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IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF PANCREATIC B-CELLS IN RATS TREATED WITH FURFURAL

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Summary. *Furfural ($C_5H_4O_2$), unsaturated cyclic aldehyde, is a well-known hepatotoxic substance that rapidly oxidizes into pyromucic acid (C_4H_3OCOOH) that is responsible for damage to liver parenchyma and development of hepatic insufficiency. In animals treated with this aldehyde, severe degenerative and necrotic alterations may occur, leading to cirrhosis development and hepatic carcinoma. Considering numerous anabolic, catabolic, detoxifying, and excretory functions of the liver, as well as a functional relation between the liver and the pancreas, we set out to examine the effect of furfural upon endocrine B-cells of the insula. An analysis was done of immunocytochemical and ultrastructural properties of pancreatic B-cells in rats treated with furfural for two months. In the treated animals, hypogranulation of the examined cells was found along with their nesidioblastosis in the exocrine pancreas. A conclusion can be drawn that furfural reduces hormone deposit in B-cells. Ultrastructural analysis of these cells shows that they contain granules of wide-ranging maturity, density, size, and shape, whilst the smooth endoplasmic reticulum is edematous and dilates tubules.*

Key words: *Furfural, insular B-cells, insulin*

Introduction

Furfural ($C_5H_4O_2$), unsaturated cyclic aldehyde, is a known hepatotoxic substance (1,2,3,4). In acute (5) and chronic (6) experiments with furfural, a change in the activity of some enzymes may occur in the liver including the increased activity of acidic phosphatase, DNase II, and glucose-6-phosphatase, and decreased activity of succinate dehydrogenase, adenosine triphosphatase, and NADH-tetrazole reductase.

In addition to enzymatic changes, furfural may cause morphologic changes in the liver (7) that in the acute experiment are manifested in diffuse necrosis associated with regeneration of hepatocytes. In the human organism and the organism of experimental animals, furfural rapidly oxidizes into pyromucic acid (C_4H_3OCOOH) (8). It is presumed that liver damage is induced parallel with initial metabolic changes in furfural, i.e., its oxidation . Changes that occur during the acute experiment terminate within a few days with liver recovery.

In the chronic experiment, furfural induces cirrhotic changes (9) concomitant with pseudolobule formation, enlargement of the portal area, and destruction of the border plaque. In the liver parenchyma, a pronounced bridging necrosis and hydropic degeneration of hepatocytes develop. At the same time, various degrees of liver insufficiency may become evident (10,11).

Given numerous anabolic (in particular the synthesis of albumin) (12,13,14,15), catabolic, detoxifying, and excretory functions of the liver, it is reasonable to presume that the liver's altered function may reflect on the endocrine and exocrine function of the pancreas (16). From this aspect and due to insufficient literature data on the effects of furfural upon endocrine B-cells of the pancreas, immunocytochemical and ultrastructural examinations of B-cells were done. The immunocytochemical procedure was used for monitoring the number, shape, size, granularity, and topography of B-cells. The ultrastructural procedure was applied for the analysis of markers of functional activity, in particular granules, mitochondria, Golgi apparatus, and endoplasmic reticulum of B-cells.

Materials and Methods

We used white male Wistar rats 150-200g of body weight. The animals were divided into two groups: control and experimental. The control comprised 10 animals, whilst the experimental group involved 40 animals. The experimental animals were treated with furfural in the following manner: in the first week, each animal was given furfural dissolved in drinking water at 20mg dose per kg/b.w.; in the second week, furfural was administered in the same manner with a difference in the dose that was raised to 30mg per b.w.; starting from

15th day and all through the final 60th day of the experiment, the animals were given higher doses of furfural, i.e., 40mg per b.w.

Both control and experimental animals were fed with the same foods. The control received drinking water without furfural. They were kept in plastic cages, each in a separate cell at 22^0C.

The first experimental group was sacrificed after 30 days and the second after 60 days of furfural treatment. Prior to sacrifice, each animal received general anesthesia and was then decapitated. The entire pancreas of each animal was then stuffed and insulated. For the immunocytochemical examination of insular B-cells, seven animals were secluded from each experimental group and control. Tissue was taken from the pancreas for the immunocytochemical analysis of B-cells (three sections from each pancreas) and Bouin's fixation. At the same time, the same tissue was taken for ultrastructural examination of B-cells. A total of 5 biopsies was made on each section and fixated in 2% glutar-aldehyde.

