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• CLINICAL RESEARCH •

Pancreatic microcirculatory impairment in experimental acute pancreatitis in rats

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Abstract

AIM: To study the feature of pancreatic microcirculatory impairment, especially the initial changes, in caerulein-induced experimental acute pancreatitis (AP).

METHODS: The pancreatic microcirculation of caerulein-induced AP model was studied by intravital fluorescence microscopy with FITC-labeled erythrocytes (FITC-RBC), scanning electron microscopy of vascular corrosion casts, and light microscopy of Chinese ink-injected/cleared tissues.

RESULTS: Animals in caerulein-treated group showed hyperamylemia (×2), pancreatic oedema, infiltration of inflammatory cells in pancreas. Constrictions of intralobular arteriolar sphincters, presence of vacuoles in all layers of sphincter, and gross irregularity in capillary network of acini were found in the AP specimens. The decrease of pancreatic capillary blood flow (0.34 ± 0.10 nl·min^{-1} *vs* 0.91 ± 0.06 nl·min^{-1} of control, $P<0.001$), reduction of functional capillary density(277 ± 13 cm^{-1} *vs* 349 ± 8 cm^{-1} of control, $P<0.001$), and irregular intermittent perfusion were observed in caerulein-induced groups.

CONCLUSION: Impairment and constriction of pancreatic intralobular arteriolar sphincter are the initial microcirculatory lesions in the early phase of acute pancreatitis, and play a key role in the pancreatic ischaemia and pancreatic microvascular failure in acute pancreatitis.

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INTRODUCTION

Etiopathology of acute pancreatitis (AP) is not fully understood[1-19]. Microcirculatory impairment has long been recognized as one of the etiological factors of acute pancreatitis[20]. Pancreatic microcirculatory disturbance may act as initiating factor or aggravating/continuing factor. However, the mechanism of microcirculatory impairment in acute pancreatitis is complex; there are questions concerning local pancreatic microcirculatory change in acute pancreatitis and the features of pancreatic microcirculatory disturbance in various stages of AP remain subject to further study[21-28]. To investigate the feature of the pancreatic microcirculatory impairment in the early-stage of caerulein-induced experimental acute pancreatitis, dynamic method of microcirculatory research combined with static method had been carried out in this study.

MATERIALS AND METHODS

Animals

48 adult male Wistar rats, weighing 250-350 g, were randomly assigned to 4 groups: (1) control group (group 1, *n*=12). (2) intravital study group, panceatic microcirculation observed with FITC-labeled RBC and intravital fluorescence microscope (group 2, *n*=12). (3) light microscopy and scanning electron microscopy study group, pancreatic microvasculature perfused with ink and methylmethacrylate (group 3, *n*=12). (4) histocellular study group (group 4, *n*=12).

Experimental pancreatitis

Caerulein used to induce acute pancreatitis was obtained from Sigma Co.. All experimental groups were injected caerulein subcutaneously 5.5 and 7.5 μg·kg^{-1} 1 and 2 h after the beginning of experiment respectively, while control group was injected physiological saline solution subcutaneously. All groups were observed 4 after the beginning of the experiment.

Erythocytes labeling

Erythocytes were labeled by fluorescein isothiocyanate (FITC, purchased from Sigma Co.) using a combined approach of the procedures of Klar (1995). The labeled cells were stored a maximum of 24h before use.

In vivo microscopy

The pancreas of the studied animal was exteriorized on a stage, then FITC-labeled RBC was intravenously injected and intravital fluorescence microscope (Olympus X-70) were used to dynamically observe the pancreatic microcirculatory indices, and the images were simultaneously picked up by high-resolution video cassette recorder.

Morphology of microvasculature

Thoracic aortas of the studied animal were cannulated for perfusion. After flushing the vessels with warmed heparinized physiological saline solution, a diluted resin mixture or China ink was injected through the cannula with an injection pressure of 12-16kPa, until the portal vein and inferior vena cava was filled with the injected resin or ink.

The pancreas of resin-injected animal was corroded overnight or longer in a hot 300-400 g·L^{-1} KOH solution, washed in running water and rinsed again several times in distilled

water, air-dried, coated with gold in a vacuum evaporator, and observed in a scanning electron miscroscope.

The pancreas of ink-injected animal was fixed overnight or longer in Bouins solution, cleared in trichloromethane, embedded in paraffin, serially sectioned (thin sections of 5-7 μm for observation of the relationship between capillaries and cells, thick sections of 50-100 μm for observation of the vessel continuation), and observed with an Olympus X-60/50 light microscope. Serial reconstruction was carried out, camera lucida tracings of photographs were made at x330 final magnification on transparent sheets and superimposed for analysis.

