PANCREATITIS. DISORDER OF MICROCIRCULATION. REVIEW

A. V. Smirnova, I. Y. Trubitsyna

Central Research Institute of Gastroenterology of the Department of Health, Moscow, Russia

Key words: pancreatitis, microcirculation, biologically active substances, pathogenesis, sanogenesis

Biological relations in the pancreas are still a mystery. Mechanisms of development of its study of inflammation throughout the world, but the data are contradictory, and experimental studies are still required to create models — ideal is not achieved. Without investigations in vivo is difficult to understand the reasons of complications from cardiovascular and bronchopulmonary systems with pancreatitis, but the simulation is still the most vulnerable link. The reason for this is a species of anatomy, embryonic and postnatal development, type of food, the associated spectrum of enzymes and bile acids, as well as species-specific structure of the molecular structures of certain cell receptors and ligands. Processes of cell-cell interactions, tissue and systemic homeostasis of biologically active substances. Study and understanding of general biological mechanisms of initiation and implementation of the pancreas inflammation require questions about how a hypoxic focus (HF) and the role of autophagy / apoptosis in this process. How different for autophagy / apoptosis in normal and pathological conditions, as well as their cell-and molecular mechanisms of formation necrosis. Issues to be resolved, hyperlipidaemia / triglitseridemia (familial form), breach of embryogenesis of the pancreatic genetics, fibrosis formation processes in the area of inflammation and the role of intercellular interaction.

Some factors in the development of inflammation of pancreas are common generic. These include the presence and action of the damaging agent of any nature to the pancreatic tissue, cytopathic effect on the cells and the appropriate cellular response to this agent. Stages of interaction have species-specific inflammatory

reaction on the enzyme and the extracellular matrix, and vascular endothelium, platelets and neutrophils. Acinar and / or stellat cells have their own species-specific features of the reaction. A combination of factors pancreas inflammation an opportunity to realize a number of molecular mechanisms, the degree of manifestation which depends on the cell type, the location of the source of inflammation in the prostate itself, proximity and speed of reaction vessels on flologene agent.

Human has several etiological factors in the development of both acute and chronic pancreatitis (AP and CP). Alcohol is the leading (30–50%) and choledocholithiasis (in animals of its elements). Much rarer cause of drugs, infectious (especially viral) diseases, iatrogenic and neoplasms; there are idiopathic pancreatitis, there are inflammatory diseases of the pancreas as an independent disease (rare) and how human-induced processes (in the experiment and / or as a result of violations of animal infectious diseases / toxicosis / and parasitic infestations etc.) in mammals. Animals do not drink alcohol without coercion, and the formation of choledocholithiasis has a different mechanism of development compared with the person. For these reasons, studies in experimental pancreatitis differ not only by induction of inflammation, but also the choice of the animal. All this puts a mark on the interpretation of data.

Currently, the most studied in vivo two causes of pancreatitis — choledocholithiasis and alcohol. There were created appropriate models — a model with elements of choledocholithiasis and models using various schemes taking alcohol animals. The base model (due to obviousness, simplicity and good repeatability) is a model of ETA alcoholic whose pathophysiological scheme is shown in Fig. 1. This is a basic model for studying in vivo models of other pancreatitis: simulating choledocholithiasis (caerulein, duodenal contents, trypsin, etc.) and / or toxic damage (acetic acid, L-arginine, cyclosporine, and dibutiltin dihlogid etc.), induction of AP and CP a concerted way. However, the mechanisms of tissue damage in the pancreas peroral alcohol use is still not fully studied.

