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OSHMYANSKA N.Y.
SI «Institute of Gastroenterology of NAMN of Ukraine», Dnepropetrovsk, Ukraine

DEATH AND REGENERATION OF THE PANCREATIC ISLETS IN RELATION TO THE DEVELOPMENT OF FIBROSIS IN PATIENTS WITH CHRONIC PANCREATITIS

Summary. *The present study was conducted on biopsy material obtained from 65 patients with complicated chronic pancreatitis and 6 samples of tissue from intact pancreas. Cellular composition, islets' death and regeneration were analyzed using histological and immunohistochemical methods depending on the fibrous transformation in the development of chronic pancreatitis. Given study provides a detailed description of the histological changes that occur in the pancreas during the development of fibrosis, along with expression patterns of pro-apoptotic nucleases Endonuclease G and DNase, and the proliferation markers PCNA and Neurogenin-3 at different stages of chronic pancreatitis.*

Key words: *chronic pancreatitis, pancreatic islets, PCNA, Neurogenin-3.*

Chronic pancreatitis (CP) — is a progressive inflammatory disease characterized by irreversible destruction of the secretory parenchyma, fibrosis, atrophy, exocrine and endocrine insufficiency [1].

More than fifteen years ago, in 1998, many scientists have reported that the prevalence of CP in many countries was in the range of 3–10 per 100 000 people, and specified that the most urgent medical problems associated with this disease include abdominal pain, steatorrhea, diabetes mellitus, and the possibility that chronic pancreatitis may be considered a precancerous condition [2].

Over the past years there has been a global tendency to increasing of the incidence of acute and chronic pancreatitis in more than 2 times, with steady growth. Every year, newly diagnosed pancreatitis recorded in 8.2–10 people per 100 000 population. The prevalence of CP in children is 9–25 cases in adults — 27.4–50 cases per 100 000 population. The detection rate of CP at autopsy ranges from 0.01 to 5.4 %, with an average of 0.3–0.4 % [3].

In the excess deposition of extracellular matrix (ECM) during CP many different mechanisms are involved such as damage to the acinar cells, necrosis, inflammation, macrophage activation, platelet aggregation, release of growth factors, activation of pancreatic stellate cells (PSC), stimulation of the ECM synthesis and reducing of its destruction.

Despite the differences in possible damaging factors, the basic pathophysiological mechanisms of CP are very close. Maintaining the secretory activity under the conditions of duct obstruction leads to ducts compensatory dilatation when pancreatic secretion infiltrates the surrounding interstitial tissue and results in formation of edema and intracellular activation of zymogens, that initiates the proteolytic bionecrosis [3].

Apoptosis and necrosis are the two major pathways of destruction in acinar cells during CP [4, 5]. Morphologically, apoptosis manifesting in «desiccation» of cells and chromatin condensation, while necrosis leads to swelling of the cell and its organelles until rupture of the cell membrane. In necrotic cells biochemical markers of apoptosis, such as activation of specific proteases and caspase-depending DNA fragmentation are usually absent. In apoptosis the cell membrane remains intact, while in necrosis it is broken, whin causes releasing of the cell contents, damage to the adjacent cells and inflammation. Thus, it should be noted that the destruction of acinar cells by apoptosis (as opposed to necrosis) may inhibit activation of PSC (as proinflam-

matory cytokines are not released, if the cell is not broken) and therefore cell death by necrosis has more severe consequences to the organ.

Initiation of apoptosis in the pancreas occurs by two ways [6]. Exogenous (membrane) initiation path associated with the activation of caspase-8 (or caspase-10), and is accompanied by activation of effector caspases (such as caspase-3). This process usually starts with «death receptors», i.e. TNF or AIF. Endogenous (non-caspase) path originates in the mitochondrial membrane, and is accompanied by the release of pro-apoptotic enzymes such as cytochrome c and activation of caspase-9, which further activates the cascade of effector caspases (in particular caspase-3) and leads to the destruction of the cell contents. Remains of the cells that died by apoptosis (apoptotic bodies) are eliminated by phagocytosis [7].

Although it can be stated that the persistent activation of the PSC promotes fibrosis, and the reverse transition to a state of rest or stimulation of apoptosis facilitates tissue repair, human pancreas not easily accessible for routine biopsiy research and very often CP is revealed to us on the stage of the formed disease, so that the factors that can reverse or inhibit the activation of the PSC or affect their «behavior» in an inactive state, have not been thoughtfully studied yet.

Materials and Methods

Material for the study has been obtained from the surgical department of the State Institution «Institute of Gastroenterology of NAMS of Ukraine». The analysis of the surgical specimens of 65 patients with CP who were treated in the forementioned medical facility in the period from 2008 to 2014 has been performed. The current study was part of the research work «Вивчити механізми розвитку фібротичних процесів при хронічному панкреатиті та удосконалити технології їх хірургічної корекції», state registration number № 0111U001065.

The age of patients ranged from 27 to 70 years with the average 45.60 ± 1.17 years, male sex more frequent (5 : 1). For monitoring and comparative analysis, 6 tissue samples of intact pancreas has been used (autopsy material).