Assays

Serum amylase level was determined and adopted as an indicator of AP. The increase in water content of pancreatic tissue served to indicate the formation and severity of pancreatic edema. The wet weight/dry weight ratio was expressed in per cent. Pancreatic tissue blocks of all groups were routinely paraffin wax-embedded, sliced, stained with hematoxylin/eosin and toluidine blue/basic fuchsin, then the sections were microscopically studied.

Statistical analysis

The results were expressed in mean ± standard deviation, and *t*-test was used to evaluate differences between control and AP groups. Difference was considered significant at the $P<0.05$ level.

RESULTS

Pancreatic edema

Gross appearance of pancreatic tissue of control group remained normal, and presented 72 % of water content. In comparison, pancreatic edema gradually appeared in Group2, 3 and 4 four hours after subcutaneous injection of caerulein, in parallel with an increase in pancreatic tissue volume. Edema of pancreatic head and body was much prominent, and the water content increased to 75 %. Inflammatory exudate accumulated in the anterior pararenal space and lesser omental sac in 50 % cases.

Morphology

Injury of intralobular arteriolar sphincter became visible 4 h after animal model established, and numerous cytoplasmic vacuoles formed; massive interstitial edema and inflammatory cell infiltration gradually emerged at 6 h. While in control group, pancreatic acini, tubules and blood vessels were normal microscopically.

Serum amylase

Serum amylase measurement in control group presented normal level (20.8 $\mu kat \cdot L^{-1}$). Serum amylase in all AP groups showed hyperamylasemia (45.0 $\mu kat \cdot L^{-1}$), significant higher than that of control group ($P<0.01$).

Light microscopy and scanning electron microscopy

Animals in the caerulein-treated group showed constriction of intralobular arteriolar sphincter 4 h after beginning of the experiment, presence of vacuoles in all the layers of sphincter, gross irregularity in capillary network of acini, reduction of capillary density, and blebs protruded from the surface of casts reflecting a substantial increase in capillary permeability.

In vivo fluorescence microscopy

Comparing with the control group, 4 hours after the start of experiment in AP groups the pancreatic microcirculatory in the caerulein-treated group showed the reduction of the velocity of FITC-labeled RBC, decrease of pancreatic capillary blood flow ($P<0.01$, Table 1), reduction of functional capillary density and arterioles diameter ($P<0.05$), and irregular intermittent perfusion of capillary network ($P<0.05$). Arterioles of pancreatic lobules and capillary density experienced significant changes at 6 h. The calibers of venules and capillaries showed no marked change in 6 h, while there was significant change by 8 h ($P<0.05$, Table 2).

Table 1 Intravital fluorescence microscopy of pancreatic microcirculation with FITC-labeled erythrocytes

t/h	Group	d(FITC-RBC)/ ($\times 10^9$cell/L)	Velocity of FITC-RBC/ (cell·min^{-1})	RBC flow/ (nl·min^{-1})	Microcirculatory blood flow/ (nl·min^{-1})
4	Control	113±5	86±3	0.28±0.01	0.88±0.06
	AP[b]	85±9	43±2	0.12±0.03	0.56±0.09
6	Control	104±4	81±4	0.31±0.02	0.99±0.07
	AP[b]	68±7	36±5	0.09±0.03	0.45±0.12
8	Control	96±6	84±5	0.29±0.04	0.91±0.06
	AP[b]	59±9	30±5	0.07±0.03	0.34±0.10

[b]$P<0.001$ *vs* control.

Table 2 Intravital observation of pancreatic microcirculation

t/h	Group	D(arteriole) /μm	D(venule) /μm	D(capillary) /μm	d(Capillary) /cm^{-1}	Capillary perfusion
4	Control	23.5±8	28±3	6.7±1.5	394±7	Stable
	AP	20.2±5.1	29.1±2	7±1.4	381±9	Unstable
6	Control	24.1±8	28±2.7	6.9±1.48	400±5.8	Stable
	AP	16.4±3.1[a]	27.5±3	6±0.3	291±16[a]	Intermittent & irregular
8	Control	23.2±5.5	27.4±1.6	7.3±1	349±8	Relatively stable
	AP	18.2±3.5[a]	29±1.5[a]	5.2±0.3[b]	277±13[b]	Intermittent & irregular

[a]$P<0.05$, [b]$P<0.001$ *vs* control.

DISCUSSION

In 1862 Panum demonstrated that acute hemorrhagic pancreatitis could be induced with wax droplets injected into pancreatic arteries. From then on, the etiological role which pancreatic ischaemia and tissue hypoperfusion plays in AP has been extensively discussed[29]. Many researches suggested that local microcirculatory disturbance, not insufficient blood flow in peripheral circulation, was responsible for perfusion failure of pancreatic tissue. In recent years, various animal models such as hemorrhagic shock, embolization of pancreatic microvasculature by minute particles and ligation of pancreatic arteries, have been used to verify that microcirculatory impairment of pancreas is the initial stage of AP. But the following questions haven't been answered conclusively: whether all types of AP are initiated by pancreatic microcirculatory impairment? What are the characteristics of early-stage pancreatic microcirculatory change? And what are the features of pancreatic microcirculatory disturbance in the natural process of AP? Insights into all these areas are crucial to the development of prevention and treatment measures.

