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FIBROTIC TRANSFORMATION OF THE RAT PANCREAS AS RESPONSE TO THE NITRIC OXIDE DEFICIENCY

Summary. *Non-specific inhibitor of nitric oxide NG-nitro-L-arginine was administrated for the duration of the experiment, and the most significant changes (discirculation and dissecretion) were observed on the 12th day, whereupon the reaction slows due to a compensatory response. On the 30th day of experiment tiny bands of fibrous tissue were formed, which also evidenced by the increase of collagen synthesis markers in blood — protein-bound hydroxyproline ($p < 0.05$). Incompetence of pancreatic cells was manifested by the reduced activity of pancreatic enzymes ($p < 0.05$), and the visible hyposecretion of acinar cells on the morphological study. The maximum decrease of nitrite/nitrate concentration was observed after the first day ($p < 0.05$), with a gradual increase to a maximum on 12th day ($p < 0.05$).*

Background

Constant attention to the problem of chronic pancreatitis (CP) results from the fact that it is a pluricausal inflammatory chronic disease of the pancreas, characterized by the progressive development of fibrosis in the setting of focal, segmental or diffuse degenerative and destructive changes of the parenchyma [1, 2].

Recently reported that the main role in triggering processes of fibrogenesis in CP is played by specific «stellate» cells, activated amid oxidative stress [3].

Nitric oxide (NO) likewise plays an important role, regulating the processes of homeostasis due to its wide range of influence.

NO has an impact on activity of the respiratory, urinary and gastrointestinal tracts by the central and autonomic nervous system. Being synthesized in various cells as an autocrine and paracrine mediator, it can affect the metabolic processes in actual and contiguous cells [4, 5].

Reinforced inactivation or insufficient synthesis of NO induces oxidative stress caused by an imbalance between the activity of endogenous prooxidative enzymes and antioxidants [6].

Under normal physiological conditions NO is synthesized from L-arginine with the help of NO-synthase (NOs), the other reaction product is L-citrulline. NOs is the only known enzyme that uses in this process simultaneously five cofactors/prosthetic groups, being thus one of the most regulated enzym in the organism [7].

Previously a model of experimental pancreatitis was established which involves blocking of this regulation by the ways of using NG-nitro-L-arginine in duration of 6 and 12 day, which leads to circulatory distress accompanied by the activation of exocrine function [8]. However, no reported data exists on the impact of NG-nitro-L-arginine on pancreatic fibrogenesis in chronic pancreatitis.

Purpose. Determine the dynamics of the fibrotic processes in the rat pancreas in response to oxidative stress caused by deficiency of NO in experimental long-term administration of non-specific inhibitor (NG-nitro-L-arginine).

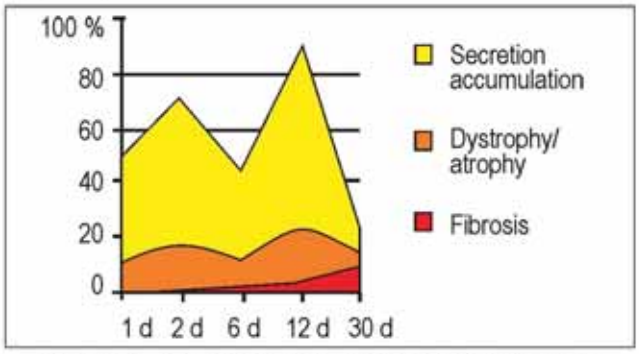

Figure 1 — Dynamics of morphological changes in rat pancreas according with the duration of administration of nonspecific NOs inhibitor NG-nitro-L-arginine (maximum values were observed after 12 days)

Material and Methods

The study was conducted in 42 male Wistar rats with weight 180–230 g NG-nitro-L-arginine, Sigma-Aldrich (USA) was everyday administrated intraperitoneal at 40 mg/kg for 1 day (n = 3), 2 days (n = 3), 6 days (n = 7), 12 days (n = 8), and 30 days (n = 6). A solution was prepared immediately before the experiment (as

