Quantitative Morphology of Experimental Fibrosis in Canine Pancreas after Laser Tunneling

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Dogs with experimental fibrosing pancreatitis were subjected to laser tunneling to assess its effect on the dynamics of some morphometric indices of the pancreas. On post-tunneling day 60, volume ratio of the parenchyma significantly increased while the volume ratio of the stroma decreased in comparison to the dogs with fibrosing pancreatitis not corrected by laser tunneling.

Key Words: *fibrosing pancreatitis; l*aser *tunneling; morphometry*

Chronic pancreatitis (CP) is a prevalent disease often leading to disability and therefore is an important social and economic problem of modern medicine. In the structure of gastrointestinal tract diseases, this pathology amounts to 5.1-9.0%, the corresponding value in overall disease profile being 0.2-0.6% [4].

Morphological changes in CP are manifested by edema of various degree, inflammation, focal necroses, strictures of the pancreatic ducts, protein plugs and calcificates in the lumens of small ducts, and pseudocysts appearing as the outcome of acute pancreatitis. The development of inflammatory and degenerative alterations, sclerosis of the parenchyma of the pancreas, and obliteration of the ductal system lead, first, to impairment of the exocrine function of the pancreas and second, to deterioration of its endocrine function. This is a phasic process with alternation of acute periods accompanied by tissue destruction in this organ and relatively quiet periods, when the damaged tissue is replaced with the connective one [3,7].

During the last decades, laser transmyocardial revascularization is employed in patients with ischemic myocardium [1,2,9,10]. We hypothesized that similar surgical intervention would revascularize fibrotic tissue of the pancreas during CP and reverse sclerotic changes in this organ.

Our aim was to study the quantitative morphology of experimental fibrosis in dogs before and after focal laser tunneling and to substantiate the possibility of using this technique for acceleration of reversion of sclerotic changes in the pancreas.

MATERIALS AND METHODS

The experiments were carried out on outbred dogs (n=21) weighing 12-13 kg. They were randomized into three groups. The first group comprised control animals (n=3). Group 2 (reference group) consisted of dogs (n=6) with experimental fibrosis of pancreas (EFP). The experimental group 3 comprised dogs with EFP (n=12) subjected to laser tunneling. EFP was induced using a routine method [8]. In narcotized dog, the abdominal cavity was opened, and the right lobe of the pancreas was ligated with No. 5 capron thread folded in two is such a way as to narrow the excretory duct by 30-40% without complete ligation. This procedure impaired secret outflow and induced ductal hypertension not accompanied by cystic alteration of the pancreas. After the phase of acute pancreatitis, the exocrine parenchyma is replaced with the connective tissue within 4 weeks with the formation of fibrosis.

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Then the dogs were repeatedly operated. The abdominal cavity was opened, and the pancreatic duct was exposed into the wound. A single-fiber quartz light guide with a diameter of 0.4 mm was used to perform laser tunneling to the depth of 1 cm at the 5-7 points on the frontal surface of the altered lobule of the pancreas, the power of laser irradiation being 13.5 W/mm^2. This irradiation was produced by an LS-0.97 diode laser (IRE-Polus) at λ=970 nm. Then the surgical wound was tightly sutured.

The dogs of the reference groups were sacrificed on days 30 and 60 after inducing EFP (3 dogs for each term). The experimental group dogs were killed on days 1, 14, 30, and 60 after laser tunneling against the background of developed EFP. On 30 and 60 days after the development of fibrosis in the reference group, the morphometric indices of the pancreas did not significantly differ, so the corresponding data were united in a single reference group.

After euthanizing the dogs, the pancreas was isolated, weighted on an analytical balance, and fixed in formalin. The specimens of the pancreas were routinely embedded in paraffin. The morphometric analysis was performed on serial sections stained with hematoxylin and eosin. To this end, a *Cito-W* computerized colored image input and analysis system (Dia Morph) was employed. It measured the diameter of acinocytes and insulocytes (in μ), the number of acinocytes in the pancreatic acinus, the number of insulocytes in Langerhans island, the volume ratio of the parenchyma and stroma (in %), and the number of excretory ducts and blood vessels per arbitrary area unit.

The data were processed statistically with the significance level set to $p \leq 0.05$.