For morphological examination biopsies, obtained during surgery from a head, body and tail of the pancreas, has been fixed in 10 % neutral buffered formalin, dehydrated in alcohols of increasing concentration and embedded in paraffin. Histological sections were stained with hematoxylin-eosin or by Mallory in Slinchenko's modification and examined using a microscope XSP-139TP (Ulab, Ukraine).

Tissue samples for immunohistochemistry (IHC) were fixed in 10 % buffered formalin (pH 7.2), embedded in paraffin according to standard methods. Paraffin sections have been applied to the adhesive-coated glass, deparaffinized by standard protocol.

The sections stained by Mallory in Slinchenko's modification or immunohistochemistry samples have been photographed at a magnification X40 and X100 of light microscope. Parenchymal area was isolated and measured using the software ImageJ 1.45S (National Institutes of Health, USA). After the recalibration of the measuring grid with a scale of ± 1 μm, visible islet area, connective and acinar tissue (with ducts), as well as focuses of lipomatosis have been measured.

In order to systematize fibrosis and atrophy after morphological and morphometric study patients have been divided into four groups. Group I included cases (15.4 %), when the relative area of acinar tissue exceed 75 % of the total biopsy area, which in most cases correspond to stage I of fibrosis by M. Stolte (interlobular fibrosis). If the relative area of acinar tissue ranged from 50 to 75 %, the case was referred to the group II (21.5 % of patients), which also corresponded to stage II of fibrosis by M. Stolte (intralobular fibrosis). Group III included 10 patients (15.4 %), in which the area of acinar tissue in the pancreas ranged from 25 to 50 % with stage III of fibrosis by M. Stolte (fibrosis fields). Group IV (47.7 % of patients) included those cases in which the relative area of acinar tissue does not exceed 25 %.

All qualitative features were evaluated with nonparametric statistical methods using the program AtteStat for Microsoft Windows, version 13.1 (Russia). The significance of differences between groups was analyzed with the test Mann — Whitney-U. $P < 0.05$ has been counted for the level of statistical significance. All quantitative features were evaluated by parametric statistical methods using the program AtteStat for Microsoft Windows, version 13.1 (Russia). The significance of differences between groups was analyzed by calculation of Student's t-test for independent samples. $P < 0.05$ has been counted for the level of statistical significance.

Results and discussion

Due to the development of CP there has been observed a significant restructuring of the pancreatic functional tissue, mainly due to the reduction of the exocrine part and the formation of fibrous tissue from the ducts, with a gradual engrossing of perilobular and intralobular spaces. Correlation analysis of morphometric parameters confirmed the presence of a strong inverse relationship between the volumes of connective and acinar tissue ($r = -0.76$; $p < 0.001$). Reduction or the acinar cells number and loss of the characteristic lobular structure depends on several basic cell death mechanisms, including necrosis, apoptosis and fatty degeneration.

Of all the analyzed cases, in 15.38 % of patients acute focal necrosis of the parenchymahas been detected. Characteristic features of it was the swelling and lysis of the acinar cells, accumulation of a small number of inflammatory cells to the necrotic zone, stromal edema (fig. 1 a). In the later stages of CP, we observed focal accumulations of lymphocytes and plasma cells in the connective tissue, away from large vessels or ducts that are likely to remain in place of destroyed lobules. It should be emphasized that necrosis was not present in the endocrine tissue regardless of the stage of fibrotic transformation, size or location of the islets. Being near the dying acinar segments, islets are isolated from inflammatory cells through fast emerging plate of connective tissue (fig. 1 b).

In the later stages of CP replacement of acinar tissue occurred also by the ways of fatty degeneration. Episodes of micro- and macrovesicular focal fatty degeneration was significantly more common in patients of group IV (64.54 %

of cases), while in 28.57 % of this cases total area of adipose tissue exceeded the area of fibrous.

According to a computer morphometry in group IV, where acinar tissue in a state of atrophy occupies (3.6900 ± ± 0.9051) % of the total area, fat tissue engross (15.5860 ± ± 4.4188) % in cases of partial atrophy of the acinar tissue and (36,5110 ± 9,8694) % in cases of total atrophy, with (24.3610 ± 5.1301) % in the whole group.

Correlation analysis of the studied parameters showed an inverse relationship between the development of fatty degeneration and acinar tissue area ($r = -0.47$; $p = 0.002$), as well as between the development of fatty degeneration and inflammation ($r = -0.44$; $p = 0.01$). In the case of complete atrophy fatty degeneration developed significantly more closer relationship ($p = 0.0419$), and the area of adipose tissue was significantly higher ($p = 0.02099$).

Under the conditions of atrophic CP fibrous tissue has a number of features. Loose and dense connective tissue has been observed. In loose structure fibrils was located around the exocrine and endocrine structures, between the fibrils PSC, rare lymphocytes, plasma cells, and blood vessels, solitary lymphatic nodules has been observed (fig. 2 a). Dense connective tissue formed thick bundles of fibrils with numerous PSC, vessels and nerves was in state or fibrosis or significantly crushed (fig. 2 b).