For the examination of topography of insular B-cells of the pancreas, we used the immunocytochemical PAP procedure. The cells were verified by the use of monoclonal antibodies on insulin at 1:1200.

The ultrastructure of B-cells was examined by electron microscopy.

Results

By using monoclonal antibodies on insulin, insular B-cells of the pancreas were isolated. In the control (Fig. 1) B-cells were localized only in the insula of their membranous part. The cell nuclei were round and small, and the cytoplasm contained dark brown, grain-like, dense deposits of insulin. Immunocytochemical polymorphism of the studied cells was not found.

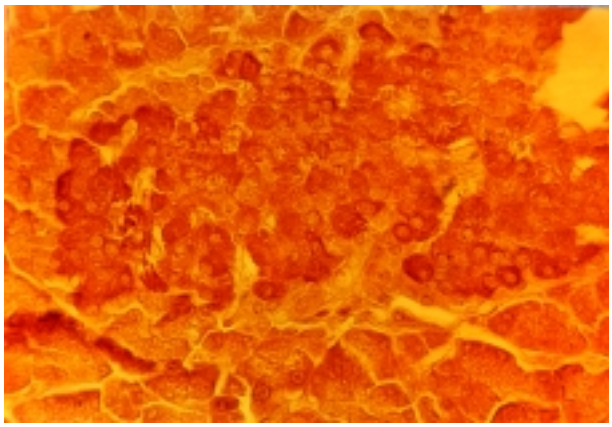

Fig. 1. Insular B-cells of control animals (PAP technique ×400)

In experimental animals treated with furfural for a month (Fig. 2), hypogranulation of B-cells was dominant, with less cellular deposit compared to the same cells in controls. Immunocytochemical polymorphism could be noticed among B-cells within the very insula. In the exocrine pancreas, a small number of B-cells were found.

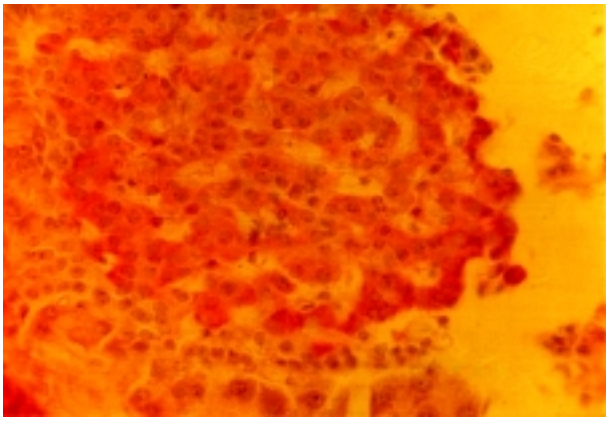

Fig. 2. Hypogranulation and polymorphism of B-cells in control animals (PAP technique ×400)

In animals treated with furfural for two months, the immunocytochemical procedure revealed significant differences in the topography of B-cells compared to controls. Namely, the number of the examined cells in the exocrine pancreas increased, leading to nesidioblastosis (Fig. 3). This change was exerted in the multiplication of B-cells in the periductural and periductal segments of the exocrine pancreas and in the presence of B-cells between the epithelial cells of larger and smaller pancreatic ducts. Hypogranulation was a constant finding in the examined B-cells in both the insula and the exocrine pancreas. In this animal group, B-cells were analyzed ultrastructurally as well and were found to contain eccentrically localized granules (Fig. 4) of wide-ranging maturity, density, size, and shape. The Golgi apparatus was less expressed and was of fissure-like lumen. Smooth endoplasmic reticulum was edematous and frequently tubules-modified (Fig. 5).