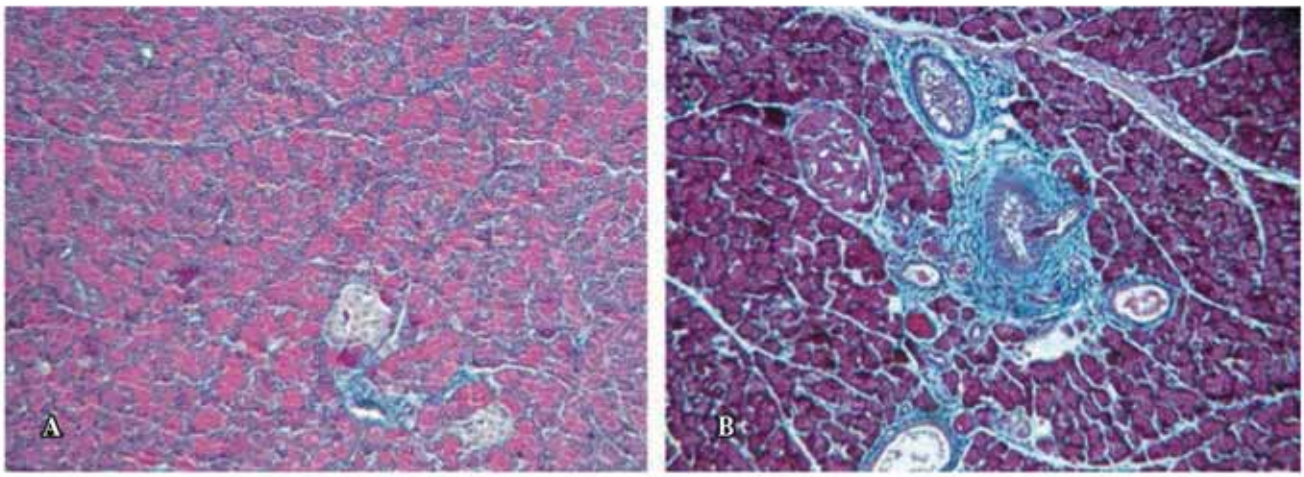

Figure 2 — Rat pancreas after 1 day (A) and 30 days (B) of administration of nonspecific NOs inhibitor NG-nitro-L-arginine. Fibrosis around vessels and pancreatic ducts, x200

recommended by the manufacturer) and intraperitoneally injected at 9–10 am. The control group (n = 15) was formed of intact rats and recieved 0.9% NaCl. Rats were sacrificed at the 1, 2, 6, 12 and 30 days.

The study was conducted following the standards of the European Convention of Bioethics (1997), European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, general ethical principles of animal experiments, approved in the law of Ukraine (№ 1759-VI of 15.12.2009) «On protection of animals from cruel treatment».

To carry out biochemical determinations and histologic study, after excision, total pancreases were excised, trimmed of fat and lymph nodes. Some small pancreatic samples from each rat were immediately fixed in 10% formalin, paraffin-embedded sections were cut at 3–5 microns and mounted on glass slides. Sections were deparaffinized and stained with hematoxylin-eosin or Mallory Trichrom.

Three groups of indicators were evaluated in three fields of view: 1) dystrophy/atrophy; 2) fibrosis (focal, segmental or diffuse); 3) accumulation of secretion in acinar cells.

Histological results of each group were expressed as a percentage, where the one field of view was taken as 100 %.

Biochemical process of fibrosis was evaluated on the content in the serum of free and protein-bound hydroxyproline [9] and hexosamines [10]. NO production was determined by the total content of nitrite/nitrate in serum using Gris test [11]. To estimate the exocrine function activity of pancreatic enzymes were measured in serum — amylase using set of Filisit-diagnosis and trypsin-α using Erlanger test with modifications of Shaternikov [10].

Results and Discussion

In one day after the administration of NG-nitro-L-arginine morphologically were observed acinar cells with inhibition of the secretion accumulation (all rats); in 2 days — the number of suchlike cells increased, and scattered degenerative changes were observed and in 50 % of animals — focal adipose degeneration.

After 6 days the percentage of acinar cells with inhibition of the secretion accumulation again began to decrease to the level of first day, while the spasm of blood vessels with stasis of blood cells and focal accumulation of lymphoid cells in the parenchyma were observed.

On the 12th day visible signs of circulatory hypoxia was developed on the background of cells degeneration and atrophy changes of acinar tissue without signs of inflammation. Acinar cells with inhibition of the secretion accumulation dominated across the whole field of view. In some cases gentle fibrosis around vessels and pancreatic ducts in the area of atrophy were developed.