Importantly, the model AP and CP also conventionally divided into "hyperthermia" and "normothermic". In hyperthermic model always stand the heat shock proteins, and using normothermic model of such processes is not observed. The first model is considered adequate for the study of AP and transplant rejection, to study the "cytokine storm", the processes of formation of necrosis, and the second — for the study of fibrosis in CP. Also used a mixed type of modeling — with the use of specific purified heat shock proteins, bacterial cell wall lipopolysaccharide and toxins. The value of these investigations is high. With age resistance and willingness to acute tissue inflammation decreases, which is the physiological norm. In this case, the AP form in vivo is a challenge that confronts researchers allocating specific "geriatric" models, which cost several times higher. It remains the question supply of experimental animals in research in gastroenterology. Homology task is waiting for a decision signaling molecules and their receptors on platelets, neutrophils, macrophages and endothelium in humans and animals. There are also models using mice (including transgenic) cell cultures (mainly for oncology, transplantation and Gerontology), tissue culture (for endocrinology), which are costly and high technology, but promising for understanding the processes of inflammation of pancreas. Why is it so important type of model? Why do you need to understand what processes they simulate? There are several reasons: each model assumes its mechanism of damage development and accompanying events — alteration, vascular reaction, adaptation / maladaptation by cells, tissue, organ or organism, as well as the completion of (resolution) of the process.

Most tolerant is to consider the development of inflammation exocrine tissue. Morphofunctional unit of the tissue is acinus. It is well supplied with blood, the acinar cells (AC) are synthesized by zymogens, they also present an inhibitor of proteolytic enzymes, which "quenches" only 30% of proteolytic activity. Proenzymes are regarded as the catalyst / trigger in the development of acute inflammation, the formation of edema and hyperemia gland tissues. Centroacinar cell are lined with the

tubules and, together with cells larger gland ducts create the conditions for activation of proenzymes and their admission into the duodenum. Important functions of these cells are the ability to regulate water and electrolyte metabolism, maintain physiological acidity and form barriers to limit inflammation in the body, to maintain cell-cell contacts, share antioxidants — vitamin A. The second type of pancreas cells are stellat (stellate) cells. Their active division can enhance damage of the pancreas. The stimulatory effect on the proliferation of these cells has a transforming factor-β (TGF-β) (significant for carcinogenesis of neo angiogenesis and platelet derived growth factor (PDGF) (significant for the formation of zones of primary and secondary ischemia, thrombosis).

Stellate cells have cytokine-inducing effect on neutrophils, activated fibrinolytic local processes, since granules contain proteases and matrix metalloproteinase synthesized. At inflammation of the effect of these substances may be twofold: in normal conditions allows you to maintain an active exocrine function of the body to form new tissue and remove dead cells, influence on angiogenesis, but also irreversibly damage acinar cell enzymes. Negative function of centroacinar stellat cells is the ability to activate the growth areas of fibrosis, expand HF and maintain long-term ischemia. At the same time creates the preconditions for the concentration of cytokines in the primary zone of ischemia.

Trypsin plays a leading role in the theory of pancreatitis. According to this theory, since the AC cytoskeletal changes under the influence of damaging factor endoenzimes begins abusing the system and digestive proenzymes, which leads to their premature intracellular activation and apoptosis triggered / autolysis / autophagy and necrosis cells.) Particular importance in this process of removing human non-pancreatic phospholipase A2 group II (phospholipase A2, group I perform digestive function without causing systemic effects. Until 1989, phospholipase A2 was the most investigated, which is abundantly present in the pancreatic juice (type IB). Then phospholipase type IIA was discovered, which is stored in secretory granules of

platelets, and its concentration is significantly increased at sites of inflammation. Chemically, these proteins are very similar to snake venom proteins, they are called serpentines. In 1994, the proteins were discovered and the type IIC V, which led to the serpentine family revision role in regulating cell functions, and new intensive search for similar proteins. In 2000 were detected other phospholipases are referred to as Type III. Also known type proteins X, IID, IIE and IIF, XII, and currently 10 types of known secretory phospholipase A2 group in a mammal. Serpentine involved in leukocyte adhesion to the vascular endothelium, non-directional movement of a mass of cells in the area of inflammation (chemotaxis and rolling), forming a zone of "cytokine storm".

Phospholipase A2 group II in the activated state violates the membrane permeability and facilitates the penetration of the prostate cell lipase, which greatly exacerbates the destructive processes in AC. Hearth of oxidative stress in tissue is formed (OS) and the increase in the concentration of metabolites in this process. OS changes the stability of cell membranes, and increase their permeability for digestive enzymes, and this provides access activated enzymes in cells of pancreas, intermediates and vascular space. Next vascular reaction seeks to limit the zone of inflammation, ischemia zone simultaneously forms and HF. If the damage AC and adjacent microvessels, platelets in large quantities produce and secrete specific factors in the activation and platelet-derived growth — PAF, PDGF.