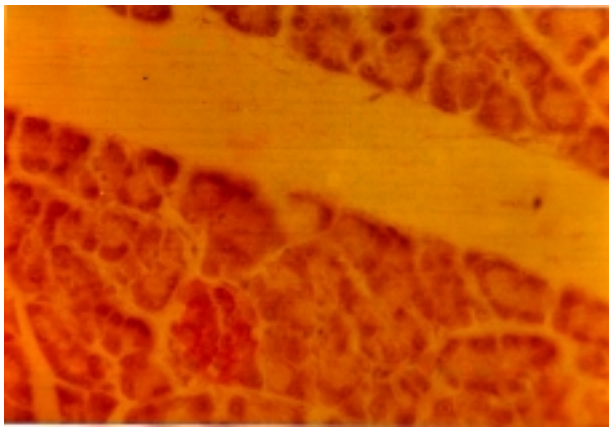

Fig. 3. Bud-like insula in acini (PAP technique ×400)

Discussion

In the organism, hepatotoxic furfural (1,2,3,4) unsaturated cyclic aldehyde, quickly oxidizes into pyromucic acid (8) which, as a pivotal intermediary product, damages the parenchyma of the liver (7). Due to a functional relation between the liver and the pancreas, the effects of

furfural are reflected on pancreatic tissue and, as shown in this experiment, on the examined insular B-cells.

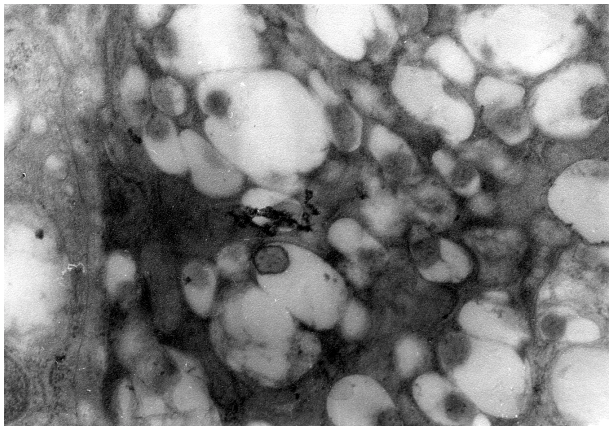

Fig. 4. Granules of B-cells of wide-ranging maturity; eccentric localization (EM ×7000)

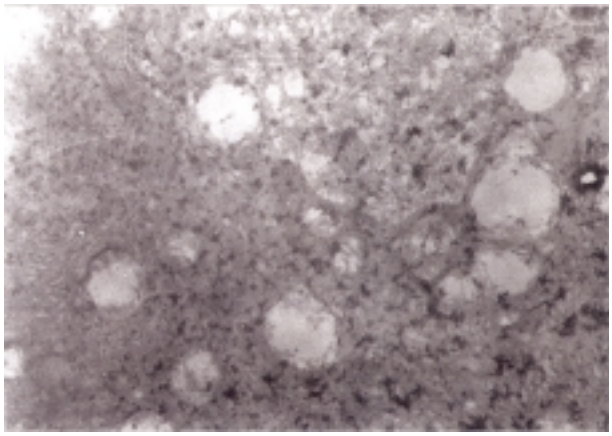

Fig. 5. Edematous smooth endoplasmic reticulum, cisternae-enlarged (EM ×5000)

However, despite the functional relation between the liver and a complex endocrine and exocrine gland such as pancreas, the effects of furfural on the pancreatic tissue have not been known till recently. Following the first reports on "empty" acini, identified on semi-fine sections (16), research was initiated into pancreatic morphology and furfural-induced function.

The liver plays a significant role in the anabolism of numerous compounds, primarily albumin compounds (11,12,13,14) and essential amino-acids. In liver damage, which in chronic experiments with furfural leads to cirrhosis (9), various degrees of hepatic insufficiency develop depending on the experiment duration (10,11). Hypoalbuminemia (17) occurs in the decompressed cirrhosis phase and is a cause of ascites development. A close relation between the pancreas and the liver is underscored by the fact that it is for the synthesis of insulin and other peptide hormones that the pancreas receives necessary substrates from the liver. On the other side, the synthetic function of the liver is stimulated by insulin via the accelerated transport of amino-acids through hepatocytes .

The functional relation between these two organs is supported by a whole family of growth factors termed insulin-like growth factors (IGFs) (18,19,20). These factors represent a family of growth-hormones-dependent peptides (21). Structurally, they are similar to insulin and have anabolic (22,23) and mitogen (24,25) effects on numerous tissues, as well as on the pancreas and the liver.