On the 30th day all cases were demonstrated multiple focal apoptosis; the number of acinar cells with inhibition of secretion accumulation were significantly decreased. Also, dilatation of pancreatic ducts and vessels were observed along with stasis of blood cells, although these features were not so pronounced as on the 12th day of experiment, which may indicate the adaptable increasing of antioxidants that counterbalance the activity of endogenous prooxidative enzymes (Fig. 1).

All animals after 30 days of experiment demonstrated the development of fibrosis tissue (Fig. 2) with varying degrees of severity, which may indicate that the physiological antioxidant reserve slowly depleted and can no longer prevent the induction of stellate cells.

Morphological signs were accompanied by the changes in biochemical parameters that characterizes metabolism of collagen. Processes of connective tissue anabolism on 30th day illustrated through content of protein-bound hydroxyproline in blood — which was increased by 1.6 times from (178.67 ± 26.39) mkmol/l (control group) to (288.92 ± 13.02) mkmol/l ($p < 0.05$) and catabolism through content of free hydroxyproline — which was increased by 1.5 times (to 14.74 ± 1.84) mkmol/l ($p < 0.05$) and 1.9 times (to 19.30 ± 0.83) mkmol/l ($p < 0.001$) at 12th and 30th days, respectively. Compared with controls (9.96 ± 0.71) mkmol/l, those values indicated increased collagen synthesis and destruction (Fig. 3, 4).

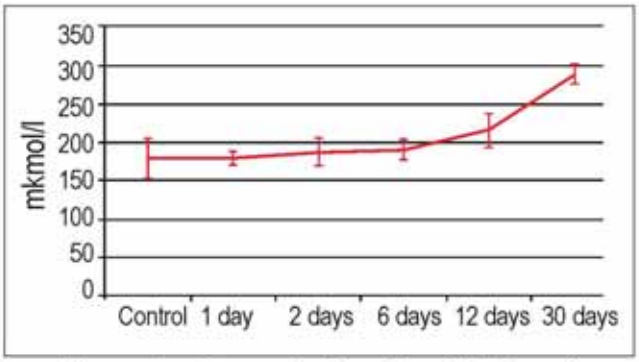

Figure 3 — Concentration of protein-bound hydroxyproline in the rat blood after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

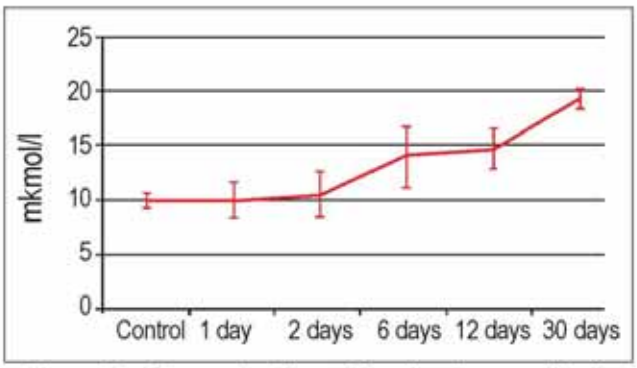

Figure 4 — Concentration of free hydroxyproline in the rat blood after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

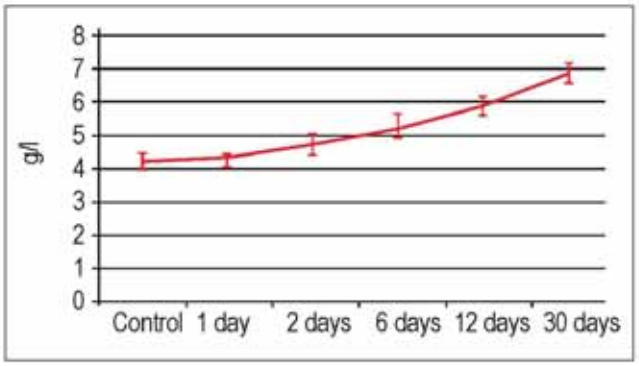

Figure 5 — Concentration of hexosamines in the rat blood after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

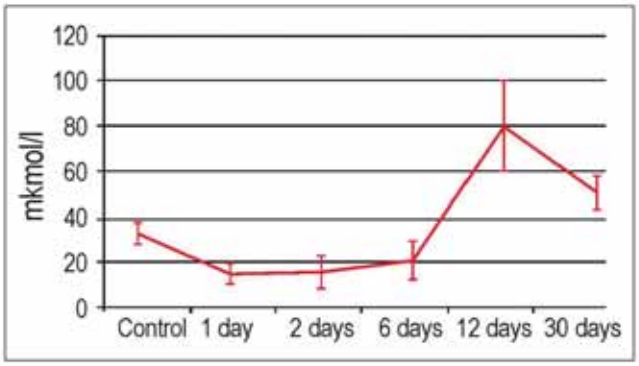

Figure 6 — Concentration of nitrite/nitrate in the rat blood after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