Animal model

Sodium taurocholate-induced experimental pancreatitis was used by many authors to investigate microcirculatory change of AP; this model can reflect soundly the pathological features of acute necrotizing pancreatitis. Since direct injury to pancreatic ductules, acini and blood vessels may happen in several minutes, the gradual evolution of early-stage pathological change of pancreas in AP cannot be explored. In addition, modulation of intraductal pressure in the process of retrograde pancreatobiliary injection of sodium taurocholate also poses a real challenge. In this study, caerulein-induced experimental pancreatitis was chosen to investigate the features of early-stage pancreatic microcirculatory change. Subcutaneous administration of caerulein is easy to operate, and can result in acute edematous pancreatitis similar to that induced by intravenous injection of caerulein. In this model, the pathological changes develop slowly and gradually, the microcirculatory and histological changes of pancreas become prominent 4 h after the beginning of experiment, and pancreatic edema reaches its zenith by 8 h. This gradual development course allows us to study the triggering factor and the features of early-stage pancreatic microcirculatory impairment without haste.

Study techniques

For decades, pancreatic microcirculatory study heavily depended on the following techniques: injection of minute particles, Indian ink and methylthionine chloride into pancreatic arteries; measurement of pancreatic blood flow through pancreatoduodenal arteries and veins; measurements of relative blood flow and tissue perfusion of pancreas with intravenous injection of nuclide Rb-86, etc. Since acute necrotizing pancreatitis is characterized with progressive regional or focal necrosis of pancreatic tissue, observation with a single method has the following disadvantages: (1) dynamic and direct observation of local microcirculatory change of pancreas is impossible, since the animal must be sacrificed at a specific time; (2) observation of local blood flow of pancreas and tissue perfusion cannot be made simultaneously on the same specimen; (3) as to traditional intravital observation of pancreatic microcirculation, quantitative study cannot be effective due to dim image. This experiment has solved the above problems by developing a new approach; this approach combined intravital microcirculation observation technique, using selective blood element fluorescent marker, with another technique-maintaining dynamic and tissue message on static specimen.

Pancreatic microcirculatory impairment in AP

In recent years, applied basic researches on the morphology of pancreatic microcirculation revealed that the blood supply of pancreatic lobule, in most cases, is provided by a single intralobular arteriole. This arteriole sends forth tree-like branches when entering pancreatic lobule; it has no anastomosis with adjacent intralobular arterioles and their branches, and can be considered end-artery[30]. This characteristic suggested that pancreatic lobules are susceptible to ischaemic injury due to spasm of intralobular arterioles, embolization of arterioles by emboli, formation of microthrombi or compression by interstitial edema. However, causative factors of early-stage ischaemia and the precise triggering factor of local microcirculatory disturbance are not evident.

This study showed that, manifested as lasting spasm of arteriolar sphincter and multiple cytoplasmic vacuoles within smooth muscle cells of sphincter, the main feature of early-stage pancreatic microcirculatory impairment of AP is injury of sphincter of pancreatic intralobular arteriole. This experiment also demonstrated that among many factors causing early-stage ischaemia, the key one is injury and spasm of sphincter of pancreatic intralobular arteriole. In this study, injury of arteriolar sphincter occurred earlier than microcirculatory impairment, which reflected that injury of intralobular arteriolar sphincter was the initial stage of pancreatic perfusion failure and local microcirculatory disturbance. Microcirculatory hypoperfusion happened almost simultaneously with injury and spasm of arteriolar sphincter, indicating that pancreatic tissue is highly sensitive to ischaemic stress and has no compensatory reserve. Since sphincter of pancreatic intralobular arteriole serves as main lockgate to control blood flow to pancreatic lobule, and intralobular arteriole has characteristics of end-artery, even sphincter spasm of very short time will quickly evoke obvious pancreatic microcirculatory impairment. Other factors causing ischaemia[31-37], such as compression from interstitial edema, microemboli or obstruction due to thombosis, tend to be secondary ones, which may happen gradually in the course of pathological change of AP. These traumatic factors help to sustain and aggravate pancreatic microcirculatory impairment. To clarify relationship between traumatic factors of pancreatic microcirculatory impairment and pathological evolution of AP has guiding value in making treatment plans for clinical AP cases of various development stages. Features of early-stage microcirculatory change of experimental pancreatitis suggested that early adoption of spasm relieving and counter-injury measures are of vital importance in prevention and treatment of local microcirculatory disturbance of AP.

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