European Review for Medical and Pharmacological Sciences 2015; 19: 2646-2650

Effect of vascular bradykinin on pancreatic microcirculation and hemorheology in rats with severe acute pancreatitis

L.-T. LIU, Y. LI, L.-Q. FAN, Q. ZHAO, D. WANG, S.-J. CHENG[1], A.-M. ZHANG[1], Y. QIN[1], B. ZHANG[2]

Department of General Surgery, The No. 4 Affiliated Hospital of Hebei Medical University, Shijiazhuang, China
[1]Department of General Surgery, The Affiliated Hospital of Hebei University, Baoding, China
[2]Department of General Surgery, The No. 2 Hospital of Baoding, Baoding, China

Abstract. – OBJECTIVE: To investigate the effect of vascular bradykinin on pancreatic microcirculation and hemorheology in rats with severe acute pancreatitis (SAP).

MATERIALS AND METHODS: Ninety male Wistar rats were randomly divided into a blank control group, an SAP group and a vascular bradykinin treatment group. The SAP model was induced by the retrograde injection of 5% sodium taurocholate in the pancreaticobiliary duct. The vascular bradykinin treatment group underwent gastrostomy, with a fine plastic tube placed in the stomach that led out of body through the abdominal wall.Vascular bradykinin was fully dissolved and administered at a dose of 20 U/kg once every 8 h. The pancreatic microcirculatory blood flow volume and velocity, microvascular permeability, hemorheology were evaluated respectively by double-channel laser Doppler flowmetry, the Evans blue leakage test, a blood rheology test instrument.

RESULTS: The pancreatic microcirculatory blood flow volume and velocity in the vascular bradykinin treatment group increased gradually after 48 h compared with the SAP group, and the changes were significantly different ($p < 0.05$). The pancreatic microvascular permeability of the vascular bradykinin treatment group was significantly reduced after 48 h compared with the SAP group ($p < 0.05$). The low shear rate blood viscosity, hematocrit and erythrocyte aggregation index of the vascular bradykinin treatment group were significantly decreased after 48 h compared with the SAP group ($p < 0.05$).

CONCLUSIONS: Vascular bradykinin can improve pancreatic microcirculation and hemorheology in rats with severe acute pancreatitis.

Key Words:
Vascular bradykinin, Severe acute pancreatitis, Pancreatic microcirculation, Microvascular permeability.

Introduction

The disorder of the pancreatic microcirculation not only contributes to the onset and exacerbation of severe acute pancreatitis (SAP), but also acts as a continuous damage mechanism throughout the course of SAP[1]. The decrease of pancreatic microcirculatory blood flow volume and velocity, and the increase of microvascular permeability lead to pancreas edema and inflammatory infiltration in the early phase of SAP together[2].

The change of hemorheology mainly includes microartery spasm, microvenous congestion, ischemia-reperfusion injury, visible blood components in adhesions, and inflammatory cytokine damage[3]. It is a late reaction in SAP, but when it become obvious, microvasculation produces massive thrombosis, and the following ischemia can cause pancreatic hemorrhage and necrosis[4-6].

Improving pancreatic microcirculation is the focus of current research of SAP, it is also an important measure and a key step in treatment[7]. As a drug that improves microcirculation, vascular bradykinin had been applied in the treatment of the fundus and acral peripheral microcirculation disorders[8,9], whether it can improve pancreatic microcirculation in SAP has not been reported. This study aimed to investigate the effect of vascular bradykinin on pancreatic microcirculation and hemorheology in rats with SAP, and provide a valuable experimental and theoretical foundation for its clinical application.

Materials and Methods

Experimental Animals and Grouping

Ninety male Wistar rats, clean grade, weight 300-350 g, were supplied by the experimental

Corresponding Author: Yong Li, MD; e-mail: yonglicn@yeah.net

animal center of the Capital Medical University. All the animals were randomly divided into 6 groups of 15 rats, and each of the 6 groups was classified as either group A or B. Group A was used to evaluate the pancreatic microcirculatory blood flow volume, velocity and microvascular permeability, and group B was used to evaluate the hemorheology. The groups were designated as follows: group A1 and B1: blank control group; group A2 and B2: SAP model group; group A3 and B3: vascular bradykinin treatment group. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the No. 4 Affiliated Hospital of Hebei Medical University.