The number of B-cells increases, whereas their size decreases, with obvious hypogranularity and an alteration in topography (nesidioblastosis, i.e., the presence of B-cells in the exocrine pancreas as well). Endocrine differentiation of "stem" cells in the exocrine pancreas can be explained by a lack of substrates (amino acids) necessary for the synthesis of pancreatic hormones, enzymes, and even receptors. This is the reason why the synthesis of hormones is decreased, which is immunohistochemically confirmed by hypogranularity of cytoplasmic B-cells. A decreased concentration of hormones in serum is probably a strong stimulus for bipotential "stem" cells to undergo endocrine differentiation. In this way, endocrine cells emerge in the exocrine pancreas as well, acting as a compensatory response to reduced synthesis of hormones and to their lower concentration in serum.

Described degenerative and necrotic changes on the organelles, in particular on the smooth endoplasmic reticulum and the nucleus, suggest a toxic effect of furfural upon the examined B-cells of the insula. The very mechanisms of these events are most complex. Here we can only point to several possibilities of the pathogenesis of the obtained results. First, furfural exerts a concurrent and direct toxic effect upon B-cells, and an indirect effect through reduced synthesis of ATP. A possibility may be left for reduced ATP synthesis due to hypoinsulinemia and hyperglycemia. Decreased energy for the functioning of Na-K pump (a result of ATP deficiency) disturbs intracellular and extracellular ionic balance, leading to swollen mitochondrias and endoplasmic reticulum, as well as separation of ribosomes and Golgi apparatus. In order to demonstrate a possible direct toxic effect upon the pancreas and the examined B-cells, further experimental studies are needed, most of all, a study that would comprise direct injection of furfural into the nutritive blood vessel of the pancreas.

However complex and insufficiently explained the mechanisms of hypofunction of B-cells (26) of the endocrine pancreas may be, it is evident that their hypofunction disturbs numerous metabolic processes in the organism (27), primarily the metabolism of carbohydrates, lipids, and proteins. Therefore, in animals treated with furfural, the endocrine function of the pancreas is most damaged, so a development of diabetes, steatoreia, and undernourishment may be expected. Furfural does not only damage the liver but the pancreas as well, thereby leading to severe metabolic disorders in the whole organism.

Conclusion

On the basis of the results obtained by the study, a conclusion may be drawn that furfural reduces the synthesis and deposition of insulin in endocrine B-cells of the pancreas. Furfural induces compensatory, non-significant hyperplasia of the B-cells as well as their nesidioblastosis, i.e., formation of the insula in the exocrine segment of the pancreas. The ultrastructural finding suggests a toxic effect of furfural upon B-cells. Degree of functional and ultrastructural damage to the examined cells correlates with progressive elevation of furfural dosage and the experiment duration.

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IMUNOCITOHEMIJSKE I ULTRASTRUKTURNE KARAKTERISTIKE B ĆELIJA PANKREASA U PACOVA TRETIRANIH FURFURALOM

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Kratak sadržaj: *Furfural ($C_5H_4O_2$), nezasićeni ciklični aldehid, je dobro poznata hepatotoksična supstanca koja se u organizmu brzo oksidiše u piromucinsku kiselinu (C_4H_3OCOOH) a ona je odgovorna za oštećenje parenhima jetre i razvoj hepatične insuficijencije. U životinja tretiranih ovim aldehidom nastaju teške degenerativno-nekrotične promene hepatocita koje dovode do razvoja ciroze i hepatičnog karcinoma. Obzirom na brojne uloge jetre (anaboličke, kataboličke, u detoksikaciji i ekskreciji), kao i funkcijski odnos između jetre i pankreasa, ispitivali smo ulogu furfurala na beta ćelije pankreasa. Ispitivana su imunohistohemijska i ultrastrukturalna svojstva beta ćelija pankreasa pacova tretiranih dva meseca furfuralom. Kod netretiranih pacova nađeni su hipogranulacija beta ćelija i porast broja ćelija egzokrinog pankreasa. Može se zaključiti da furfural redukuje depoe hormona u B ćelijama. Ultrastrukturna analiza ovih ćelija pokazuje da one sadrže granule različite zrelosti, gustine, veličine i oblika dok je glatki endoplazmatski retikulum edematozan sa dilatiranim tubulima.*

Ključne reči: *Furfural, B ćelije insula, insulin*