. Recent findings have shown that deferoxamine is taken up via endocytosis only.[34] This implies that when deferoxamine is added to cells in culture it chelates iron and inhibits oxidant damage within the vacuolar apparetus only. Cells contain several effective anti-oxidative defense mechanisms and, consequently, cell death due to hydrogen peroxide-mediated mechanisms will occur only under elevated pathological levels of hydrogen peroxide. Since this compound is constantly produced, albeit normally in low concentrations, the "anti-oxidative shield"[35] preventing its entrance into the lysosomal compartment is of great importance to cellular homeostasis. We have previously suggested that a small, or even moderate, leakage of lysosomal enzymes into the cell sap is a situation that cells may be able to cope with and repair by autophagocytosis of damaged parts.[31] Another factor contributing to cell survival in the case of redistribution of lysosomal hydrolytic enzymes is that lysosomal cathepsins have their pH optima in the acidic range with reduced activity at the near-neutral pH of the cytosol.

In conclusion, we suggest that the intralysosomal occurrence of reactive iron will generate hydroxyl radicals in the presence of hydrogen peroxide and, in turn, cause damage to the lysosomal membrane. The sequence of events leading to the ultimate death of cells after exposure to hydrogen peroxide is probably not attributable to one factor only. We would, however, like to point out that damage to the lysosomal membrane with loss of lysosomal integrity and consequent uncontrolled autolysis may be a hitherto not described cause of hydrogen peroxide-induced cell degeneration and death.

Acknowledgements

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