RESULTS

On days 30 and 60 after EFP induction, the pancreas decreased in size, its consistency became dense, and its weight was 21.3±1.2 g, which significantly differed from the control value 26.6±1.7 g. The capsule was non-uniformly thickened and its color being whitish. On section, the pancreas was pale rose with a lobular structure; histological examination revealed the development of atrophic and sclerotic processes. Morphometry revealed significant decrease in the number of insulocytes and blood vessels and an increase in the number of excretory ducts per unit area. These alterations were paralleled by significant increase in the volume ratio of the stroma and decrease in volume ratio of the parenchyma compared to the corresponding indices in the control group (Table 1).

On post-tunneling day 1, visual inspection of the pancreas revealed small black retracted foci ~2 mm in diameter. On sagittal section, an area of laser-produced lesion of conic shape with a depth of 1 cm and the cone basis facing the irradiation source was seen. On this section, the tissue was rosy-blue and lobular. The weight of the pancreas was 21.2±1.1 g ($p<0.05$ compared to the control group, but $p>0.05$ in comparison with untreated EFP group).

Histological examination of pancreas specimens revealed eschar foci of yellow-black color surrounded with coagulation necrosis area. In these specimens, the inflammatory reaction was minor. There was vascular coagulation at the necrotic boundary characterized with obturation of the lumen with hyaline-like thrombi and coagulation of the excretory ducts with obturation of the lumen with eschars. Quantitative study showed that volume ratio of the stroma and the number of excretory ducts in the conventional area were significantly higher, while the volume ratio of the parenchyma and the number of insulocytes were significantly lower than the corresponding control values (Table 1). However, these parameters did not significantly differ from those in EFP (Table 1).

On day 14, the weight of the pancreas was 22.9± 1.2 g ($p>0.05$ compared to the control, the EFP reference group, and with the data of the experimental group obtained in previous examination day). Histological examination of pancreatic specimens revealed replacement of necrotic foci with the connective tissue paralleled by proliferation of small excretory ducts and endotheliocytes forming capillaries. Quantitative study showed that diameter and number of acinocytes and insulocytes, volume ratio of the stroma, number of blood vessels and excretory ducts per unit area were far higher, while the volume ratio of the parenchyma was below the control values (Table 1). At the same time, the diameter and number of acinocytes and insulocytes, the number of blood vessels and the excretory ducts per unit area significantly increased compared to the corresponding parameters in the reference EFP group. Moreover, the diameter and number of acinocytes and the number of blood vessels per unit area significantly increased compared to the corresponding parameters in this experimental group at the previous term.

On day 30, the weight of the pancreas was 24.5± 0.9 g ($p>0.05$ in comparison with the control group, reference EFP group, and experimental group at the previous examination day). Histological examination of pancreas specimens taken in the lesion area, cicatrical tissue, and its border revealed the acinar parenchymal islets, the excretory ducts, and the blood vessels. The morphometric study showed that the number of acinocytes and insulocytes, volume ratio of the stroma, number of blood vessels and excretory ducts per unit area were significantly higher, while volume ratio of the parenchyma was lower in comparison with control values (Table 1). Moreover, the diameter and number

TABLE 1. Morphometric Parameters of the Pancreas in Dogs with Experimental Fibrosis in Various Terms after Laser Tunneling (*M*±*m*)

Index	Control (*n*=3)	EFP (reference group, *n*=6)	Laser tunneling of fibrotic pancreas			
			day 1 (*n*=3)	day 14 (*n*=3)	day 30 (*n*=3)	day 60 (*n*=3)
Diameter, μ						
acinocytes	8.3±0.8	6.6±0.8	7.3±1.4	14.3±0.3*+	9.6±0.6+	8.6±0.6
insulocytes	8.6±1.4	7.3±1.4	8.3±0.8	15.3±1.4*+	9.3±0.6	8.3±1.2
Number of acinocytes in acinus	7.3±1.4	4.3±0.8	5.6±0.3	15.3±1.4*+	12.6±0.6*+	11.3±0.8*+
Number of insulocytes in pancreatic islet	47.3±0.8	35.3±1.4*	39.6±1.2*	57.6±1.7*+	52.3±1.4*+	50.6±1.7+
Volume ratio, %						
parenchyma	94.3±1.2	81.3±1.4*	81.3±2.1*	82.6±3.1*	85.0±2.3*	89.0±2.3+
stroma	5.6±1.1	18.6±1.3*	18.6±2.0*	17.3±3.0*	15.0±2.2*	10.0±2.2+
Number per unit area						
excretory ducts	6.0±0.5	8.3±0.8*	8.3±0.3*	13.3±1.4*+	16.0±1.7*+	13.6±0.6*+
blood vessels	9.3±1.4	5.3±0.8*	7.0±1.1*	15.0±1.7*+	19.0±1.1*+	16.0±1.7*+