Preparation of the SAP and Gastric Fistula Model

Using Aho's method[10], 5% sodium taurocholate (Sigma, San Francisco, CA, USA) was retrograde injected into the rats in groups A2, B2, A3 and B3 through the pancreatic duct with an infusion pump at a rate of 0.2 ml/min and 15 μl/kg. Before preparing the model, food and water were withdrawn for 12 and 4 h respectively. Immediately after the model was established, 5 ml saline was injected into the left subcutaneous tissue. Under the same conditions, saline was retrograde infused into the rats in group A. The rats in group A3 and B3 underwent gastrostomy after establishment of the model. To accomplish this, a fine plastic tube was placed into the stomach, which led out of the body through the abdominal wall, and the wounds were sutured temporarily. Drug delivery: vascular bradykinin was fully dissolved and administered through the tube at 20 U/kg, and the tube was clipped once every 8 h.

Pancreatic Microcirculatory Blood Flow Volume and Velocity

The pancreatic microcirculatory blood flow volume and velocity in group A were measured by flowmetry using the Preflux-4001 double-channel laser Doppler (Perimed, Stockholm, Sweden) at 12, 24 and 48 h after the model was established. The pancreatic head and tail were measured first, and two equidistant points between the head and tail were, then, selected, avoiding large blood vessels or hematomas. The arithmetic mean of these readings was recorded.

Pancreatic Microvascular Permeability

Using Keiji's method with improvements[11], 1% Evans blue (Sigma, San Francisco, CA, USA) was injected into the femoral vein of rats in group A at 2 ml/kg 48 h after the model was established. Then, the entire pancreas of every rat was removed, and the wet weight was recorded after 30 min. Approximately 150 mg of pancreatic tissue was clipped and placed into a 5 ml test tube. A total of 0.03 mg formamide was added, and 1 ml of the solution was collected after 24 h. The Evans blue concentration was determined with a spectrophotometer, and the Evans blue leakage volume was calculated. The remaining tissue was placed in a 160°C galvanothermy dry box, the dry tissue was weighed after 24 h, and the dry weight of 150 mg pancreatic tissue was calculated according to the wet/dry weight ratio. The Evans blue leakage volume/pancreatic tissue dry weight (μg/g) represented the microvascular permeability.

Hemorheologic Changes

A sample of 6 ml of blood was collected and strained through the inferior vena cava from every rat in group B 48 h after establishing the model, and the hemorheology was determined after adding the blood to a condensate tube.

Statistical Analysis

The statistical analysis was performed using the SAS 8.0 software package. The results of pancreatic microcirculatory blood flow volume and velocity, microvascular permeability and hemorheology were quantitative data and presented as the mean±SD. Multiple-group comparisons were performed using variance analysis. $p < 0.05$ was considered to be statistically significant.

Results

Comparison of Pancreatic Microcirculatory Blood Flow Volume and Velocity

The pancreatic microcirculatory blood flow volume and velocity in group A2 decreased gradually at 12 h (283.31ml/min vs. 355.72 ml/min and 86.63 cm/s vs. 101.91 cm/s) ($p < 0.01$ and $p < 0.05$), 24 h (279.57 ml/min vs. 328.02 ml/min and 72.72 cm/s vs. 87.05 cm/s) ($p < 0.01$ and $p < 0.05$), and 48 h (265.92 ml/min vs. 301.45 ml/min and 71.91 cm/s vs. 83.17 cm/s) ($p < 0.01$ and $p < 0.05$) compared with group A1, and these differences were statistically significant. The

pancreatic microcirculatory blood flow volume and velocity in group A3 increased gradually after 48 h (285.97 ml/min vs. 265.92 ml/min and 79.50 cm/s vs. 71.91 cm/s) ($p < 0.05$) compared with group A2, and these differences were statistically significant (Figure 1).

Comparison of Pancreatic Microvascular Permeability

The pancreatic microvascular permeability of rats in group A2 increased significantly after 48 h in comparison with rats in group A1 (1483.92 ug/g vs. 1375.41 ug/g) ($p < 0.01$). The pancreatic microvascular permeability of rats in group A3 was significantly reduced after 48 h compared with rats in group A2 (1404.17 ug/g vs. 1483.92 ug/g) ($p < 0.05$) (Figure 2).

