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X-ray induced L02 cells damage rescued by new anti-oxidant NADH

Fa-Quan Liu, Ji-Ren Zhang

Fa-Quan Liu, Ji-Ren Zhang, Department of Oncology, Zhujiang Hospital, First Military Medical University 510282, Guangdong Province, China

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Correspondence to: Fa-Quan Liu, Department of Oncology, Zhujiang Hospital,First Military Medical Unive Guangzhou, 510282, China. liufaquan@163.net
Telephone: +86-20-85143202 Fax: +86-20-85143200

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Abstract

AIM: To explore molecular mechanism of nicotinamide adenine dinucleotide (NADH) antagonization against L02 cells damage.

METHODS: L02 liver cells were cultured in RPMI 1640, exposed to X-ray irradiation and continued to cultur presence or absence of NADH. Cellular viability was analyzed by routine MTT methods. The percent age of and positive expressions of p53, bax and bcl-2, fas, fasL proteins were determined by FCM. Level of intrace determined by confocal microscope scanning. Morphological change was detected by scanning electron mi

RESULTS: The viability of L02 cells was decreased with increasing dose of X-ray irradiation. NADH could no the apoptosis induced by X-ray irradiation, but also up-regulate expression of bcl-2 protein and down-regul of p53, bax, fas and fasL proteins (P<0.05). At the same time, NADH could reduce level of intracellular RO L02 cells.

CONCLUSION: NADH has marked anti-radiation effect, its mechanism may be associated with up-regulatio expression and down-regulation of p53, bax fas and fasL expression, as well as decline of intracellular ROS further investigation of its mechanism is worthwhile.

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INTRODUCTION

Recent radiobiological studies have demonstrated that ionizing radiation can induce cell death. Exposure of radiation over a wide dose range results in activation of cellular response pathways, including p53-depende independent ways[1,2]. At the same time, apoptosis resulted from a coordinate sequence of biochemical ev leads to cell death. Among these,the generation of ROS with perturbation of prooxidant/antioxidant ratio, a mitochondria structure and $\triangle\psi m$, and diminutions of plasma membrane potential have been investigated[3,

stabilized electrochemical gradient relies on a functional ion exchange via the electrogenic transporter Na+ Na+/K+-ATPase is an energy hungry process which consumes a major of cellular ATP production. Therefor be a decrease of ATP level when apoptosis starts within a few minutes after ionizing irradiation. NADH, an coenzyme, participates in three carboxyl cycles and ultimately produces ATP molecules. We added NADH tc undergoing X-ray irradiation *in vitro*, and observed change of survivals and apoptosis as well as radiation a proteins which take part in signal transduction of apoptosis.

MATERIALS AND METHODS

Reagents

NADH (purity: 97 %) was gifted by Professor Birkmay from Chemical Department of Graz University. Mono anti-p53, bcl-2, bax antibodies and rat anti-Fas, FasL antibodies were from Beijing Zhongshan Biotechnolog PI/Annexin V kits were purchased from Immunotech (France).High FITC-labeled goat anti-mouse antibodie Zhongshan Company. H2DCF probe was purchased from America Molecular Probe Company.

Cell lines and culture

Normal human liver cell line L02 was purchased from Shanghai Institute of Cell Biology, Chinese Academy The cells were cultured in RPMI 1640 supplemented with streptomycin (50 U/ml), glutamate (2 mM) and 1 bovine serum.

Induction of apoptosis

The L02 cells were seeded in 6-well tissue cultured plates, the supernatant was discarded and 0.01 M PBS added. The L02 cells were X-ray-irradiated with 2.5, 5.0 and 7.5 Gy. The cells were then cultured in a com in the presence or absence of NADH at a concentration of 400 ug/ml, respectively. Non-irradiated culture s control.

MTT cell viability assay

The cells were seeded into 96-well dishes (5×10^3 cells/well),incubated for 24 h to allow attachment, treate 5.0 Gy, and continued to culture for 12, 24, 36, 48 h in the presence or absence of NADH. Absorbance was nm by using DG3022 ELISA according to the routine MTT methods. The cellular viability was calculated as MTT uptake[5].