The density of fibrous tissue often depends not on the duration of its formation, but on various additional factors. Thus, in our selected 15 cases of autoimmune pancreatitis type I and II, regardless of the stage of transformation was observed dense fibrosis with characteristic periductal infiltrate and obliterative phlebitis. In other cases, the fields of dense fibrosis interspersed with loose ones.

Excessive deposition of extracellular matrix and reduced rate of its destruction has the most negative impact on the endocrine cells. The latter stages of CP demonstrated the partial destruction of the majority of islets, which was located in the fibrous tissue. At the same time, along with a decrease in the amount and ratio of the hormone-producing cells, there was a marked dilatation and fibrous of intainsular capillary network.

At all stages of the fibroutic transformation of the pancreas we encountered sporadic apoptotic acinar cells, which

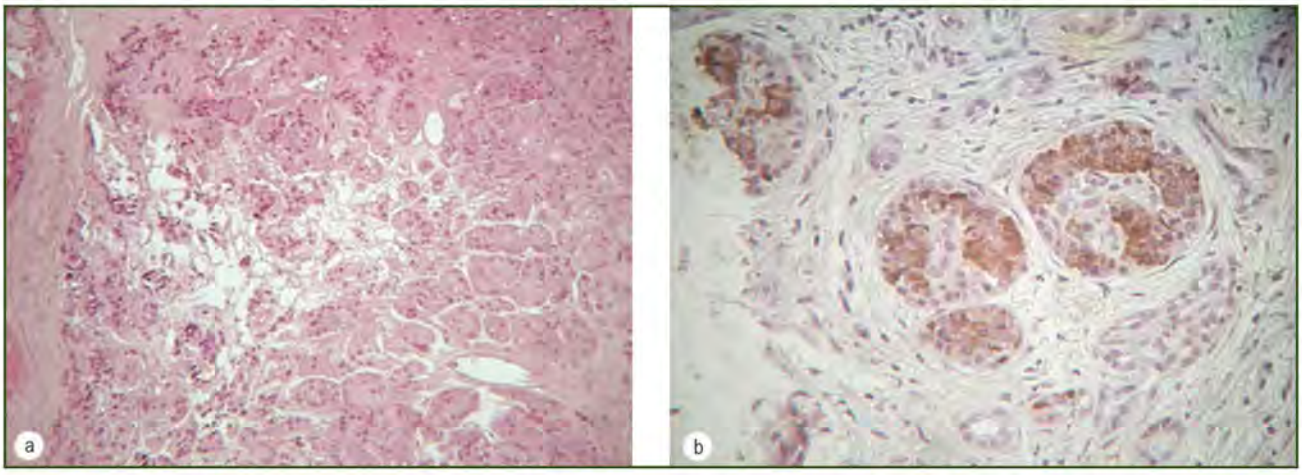

Figure 1 — Pancreas of patient with CP (III group): a) focal necrosis. Remains of acini and ducts between fields of connective tissue. Hematoxylin and eozin, × 100; b) fibrous tissue surrounds the islet. Immunohistochemical staining of insulin, × 200

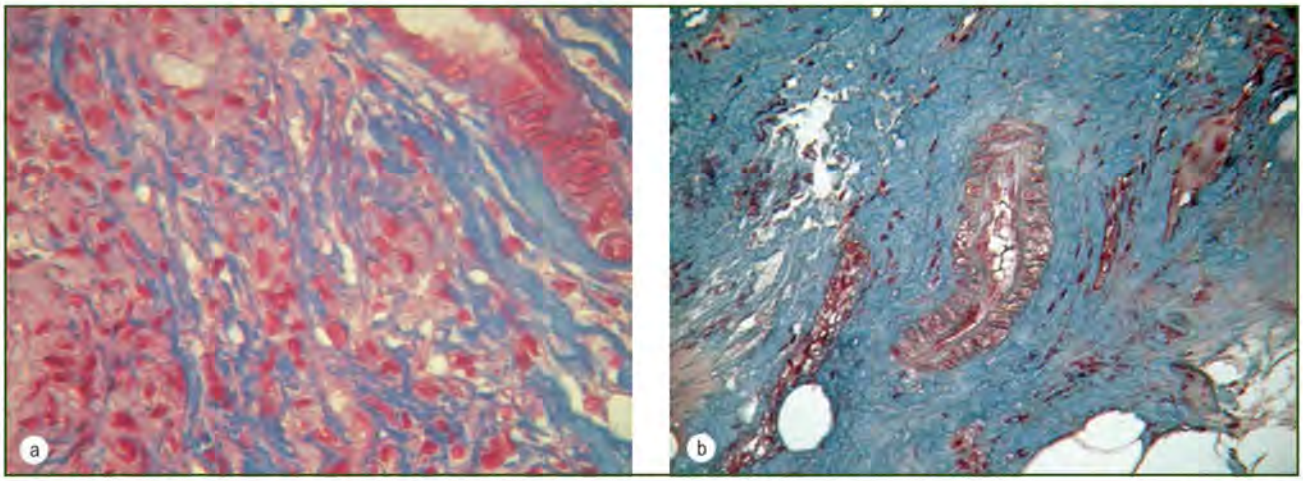

Figure 2 — Pancreas of patient with CP (IV group): a) the loose fibrous tissue infiltrates remaining atrophied lobules. Mallory's trichrom, × 200; b) a dense connective tissue surrounds the ducts of all sizes. Mallory's trichrom, × 200

indicates a constant cell death in CP. These cells are eosinophilic, have a polygonal shape, with a wrinkled overly basophilic nucleus.

