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A possible role of oxidative stress in the switch mechanism of the cell death mode from apoptosis to necrosis – studies on ρ^0 cells

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Abstract

Apoptosis is induced not only during morphogenesis and embryogenesis but also under various pathological conditions, especially related to oxidative stress. Apoptotic cells are phagocytized by neighboring cells while necrotic cells cause local and general reactions sometimes lethal to our bodies. Data have been accumulated to demonstrate that the switch of the cell death mode from apoptosis to necrosis does occur. However, detailed mechanisms involved in the switch mechanism remain unsolved although decreases in the intracellular level of ATP and a burst in the cellular level of reactive oxygen species (ROS) have been proposed. Recently, we have shown that the population of apoptotic cells reaches maximum in human osteosarcoma 143B cells treated for 6 h with menadione (MEN) while necrotic cells become predominant at 9 h of the treatment. In the present study we have attempted to clarify the role of cellular ATP in the switch mechanism using ρ^0 cells derived from human osteosarcoma ρ^+ cells. Results are summarized as follows: (1) Apoptotic and necrotic changes in ρ^0 cells are much faster than ρ^+ cells after the treatment with MEN. (2) Cellular level of ATP in ρ^0 cells remains essentially in the same level before and after the MEN-treatment while intracellular levels of superoxide continuously increase after the MEN-treatment. (3) ρ^+ cells treated with MEN in the presence of antimycin A plus oligomycin show similar changes to those of MEN-treated ρ^0 cells. (4) MEN-induced increases in the cellular level of superoxide are distinctly suppressed by inhibitors of NADPH oxidase. These results suggest that the intracellular level of superoxide may be a key factor directly related to the switch mechanism from apoptosis to necrosis, and that decreases in cellular level of ATP accelerate both apoptotic and necrotic changes of the cells.

Keywords: Cell death mode; Menadione; ρ^0 cells; ROS; ATP; Flow cytometry; Electron microscopy

1. Introduction

One of the hottest topics in the research field of apoptosis is the switch mechanism of the cell death mode from apoptosis to necrosis. Apoptotic cells are phagocytized by neighboring cells whereas necrotic cells cause local and general reactions. Apoptosis plays a central role not only in embryogenesis and morphogenesis during development, but also is induced under various pathological conditions, especially related to oxidative stress. A body of experimental data is available in the literature demonstrating that some chemicals cause apoptotic changes followed by necrosis (Ledda-Columbano et al., 1991; Sun et al., 1997). In some cases on chemical at low concentrations causes apoptosis, while it causes necrosis at high concentrations (Sata et al., 1997). Recently, we examined menadione (MEN)-induced cell injury processes in human osteosarcoma 143B cells using flow cytometry, confocal microscopy and electron microscopy (Kamiński et al., 2004) (Fig. 1). It has turned out that the population of apoptotic cells detected

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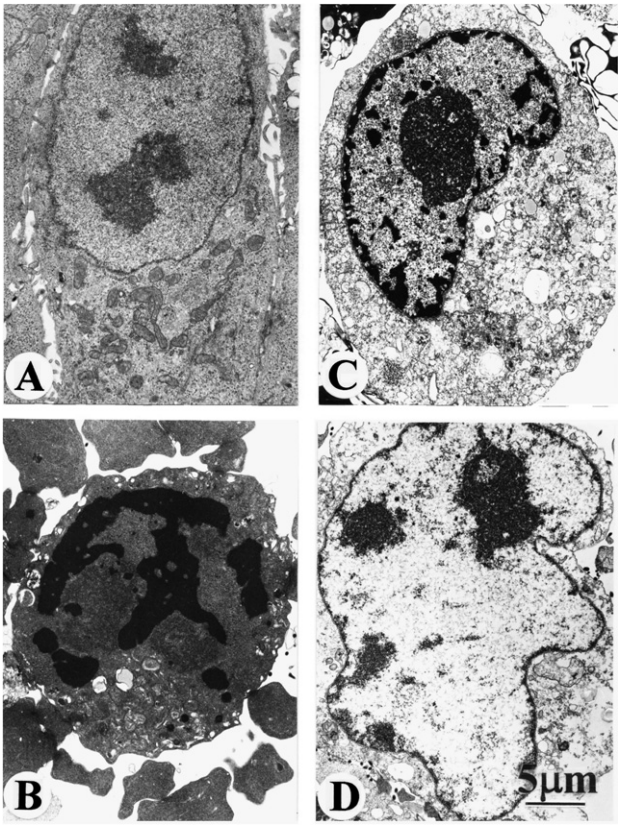

