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Coenzyme Nicotinamide Adenine Dinucleotide Hydrogen: Therapeutic Potential as a Cardioprotective Agent

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Anthracycline anticancer agent Doxorubicin (Dox) is widely used in chemotherapy against human tumors. However, high doses of the Dox can induce cardiomyopathy that is progressive and nonreversible. Here, we report a strategy that may help preventing Dox-induced cardiac lesions by administering Dox in combination with coenzyme nicotinamide adenine dinucleotide hydrogen (NADH). NADH plays a vital role in the generation of adenosine triphosphate and in many metabolic reactions in cells. We examined the effect of NADH on Dox toxicity in rats and mice. Cardiac function, mitochondrial oxidation and phosphorylation were also measured in rats. Nude mice were implanted with human nasopharyngeal carcinomas treated with either Dox or NADH/Dox. We found that NADH inhibits Dox-induced apoptotic damage in cardiac myocytes, improves mitochondrial oxidative phosphorylation and reduces the concentration of Dox in myocyte. NADH can improve cardiac function and prevent the cytotoxicity of Dox without affecting its antitumor activity. In tumor bearing mice, higher concentration of NADH in cardiac tissue may explain its cytoprotective effect. These results suggest that NADH may be used in conjunction with Dox to reduce the toxic effects of Dox on heart tissue.

Introduction

Chemotherapy plays an important role in the comprehensive treatment of cancer. Chemotherapy has provided better clinical remission and cure for patients with some malignant tumors, such as skin cancer, chorioepithelioma and retioblastoma. However, chemotherapy often has severe side effects that limit its efficacy, such as myelosuppression, cardiotoxicity and nephrotoxicity (1). Cardiomyopathy caused by high doses of Dox is progressive and nonreversible (2). If response rates to antineoplastic agents are to be improved, normal tissues need to be protected from the immediate and delayed effects of cytotoxic agents. One way to reduce the toxic effects of Dox on heart tissue is in conjunction with a cardioprotective drug (3-5). NADH is the most biologically potent antioxidant in nature and has the strongest positive effect to reduce free radical (6,7). In patients with Parkinson's disease, NADH increases the production of depleted brain neurotransmitter levodopa, alleviates the impairment in motor skills and effectively improves the corresponding cognitive dysfunction (8).

Many antitumor drugs, including Dox, induce cell death by means of apoptosis and the toxic effects are thought to arise due to mitochondrial damage (9). Mitochondria are known as important in the apoptotic cascade and anti-apoptotic molecules and function as mitochondrial membrane stabilizing agents. Caspases zymogens are seen to be activated during apoptosis whereas specific caspase inhibitors prevent the apoptosis (10). Molecules that are able to control or reduce apoptosis may prove beneficial as an adjunct to chemotherapy-induced apoptotic damage.

This study intended to clarify the mechanisms of coenzyme NADH in cytoprotection against Dox-induced apoptotic damage. The apoptotic damage of Dox, the mitochondrial regulation of NADH in cytoprotection and the action of NADH against Dox-induced cardiotoxicity are studied.

Materials and methods

Primary Cardiac myocyte culture

Hearts were rapidly excised from 3-day-old neonatal C57BL/6J mice. The ventricles were minced and placed in a dissociating solution (Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution containing 0.5 mg/l trypsin and 10 mM HEPES). Harvested cells were centrifuged, and the sediment was resuspended in RMPI-1640 containing 20 U/ml penicillin and 10% newborn calf serum. The fibroblast content of the cell suspension was reduced with a differential attachment technique (11). Cardiac myocyte medium was placed in collagen-coated multiwell plate dishes that had been prepared for patterned cell growth. Culture medium was changed after 24h and every 2d thereafter with RMPI-1640 containing 5% neonatal calf serum. Exponentially growing cardiac myocytes in log phase were treated as follows: (1) the group of Dox, cells were incubated in medium containing 0.5μM Dox for 48h; (2) the group of NADH/Dox, cells were incubated in medium containing 0.8mM NADH for 6h and then 0.5μM Dox for 48h; (3) the group of control, cells were incubated in RPMI-1640 medium for 48h.

Assessing apoptosis by detection of sub-diploid population

Cells were harvested and single cell suspension was prepared in PBS buffer. Cells were then washed and aliquoted in a 15 ml polypropylene, V-bottomed tube. 3 ml cold absolute ethanol was added. Cells were fixed for at least 1h, washed in PBS and resuspended in 0.5 ml PBS and 0.2 ml DNA extraction buffer added (12). The cell suspension was then incubated at room temperature for 5 min and centrifuged. One ml of propidium iodide staining solution was added to cell pellet and then mixed well. Finally, 50μl of RNase stock solution was added and cells were incubated for 30 min at room temperature. Apoptosis rate (sub-diploid population) was analyzed by flow cytometry.

Measurements of cardiac function and electrocardiogram changes

Healthy SD rats (150-200g) (the Central Animal Laboratory, Hong Kong University) were assigned into Dox and NADH treatment groups: 1) the group of Dox, 8 rats were given 2 mg/kg of Dox i.p. every other day up to a maximal cumulative dose of 14mg; 2) the group of NADH/Dox, 8 rats were given 5 mg/kg NADH and then 7 times 2 mg/kg of Dox i.p. every other day; 3) the group of NADH, 8 rats were given 7 times 5 mg/kg NADH i.p. every other day; 4) the group of control, 6 rats were given 7 times 0.5 ml saline i.p. every other day. All rats were anesthetized with pentobarbital sodium (60 mg/kg ip) on 7d after the last Dox injection. A carotid artery was isolated and cannulated with a microtip catheter connected to a pressure transducer (power lab/4sp, ADI Inc, England). The catheter was advanced into

the left ventricle to record ventricular pressure for a brief period of time. LV systolic and end-diastolic pressures (LVSP), the maximum and minimum rate of LV systolic pressure (±dp/dt max) were monitored and recorded on a chart-strip recorder.

old BALB/c nude mice, the Central Animal Laboratory, Hong Kong University). When the tumors reached 1 cm in diameter, 15 mice were divided randomly into 3 groups containing 5 mice each: 1) Dox-treated, mice were given 1.5 mg/kg of Dox i.p. every other

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