Analysis of apoptosis via reticular pathway (caspase 12) in L-arginine induced cell death in rats' hepatocytes

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Abstract

The aim of present study was assessment of reticular pathway of nitric oxide induced programmed cell death. In intrinsic pathway if apoptosis the mitochondria or endoplasmic reticulum are involved. This is the result of cellular stress and destruction endoplasmic membranes in the cell. In experiment we used rats divided into two groups. Animals from experimental group received L-arginine -- precursor of nitric oxide. Specimens taken from liver of decapitated rats was prepared to immunohistochemical method detected caspase 12. Caspase 12 is the enzyme in endoplasmic reticulum. We used standard three step method. In the slides of the liver of rats from experimental and control group we didn't detect overexpression of caspase 12.

Keywords: Liver, caspase 12, programmed cell death, NO

Introduction

Caspase 12 is the enzyme from family of cysteine protease proteins known as caspases. Some members of this family are part of a proteolytic cascade that plays a main role in cell death by apoptosis. Inactive Caspase-12 is localized in the endoplasmic reticulum in the cell. The endoplasmic reticulum (ER) consists of some membranes This organellum is responsible for the production of the protein and lipid components of cell's organelles. There are two types of ER - rough, which contains ribosomes, and smooth one. Rough ER is the surface of protein synthesis. The main role of smooth ER is detoxication of ethanol. Caspase 12 is activated by perturbation of endoplasmic reticulum calcium homeostasis, hypoxia, glucose starvation, treatment of cells with ER stress inducers and accumulation of excess proteins.

There are two main pathways leading to cell apoptosis [1]: Extrinsic pathway is induced by an external signal and is connected with the death receptors on cell's membrane or with attack of cytotoxic T lymphocytes. In intrinsic pathway the mitochondria or endoplasmic reticulum are involved. Sometimes amplification of proapoptotic signal is observed when both pathways are induced.The intrinsic pathway is activated by cellular stress [2, 3]. Cellular stress may occur during the cell exposure to radiation, chemical substances, viral infection. Moreover, it may result from growth factor damage or oxidative stress. The intrinsic pathway initiates apoptosis via involvement of mitochondria or endoplasmic reticulum. The damage of ER leads to the activation of caspase 12 located inside the reticulum. The activation of caspase 12 triggers the caspase cascade [4].

The last activated caspase is caspase 3. Its action results in: the damage of nuclear enzymes, protein in cytoskeleton and fragmentation of DNA between individual nucleosomes.

The dying cell is divided into small pices - apoptotic bodies. The apoptotic bodies are recognized by the adjacent cells, macrophages responsible for their phagocytosis thanks to the receptors on their surface. Newer and newer kinds of receptors are being discovered. Recently, the phosphatidylserine receptor (PSR) protein has been discovered on the surface of the immune cells responsible for engulfing apoptotic cells, which binds phosphatidylserine present on the apoptotic cell surface. It has been observed that the cells lose their ability to phagocytose the apoptotic cells when PSR proteins are blocked by antibodies while the cells to which the PSR protein encoding gene has been introduced recognize and engulf the apoptotic cells [5,6]. The other discovered receptors located on the apoptotic cells include: complement receptor 3 (CR3) [7], complement receptor 4 (CR 4) [8], vitroectin and thrombospondin receptors, CD 14. These receptors are recognised by macrophages and other cells which phagocytose the apoptotic bodies.

In present study we analyzed caspase 12 activation stimulated by L-arginine- induced damage of endoplasmic reticulum in hepatocytes of rats' liver.

Material and methods

The rats used in present study were divided into 2 equal groups. The rats in experimental one were adminis-

tered L-arginine every other day in the amount of 40 mg/kg body weight for 2 weeks. The rats in control one received 2 ml of distilled water every other day for 2 weeks. The animals were decapitated after 3 weeks of the experiment.

After decapitation the hepatic specimens collected for immunohistochemical examinations were fixed in 10% formalin, dehydrated in the alcohol series and embedded in paraffin blocks. The slides was made. Next paraffin was removed in xylene and in a graded alcohol series. The specimens were subjected to thermal preparation in the acid medium. Next endogenous peroxidase was blocked and after that the specimens were incubated with rabbit primary antibody caspase 12 (Lab Vision RB-9259-PO) in 1% TBS/BSA, dilution 1/100. Then the Dako-Cytomation kit was used for immunohistochemical reactions, which included: biotinylated secondary antibody, streptavidin conjugated with horse-radish peroxidase and AEC substrate. After chromatogen staining the specimens were placed in the haematoxylin solution and rehydrated. The photographic documentation was prepared using the computer-guided Colour Video Camera CCD-IRIS(Sony).

The results of immunohistochemical examinations were subjected to qualitative evaluation and quantitative evaluation using the Analysis-pro software, version 3 (Soft Imaging System GmbH, Germany). The microscopic images, magnification x125 were analysed assessing the protein expression in 3 randomly chosen areas, 781193.35μm^2, each. The surface area of cells with positive reaction (+) was calculated.