In 55.4 % of cases CP was accompanied by complications in the form of pseudocyst. In these cases it was found that pseudocyst formed in damaged parenchyma, accompanied by focal accumulations of lymphocytes and neutrophils followed by destructive changes in the parenchyma. Later at the place of injury granulation tissue was formed, with lots of blood capillaries. The main feature of this formation was the absence of the epithelial lining in the wall of such cysts, which were formed from coarse fibrous tissue and filled with a liquid matrix. In the context of main duct obturation, excretory ducts was enlarged, and sometimes cysts was partially lined with ductal epithelium, which indicates their ductal origin. Apoptosis took an important part in the formation of these cysts.

While analysing insular apparatus of the pancreas irregular arrangement of islands came to our attention — in large areas of fibrotic tissue only small islets were observed (fig. 3 a), consisting of a few cells, while large and medium-sized islets were located next to each other in the form of groups or conglomerates, and were relatively rare. This was most noticeable when in the IV group with significant atrophy of acinar tissue. Furthermore, it should be noted that in areas with severe steatosis no islets were observed. All this, as well as single apoptotic bodies on the periphery of some islets, suggests that the process affects them too. Small islets (diameter < 100 micrometers) occur significantly more frequently with an increase in the area of fibrosis and may also be the remains of larger ones, in which some part of cells was destroyed.

In immunohistochemical study we determined the expression of pro-apoptotic nucleases Endonuclease G and DNase I. DNase I wsa observed in the cytoplasm of acinar cells of the pancreas in both the early and the late stages of the CP development. Translocation of DNase into the nucleus was more frequently observed during the later stages of CP in the foci of acinar tissue destruction, as well as in the tubular complexes, indicating a relatively lower life expectancy and active cell renewal. Due to the morphological similarity of centoacinar ducts cells and of acinar cells it seems that DNase was expressed directly in the ducts, but we found no ductal Dnase + cells outside the tubular complexes.

In the nuclei of the cells of degenerated ductal epithelium in the cases of cysts formation in the pancreas pronounced Endonuclease G expression was observed. In addition, EndoG + cells are most often located on the periphery of the islets, near the fibrous tissue (fig. 3 b). This localization corresponds to the location of apoptotic cells, which are a rare findings in the light microscopy. Although we have not found significant differences between the occurrence of EndoG + cells in the islets at different stages of the fibrotic transformation of the pancreas, but in the same biopsy, they are less common in the islets, located among the remains of acinar tissue.

Due to progressive fibrosis in CP the structure of the ductal systemis significally changed. In the context of the fibrotic transformation of the pancreas in 100 % of cases, there was epithelial hyperplasia of the interlobular and main ducts. Unlike normal cuboid epithelium hyperplastic epithelium was closer to the prismatic, with an increase in nuclear-cytoplasmic ratio and closely spaced nuclei.

At the same biopsy two types of ducts changes has been observed: medium and large ducts was extended, with a deformed wall (fig. 4 a), and numerous ducts of small diameter, which are the center of death and regenerating of acinar tissue. Formation of tubular and tubulo-islet complexes has been observed in the parenchyma — it appears as the area of small proliferating ducts with epithelial dysplasia in close association with endocrine islets. In cases of partial atrophy of acini tubular complexes are more common on the periphery of lobules, which is also the characteristic localization of islet tissue for this cases.

Immunohistochemical staining for proliferating cell nuclear antigen (PCNA) showed the results in the ducts, acinar and endocrine tissues. PCNA-positive cells with dark brown nuclei has been interpreted as S-phase of the cell