Platelet-derived growth factor PDGF (platelet derived growth factor) — a polypeptide which has a special role in the chain of interactions between effectors and mediators of inflammation, angiogenesis and fibrogenesis. It is found in α-granules of platelets and is synthesized by megacaryocytes. Each platelet is about a thousand molecules of the substance, the effect of which is aimed at stimulating tissue repair. Receptors thereto are situated in the vascular wall, fibroblasts and smooth muscle cells, which stimulates the proliferation of PDGF and creates a zone of fibrosis. Species-specific receptors, but have a high degree of homology. Furthermore, PDGF

increases the production of connective tissue components (glycosaminoglycans, collagen, etc.).

It should be noted that, despite the fact that it is one of the first isolated and studied growth factors, its biological activity has not been studied until now full. It is known that a family of molecules of protein variants include peptides 4 — A, B, C and D, which are homo-or heterodimers to each other. Their receptors are highly homologous and represented by α and β-chains. With the α-chain of the receptor will specifically bind PDGF A, B and C, but with β-chain — only a PDGF-D. PDGD-B is involved in the processes of cell transformation, to form areas of fibrosis in CP, and PDGF-D — in the migration and angio genezgenez. In CP role of these factors is difficult to underestimate, because of them formed the basic conditions for the beginning of cyst, and then the development of carcinoma, the reaction of the extracellular matrix (the process is shown in Fig. 2).

The second important factor in the disturbance of microcirculation in the pancreas is FAT, or platelet-activating factor. PAF (platelet-activating factor, PAF) — a strong phospholipid mediator of inflammation, which is concentrated mainly in the pancreas at the site of the white thrombus. It is produced by many types of cells: neutrophils, basophils, platelets and endothelial cells, inflammation and participates in platelet aggregation plays a role in the pathogenesis of anaphylactic shock. Its role is to examine and complications of pancreatitis. Molecule platelet activating factor has several features — different alkyl chain length and isomer residues, which may explain its unequal activity against cells of different origin. PAF is synthesized from lysophosphatidylcholine and acetyl-coenzyme A, lysophosphatidylcholinepheras enzyme that makes it a very important object of study for biliary pancreatitis etiology. Degradation of PAF PAF-acetylhydrolase is performed (superfamily phospholipase A2), which is important to consider the study of disorders of calcium metabolism. Nowadays, it is believed that stellat and centroacinar cells can regulate the level of this substance in the inflammation of a number of activation of nuclear factor

involved in intracellular Ca^{2+}-exchange, and thanks to metabolize vitamin A. It is known that PAF promotes platelet aggregation and vasodilation in a concentration of 1 pmol / l causes acute inflammation of the bronchial tree, which explains the number of complications ETA. Various toxins (fragments of destroyed bacteria entering the pancreas and as a consequence of inflammation) stimulate the synthesis of PAF. Also been shown that the action of substances on the portal vein and hepatocellular circulatory system, role in the formation of HF in the myocardium. In addition to the induction of degranulation of platelets and leukocytes PAF has a strong influence vazodilated influence that leads to violations of the peripheral and central hemodynamics. Currently, as one of the factors etiopathogenetic pancreatitis considered violations hemostatic function of blood plasma.

Hemostatic system in AP study as such, and the main reactions — vascular, cellular, fibrin. If the system is working properly, then, the principle of "biological tissue preservation" and inflammation docked in the shortest possible time. However, for these pancreatic intensive processes may cause the development of acute (often edematous or gemorragical) inflammation. For AP are important the formation of microcirculation disturbances HF areas of thrombosis, which enhances tissue hypoxia. CPs more meaningful consider the processes of formation of new arteriovenous anastomoses in place thrombosed reaction vessel and antigemostaz in it, which are aimed at creating conditions for the blood supply to the ischemic area of the pancreas.

