ELECTRON MICROSCOPE EVALUATION OF IRREVERSIBLE ELECTROPORATION ON THE LIVER IN A PORCINE MODEL

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Introduction: Irreversible electroporation (IRE) involves application of electric field pulses to cells leading to induced permanent nanopores on the plasma membrane providing a non-thermal cell death. The proposed mechanisms of apoptotic or necrotic cell death due to IRE include disruption of cell membrane caused by pore expansion and osmotic or chemical stress. In recent years, IRE has emerged as a new method of tumor ablation. In addition, IRE has been widely tested and per formed in several organs and tumors. Despite these promising developments, the fundamental mechanisms leading to cell death remain unknown. In this ultrastructural study, those modifications (from cell death to regeneration) occurring in different cellular types forming functional hepatic unit are assessed by using porcine livers under different experimental conditions.

Material and methods: In this study, 3 pigs (40 kg) were treated using parallel-plate electrodes separated by the width of the liver (i.e. 1-1.5 cm) and applying 100 pulses of 100 µs, with a frequency of 0.5 Hz to avoid thermal damage. Three electric field intensities were tested, one in each of the three used liver lobules: 1000 V/cm, 1500 V/cm, and 2 000 V/cm. Post-operative biopsies were performed at 3 h and 3 days after the stimulation. Samples were routinely processed for transmission and scanning electron microscopy visualization.

Results: Three hours after IRE application, alterations of cell membrane in the electroporated area were observed. They ranged from nanometric pores to wide disruption with spreading of organelles in the extracellular space. Cellular junctions between hepatocytes were not maintained. Dilated granular endoplasmic reticulum and mitochondria were observed. Autophagic processes initiated since presence of autophagolysosomes was evidenced. Surprisingly, Kupffer as well as Ito cells, erythrocytes and neutrophils showed conserved membranes. Fenestrated endothelia of Disse space also showed intact membranes. Three days after IRE application some hepatocytes showed nuclear fragmentation without disruption of cell membrane, suggesting apoptosis. However, the most of these hepatic cells showed necrosis, being then removed by macrophages. Subsequent changes demonstrating the beginning of regeneration of hepatocytes and *de novo* vascular formation were found.

Conclusion: Experimental data showed that ultrastructural study is an indispensable tool for reliable assessment of IRE ablation.

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