The results were presented as means and standard deviation of the mean using the ANOVA, t-student tests. 5% error risk and statistical significance at $p \leq 0.05$ were accepted.

Results

The caspase 12 reaction with the antibody was was focal and concerned the cytoplasm which it filled evenly. The adjacent hepatocytes were characterized by various intensity of positive caspase 12 reaction.

Quantitative evaluation of immunohistochemical reaction in experimental group was comparable with the control group.(Fig. 1, 2, Tab. 1).

Discussion

Procaspase 12 is proapoptotic enzyme activated by many factors. One of them is caspase 7 [9]. This is an effector caspase, which is involved in or initiate the destruction of cellular DNA, which leads to cell destruction. Caspase 7/Mch3/ICE/LAP3/CMH-1 (Mammalian CED-3 homologue 3/ICE-like apoptotic protease 3); in its structure and function is similar to caspase 3 [10]. Bitko and Barik [11] have demonstrated that apoptosis induced by

Fig.1. CONTROL GROUP. Medium-intensive caspase 12 reaction in the rat liver section. AEC+H staining. Magnification about x 140.

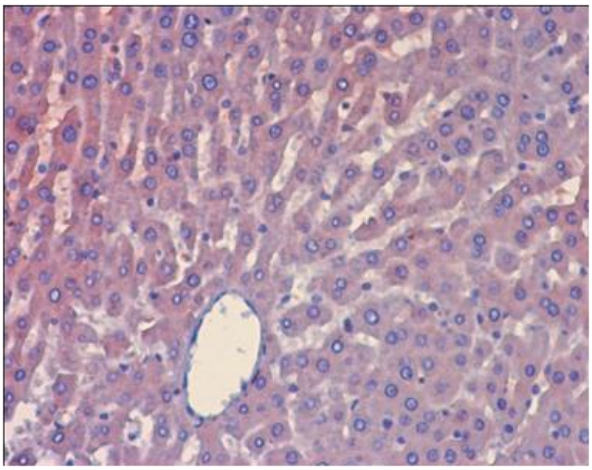

Fig.2. EXPERIEMNATL GROUP. The liver section of the L-Arg-treated rat. Medium intensive caspase 12 reaction. AEC+H staining. Magnification about x 280.

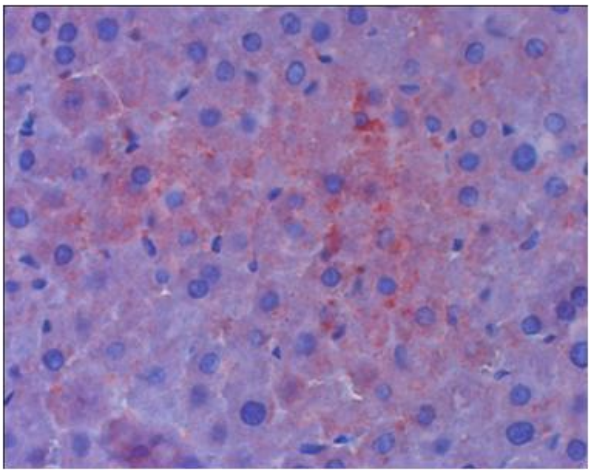

Table 1. Mean area occupied by caspase 12 reaction in the rat liver in control and experimental group. Statistical significance of differences. ONE WAY ANOVA test

Group	Control	Experimental	ONE WAY ANOVA
Caspase 12	302.8μm^2 +/-218.89	164.63μm^2 +/-169.81	p=0.36

infection with Respiratory Syncytial Virus in the lung epithelial cell line A549 occurs through an endoplasmic reticulum stress response that activates caspase-12.

Apoptosis is important for correct development of multicellular organism. This cellular death is helpful in keeping of tissue homeostasis. Too much or too little apoptosis can be cause of many diseases. Stress in the endoplasmic reticulum (ER) can result in apoptosis. ER contains procaspase-12 which is activated by ER stress, including disruption of ER calcium homeostasis and ac-

cumulation of excess proteins in ER, but not by membrane- or mitochondrial-targeted apoptotic signals [4].

TRAF2 has been shown to couple ER stress sensors with activation of caspase-12 by interacting with procaspase-12 and promoting procaspase-12 activation [12].

Hetz et al.[13] proved that misfolded prion protein (PrP^{Sc}) in prion diseases toxicity is associated with an increase of intracellular calcium released from endoplasmic reticulum (ER).

Activation of death receptors and mitochondrial damage are well-described common apoptotic pathways. [14]. Mehmed H [15] showed that if during apoptosis the caspases were activated near the cell membrane (extrinsic, receptor pathway) or mitochondrial membrane (mitochondrial pathway), caspase 12 was not activated.

Liu et al. noticed that, the caspase 12 plays an important role in cisplatin-induced apoptosis. It is proposed that the oxidative stress that results from the interaction of cisplatin with the ER cytochrome P450 leads to activation of procaspase 12, resulting in apoptosis.[16]

Apoptosis induced by adriamycin via ER pathway was also described by Jang YM and colleagues [17].

In present study we didn't observe an increase of caspace 12 activation connected with L-arginine induced apoptosis.

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