In etiologically different pancreatitis platelets are one of the connecting links and important — without their participation cannot be formed primary "white" thrombus, do not form conditions for the development of NO-mediated tissue damage (OS version) does not stimulate the release of inflammatory mediators, chemokines, cytokines and growth factors. An important effect of this system is the activation of cytotoxic proteases, which should have been allocated, including for the formation of new blood flow in the vessel, but the side effect is damage to normal pancreatic tissue. This fact allows some authors suggest that damage to the pancreas precedes

normothermic violation of the platelet system and its disregulation. This view of the role of platelets is a relatively new. To conduct such studies should use the new detection system: in vivo-labeling platelets, leukocytes, and other cells, special "metabolic" camera for observing animals, etc.

At pancreatitis occurs at the tissue level cascade of tissue-specific responses. Along with disregulation homeostasis isolated reaction system white blood cells that are associated with platelet responses. Reaction of Rolling leukocyte adhesion and species-specific, they are responsible for the development of immunological cascade of inflammatory reactions and the formation of many cell-cell interactions — adhesion, proliferation / necrosis / apoptosis / transformation and. etc. Leukocytes (neutrophils) in the pancreas by factors released from platelets, quickly form a zone of regional standing in the arterial vessels, and create conditions for the development of HF and OS. Ischemia, HF and OS slow recovery process of intercellular contacts and polarity AC membrane that is because platelets of inflammatory molecules activate endothelial arterioles on the expression of specific adhesion molecules for neutrophils. Normally, such a system should serve as an insulating barrier, but because of the structural features of the pancreas, this process causes inflammation cascade mediated by cytokines and necrosis factors (a family of proteins TNF, CD95 Fas \ ApoL System) which affect AC. If the marginal position is delayed, then formed a secondary focus of ischemia and the process repeats. Depleted tissue formed hearth of overconcentration cytokines (tissue, the local "cytokine burst" or "cytokine storm") that express neutrophils and possibly stellat cells. Under the influence of cytokines IL-6, IL-8, IL-1a and TNFα, inflammatory mediators, and platelet activating factors, free radical damage secondary process is already pancreatic OS metabolites. As described above, the cause of these events is the expression of adhesion molecules on vascular endothelial cells and associated receptor system for platelets and neutrophils (leukocytes) (Table 1). Neutrophil interaction with the vessel wall causes a cascade of reactions, physiological purpose is obturation damaged vessel thrombus and creation

of collaterals or arteriovenous anastomoses. This is a good reason to create a visual model to study the interactions of leukocytes, platelets and vascular wall between themselves in acute pancreatitis. This work was done in Germany group D. Uhlmann in order to understand the mechanisms of inflammation of pancreas transplant.

Neutrophils activated platelets together with a number of cytokines and inflammatory mediators, it is necessary for the chemotactic inflammatory zone in new cell units, so-called stem cells. Coordinated work of the "platelet-neutrophil" provided that:

- neutrophil is able to adhere to the endothelial wall;
- form a tight contact;
- rolling — to migrate to the site of damage / inflammation.

This process involves three families of adhesion molecules: selectins, b-2integriny immunoglobulins.

Table 1

Adhesion factors (according to D. Uhlmann, 2012)

Adhesion receptors	Ligands	Molecule is on	Functions
a4b7 (non-activated) a4b1 (non-activated)	MadCAM-1 VCAM	Leukocytes and vascular endotlelia	Neutrophil «Rolling»
PSGL-1 L-selectin	P-selectin P-selectin, E-selectin, MadCAM-1	Platelets, vascular endothelium, leukocytes	Neutrophil «Rolling, adhesion and cell-cell interactions
a4b7 (activated) a4b1 (activated)	VCAM\MadCAM1 VCAM1	Leukocytes and vascular endothelium	Tight cell-cell contacts
CD11a\CD18 CD11b\CD18 PECAM1	ICAM1,2 ICAM1 PECAM1	Endothelium of small vessels Platelets and endtelia arterioles	Tight cell-cell contacts, migration Emigration

Selectins are involved in the early stage of adhesion. L-selectin is required for the formation of new blood flow path in the vessel. L-selectin binds to P-selectin which is present on neutrophils and endothelium. After the reaction mechanisms

include oxidative damage to the cell / tissue oxidative stress (OS), hypercalcemia, and the binding to thrombin and / or serum complement are activated and start the program for the formation of a thrombus. This process may be disrupted.

