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Original Contribution

Hydrogen peroxide-induced activation of defense mechanisms against oxidative stress in rat pancreatic acinar AR42J cells

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Abstract

Oxidative stress has been implicated in the pathogenesis of acute pancreatitis. Generally, cells respond to oxidative stress with adaptive changes in gene expression aimed at preventing cellular damage and increasing their survival. However, the overall extent of these genetic changes remains poorly defined. This issue was, therefore, examined in the current study. Following exposure of rat pancreatic AR42J cells to 0.08 mM hydrogen peroxide (H_2O_2), a concentration failing to induce necrotic cell death, the expression of 96 stress-related genes was monitored by cDNA microarray analysis. H_2O_2 provoked a time-dependent reorientation of 54 genes. In particular, at 6 and 24 h, 27 and 11 genes were induced, whereas 10 and 6 genes were suppressed, respectively, showing that the degree of change was stronger at the early time point, and that the number of up-regulated genes was obviously larger than the number of down-regulated genes. Reverse transcription-PCR for selected genes confirmed the gene expression pattern. Many of the differentially up-regulated genes can be related to the antioxidant enzymatic defense system, to cell cycle arrest, to repair and/or replacement of damaged DNA, to repair of damaged protein, and to activation of the NF-κB pathway. The results suggest that AR42J cells respond to sublethal oxidative stress with transient transcriptional activation of multiple defense mechanisms that may be an indication for a complex adaptation process. An understanding of the cellular stress responses may lead to new insights into the pathogenesis of oxidative stress-related diseases including acute pancreatitis.
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Keywords: Pancreatic acinar cells; Oxidative stress; cDNA microarray; Stress response; Antioxidant defense mechanisms; Apoptosis; Adaptation

Introduction

Exposure of cells to various types of environmental stress (hyperthermia, oxidants, heavy metals) and physiological stress (ischemia, inflammation) leads to transcriptional activation of genes encoding proteins with damage-preventing or damage-repairing functions referred as to stress proteins, whereas the expression of potentially harmful genes is down-regulated. This process known as stress response may be critically important in protecting cells from injury or helping cellular recovery.

Evidence suggests that the pancreas has the ability to rapidly start defense mechanisms when submitted to stress. Thus, whole-body stress of rats induced by heat and water immersion has been demonstrated to lead to up-regulation of the heat shock proteins HSP70 and HSP60 in the pancreatic tissue which was associated with protection against different forms of acute pancreatitis [1–3]. Cellular stress promoted by experimental acute pancreatitis has been reported to induce a variety of stress proteins including the transcription factor p8, pancreatitis-associated protein 1 (PAP-1), and the glycoprotein clusterin [4–6] and to down-regulate genes encoding the potentially harmful

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GSR, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; GSH, glutathione; HSP, heat shock protein; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; MT, metallothionein; NF-κB, nuclear factor-κB; PAP-1, pancreatitis-associated protein 1; PI, propidium iodide; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase; TAS, total antioxidant status.

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digestive pancreatic enzymes such as chymotrypsinogen and elastase [6]. This reorientation of gene expression pattern was found to exert anti-inflammatory and antiapoptotic effects [4].