Note. $p<0.05$-0.01 compared to: *control, +EFP groups.

of acinocytes and insulocytes, number of blood vessels and excretory ducts per unit area were significantly higher than in the reference EFP group. In addition, the diameter of acinocytes and insulocytes was significantly lower than in the same experimental group at the previous examination day.

On day 60, the weight of the pancreas was 25.3± 0.8 g, which did not significantly differ from the corresponding parameter in the same experimental group at the previous examination day (24.5±0.9 g, $p>0.05$) or from the corresponding control value (26.6±1.7 g, $p>0.05$), but significantly surpassed the weight of the pancreas in the reference EFP group (21.3±1.2 g, $p<0.05$). Histological examination of pancreatic specimens taken in the lesion area revealed small vascularized scars. Morphometry showed that the numbers of acinocytes, blood vessels, and excretory ducts per unit area were above the control values, while the volume ratio of the parenchyma and stroma did not differ from the control. At the same time, the numbers of acinocytes and insulocytes, volume ratio of the parenchyma, number of blood vessels and the excretory ducts per unit area were higher, while the volume ratio of the stroma was lower than in the reference EFP group. It is worthy to note that all examined indices did not significantly differ from the corresponding values in the same experimental group at the previous examination day. It should be stressed that there were no bleeding or pancreatic juice outflow in the laser burn area in fibrotic pancreas, probably because blood vessels at the border of the necrotic area, were coagulated and plugged with hyaline-like thrombi, while the excretory ducts were coagulated and obturated with eschars. These alterations observed as early as at the end of the first day prevented the development of acute pancreatitis and peritonitis, which negated the necessity of additional surgical treatment of the laser wound.

Of great practical importance is solution of the problem of reversibility of the sclerotic alterations in the pancreas. It was firmly established that stimulation of regeneration by resection of pancreatic lobes dramatically accelerates resorption of excessively developed fibrotic tissue [6]. Similar process was induced in our experiment by focal laser tunneling of sclerotically modified pancreas in dogs [5]. This fact is also corroborated by significant decrease in the volume ratio of the stroma and increase in the volume ratio of the parenchyma by day 60 (compared to the corresponding parameters in the reference EFP group). Most researches explain sclerosis reversibility by complex biochemical processes playing between the proliferating parenchyma and surrounding fibrotic tissue, as well as by the development of feedbacks between collagen catabolism and synthesis in the cicatrical tissue [6].

Some studies demonstrated that cells in Langerhans islets and in the excretory ducts were characte-

rized with higher proliferation activity (cell regeneration) and far lesser capacity to hypertrophy, *i.e.* to intracellular regeneration [6]. This hypertrophy seems to play the leading role in compensation of the disturbed pancreatic functions [6]. We revealed a pronounced increase in the number of acinocytes and its diameter on day 14 after laser irradiation of sclerotic pancreas compared to control values and the corresponding parameters in the reference EFP group. At the same period, the number of insulocytes increased. Moreover, the period of 14-60 days after laser was characterized by pronounced increase in the number of excretory ducts and blood vessels per unit area compared to the control values and the corresponding indices in the reference EFP group. Similar morphological alterations characterized by proliferation of the excretory ducts, hypertrophy of Langerhans islet, and improvement of circulation were observed at various terms after laser-produced resection of the pancreatic lobes in dogs [5].

Thus, our results attest to acceleration of reversibility of the sclerotic alterations in the pancreas due to stimulation of the regeneratory processes by focal laser tunneling. They also open the way to apply this method in clinical practice to treat the patients with chronic fibrotic pancreatitis.

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