OS, platelet activating factor, leukotrienes neutrophils stimulated by b2-Integrin expression of intracellular granules. These molecules on the cell membrane protein family interact with ICAM-1 and PECAM-1 and to trigger the activation cascade of cytokines: TNF-α, and IL-1. Super-concentration of these cytokines starts the apoptosis / necrosis of tissue.

Cytokines trigger mass death of neutrophils, resulting in intracellular granules are released, which contain biologically active substances (proteases, collagenase, elastase, lipoxygenase) to form a new channel for blood flow through the damaged vessels. These proteolytic enzymes pancreas damage cells, forming a secondary focus on the sore spot already available. This process is cascaded, and proceeds cyclically, and will continue until the complete exhaustion or cell or microorganism death to the same manner, formed necrosis.

Choledocholithiasis is the second most common cause of pancreatitis. To study it as a factor in the study etiopathogenetic AP and CP use different experimental schemes in vivo. In recent work with rats increasingly abandoning the common bile duct ligation, since it is an important element in the metabolism of bile acids. Ethics committees of many research centers operation ligating the choledoch regarded as crippling. For studies animals are introduced into and / or chemically pure prostatic and / or obtained from patients / healthy animals / humans cholic acid, their mixtures and / or their chemical antagonists, drugs which activate specific inflammatory molecules, cytokines and antibodies thereto blockers OS, and bacterial lipopolysaccharide shell etc.

At choledocholithiasis an important role in the development of pancreatitis withdrawn to violations of regulation of biosynthesis of bile acids. It is believed that many of the functions of bile acids feasible mediated system growth factors, the main

violations which bind with protein molecules infringement action of fibroblast growth factor-21-FGF-21. For studies of this system have been developed in transgenic model animals. These studies were developed in Gerontology.

In CP pathophysiology role of platelets is significant. Thrombocytic link hemostasis is spoken as a possible target for prevention and treatment of exacerbations of CP. In particular, in 2008 in Poland was conducted in vivo study of the possibility of heparin to prevent pancreatitis ischemia / circulatory disorders in the pancreas and prevention of intravascular coagulation dissymmetric syndrome. It has been shown that heparin in combination with antitrombnom III and / or co-factor II for decreases heparin activity of trypsin, chymotrypsin and inhibits trypsinogen cascade of biochemical reactions in trypsin. Also obtained data on the anti-inflammatory effects of heparin, which is realized by reducing the number of neutrophils in the area of inflammation and down-regulation of TNF-α at Rolling leukocytes in the area of massive cell damage, which significantly limits the spread of the cytokine storm. There is evidence, and that due to the action of the "antithrombin III-heparin" decreases the activity of elastase and cathepsin G platelets. All these factors make the use of different types of heparins interesting enough direction and practical importance.

References

1. Хендерсон Д. М. Патофизиология органов пищеварения : пер. с англ. / Д. М. Хендерсон. — 3-е изд., испр. — М. : БИНОМ, 2012. — 272 с.
2. Animals models for investigation chronic pancreatitis / A. A. Aghdassi, J. Mayerle, S. Christochowitz [et al.] // Fibrogenesis Tissue Repair. — 2011. — Vol. 4. — P. 26.
3. Apte M. The fibrosis of chronic pancreatitis: new insight into the role of pancreatic stellate cells / M. Apte, R. Pirola, J. Wilson // Antioxid. Redox. Signal. — 2011. — Vol. 15, No 10. — P. 2711–2722.
4. Beenken A. The FGF family: biology, pathology and therapy / A. Beenken, M. Mohammadi // Nat. Rev. Drug Discov. — 2009. — Vol. 8, No 3. — P. 235–253.
5. Bhatia M. Apoptosis versus necrosis in acute pancreatitis / M. Bhatia // Am. J. Physiol. Gastrointest. Liver Physiol. — 2004. — Vol. 286, No. 2. — P. G189–G196.
6. Epidermal growth factor receptor regulated pancreatic fibrosis / S. A. Blane, K. C. Ray, K. M. Branch [et al.] // Am. J. Physiol. Gastrointest. Liver Physiol. — 2009. — Vol. 297, No 3. — P. G434–G441.
7. Fortunato F. Immune-compromised state in the rat pancreas after chronic alcohol exposure: the role of peroxisome proliferator-activated receptor gamma / F. Fortunato, I. Berger, M. L. Gross // J. Pathol. — 2007. — Vol. 213, No 4. — P. 441–452.
8. Jakob M. Stromal cells and tumor milieu: PDGF et al. / M. Jakob, E. Puré // Cancer genome and tumor microenvironment / Ed. A. Thomas-Tikhonenko. — Philadelphia : Springer, 2009. — 480 p.
9. Jonhston C. L. Fibroblat growth factor 21 reduces the severity of cerulein-induced pancreatitis in mice / C. L. Jonhston, J. Y. Weston, S. A. Chadi // Gastroenterology. — 2009. — Vol. 137. — P. 1795–1804.