Comparison of Hemorheology

Compared with group B1, the low shear rate blood viscosity, hematocrit and erythrocyte aggregation index in group B2 increased after 48 h (48.50 mPa.s vs. 30.25 mPa.s, 53.42% vs. 34.45% and 4.34 vs. 2.43) ($p < 0.01$). Compared with group B2, the low shear rate blood viscosity, hematocrit, and erythrocyte aggregation index of group B3 were significantly decreased (40.53 mPa.s vs. 48.50 mPa.s, 49.86% vs. 53.42% and 3.97 vs. 4.34) ($p < 0.05$), and there was a significant difference between each group (Figure 3).

Discussion

Pancreatic microcirculatory disorder is an important aspect of pathogenesis and pathophysio-

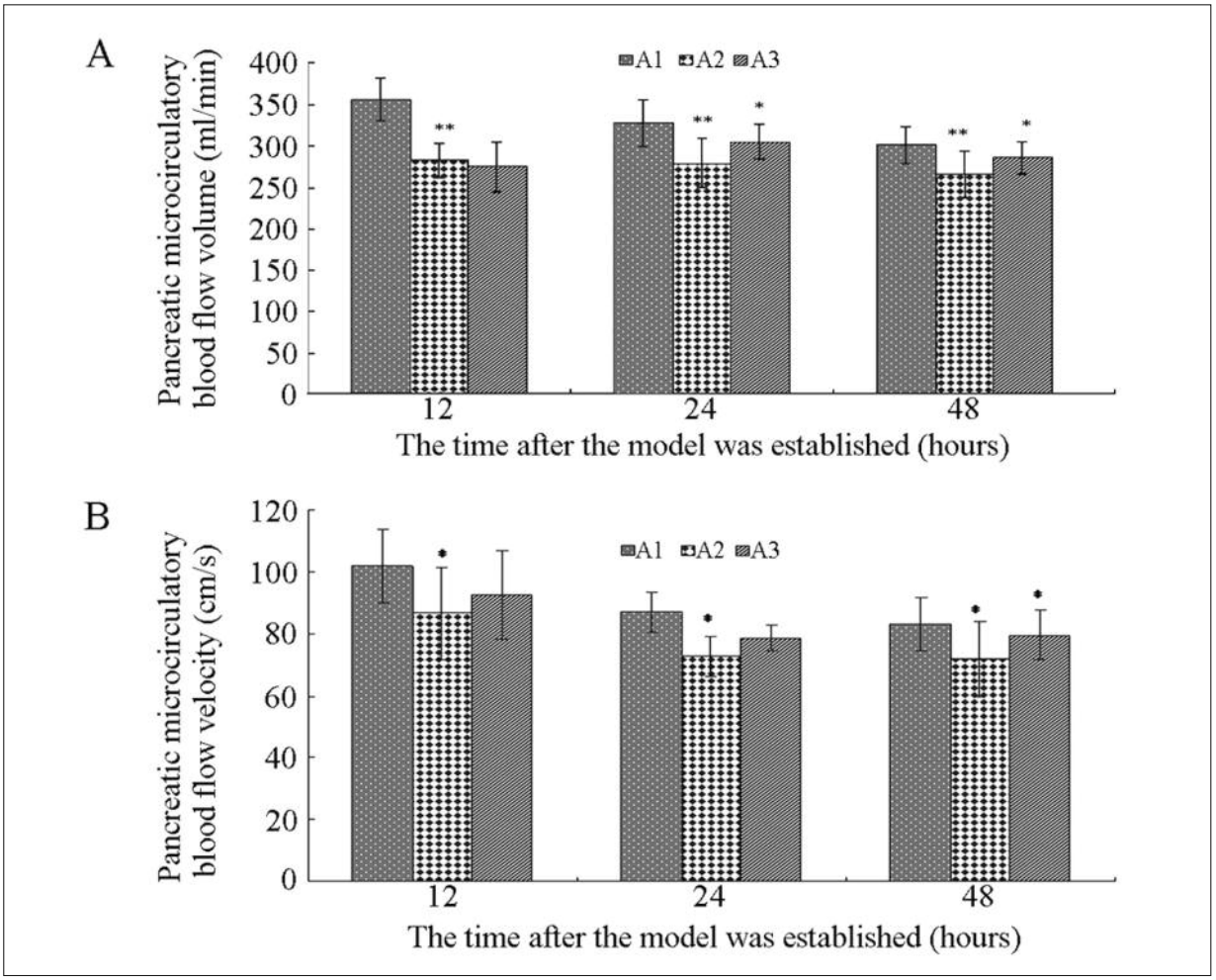

Figure 1. The pancreatic microcirculatory blood flow velocity in group A3 increased gradually after 48 h compared with group A2 (79.50 cm/s versus 71.91 cm/s), and these differences were statistically significant ($p < 0.05$). The pancreatic microcirculatory blood flow volume in group A3 increased gradually after 48 h compared with group A2 (285.97 ml/min versus 265.92 ml/min), and these differences were statistically significant ($p < 0.05$). *$p < 0.05$, **$p < 0.01$.

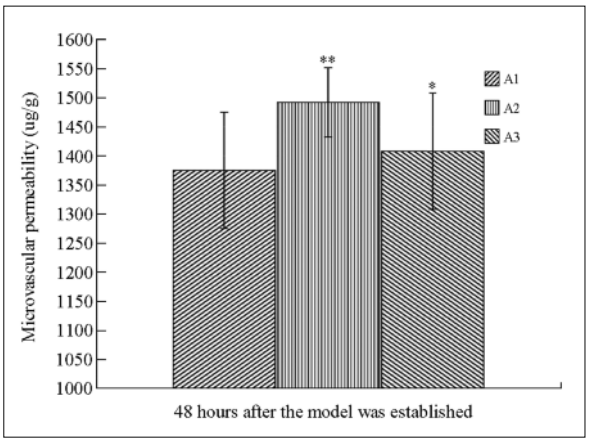

Figure 2. The pancreatic microvascular permeability of rats in group A3 was significantly reduced after 48 h compared with rats in group A2 (1404.17 ug/g versus 1483.92 ug/g), and the differences were statistically significant ($p < 0.05$). *$p < 0.05$, **$p < 0.01$.

logical manifestations in severe acute pancreatitis. The pancreatic microcirculatory blood flow volume and velocity decreased gradually in severe acute pancreatitis. Our experiment showed the pancreatic microcirculatory blood flow volume and velocity improved after 48 h in the group treated with vascular bradykinin. Vascular bradykinin is a proteolytic enzyme that is extracted from animal pancreas,this enzyme can improve the activity of the fibrinolytic system, reduce blood viscosity, prevent platelet aggregation and act as an antithrombotic[12], these effect can prevent microvascular thrombosis and alleviate pancreatic hemorrhage and necrosis in SAP.