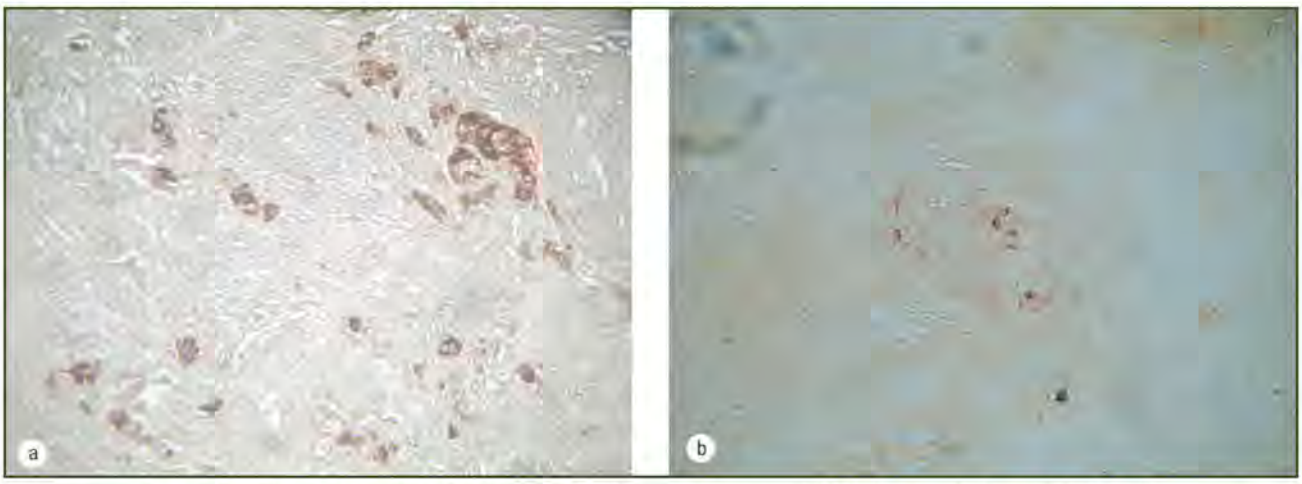

Figure 3 — Pancreas of patient with CP: a) numerous small islets consisting of 1–2 cells. Immunohistochemical staining of insulin, × 400; b) endonuclease G in the nuclei of islet cells. Indirect immunoperoxidase reaction, × 200

cycle. Based on this criterion, in the III and IV group the majority of cells in S-phase were ducts cells, and those in the tubular complexes (fig. 4 b), where they were much more common than outside of it.

Islets, located in the pancreas of patients in the control group, consisted predominantly of insulin-positive cells ((74.72 ± 2.23) %), with glucagon-positive and somatostatin-positive cells occupy (36.26 ± 2.02) % of total area with little scatter in values.

With the development of chronic pancreatitis insulin/glucagon ratio in the islets significantly changed: the number of glucagon-positive cells slowly grew ($r = 0.37$; $p = 0.0499$), and in group III–IV on the background of marked fibrosis cells of this type often dominate ((54.79 ± 4.52) %). Percent of insulin -positive cells to total demonstrated a tendency to decrease ($r = -0.46$; $p = 0.0004$), with the lowest values in group III ((56.29 ± 3.13) %). Analysis of the data using the Student's t test showed the greatest significance of differences between the control and the values obtained in groups II and III ($p = 0.0002$ for insulin-positive and $p = 0.0499$ for glucagon-positive cells).

At the same time, in the early stages of fibrosis (groups I and II) values showed a considerable scatter, and often islands, consisting almost of 100 % insulin- or glucagon-positive cells has been observed along with the small islets consisting of 1–2 cells of only one type in the later stages (groups III and IV).

While the total area of the insulin-positive cells and their relative contents in islet decreases, total islet area in mm² and the amount of large islets (diameter > 500 micrometers) remains virtually constant, whereas the density of medium islets was significantly reduced in group IV, and density of small (diameter < 100 micrometers) — significantly increased. In the ducts, and especially in tubular complexes solitary endocrine cells has been found. These cells were immunohistochemically stained for insulin, and morphologically demonstrate round nucleus and abundant cytoplasm filled with a few secretory granules. In the same area rare Ngn3-positive cells has been found, which can be regarded as precursors of the aforementioned β-cells (fig. 5 a, b).

It is thus has been borne in that by the histological point of view CP manifested in the form of two opposing interde-