Fig. 1. Typical features of electron microscopic appearances of MEN-treated cells. Cells were treated with 100 μM MEN for 24 h. (A) Control; (B) apoptotic cells; (C) intermediate cells; (D) necrotic cells. Magnification, 8750×.

by Annexin V and PI double staining in the cells treated with MEN for 6 h reaches maximum followed by distinct decreases thereafter while that of necrotic cells continuously becomes increased. Necrotic cells judged by Annexin V and PI double staining were characterized electron microscopically by the presence of condensed nuclei and swollen cytoplasm suggesting that they became necrotic via the apoptotic processes (Kamiński et al., 2003). Intracellular level of superoxide reaches maximum at 6 h of the MEN-treatment followed by a distinct decrease thereafter. Intracellular level of ATP decreased immediately after the treatment with MEN, stayed at almost the same level for up to 9 h, and became further decreased thereafter (Kamiński et al., 2004). Thus, we could not conclude which factor was primarily involved in the switch mechanism.

Two major proposals have been made as to the switch mechanism of the cell death mode from apoptosis to necrosis: decreases in the cellular level of ATP (Leist et al., 1997; Ferrari et al., 1998; Ha and Synder, 1999) and a burst in the intracellular level of reactive oxygen species (ROS) (Nobel et al., 1997; Hampton and Orrenius, 1997).

In the present study we examined MEN-induced cell injury processes in ρ^0 cells derived from 143B cells if intracellular level of ATP is directly related to the switch from apoptosis to necrosis. ρ^0 cells have much less ability to generate ATP and depend on energy sources derived from glycolysis. We also followed MEN-induced cell injury processes in the presence of antimycin A, an inhibitor of electron transfer chain, and oligomycin, an inhibitor of ATP synthesis mimicking ρ^0 cells.

Data have been accumulated to demonstrate that NADPH oxidase, detected originally in the plasma membrane of leukocytes, is also detected in various mammalian cells (Xia et al., 2002). In neurons, the rate of the generation of superoxide from NADPH oxidase has been reported to be 6- to 10-fold higher than that from mitochondria (Martín-Romero et al., 2002). Thus, we examined a possible contribution of NADPH oxidase to MEN-induced switch of cell death mode from apoptosis to necrosis.

2. Materials and methods

2.1. Cell culture

The ρ^0 143BTK$^-$ cells were obtained from 143BTK$^-$ osteosarcoma (ρ^+) (ATCC CRL8303). The ρ^0 and ρ^+ cells were kindly provided by Dr. M. Tanaka, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan. The ρ^0 and ρ^+ cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in Dubecco's modified Eagle's medium (Nissui CO., Tokyo, Japan) containing 1 mM pyruvate supplemented with 10% bovine serum and 50 μg/ml kanamycin and 4500 μg/ml glucose. Uridine (50 μg/ml) was added to the culture medium for ρ^0 and ρ^+ cells.

2.2. Treatment of cells with various chemicals

Cells were treated with various chemicals specified below: MEN (100 μM) (Sigma Chemical CO., St. Louis, MO), antimycin A (50 μM) (Sigma), oligomycin (3.5 μg/ml) (Sigma), carbonylcyanide-*m*-chlorphenylhydrazone (CCCP, 100 μM) (Sigma). Inhibitors of NADPH oxidase: diphenyleniodonium chloride (DPI), apocynin (AP), *N*-vanillylnonenamide (N-VNA). Other chemicals were of analytical grade. All reagents were prepared as 1000×-concentrated stock solutions dissolved in DMSO except for MEN that was dissolved in MilliQ water and stored at −20 °C.

2.3. Annexin V and PI-binding assay

Viability of cells was assayed using FITC-conjugated Annexin V (BioSource Int., CA) and propidium iodide (Molecular Probes) double staining using Coulter FACSCAN, as described before (Kamiński et al., 2003).

2.4. Intracellular levels of ATP

Intracellular level of ATP was measured by high performance liquid chromatography (HPLC). Extraction of nucleotides from the cells was carried out essentially according to the method of Kalsi et al. (1999), as described before (Kamiński et al., 2003). Application of samples to HPLCV was performed according to the method of