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Electronomicroscopic study of the influence of low frequency impulse magnetic field on experimental D-galactosamine hepatitis

KEYWORDS: Galactosamine; Hepatitis, Toxic; Electromagnetic Fields; Rats, Wistar; Microscopy, Electron

ABSTRACT

Experimental D-galactosamine (D-GAL) hepatitis has micromorphologic and ultrastructural similarities with human viral hepatitis. Magnetotherapy is nowadays widely applied in different chronic inflammatory conditions, however the data concerning the use of impulse magnetic field (IMP) in D-GAL hepatitis are scant. The aim of this study was to study electronomicroscopic changes of animal hepatocites treated by D-GAL with subsequent IMP therapy. This study was performed on adult laboratory Wistar rats divided in four groups. D-GAL was applied intraperitoneally in 400 mg/ml single doses. Experimental animals were treated with low frequency IMP using ELEC SYSTEM (8 mT, 12 Hz, 30 minutes per day). During spontaneous regeneration and IMP therapy electron microscopy indicated on the increase of nucleoli with fibrilar and granular component, clearly visible lacunar system, increase of glycogen particles, number of free ribosomes, while cells with dilatation of endoplasmic reticulum and variation of size and shape of mitochondria and autophagous vacuoles were rare. These results indicate that IMP has positive effects with decrease of intensity and volume of degenerative lesions of hepatocites, activation and stimulation of Kupffer cells, and thus improvement of regenerative processes of liver parenchyma with marked restoration of liver architectonics.

INTRODUCTION

D-galactosamine is an indirect hepatotoxin with cytotoxic effect on parenchymatous liver cells. Experimental rat D-galactosamine hepatitis is very important because of micro morphologic and ultrastructural similarities to human viral hepatitis. A reduced amount of UTP is the primary cause of biochemical alterations that lead to inhibition of RNA synthesis and protein synthesis with consequent necrosis (1,2). Ultrastructural changes of hepatocytes after induction of D-galactosamine hepatitis are well studied. They include nucleolar microsegregation and spherical forms, dilatation and ribosomal separation in endoplasmatic reticulum (3). Magnet field therapy is widely used for different chronic inflammatory processes, degenerative diseases of locomotor system, trauma, pain, and others. However, the exact mechanism of magnet field action and its physiologic action are not completely explained. Positive effect of impulse magnetic field (IMF) was verified in experimental in vitro and in vivo conditions. There are no data concerning therapeutic action of IMF in hepatitis. The aim of this study was to analyze electron microscopic changes of hepatocytes in D-galactosamine hepatitis with subsequent IMP therapy.

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MATERIAL AND METHODS

The study was performed on adult laboratory Wistar male rats, weighting 19010 g, from vivarium of Medical Faculty Nis. There were 8 control and 24 experimental animals divided in three groups. The first experimental group was treated by intraperitoneal application of D-galactosamine ("Serva") during three days with individual dose of 400 mg/kg BW in 0.5 ml of physiologic saline. The second group was after the same D-galactosamine administration was on regular feeding procedure for 12 days. The third group was after D-galactosamine on impulse magnetic field therapy for 12 days. Magnetic field therapy was applied using ELEC system (GMT Ag, Switzerland) with 8 mT, 12 Hz, 30 minutes daily.

For transmission electron microscopy (TEM) liver tissue samples with size 1mm3, fixed in McDowell solution for 24 hours, rinsed in Milling buffer, post-fixed one hour in osmium tetroxide, dehydrated in alcohol and propylene oxide, mounted in peon araldite. Ultra thin sections were contrasted with 4% alcohol solution of uranyl acetate and lead citrate and analyzed on microscope TEMSCAN 100 CX (Jeol), 80 kV.

RESULTS AND DISCUSSION

Hepatocytes with hydropic degeneration have dilatation and microvesicular transformation of smooth endoplasmatic reticulum, proliferation and concentric stratification of smooth membrane with myelin forms, depletion of ribosomes and glycogen granules. Lipid particles, atypical dense bodies, different mitochondrial alterations, microsegregation of nucleolus, and other organelles are seen.

Hepatocytes with acidophilic degeneration are seen as electron dense cells with reduced condensed cytoplasm, and scant membrane of organelles.

Ultrastructural changes of hepatocytes with IMF therapy (the third group) have significant differences. Nucleoli are much bigger, with fibrilar and granular component and lacunar system. Chromatin is filamentous or granular, without marked condensation. Cytoplasm has autophagous vacuoles and residual bodies, increased number of glycogen granules as and particles. Endoplasmatic reticulum resembles control with dense ribosomal granules. Mitochondria have different size, but matrix is regular.

Electron microscopy gives important data on pathology and pathogenesis of D-galactosamine hepatotoxicity. It indicated that the primary ultrastructural changes affect nucleoli caused by depletion of UTP and inhibition of RNA synthesis (4-6). Microsegregation of nucleoli indicates on greater need for nucleolar RNA and partial inhibition of transcription. In subacute D-galactosamine intoxication microsegregation is considered a sign of direct and indirect toxic effects. Nucleoli are consequently transformed in little dense particles. Lipid metabolism is also altered (7,8). In references dealing with electron microscopic changes in hepatocytes during spontaneous recovery from D-galactosamine hepatitis (9,10) there are no data about the influence of IMF on this process.

Hepatocytes after toxic damage and spontaneous recovery or therapy with IMF show enlarged nucleoli with granular and fibrilar material, discernible lacunar system, restoration of glycogen granules as and particles, regular orientation of cistern of rough endoplasmatic reticulum with external ribosomes, increased number of free ribosomes, autophagous vacuoles and moderate variation in mitochondrial size with regular matrix density and crystas. Some cells exhibit bigger number of osmiophylic lipids with variable

diameter that is smaller after IMF therapy. Free ribosomes indicate on increase of synthesis of structural and functional proteins, and structure of endoplasmatic reticulum shows active synthesis of protein products for secretion. There was no important difference in cells with small lipid particles, neither lipid mobilization, nor reactive steatosis. IMF therapy led to absence of osmiophylic lipids. Analysis of rat liver during recovery period and therapy with IMF indicate the presence of positive effects with reduced intensity of degenerative lesions of hepatocytes and stimulation of Kupffer cells with restoration of liver histoarchitectonics.

CONCLUSION

Electronomicroscopic study of subacute D-galactosamine hepatitis confirmed important ultrastructural alterations that are significantly less after spontaneous recovery and especially after therapy with impulse magnetic field. Nucleoli are increased with fibrilar and granular component, lacunar system, more glycogen particles and number of free ribosomes.

The results of this study indicate that application of IMF after D-galactosamine gives positive and simulative effect on regenerative and proliferative changes with reduction of degenerative alterations.

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