Hemolymphomicrocirculatory Bed of the Pancreas during Acute Experimental Pancreatitis

Yu. I. Borodin, M. B. Vasilyeva, P. M. Larionov, V. V. Astashov, and E. V. Yankaite

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 4, pp. 478-480, April, 2006
Original article submitted December 6, 2005

Histochemical study of rat pancreas was carried out on days 14 and 30 of acute pancreatitis. The area of the stroma increased after damage and the area of lymph microcirculatory component increased in comparison with the blood circulation component.

Key Words: *acute pancreatitis; lymph endothelium; 5'-nucleotidase; alkaline phosphatase*

Mortality in acute pancreatitis is 30-70%, sometimes it reaches 100%. The importance of the problem increases because of increasing incidence of destructive forms and absence of a trend to stabilization or reduction of this parameter, and increased incidence of the disease in young and middle-aged subjects [8].

Disorders in the structural organization of the microcirculatory system play an important role in the pathogenesis of many diseases, including AP [3, 5,13]. Changes in the hemomicrocirculatory system of the pancreas in AP are described in detail [4,6].

The lymph system, the main collector of interstitial fluid, participates in the regulation of cell metabolism and tissue metabolic processes [2].

In addition to the immunohistochemical methods, enzymatic methods are used for identification of the endothelium type (blood or lymph). They help to detect the details of the structure and development of the blood and lymph capillaries [10].

The aim of this study was visualization of the hemolymphomicrocirculatory bed of the pancreas in health and during acute experimental pancreatitis (AEP) by enzymatic methods.

Department of Preventive and Ecological Lymphology, Institute of Clinical and Experimental Lymphology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** vasilievam@yandex.ru. M. B. Vasilyeva

MATERIALS AND METHODS

The study was carried out on outbred adult male Wistar rats (200-250 g). The animals were divided into groups, 10 per group: 1) intact controls; 2) animals with AEP (material collected on day 14); and 3) animals with AEP (material was collected on day 30).

Acute experimental pancreatitis was modeled using an original method developed on the basis of combination of alcoholic [12] and subsequent ischemic injury of the pancreas [11]. The animals received 10% ethanol (2 ml/100 g) through an intragastric tube [12]. After 30 min median laparotomy was carried out under ether narcosis and the pancreas was exposed. The splenic artery and vein were clamped with a microsurgical vascular clamp for 60 min, after which the clamp was removed and the abdominal wall was sutured hermetically layer-by-layer.

The development of pancreatitis was confirmed by histological studies. Changes of the acute edematous pancreatitis type with manifest interlobular edema and microclots were detected in pancreatic tissue specimens collected from animals with AEP on day 3. Solitary foci of predominantly neutrophilic infiltration, partial damage to the acinuses with emergence of fibrinoid necroses were detected. Hyperplastic processes were detected in the

0007-4888/06/1414-0491

insular islet cells, and reactive vasculitis phenomena were seen.

The animals were decapitated under deep ether narcosis on days 14 and 30 of the experiment. Tissue fragments for histological and histochemical studies were collected from the proximal part of the pancreas. Fresh tissue specimens were rapidly frozen in liquid nitrogen. Sections (7 μ) were made on a cryostat microtome (Microm HM 550) and postfixed in acetone:ethanol (95%) mixture for 5 min. Ready pancreatic tissue sections were stained with hematoxylin (by the standard method) and eosin after Mayer, and two-staged staining for 5'nucleotidase and alkaline phosphatase (AP) was carried out [10].

Morphometry of pancreatic sections was carried out using a square 289-dot reticular ocular insert; areas of the parenchyma and stroma per unit of area were determined at a final magnification of ×200 as described previously [1]. Relative area of the blood and lymph capillaries was determined in histochemically stained pancreatic sections. The data were processed using Fisher—Student test. The results were considered significant at $p<0.05$.

RESULTS

On day 14 of the experiment the content of stromal tissue in group 2 increased 1.9 times in comparison with the control (9.46±0.59%; $p<0.05$). On day 30 the area of the stromal component increased by 26% more (in comparison with day 14: 18.88± 0.79; $p<0.05$).