The pancreatic microvascular permeability increased significantly after 48h in the early phase of SAP, and this change is expected to increase pancreatic edema. After the administration of vascular bradykinin, the pancreatic microvascular permeability decreased, which alleviated pancreatic edema. Vascular bradykinin can protect the microvasculature and maintain endothelial cell structure and integrity[12], this effect may be benefit to improve the pancreatic microvascular permeability.

Our experiment showed that the low shear rate blood viscosity, hematocrit, erythrocyte aggregation index and erythrocyte rigidity index increased in the SAP model, which means that the blood viscosity increased, the mobility decreased, erythrocyte deformability was reduced, blood resistance increased, pancreatic microcirculation stabilized, the blood flow was reduced and ischemia and hypoxia of the pancreatic tissue increased the degree of pancreatic necrosis. After treatment with vascular bradykinin, the blood low shear rate blood viscosity, hematocrit and erythrocyte aggregation index were significantly reduced, demonstrating that this treatment can reduce blood viscosity, pancreatic microcirculatory stasis, cellular ischemia and hypoxia, reducing pancreatic hemorrhage and necrosis in SAP. The reasons are likely related to the ability of vascular bradykinin to prevent blood coagulation and thrombosis, decrease blood viscosity and prevent platelet aggregation.

If we varied the dose and intervention time and use different observational indexes, maybe we could obtain more valuable information to improve pancreatic microcirculation and hemorheology.