10. Kharitonenkov A. FGFs and metabolism / A. Kharitonenkov // Curr. Opin. Pharmacol. — 2009. — Vol. 9. — P. 805–810.
11. Mihaljevic A. L. Molecular biology, models and histopathology of chronic pancreatitis and pancreatic cancer / A. L. Mihaljevic, I. Esposito, J. Kleff // Eur. Surg. — 2009. — Vol. 41, No 6. — P. 250–267.
12. Nandy D. Growth factor mediated signaling in pancreatic pathogenesis / D. Nandy, D. Mukhopadhyay // Cancers. — 2011. — Vol. 3, No 1. — P. 841–871.
13. Pathophysiological role of platelets and platelets system in acute pancreatitis / D. Uhlmann, H. Lauer, F. Serr, H. Witzigmann // Microvasc. Res. — 2008. — Vol. 76, No 2. — P. 114–123.
14. Phosphatidylinositol 3-kinase faciliates bile acid-induced Ca(2+) response in pancreatic acinar cells / L. Fischer, A. S. Gukovskaya, J. M. Penninger [et al.] // Am. J. Physiol. Gastrointest. Liver Physiol. — 2007. — Vol. 292, No 6. — P. G875–G886.
15. Protective and therapeutic effects of heparin in acute pancreatitis / P. Ceranowicz, A. Dembinski, Z. Warzecha [et al.] // J. Physiol. Pharmacol. — 2008. — Vol. 59, Suppl. 4. — P. 103–125.
16. The role of heme in hemolysis-induced acute pancreatitis / M. Saruç, H. Yuceyar, N. Turkel [et al.] // Med. Sci. Monit. — 2007. — Vol. 13, No 3. — P. BR67–BR72.
17. Thrower E. C. Molecular and cellular mechanisms of pancreatic injury / E. C. Thrower, F. S. Gorelik, S. Z. Husain // Curr. Opin. Gastronterol. — 2010. — Vol. 26, No 5. — P. 484–489.
18. Thrower E. Molecular basis for pancreatitis / E. Thrower, S. Husain, F. Gorelic // Curr. Opin. Gastroenterol. — 2008. — Vol. 24, No 5. — P. 580–585.

19. Uhlmann D. Microcirculatory disturbances in the pathogenesis of acute pancreatitis / D. Uhlmann // Acute pancreatitis / Ed. L. Rodrigo. — [s.l.] : InTech, 2012.
20. Vonlaufen A. Molecular mechanisms of pancreatitis : current option / A. Vonlaufen, J. S. Wilson, M. V. Apte // J. Gastroenterol. Hepatol. — 2008. — Vol. 23, No 9. — P. 1339–1348.

Pancreatitis. Disorder of microcirculation. Review

A. V. Smirnova, I. Y. Trubitsyna

Central Research Institute of Gastroenterology of the Department of Health, Moscow, Russia

Key words: pancreatitis, microcirculation, biologically active substances, pathogenesis, sanogenesis

Review presents recent data on the role of relationships of biologically active substances in the pancreatic tissue during the patho- and sanogenesis of acute and chronic pancreatitis. Lesion and fibrosis in the pancreas depends on the combination of regulatory molecules and their ligands and signaling pathways that are involved in the formation of the pathological process.