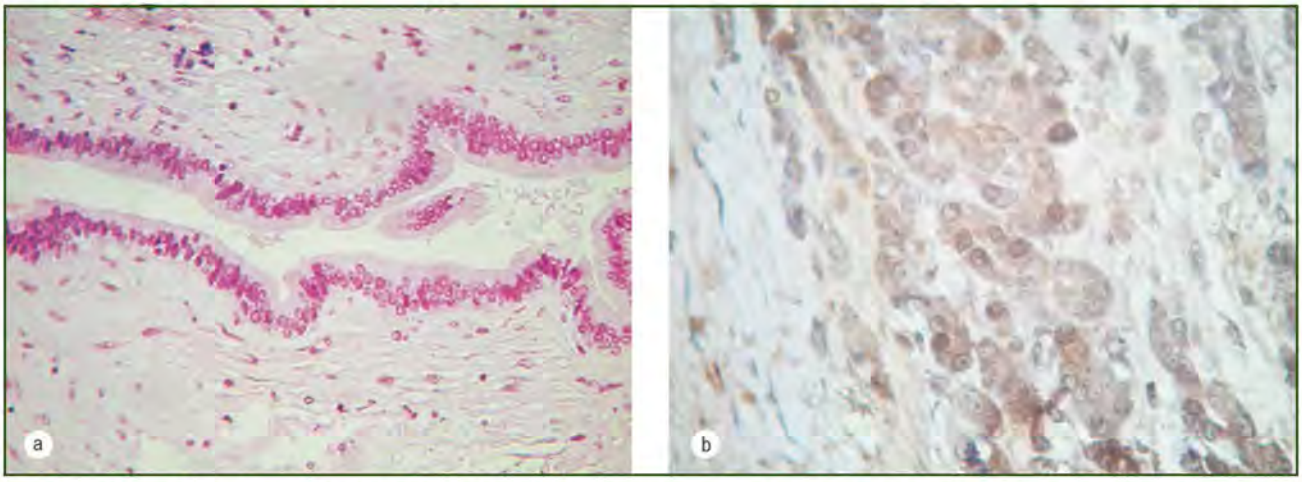

Figure 4 — a) IV group, subtotal fibrosis of the pancreas. Hyperplastic epithelium of the interlobular duct surrounded by loose fibrous tissue. Hematoxylin and eozin, × 100; b) typical tubular complex with PCNA-positive cells. Indirect immunoperoxidase reaction, × 200

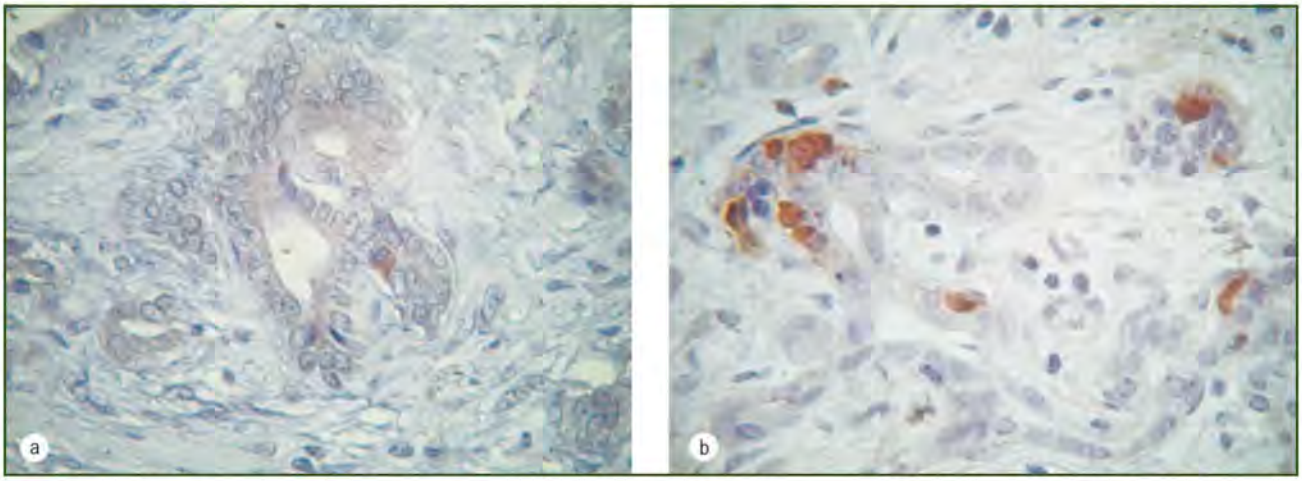

Figure 5 — Pancreas of a patient with severe atrophic-fibrotic pancreatitis (III group). The formation of tubular complexes. Indirect immunoperoxidase reaction, × 200: a) single Ngn3-positive cell; b) insulin-positive cells in the epithelium of hyperplastic duct

pendent processes: the fibrous transformation of the pancreas and atrophy of functional tissue. At the cellular level, we observed the processes such as necrosis, apoptosis, and fatty degeneration.

Mareninova O.A. et al. [4] showed that the severity of pancreatitis is directly correlated with necrosis and reverse correlated with apoptosis. Endonuclease G is released from mitochondria with CAD and AIF during apoptosis and its translocation to the nucleus relate to the later events that mark the irreversible cell death [8]. In our study, Endonuclease G was determined in ducts' cells, especially in cases of severe dilatation or cyst formation. Besides, Endonuclease G + cells appears in the islets, most likely at the periphery, near the fibrotic tissue. This localization corresponds to the location of apoptotic cells, which are a rare finding in the light microscopy.

DNase is a Ca- and Mg-dependent apoptotic endonuclease, which is produced mainly in the salivary glands, kidney and pancreas. DNase participates in the process of apoptosis in normal and its deficiency may cause a shift of apoptotic-necrotic index to the necrosis, which apparently occurs in some cases of acinar tissue atrophy during the development of chronic pancreatitis [9].

Immunohistochemical study showed us DNase in the cytoplasm of acinar cells on both early and late stages of CP development, especially in the area of tubular complexes, where under light microscopy many apoptotic bodies has been found. This, together with other features of apoptosis, indicates relatively lower life and active regeneration of cells in this region.

Such system of distribution of pro-apoptotic nucleases is apparently one of the main (but not only) factor protecting the islets in the development of CP and confirms the hypothesis of an independent mechanism of endocrine tissue apoptosis.