A variety of studies indicate that oxidative stress may play an important role in the initiation and development of acute pancreatitis [7,8]. This assumption is based on the detection of oxidative stress in the pancreatic tissue of rats suffering from acute pancreatitis [9] and on the observation that antioxidant treatment decreased the severity of pancreatic damage in different models of experimental acute pancreatitis [7,10,11]. Oxidative stress results when the generation of reactive oxygen species (ROS) exceeds the capacity of the cellular antioxidant defense systems that naturally neutralize and eliminate them. ROS can cause oxidative damage to macromolecules resulting in lipid peroxidation, oxidation of amino acids, formation of protein–protein cross-links and protein fragmentation, DNA damage, and DNA strand breaks [12]. High concentrations of ROS are cytotoxic leading to necrotic cell death [12], whereas pretreatment of cells with sublethal ROS levels activates antioxidant defense and/or repair systems, leading to temporary adaptation to oxidative stress [13–15]. Previously, in the pancreatic acinar cell, moderate oxidative stress has been reported to activate the transcription factor nuclear factor (NF)-κB and to stimulate the transcriptional up-regulation of PAP-1; both parameters have been identified to belong to the acinar defense system against apoptosis [16,17]. All in all, little is known what defense systems against oxidative stress are activated in the pancreatic acinar cell in response to moderate oxidative stress. Extrapolation of previous studies in other cell types to the pancreatic acinar cell may lead to false conclusions because the toxicity of ROS varies with cell type studied [18]. In addition, the pancreatic acinar cell is distinctly different from almost all other cell types [19]. Therefore, the aim of the present study was to profile the stress gene expression pattern in the acinar cell associated with moderate oxidative stress using the Stress/Toxicity PathwayFinder GEArray from SuperArray to monitor the expression of 96 stress genes, the pancreatic acinar cell line AR42J as model system, and hydrogen peroxide (H_2O_2) to generate oxidative stress. An understanding of cellular responses to oxidative stress may lead to new insights into the pathogenesis of oxidative stress-related diseases and to new therapeutic strategies for these pathologic conditions including acute pancreatitis.

Our results suggest that AR42J cells respond to sublethal H_2O_2 with activation of multiple defense systems including antioxidant defenses, growth arrest, and damage removal and repair systems that may at least in part render them resistant to oxidative stress-induced cell death.

Materials and methods

Materials

Heat inactivated fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and PCR primers were obtained from Life Technologies (Eggenstein, Germany). Dexamethasone (Fortecortin Mono 4) and H_2O_2 were purchased from Merck (Darmstadt, Germany), penicillin and streptomycin from Biochrom Seromed (Berlin, Germany) and Biotin-16-dUTP and RNase A from Roche Diagnostics (Mannheim, Germany). MMLV reverse transcriptase was obtained from Promega (Mannheim, Germany). Trypan blue solution and most other chemicals used were from Sigma-Aldrich (Deisenhofen, Germany).

Cell culture and treatments

Because of the short lifetime of dispersed rat pancreatic acinar cells, the rat pancreatic AR42J cell line (American Type Culture Collection, Rockville, MD) was used for the present experiments. Dexamethasone treatment has been found to convert these cells into exocrine cells [20]. AR42J is as yet the only cell line that exhibits many characteristics of normal pancreatic acinar cells such as synthesis and secretion of digestive enzymes. Thus, this cell line has been used to study secretion, growth, proliferation, and oxidant-induced apoptosis of exocrine pancreatic cells [21].

Cells were used between passages 21 and 31. They were seeded at a density of $2\times10^4/cm^2$ in 75 cm^2 culture flasks and routinely grown for 72 h in DMEM supplemented with 10% (v/v) FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Growing cells were checked for viability and confluence by light microscopy. After incubation with 100 nM dexamethasone for 48 h, the cells were washed with PBS and incubated in fresh medium. The experiments were started by the addition of H_2O_2 at different concentrations or medium. After 6 and 24 h, cells were harvested for further analysis.

Cell viability

Cell viability was assessed by measuring the release of lactate dehydrogenase (LDH) into the culture medium using the LDH test kit and Synchron LX20 analyzer from Beckman Coulter (Krefeld, Germany). In addition, the trypan blue exclusion assay was used. Briefly, at the end of the experiments, cells were harvested and an aliquot of the cell suspension was diluted 1:1 with 0.4% trypan blue solution. Cell number was determined using a Neubauer chamber for blood cell counting. All cells excluding trypan blue were considered viable.

Electron microscopic evaluation

Cells were fixed with 4% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0) for 1 h, washed in the same buffer, and postfixed in 1% osmium tetroxide. Thereafter, they were embedded in 0.1% liquid agar. Small cubes of the samples were dehydrated in a graded series of ethanolic solutions and embedded in Durcupan ACM. Ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and imaged using an electron microscope EM 902 A (Zeiss, Oberkochen, Germany). The evaluation was performed by a pathologist blinded to the experimental protocol. In each group, 500 cells were assessed. According to the severity of cell damage, a score of 1–4 was

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