Assessment of apoptotic cells

The L02 cells were seeded at 5×10^4-5×10^5/ml in 6 well tissue cultured plates and cultured for 48 hours in F medium containing 10 % FBS. Apoptosis was induced by X-ray irradiation, the LO2 cells were continued to h. A total of 5×10^5 -5×10^6/ml cells were collected by centrifugation at 200 g (5 min) and washed twice with (pH 7.4). Percent age of apoptosis was detected by flow cytometry according to PI/Annexin V kits.

Scanning electron microscope

24 hours following exposure to 2.5 Gy X-ray radiation, the L02 cells were fixed for 1 hour at room tempera glutaraldehyde in PBS and proceeded for scanning electron microscopy as routine methods.

Protein expression of bcl-2, bax, fas, FasL and p53

The L02 cells were collected and then washed twice with ice-cold PBS, followed by fixation in 0.5 % parafo 4 °C for 30 min. The fixated cells were treated with PBS containing 0.1 % Triton-100 and washed twice. Th of every tube were divided into five tubes and washed. The supernatant was aspirated. The antibodies aga p53, fas, FasL were added into each tube, mixed and incubated for 1 h at 37 °C. The cells were washed tw FITC-labeled second antibodies were added for 30 min at 37 °C. Then, the cells were washed twice with i resuspended with 500 μl PBS. 10 000 events were analyzed and the positive rate of protein expression was

FCM.

Determination of intracellular ROS concentration

The cell suspension was dispensed into special culture plates at a density of 2×10^4 cells per ml and incubat % CO_2 for 48 h. The supernatant was removed and replaced with Hank's solution , then exposed to 2.5 Gy radiation. The supernatant was discarded at once, and replaced with RPMI-1640 medium with or without N concentration of 400 ug/ml for 4 h, respectively. Non-irradiated culture served as control, followed by wash with Hank's solution. Measurement of intracellular ROS concentration was described in literature. Briefly, th loaded with 0.5 ml H2DCF in DMSO solution at 5 μg/ml and incubated at 37 °C for 30 min. After washed th PBS, 0.5 ml PBS was loaded and the change of intracellular ROS was detected by scanning fluorescence int confocal microscope.

RESULTS

X-ray treatment inhibited growth of L02 cells

The L02 cells were treated with different doses of X-ray irradiation. Cell survival was determined after 12, 2 Inhibition of growth in X-ray treated cells occurred in a dose-dependent manner (Figure 1). Survival of L02 as the dose of X-ray increased. It was most obvious at 24 h post-irradiation.

Figure 1(PDF) X-ray induced inhibition of growth of L02 cells.

NADH antagonized apoptosis of X-ray treated L02 cells

The L02 cells were treated with 2.5, 5.0, 7.5 Gy X-ray irradiation, then post-incubated in fresh complete RP medium containing NADH or NADH free drug for 24 h. Percent age of apoptosis was determined by FCM us V stain method. The results showed that NADH diminished apoptosis of L02 cells exposed to X rays. The pe apoptosis was (7.08±2.34) %, (28.16±2.46) %, (47.30±3.43) % in the absence of NADH. However, it was (8.25±1.64) %, (15.30±1.98) % in the presence of NADH. The difference was significant between L02 cells 5.0 Gy,7.5 Gy X rays and cultured for 24 h in the presence of NADH and L02 cells cultured in the absence o (P<0.05). These findings suggested that NADH was involved in cytoprotection by blocking the induction of

NADH rescued L02 cells damage from X-ray radiation

X-ray radiation could induce L02 cells damage. As shown in Figure 2, Part(b) and part(c) represented differ morphologic changes of 2.5 Gy X-ray radiated L02 cells in the absence or presence of NADH. Part(a) repres shamly irradiated L02 cells, which had normal liver cell surface structure with normal protuberance and vol (b) had decreased protuberance and atrophy. However, degree of damage in L02 cells of part(c) group wa than that of part(b). These suggested that NADH could rescue L02 cells damage from X-ray irradiation.

Figure 2 The result of scan electron microscopy. (a) Normal liver cell surface structure. (b) Irradiated hep atrophy and decreased protuberance. (c) Morphological changes of hepatocytes irradiated and incubated in of NADH.