Increased concentration of hexosamine (HA) in the blood indicates increased catabolism of carbohydrate-protein components of connective tissue, as HA forms part of the proteoglycans and its components — glycoproteins. Aside from that increasing of HA concentration is a factor that suggest inflammation, while long-term inflammation of the pancreatic tissue may cause its destruction. Leading role in the pancreatic tissue destruction play proteolytic enzymes of polymorphonuclear leukocytes, which induce disintegration of macromolecular complexes containing HA. Probably HA may instigate processes of fibrosis and increasing of its concentration is first indication of changes among other parameters that characterize the functional state of the connective tissue. In this specific experiment, the content of HA in rat blood after 12 days of NG-nitro-L-arginine administration is increased by 1.4 times, to (5.90 ± 0.25) g/l ($p < 0.001$), and after 30 days — by 1.6 times, to (6.84 ± 0.31) g/l ($p < 0.001$) compared with the control group — (4.27 ± 0.18) g/l mcM (Fig. 5).

One day after the introduction of NG-nitro-L-arginine significant decrease of nitrite nitrate concentrations was observed in blood — by 2.2 times, to (14.85 ± 4.77) mkmol/l ($p < 0.05$) in comparison with the control group (32.61 ± 4.55) mkmol/l, whereas at 12th day there was a significant increase by 2.5 times, to (80.22 ± 19.91) mkmol/l ($p < 0.05$). Hereon those concentrations remained increased till the 30th day by 1.6 times, to ($50.56 \pm \pm 7.12$) mkmol/l ($p < 0.05$) (Fig. 6).

After the first day of experiment significant decrease in the activity of α-amylase was noted — by 2.6 times, from (96.02 ± 20.30) mg/s • l (control) to (36.73 ± 1.19) mg/s • l ($p < 0.05$), with maximum decrease on the 2nd day — by 6 times (16.03 ± 3.02) mg/s • l ($p < 0.01$). Upon 6 days of NG-nitro-L-arginine administration activity of this enzyme in serum increased by 3.2 times, to (311.26 ± 37.39) mg/s • l ($p < 0.001$). After 12 days there was a gradual decreasing by 2.1 times (205.49 ± 31.47) mg/s • l ($p < 0.05$), but the activity of α-amylase was still higher than the control group and after 30 days was still decreased by 1.3 times (72.78 ± 14.80) mg/s • l compared with controls (Fig. 7).

Trypsin is the best marker for the detection of pancreas pathology, as it is specific to this organ. Significant decrease of enzyme activity by 5.4 times from (4.19 ± 0.92) mkmol/ml • min (control group) to (0.77 ± 0.17) mkmol/ml • min ($p < 0.01$) observed after the first day, followed by the maximum increase at 2nd day of NG-nitro-L-arginine administration by 3.1 times (13.17 ± 2.24) mkmol/ml • min ($p < 0.01$), upon 6th day by 2.5 times to (10.45 ± 1.76) mkmol/ml • min ($p < 0.01$) and with a following decrease to the levels of the control group at 30th day of the experiment (Fig. 8).

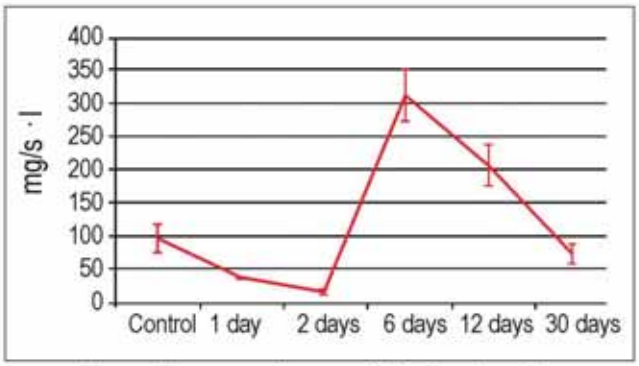

Figure 7 — α-amylase activity in rats after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