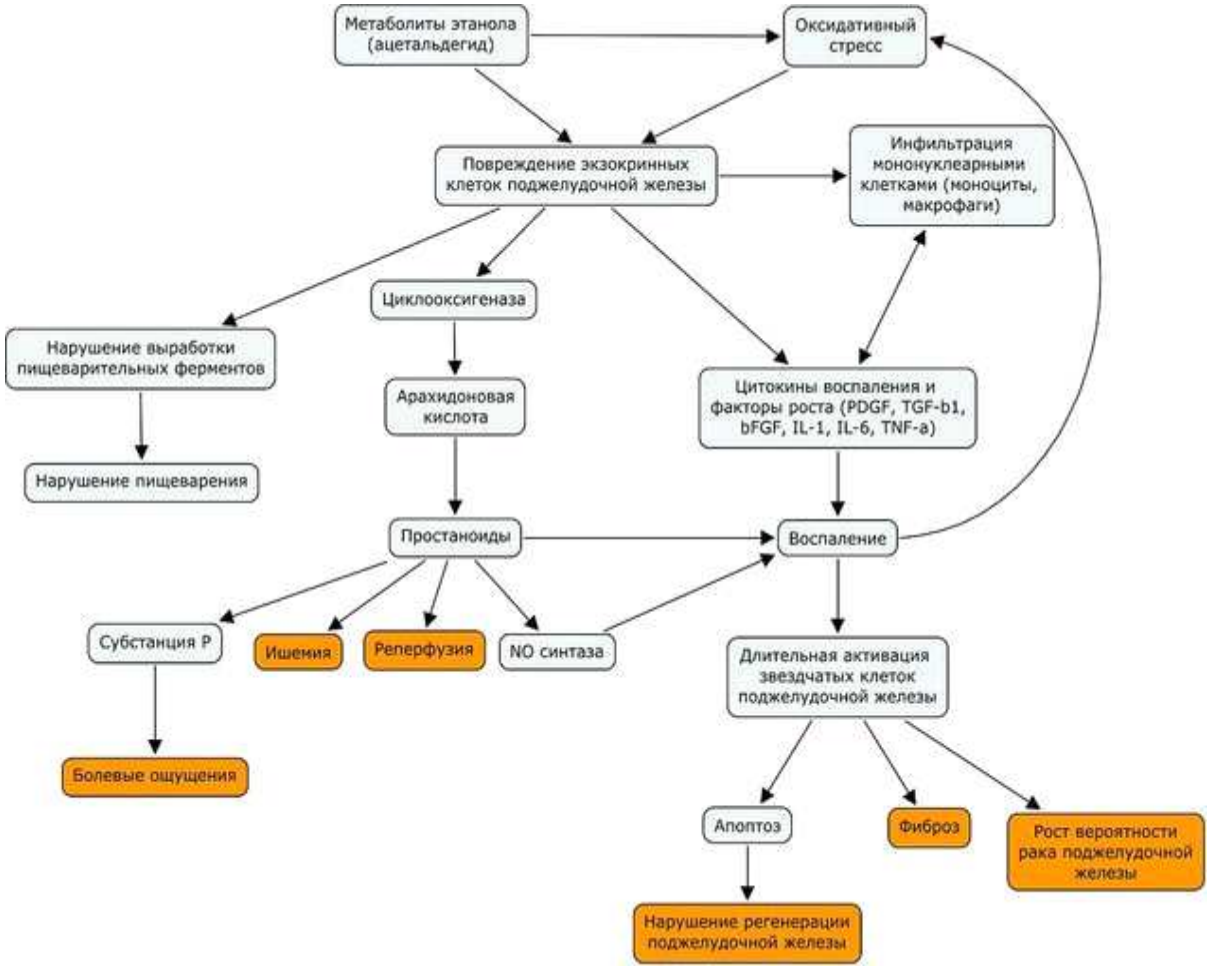

Fig. 1. Scheme of the development of alcoholic pancreatitis in vivo (with the permission of A. A. Moskalenko)

Fig. 2. Scheme of PDGF-polypeptides' action upon CP