The ratio of areas of blood and lymph capillaries also changed (Table 1).

The area of blood vessels decreased significantly (by 10.8%) in group 2 compared to the control on day 14 of the experiment. On day 30 it increased by 30.9% *vs.* day 14 and surpassed the control value by 16.7%. The area of lymph capillaries in group 2 increased by 72.6 on day 14. On day 30 their area increased 1.4 times compared to that on day 14 ($p<0.05$).

These changes can suggest that natural course of AEP leads to gradual growth of stromal elements by days 14 and 30, which is in line with the classical concepts of inflammation and regeneration processes [9].

We failed to find statistical data on the ratio between the blood and lymph capillaries in tissues. Some authors studied hemomicrocirculatory bed of the pancreas, but only during the first 24 h after initiation of the process [5,7].

The increase in the area of lymph capillaries can be explained by their important role in elimination of degradation products from the damaged zone during pathological process [2]. Presumably, the lymph capillary system is involved in the inflammatory process over the entire evolution of the inflammatory focus from the moment of tissue injury until liquidation and development of fibrosis [9].

TABLE 1. Areas of Blood and Lymph Capillaries in the Pancreas in AEP (%; *M*±*m*)

Group	Capillaries	
	blood	lymph
1 (control)	9.41±0.43	4.92±0.32
2 (AEP, 14 days)	8.39±0.11*	8.49±0.11*
3 (AEP, 30 days)	10.98±0.28*+	11.76±0.33*+

Note. *$p<0.05$ compared to the control, +$p<0.001$ compared to AEP group, 14 days.

Hence, under conditions of normal blood and lymph microcirculation the area of blood capillaries in pancreatic tissue of random-bred male Wistar rats is larger than the area of lymph capillaries; in AEP the involvement of the blood and lymph capillaries in the drainage of the pancreas at early and later stages of experiment is equally pronounced, which is confirmed by approximately equal areas of the blood and lymph capillaries.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry. Manual* [in Russian], Moscow (1990).
2. Yu. I. Borodin, M. S. Lyubarskii, A. V. Efremov, *et al.*, *Clinical Pathogenetic Approaches to Lymph Correction* [in Russian], Novosibirsk (1997).
3. R. V. Vashetko, A. D. Tolstoi, A. A. Kurygin, *et al.*, *Acute Pancreatitis and Pancreatic Injuries* [in Russian], St. Petersburg (2000).
4. Yu. S. Vinnik, D. V. Cherdantsev, A. A. Vakhrunin, *et al.*, *Methodology of Flowmetry* [in Russian], (1997), pp. 93-106.
5. I. V. Gaivoronskii, S. V. Petrov, L. P. Tikhonova, and A. L. Efimov, *Regional'noe Krovoobrashchenie i Mikrotsirkulyatsiya*, No. 4, 70-77 (2004).
6. K. A. Dyubenko, *Klin. Khir.*, No. 11, 30-31 (1985).
7. N. A. Efimenko, M. E. Cherenekhovskaya, T. A. Fedorova, and V. K. Shichlo, *Microcirculation and Methods of Its Correction* [in Russian], Moscow (2003).
8. V. S. Savel'ev, M. I. Filimonov, S. Z. Burnevich, and P. A. Sobolev, *Ann. Khir.*, No. 1, 34-39 (1998).
9. A. I. Strukov, *Arkh. Patol.*, No. 9, 73-76 (1983).
10. S. Kato, *Stain. Technol.*, **65**, No. 3, 131-137 (1990).
11. R. Obermaier, S. Benz, B. Kortmann, *et al.*, *Clin. Exp. Med.*, **1**, No. 1, 51-59 (2001).
12. M. Siech, H. Weber, G. Letko, *et al.*, *Pancreas*, **14**, No. 1, 32-38 (1997).
13. J. Werner, T. Dammrich, T. Hackert, *et al.*, *Pancreatology*, No. 2, 217-361 (2002).