ULTRASTRUCTURAL EVALUATION OF PANCREATIC PARENCHYMA IN THE COURSE OF INTOXICATION WITH ETHANOL

KATARZYNA KOT, ZBIGNIEW BORATYŃSKI, AGNIESZKA PEDRYCZ, JACEK MENDOCHA, JERZY RUBAJ, BEATA CICHACZ, AND WIOLETA PLASKOTA

Department of Animal Anatomy and Histology, Faculty of Veterinary Medicine
University of Life Sciences in Lublin, 20-934 Lublin, Poland
zbyszek.boratynski@up.lublin.pl

1Department of Histology and Embryology with the Laboratory of Experimental Cytology, Medical University of Lublin, 20-080 Lublin, Poland
apw4@wp.pl

2Oncology Hospital in Lublin, 20-090 Lublin, Poland

3Emergency Department at University Hospital No. 4 in Lublin
20-090 Lublin, Poland

Received for publication July 08, 2008

Abstract

The experiment was performed on rats that were given ethanol ad libitum for 4 weeks. After decapitation of the animals, pancreatic sections were prepared for an electron microscopy. A significant broadening of the rough endoplasmic reticulum cisterns in the vesicular cells was observed as well as the presence of nuclei with irregular outlines resulted from placation of the nuclear membrane, which is worth of attention. Such a picture suggests that the examined cells had a lowered metabolic ability.

Key words: rats, pancreas, ethanol, electron microscopy.

Ethyl alcohol is a widely applied solvent used in industry, for consumption, and as a disinfecting agent. From 2% to 10% of the consumed ethanol is excreted in an unchanged form by the kidneys and lungs. The remaining amount undergoes oxidation, mainly in the liver.

Ethanol particles are small and hydrophilic; this is why ethanol is quite quickly absorbed from the alimentary tract and from the alveoli. A small amount of it is already absorbed in the oral cavity. The ethanol easily passes through the skin and placental barrier.

The biotransformation of 85%-90% of the ethanol proceeds in the organism with the participation of the liver enzymatic systems: alcohol dehydrogenase (ADH), microsomal ethanol-oxidisation system (MEOS), and catalase.

Chronic administration of ethanol to experimental animals leads to neurological disorders and organ dysfunctions concerning the alimentary tract, liver, kidneys, heart, and pancreas, accelerates atheromatous changes, and causes alcoholic damage of the foetus (7).

The pancreas is exceptionally sensitive to the influence of ethanol and its damage can lead to the disturbances in insulin secretion. Because of its enzymatic-hormonal secretion, the pancreas is a necessary organ for living organisms and its functions in digestion processes must be retained on a proper level.

At present, ethanol intoxication is considered to be the main risk factor for the occurrence of a chronic pancreatitis in 80% of cases (5, 6).

The present study is a continuation of the research on the post-alcoholic fibrogenesis of the pancreas parenchyma (8). Its purpose is an ultrastructural evaluation of the pancreas in the course of intoxication with ethyl alcohol.

Material and Methods

The material for the ultra-structural research was obtained from the experiment described earlier by the authors (8). The permission for the experiment was given by the Ethical Commission at the Medical University in Lublin (No. 23/2000).

The study was conducted on white male Wistar hybrid rats, weighing on average 250 g, and being three months of age. During the whole experiment, the animals were fed a standard LSM feed. The animals were randomly classified into control and experimental group, comprising five animals each. The control group was given water ad libitum, while the experimental
group was given a 20% ethyl alcohol solution *ad libitum*, instead of water.

Four weeks after the start of the experiment, the animals were decapitated.

Pancreatic samples (about 8 mm³) for ultrastructural examination were fixed in 4% glutaraldehyde in 0.1M phosphoric buffer, pH 7.4, with the addition of 2% saccharose. Next, the samples were osmosed, dehydrated in a number of ethyl alcohol solutions of gradually increasing concentrations, and embedded in Spurr resin. The blocks were cut with the Reichert Ultracout S. ultramicrotome. The obtained ultra-thin sections were contrasted with the use of uranyl acetate and lead citrate by the Reynolds method. The photographic documentation was prepared using the Tesla BS-500 electron transmission microscope.

**Results**

In pancreatic preparations coming from rats of the control group, vesicular cells with large nuclei were observed in the exocrine part. They had a regular nuclear membrane with a distinct nucleolus. In the basal part of the cells, a great amount of the membranes of rough endoplasmic reticulum was observed and in the top part, a great amount of zymogen grains (Fig. 1).

![Fig. 1. Rat's pancreas. The control group. TEM. 6000x.](image1)

![Fig. 2. Rat's pancreas. The experimental group. TEM. 6000x.](image2)

![Fig. 3. Rat's pancreas. The experimental group. TEM. 8000x.](image3)
In pancreatic preparations coming from rats of the experimental group, the broadening of the rough endoplasmic reticulum cisterns in vesicular cells was observed. In the neighbourhood of basal membranes of these cells, the accumulation of the bands of collagen fibres was observed. In their cytoplasm, numerous nuclei were detected. They had irregular outlines resulting from plication of the nuclear membrane. In the area of the nuclei, large clusters of heterochromatin that resulted from placation of the nuclear membrane. In the central part the nucleolus was present (Figs 2, 3). The thickening of the basal membranes of the epithelium that lines interlobular ducts and the vessel endothelium was also observed.

Discussion

The mechanisms leading to a chronic pancreatitis have not been fully recognised so far (4). It is known, however, that alcohol plays a key role in this process, because it stimulates pancreatic juice secretion, which causes an excessive accumulation of protein and, in turn, a calculi formation. The calculi can cause a blocking of the fine endo- and perilobular ducts and even the main pancreatic ducts (5). This may result in a damage of the epithelium cells of the tubules and of vesicular ones as far as the pancreatic islets disappear and the reactive development of perilobular connective tissue takes place.

In the discussed experiment, the electron microscope study with the experimental group revealed an accumulation of bands of collagen fibres in the neighbourhood of the basal membranes of vesicular cells. In their cytoplasm, numerous nuclei with irregular outlines - being the result of plication of the nuclear membrane - were observed. Such a state may be an expression of deterioration of cell metabolic ability, resulting from the damage caused by ethanol.

At present, it is assumed that chronic pancreatitis is a consequence of acute pancreatitis, which triggers the attraction of inflammatory cells of an early phase (lymphocytes, neutrophils) giving signals to cytokines: TGFβ, TNFα, MIF, and IL10 (3, 6, 10). An increase in mRNA expression for these cytokines caused by a four-week administration of alcohol raises the level of free radicals in the blood and activates the inflow of stellate cells.

Thus, alcohol, acute pancreatitis episodes, as well as chronic oxidative stress play a significant role in the aetiology of chronic pancreatitis (1, 2).

In the presented experiment, the thickening of the basal membranes of epithelial cells that line the interlobular ducts and vessel endothelium was also observed in the pancreas of the experimental animals.

In literature, it is said that already the first dose of ethanol causes an increase in cellular membranes liquidity, which favours the later accumulation of cholesterol in the basal membrane. Successive doses of ethanol fix cellular membranes leading to the occurring of biochemical changes in them. It mainly concerns membrane enzymes and includes the decrease of Na’/K’ ATPaza (9).

The changes in the pancreatic parenchyma caused by chronic ethanol intoxication, observed under a light microscope (described in our previous study) as well as under an electron microscope (also described in the present study), indicate the toxic effect of ethyl alcohol leading to chronic pancreatitis, which is followed by the organ’s dysfunction. A histological observation of the above-mentioned changes makes it easier to understand the mechanisms of their pathogenesis.

References