HISTOMORPHOLOGICAL EVALUATION OF FIBROGENESIS IN THE PANCREAS AFTER AN EXPERIMENTAL ADMINISTRATION OF ETHANOL

AGNIESZKA PEDRYCZ1, ZBIGNIEWS BORATYŃSKI, KATARZYNA KOT1, JACEK MENDOCHA2, JERZY RUBAJ3, BEATA CICHACZ1, AND WIOLETA PLASKOTA1

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, 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 of University Hospital No. 4 in Lublin, 20-090 Lublin, Poland

Received for publication July 08, 2008

Abstract

The purpose of the presented study was the analysis of post-ethanol fibrogenesis in the pancreas, being one of the causes of exo- and endocrine pancreatic insufficiency. The study was performed on rats classified into a control and experimental group. The animals from the experimental group were given 20% ethyl alcohol solution ad libitum for 4 weeks. After decapitation of the animals, pancreatic segments were collected and histological sections of the segments stained with haematoxylin and eosin and with Masson’s method for connective tissue were prepared. In the sections from the experimental group, a significant increase in the number of connective tissue fibres was observed. They were observed not only around the lumen of glandular ducts (as it was the case in the control group), but also among some vesicles and on the border of the pancreatic lobules. Their accumulation was also observed around the cells forming the pancreatic islets.

Key words: rats, pancreas, ethanol, fibrogenesis, histocytological analysis.

Apart from the liver and kidneys, the pancreas is the organ most sensitive to the influence of ethanol, which is most often manifested in acute or chronic pancreatitis with subsequent enzymatic and hormonal disorders (5).

Ethanol overuse is one of the main risk factors of the occurrence of a chronic pancreatitis. It is a progressive irreversible process of the destruction of pancreatic parenchyma with a heterogenous development of a fibrous connective tissue, which results in gradual exo- and endocrine pancreatic insufficiency.

Acetaldehyde, to which ethyl alcohol is metabolised, is responsible for a toxic influence of ethanol on pancreatic cells as well as the appearance of pathological changes. It mainly happens in the liver and pancreas.

Post-alcoholic macroscopic changes in the pancreas manifest themselves in a decrease in the glandular parenchyma of the organ. A few histological post-alcoholic changes in the pancreatic parenchyma were described. They mainly include peri- and intralobular fibrosis and formation of protein debris undergoing calcification. These calculi lead to the obstruction of the fine intra- and perilobular ducts, and even main pancreatic ducts. This results in the damage of vesicular cells as well as epithelial cells in the expanded interlobar ducts. The number of lisosomes, zymogen grains, and vacuoles increases in vesicular cells. Additionally, the amount of the endoplasmic reticulum increases. A developing inflammatory response causes the accumulation of lymphocytes and neutrophils, and in a later phase the accumulation of stellate cells (1, 8).

In literature, a post-alcoholic atrophy of the pancreatic islet parenchyma and the reactive development of perilobular connective tissue that follows it were described. From a histopathological point of view, fibrogenesis is an interesting phenomenon, worth analysing and understanding the mechanisms that deal with it. In the presented experiment, a post-ethanol fibrogenesis model that undergoes in the pancreas was described.
Material and Methods

The material for the histomorphological research was obtained from the experiment for which permission (No. 23/2000) of the Ethical Commission at Medical University in Lublin was given.

The research was done on Wistar male rats, with an average mass of 250 g and at the age of 3 months. During the whole experiment, the animals were given LSM standard feed. The animals were randomly classified into a control and the experimental group, comprising five animals each.

The rats from the control group were given water ad libitum. The rats from the experimental group were given a 20% solution of ethyl alcohol ad libitum.

Four weeks after the beginning of the study, the animals were decapitated. Pancreatic segments were fixed in Baker’s fluid (formalin, calcium chloride, distilled water), and then 7µm paraffin sections stained with Mayer’s haematoxylin and eosin, and additionally with Masson’s method for connective tissue were prepared. Photographic documentation of the sections was prepared by means of a light microscope of the Jenamed Company with an MF microphotographic attachment produced by Carl Zeiss Jena.