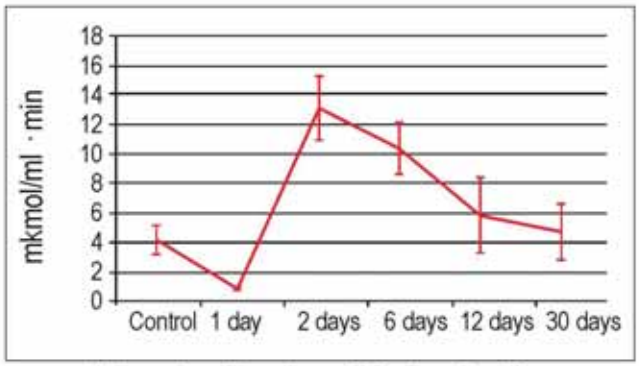

Figure 8 — Trypsin activity in rats after administration of nonspecific NOs inhibitor NG-nitro-L-arginine

Conclusions

1. Experimental administration of nonspecific NOs inhibitor NG-nitro-L-arginine leads to suppression of secretion accumulation in the acinar cells of rat pancreas and can inflict degenerative and atrophic changes in the parenchyma. Oxidative stress caused by the accumulation of endogenous prooxidative enzymes stimulated the formation of fibrous tissue.

2. The most notable circulatory changes were observed at 12th day, whereupon the reaction markedly decreased through compensatory response of the organism. On the 30th day after the administration of NG-nitro-L-arginine bonds of fibrosis tissue were forming around vessels and pancreatic ducts with a relevant increase of collagen synthesis markers in blood — protein-bound hydroxyproline ($p < 0.05$).

3. Prolonged administration of NG-nitro-L-arginine have influence upon development of functional failure in rat pancreas, as indicated by decreasing activity of pancreatic enzymes — α-amylase and trypsin — in blood on 30th day of the experiment and was accompanied by changes in the functional state of acinar cells (inhibition of the secretion accumulation).

4. Concentration of nitrite/nitrate after the first day of experiment was decreased ($p < 0.05$); later demonstrates gradual growth, and after 12th days remains higher than original characteristics ($p < 0.05$).

Prospects for further research. The results indicate the viability of further study of the pancreas structure characteristics in state of fibrous transformation and regeneration opportunities in adenocarcinoma.

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ФИБРОЗНАЯ ТРАНСФОРМАЦИЯ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ КРЫС В УСЛОВИЯХ ДЕФИЦИТА ОКСИДА АЗОТА

Резюме. При экспериментальном введении неспецифического ингибитора оксида азота NG-нитро-L-аргинина наиболее значительные дисциркуляторные и диссекреторные изменения наблюдались на 12-е сутки, после чего реакция заметно замедлялась в связи с компенсаторным ответом организма. На 30-е сутки в перидуктулярной зоне формировались тяжи фиброзной ткани, о чем также свидетельствовало увеличение в крови маркера синтеза коллагена — белковосвязанного оксипролина ($p < 0,05$). Функциональная недостаточность поджелудочной железы проявлялась снижением в крови активности панкреатических энзимов ($p < 0,05$), а при морфологическом исследовании — гипосекрецией ацинарных клеток. Максимальное снижение концентрации нитритов/нитратов наблюдалось после первых суток ($p < 0,05$) с постепенным максимальным повышением на 12-е сутки ($p < 0,05$).

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ФІБРОЗНА ТРАНСФОРМАЦІЯ ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ В УМОВАХ ДЕФІЦИТУ ОКСИДУ АЗОТУ

Резюме. При експериментальному введенні неспецифічного інгібітору оксиду азоту NG-нітро-L-аргініну найбільш значні дисциркуляторні й диссекреторні зміни спостерігалися на 12-ту добу, після чого реакція помітно уповільнювалася у зв'язку з компенсаторною відповіддю організму. На 30-ту добу в перидуктулярній зоні формувалися тяжі фіброзної тканини, про що також свідчило збільшення в крові маркера синтезу колагену — білковозв'язаного оксипроліну ($p < 0,05$). Функціональна недостатність підшлункової залози проявлялася зниженням у крові активності панкреатичних ензимів ($p < 0,05$), а при морфологічному дослідженні — гіпосекрецією ацинарних клітин. Максимальне зниження концентрації нітритів/нітратів спостерігалося після першої доби ($p < 0,05$) із поступовим максимальним підвищенням на 12-ту добу ($p < 0,05$).