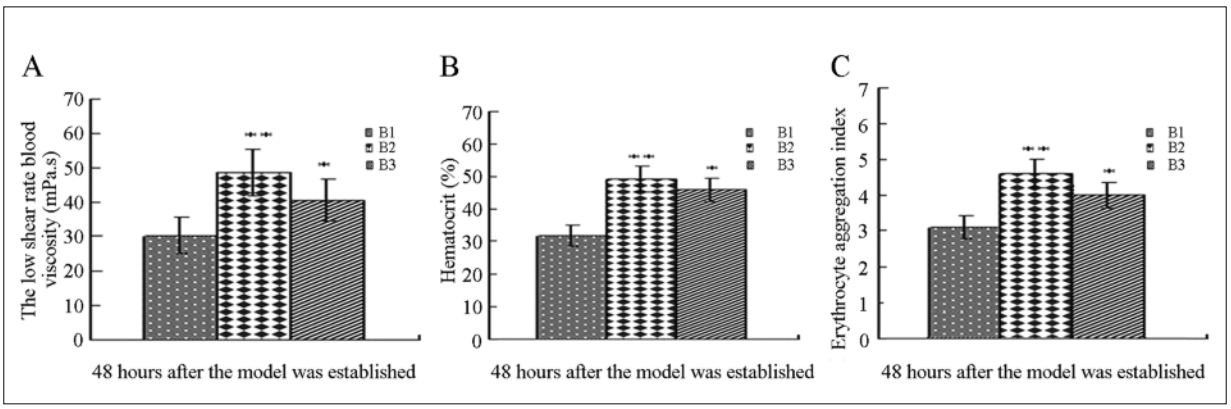

Figure 3. ***A, B,*** Compared with group B2, the low shear rate blood viscosity, hematocrit, and erythrocyte aggregation index of group B3 were significantly decreased after 48 h (40.53 mPa.s versus 48.50 mPa.s, 49.86% versus 53.42%, 3.97 versus 4.34), and these differences were statistically significant ($p < 0.05$). *$p < 0.05$, **$p < 0.01$.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Mann O, Kaifi J, Bloechle C, Schneider CG, Yekebas E, Kluth D, Izbicki JR, Strate T. Therapeutic small-volume resuscitation preserves pancreatic microcirculation in acute experimental pancreatitis of graded severity in rats. Pancreatology 2009; 9: 652-661.
2) Zhang XP, Li ZJ, Zhang J. Inflammatory mediators and microcirculatory disturbance in acute pancreatitis. Hepatobiliary Pancreat Dis Int 2009; 8: 351-357.
3) Lu F, Huang H, Wang F, Chen Y. Intestinal capillary endothelial barrier changes in severe acute pancreatitis. Hepatoqastroeaterology 2011; 58: 1009-1017.
4) Wu BU, Johannes RS, Conwell DL, Banks PA. Early hemoconcentration predicts increased mortality only among transferred patients with acute pancreatitis. Pancreatology 2009; 9: 639-643.
5) Gardner TB, Robertson DJ. Influence of baseline hematocrit on traditional calculations of hemoconcentration to predict severity in acute pancreatitis. Pancreas 2008; 36: 209-210.
6) Gardner TB, Olenec CA, Chertoff JD, Mackenzie TA, Robertson DJ. Hemoconcentration and pancreatic necrosis: further defining the relationship. Pancreas 2006; 33: 169-173.
7) Zhang X, Tian H, Wu C, Ye Q, Jiang X, Chen L, Cai Y, Xu R, Yuan W. Effect of baicalin on inflammatory mediator levels and microcirculation disturbance in rats with severe acute pancreatitis. Pancreas 2009; 38: 732-738.
8) Webb JG. The kallikrein/kinin system in ocular function. J Ocul Pharmacol Ther 2011; 27: 539-543.
9) Nakamura S, Morimoto N, Tsuruma K, Izuta H, Yasuda Y, Kato N, Ikeda T, Shimazawa M, Hara H. Tissue kallikrein inhibits retinal neovascularization via the cleavage of vascular endothelial growth factor-165. Arterioscler Thromb Vasc Biol 2011; 31: 1041-1048.
10) Zhang X, Chen L, Luo L, Tian H, Feng G, Cai Y, Xu R, Wang K, Wang Z. Study of the protective effects of dexamethasone on ileum mucosa injury in rats with severe acute pancreatitis. Pancreas 2008; 37: e374-382.
11) Keck T, Jargon D, Klünsch A, Thomusch O, Richter S, Friebe V, Adam U, Hopt UT. MMP-9 in serum correlates with the development of pulmonary complications in experimental acute pancreatitis. Pancreatology 2006; 6: 316-322.
12) Oshima K. A review on the development of Kallikrein (Kallidinogenase). Yakushiqaku Zasshi 1994; 29: 498-507.