Acinar cells in CP (as opposed to the norm) express receptors for TNF-related apoptosis-inducing ligand (TRAIL). Activated PSC express TRAIL, and it has been hypothesized that this is what causes the decrease in the number of acinar cells in areas of fibrosis. PSC, in turn, express the death receptor domains P75 to the NGF ligand which is expressed in acinar cells during CP [10]. This confirms the crosslink between the activation of the PSC and damage of acini.

The connection of endocrine islets with UCS is also of interest. It is known that insulin is an activator of stellate cells via the classical receptors for insulin, insulin-like growth factor (IGF) or both at once. Furthermore, it was shown that the insulin and IGF inhibit apoptosis of PSC [11].

This explains the fact that in our study, even in the early stages of CP, we often observed islets shrouded in a thin plate of fibrous tissue that protects them from the effects of necrotizing parenchymal factors.

In addition, the development of atrophy of the acinar tissue in the pancreas results in relative abundance of insulin, which forms a kind of «circulus vicious» — PSC activation exacerbates the CP, the progression of fibrosis causes further atrophy of acini, which leads to an even greater excess of insulin.

We detected the presence of an inverse relationship between the development of fatty degeneration and acinar tissue area ($r = -0.47$; $p = 0.002$), while, in the case of complete atrophy it was significantly more expressed ($p = 0.0419$), and the relative area of adipose tissue was significantly higher ($p = 0.02099$).

It is known that inactive PSC accumulate fat droplets, the content of which is inversely proportional to the ECM synthesis [12]. We hypothesized that fatty degeneration of the pancreas, which accompanies the development of CP in the later stages, is not a consequence of the degeneration of acinar cells, most of which die by apoptosis, but has been the deposition of inactive (inactivated) PSC.

In the embryogenesis, the cells of the pancreas are derived from multipotent progenitors, but maintaining of their weight and proliferation in postnatal life depends mainly on the dissemination of existing differentiated cells. Normally exocrine acinar cells and islet endocrine cells both regenerated this way [13]. However, under certain circumstances, when the regenerative capacity of the existing cells is insufficient, other sources could be involved. The relative contribution of a specific cell type in an alternate way of regeneration is likely dependent on the stimulus, which may be toxic (e.g., alcoholic pancreatitis), and traumatic (e.g., occlusion of ducts, selective ablation and others). In the context of the fibrous transformation of the pancreas in CP 100% of the cases, observed by us, has been demonstrated the epithelial hyperplasia of the interlobular and main ducts with the formation of tubular and tubulo-insular complexes, which appear to observer as regions of many small proliferating ducts, in which acinar cells contained fewer zymogen granules compared to the acinar cells in other areas. That, and the fact that the PCNA-positive cells (S-phase of the cell cycle) in tubular complexes has been observed more often than outside of it, allows us to assume that it is the area of acinar tissue regeneration in CP.

In cases of partial atrophy tubular complexes are more common on the periphery of lobules, along with small islets, consisting only of β-cells. In a few cases in these islets specific endocrine transcription factor Ngn3 was discovered by us. It should be noted that the Ngn3-dependent path of the ducts-to-islets genesis has already been described by other researchers in experiments on animals, ande those authors also showed that each Ngn3+ cell can act as a progenitor for only one endocrine cell [14]. In this regard, although the solitary endocrine cells that we detected in tubular complexes are likely to be the end result of the transformation of ductal cells, a comparatively low rate of replication can not provide sufficient recovery process for β-cells of the islets in CP.

According to the literature [15] summarized results of several independent studies using laser scanning confocal microscopy indicate that the architecture and cellular composition of human islets is significantly different from rodents, which are most often used in experiments to study the CP. In particular, the amount of α-cells in human islet are higher (up to 40 % of total cells) and they are not necessarily located on the periphery. In the control group we obtained results, which are consistent to the literature; we

also noted that although α-cells cells were often located on the outer side, and β-cells closer to the center, this order is not required, especially for the larger islets. At the same time, in the early stages of fibrosis (groups I and II), we noted a significant spread of values, and often observed islets, consisting of 100 % α or β-cells, while in the later stages (groups III and IV) the number of small islets consisting of 1–2 cells (only one cell type) has been significally higher.

Two types of the endocrine disorders associated with the progression of CP is known. In the initial stages, when the functionality of the pancreas still saved, there may be effects of hyperinsulinism with clinical hypo-glycemic states (hunger, tremors throughout the body, cold sweats, weakness). In the later stages of the disease pancreatogenic diabetes is described [16, 17]. However, early identification of endocrine insufficiency by the basal blood glucose levels or C-peptide has been proved oneself as unrepresentative [17] due to the fact that normally a significant portion of insulin that is released by the islets is absorbed by exocrine cells of pancreas. It can be assumed that in the development of CP, due to progressive atrophy of the exocrine tissue, insulin requirement became lower and accordingly to it, the effect of reducing the mass of islets is not visible.

Researchers have noted that in patients with clinical manifestations of pancreatic diabetes, the number of β-cells in the islets is lower and α-cells higher than in patients with chronic pancreatitis, but without diabetes [18]. However, the area of β-cells seems unable to predict the severity of diabetes.