Results

Typical triangle-shaped lobules consisting of spherical vesicles formed by endocrine cells were observed in the control sections stained with haematoxylin and eosin. A basal part of the cells showed a basophilic character, while the top part placed at a lumen of the vesicle was acidophilic. In most vesicles, basally placed nuclei were visible and quite numerous zymogen grains in top parts also showed acidophilic properties (Fig. 1). Single Langerhans islets were visible in the preparations. They were oval in shape, and were showing a slight affinity to haematoxylin and eosin. Additionally, fine glandular ducts of the gland were present.

In the control preparations stained with Masson’s method, blue connective tissue fibres were observed. These fibres were localised around the lumen of the interlobular ducts and around the intercalated ducts. The fibres had an undular course and formed slightly marked septa between pancreatic lobules.

In the experimental group, in the preparations stained with haematoxylin and eosin, there were observed vesicles with glandular cells, which showed a strong affinity to eosin but a much weaker one to haematoxylin. The basophilic basal part of the cells was significantly reduced for the sake of the acidophilic part. The nuclei in vesicular cells were clearly visible. A considerable broadening of blood vessels and their overflowing with erythrocytes was observed. The sinusoids within the islets were also broadened (Fig. 2).

In the preparations from the experimental group, stained with Masson’s method, a significant rise in the number of the connective tissue fibres was observed. These fibres were present not only around the lumen of glandular ducts but also among some vesicles as well as on the border of the pancreatic lobules and around the cells forming pancreatic islets. Steatosis in pancreatic parenchyma was also observed (Fig. 3).
**Discussion**

A type I collagen is responsible for the post-ethanol fibrosis of the pancreas. Reports say that chronic administration of ethanol to experimental animals leads to an increase of procollagen α 1(I) mRNA expression in pancreatic stellate cells (4, 7, 9). This type of collagen is also responsible for post-alcoholic fibrosis in the liver.

It was proved that alcohol and acetaldehyde activate three MAP kinase classes in the pancreatic stellate cells. They are kinases: ERK1/2, JNK/SAPK, and p38MAP.

Kinase p38MAP is said to play the main role in the processes of pancreatic fibrosis. As it was shown, antioxidants have the ability to block the activity of this enzyme, that points at the participation of oxidation stress in this process (7).

The activation of class MAP kinases participates in the production of free oxygen radicals, which play a key role in oxidation stress in the course of ethanol to aldehyde change, thus intensifying the process of lipid peroxidation in cells (2, 3).

In the liver, TGF-β is responsible for the formation of type I collagen. It directly stimulates the change of stellate cells to myofibroblasts. The latest research suggests that a similar phenomenon also takes place during fibrosis in the pancreas; where in the formation of TGF-β, angiotensin II (9) contributes. It was proved that a long-lasting ethanol diet caused a significant increase in TGF-β mRNA expression in pancreatic cells in the experimental animals group (9).

The fibrogenesis process in the pancreas has a similar course as in the liver. Stellate cells are considered to be the key mediators of fibrosis as well as collagen sources (6). Among the regulating factors induced by the ethanol pancreatic stellate cells activation, there are MAPK (myogen-activated protein kinase), PI3K (phosphatidylinositol-3-kinase), PKC (protein kinase C), and AP-1(activator protein-1), as well as ethyl alcohol, its metabolite acetaldehyde, and oxidation stress as reported by Apte et al. (1).

Activated stellate cells gain the ability to activate and attract inflammatory cells through ICAM-1 and MCP-1 expression – the important mediators of leukocytes. Ethyl alcohol does not have such ability (7).

When proliferating, activated myofibroblasts produce collagen fibres, fibronectin as well as α-actin of smooth muscles, which for the reason of expression increase is considered to be a marker of pancreatic stellate cells activation.

**References**