Expression of p53, bax, bcl-2, Fas and Fas-L in L02 cells

The results of FCM analysis for p53, bax, bcl-2, Fas and FasL protein expression in X-ray irradiated and mo L02 cells are summarized in Table 1. Significantly high levels of p53, bax, Fas and Fas-L protein expression in cells irradiated and cultured in the absence of NADH than in those cells cultured in the presence of NADH irradiated, but expression of bcl-2 protein tended to be low in L02 cells. Our results showed that NADH up-expression of bcl-2 protein and down-regulated expression of p53, bax, bcl-2, Fas and FasL protein in L02 undergoing X ray irradiation. It might be one of the mechanisms that NADH rescues L02 cells injury from ic irradiation.

Table 1 Effect of NADH on regulation of apoptosis associated proteins in L02 cells treated with X-ray (n=

Group	p53	Fas	FasL	bcl-2	
Mock IR	22.40±0.91	7.01±0.21	66.66±1.60	5.27±0.12	
IR	37.4±1.11[a]	13.40±0.78 [a]	74.40±1.09 [a]	2.22±0.18 [a]	8
IR+NADH	26.93±6.73	11.29±1.40	68.93±1.88	3.62±1.34	

Based on t test, "Mock IR" represented L02 cells of non-irradiated group. "R+NADH" represented L02 cells continued to culture in the presence of NADH. [a]P<0.05 vs Mock IR group and IR+NADH group.

Figure 3A(PDF) The change of intracellular H_2O_2 production in L02 cells cultured with or without NADH fo ray irradiation. (a) Sham irradiation group. (b and c) X-ray treated (2.5 Gy) L02 cells were respectively cult absence or presence of NADH. Mean value of fluorescence was calculated according to the number of L02 compared with sham irradiation (a) and test group (c).

Determination of intracellular ROS

Figure 3A and Figure 3B show that 2.5 Gy X-ray irradiation increased the level of intracellular ROS after 4 h L02 cells compared with that in L02 cells of sham irradiation group. However, NADH could reverse the effe irradiation.

Figure 3B The graph showed the cell fluorescence change of intracellular ROS by confocal microscope sc cells of different treatment. a b c

DISCUSSION

When a cell exposed to ionizing irradiation, at least two signal-generating targets are activated, one at the the other at the DNA. Signal may also originate in cytoplasm[6,7]. These signal targets ultimately result in c non-death stress response. Apoptosis, also called programmed cell death (PCD),is a peculiar form of cell de characterized by several morphological and biochemical aspects which are different from necrosis, an othe X-ray irradiation is one of the ionizing radiations, which can cause both membrane and DNA damage to cel cell apoptosis. How ionizing radiation triggers apoptosis is not known. It was reported that apoptosis media damage occurred via p53-dependent and p53-independent pathways[1,2]. However, several pathways of ap been reported. One of these is the Fas/FasL pathway, which involves binding of a death receptor to a deat initiating a cascade of proteases that leads to cell apoptosis[8]. In the present study, we tested whether ap by X-ray irradiation occurred via DNA damage or Fas/FasL pathway. Our results demonstrated that X-ray ir cell apoptosis by increasing positive rate of L02 cells expressing p53 and bax proteins, and decreasing posi 2 protein. At the same time, Fas and FasL expression in L02 cells exposed to irradiation was up-regulated a with that in mockly irradiated-cells.

Cellular sensitivity to radiation reflects a culmination of distinct molecular pathway including DNA repair checkpoint fidelity, and particularly apoptosis. Several oncogenes and tumor suppressor genes play a pivot modulating the response of cells to radiation. An important molecule, p53, initiates responses to DNA dama the sensitivity of cells to apoptosis. Functional inactivation of p53 is associated with resistance to radiother Overexpression of wtp53 gene was found to be associated with increased cellular sensitivity to apoptosis in ionizing irradiation[9,10]. Bcl-2, an important regulator of apoptosis, was found to be associated with anti-ap response. Over expression of bcl-2 by transferring bcl-2 gene into deficient cells has been associated with cellular resistance to induction of apoptosis by a variety of DNA-damaging agents including ionizing irradiat However, bax, another member of bcl-2 family, as an inhibitor of apoptosis, can bind bcl-2 to form homo- a heterodimers. Rate of bcl-2 to bax may determine the extent to which apoptosis is induced or suppressed

of cell increased. Our observations provide evidence that NADH is a new kind of radiation protector.

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