This suggests that the symptoms of hyperglycemia can be caused both by the decrease in the density of β-cells in the islet, and the increase in the density of the α-cells. In our study, the amount of α-cells cells was slowly increased with the progression of CP ($r = 0.37$; $p = 0.0499$), and in III-IV groups against the background of pronounced fibrosis cells of this type often dominate. The percentage of β-cells to the total number of cells show a downward tendency, with the lowest values in group III.

It may be reasonable to conclude that under the conditions of CP changes in islets can be divided into three periods. In the initial stages, when the atrophy of functional tissue is not pronounced and the area of fibrous tissue does not exceed 50 % protective mechanisms listed above provide comparative safety of endocrine tissue. But even then the process of restructuring already begins, which is reflected in the increase in the relative and absolute amounts of α-cells in the islets. Acinar atrophy of the pancreas gradually affects the islet tissue, but is compensated by activation of regeneration, In course of which α-cells are regenerated mainly through proliferation of existing ones, while the recovery mechanism of β-cells is more complex and includes Ngn3-mediated genesis from the ductal cells. Inequality in the implementation of the regeneration mechanisms leads to an even greater shift of α-cells/β-cells ratio in the islets, and at this stage may be clinically manifested as a relative hyperglycemia.

Finally, under restrictions of total and subtotal atrophy of the acinar tissue regenerative and protective potential of endocrine islets is depleted, which leads to their imminent death and the development of pancreatic diabetes.

Conclusions

1. Immunohistochemical study of pro-apoptotic nucleases in cases of fibrotic transformation of the pancreas associated with the development of chronic pancreatitis, showed that the proapoptotic Endonuclease G is expressed in the nuclei of ducts cells, especially in cases of severe dilatation or cyst formation. Besides, Endo-G + cells was found in the islets, most often on the periphery, near the fibrotic tissue. This localization corresponds to the location of apoptotic cells, which are a rare finding in the light microscopy. DNase I was expressed in the cytoplasm of acinar cells on both early and late stages of CP development, especially in the area of tubular complexes, indicating relatively lower life and active regeneration of cells in this region.

2. The process of regeneration of the islets of Langerhans in the development of CP is cocurrent to the atrophy of the acinar tissue and the development of fibrosis, with the involvement of several different mediators in the process of tissue repair. Thus, α-cells regenerated mainly through proliferation of existing ones, while recovery mechanism of β-cells is more complex and includes Ngn3-mediated genesis from the ductal cells. Inequality in the implementation of the regeneration mechanisms leads to an even greater shift of α-cells/β-cells ratio in the islets, and at this stage may be clinically manifested as a relative hyperglycemia.

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Ошмянская Н.Ю.
ГУ «Институт гастроэнтерологии НАМН Украины, г. Днепропетровск

ГИБЕЛЬ И РЕГЕНЕРАЦИЯ ПАНКРЕАТИЧЕСКИХ ОСТРОВКОВ И РАЗВИТИЕ ФИБРОЗА У БОЛЬНЫХ ХРОНИЧЕСКИМ ПАНКРЕАТИТОМ

Резюме. На биопсионном материале 65 больных с осложненным хроническим панкреатитом и 6 образцах неизмененной ткани поджелудочной железы с использованием гистологических и иммуногистохимических методов изучен клеточный состав, пути регенерации и гибели островков Лангерганса в зависимости от фиброзной трансформации при развитии хронического панкреатита. Описаны гистологические изменения, которые сопровождают развитие фиброза в поджелудочной железе, проанализированы особенности экспрессии проапоптотических нуклеаз Endonuclease G и DNase, а также маркеров пролиферации PCNA и Neurogenin-3 на различных этапах развития хронического панкреатита.

Ключевые слова: хронический панкреатит, островки поджелудочной железы, PCNA, Neurogenin-3.

Ошмянська Н.Ю.
ДУ «Інститут гастроентерології НАМН України, м. Дніпропетровськ

ЗАГИБЕЛЬ І РЕГЕНЕРАЦІЯ ПАНКРЕАТИЧНИХ ОСТРІВЦІВ ТА РОЗВИТОК ФІБРОЗУ У ХВОРИХ НА ХРОНІЧНИЙ ПАНКРЕАТИТ

Резюме. На біопсійному матеріалі 65 хворих на ускладнений хронічний панкреатит і 6 зразках незміненої тканини підшлункової залози з використанням гістологічних та імуногістохімічних методів вивчено клітинний склад, шляхи регенерації та загибелі острівців Лангерганса залежно від фіброзної трансформації при хронічному панкреатиті. Описано гістологічні зміни, що супроводжують розвиток фіброзу в підшлунковій залозі, проаналізовано особливості експресії проапоптотичних нуклеаз Endonuclease G і DNase, а також маркерів проліферації PCNA та Neurogenin-3 на різних етапах розвитку хронічного панкреатиту.

Ключові слова: хронічний панкреатит, острівці підшлункової залози, PCNA, Neurogenin-3.