



The Effect of Vascular Endothelial Growth Factor in the Improvement of Microcirculation Disturbance in Rats with Severe Acute Pancreatitis

Palikhe M¹, Zhan J^{1*}, Jha RK² and Zhang X²

¹Department of Gastroenterology, The Second Affiliated Hospital of Xi'an Jiaotong University, China

²Department of Gastroenterology, Xi'an Medical University, China

Abstract

Objective: To observe the function of Vascular Endothelial Growth Factor (VEGF) in the improvement of microcirculation disturbance in rats with Severe Acute Pancreatitis (SAP).

Methods: 120 adult male Sprague-Dawley (SD) rats were randomly divided into four groups, the Sham Operation (SO) SAP VEGF treatment or VEGF antibody (anti-VEGF) treatment group. The mortality rates were counted at 24 hr; the dry/wet ratios of tissues were measured in the lung, small intestine, and liver; changes in the ultrastructure of the microcirculation in pancreatic and lung tissue were observed using a Transmission Electron Microscope (TEM), and the levels of VEGF, Nitric Oxide (NO), Endothelin (ET), and trypsin were measured using an Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The mortality in SO group at 24 hr was 0%, SAP group was 90% (9/10), anti-VEGF group was 80% (8/10), and VEGF group was only 10% (1/10). The dry/wet ratios of all tissues in the VEGF group were significantly lower than those in the SAP and anti-VEGF groups ($P < 0.05$). Results from the TEM showed that the SAP and anti-VEGF groups exhibited obvious pathological changes. The serum ET, NO, and trypsin levels in the SAP and anti-VEGF groups were significantly higher than those in the SO group ($P < 0.05$).

Conclusion: VEGF could improve microcirculation disturbance in SAP rats through the repair of the vascular endothelial barrier and maintenance of vascular endothelial function to further reduce the mortality of rats, which might become a new breakthrough in SAP treatment in the future.

Keywords: Severe acute pancreatitis; Vascular endothelial growth factor; Microcirculation disturbance; Multiple organ damage

Background

Acute Pancreatitis (AP) is an acute inflammatory process of the pancreas [1]. It is a potentially life-threatening disease characterized by tissue edema, necrosis, and hemorrhage in the pancreas [2]. Severe Acute Pancreatitis (SAP) has a poor prognosis and may lead to degradation of the capillary endothelial basement membrane and alter capillary endothelial cell connections [3,4]. The presence of microcirculation disturbance is an early event in SAP and penetrates through the whole process of SAP development [5], finally causes Multiple Organ Dysfunction Syndrome (MODS) [6]. A large number of clinical observations and animal experiments have confirmed that the major presentations of microcirculation disturbance in SAP are microvascular permeability changes and hemorheological changes [7,8]. Therefore, the early pathophysiological change in pancreatitis is the systemic inflammatory response centered on blood vessels [9]. Currently, based on the above theory, how to improve microcirculation disturbance to further increase the cure rate of SAP patients has become a hot research issue in SAP treatment. At present, therapies for SAP, such as corticosteroids or non steroid anti-inflammatory agents, attempt to reduce the microcirculatory disturbance [10].

Vascular Endothelial Growth Factor VEGF (VEGF) families of proteins are key regulators of physiological systems [11]. VEGF has the functions of protection of vascular endothelial cells and maintenance of vascular endothelial functions as well as the promotion of angiogenesis and anti-inflammation and anti-thrombosis activities [12]. It is currently the substance with the strongest known function in the promotion of angiogenesis and protection of the vascular endothelium

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*Correspondence:

Zhang Jun, Department of Gastroenterology, The Second Affiliated Hospital of Xi'an Jiaotong University, No.157, Xi Wu Road, Xi'an, China, E-mail: jun3z@163.com

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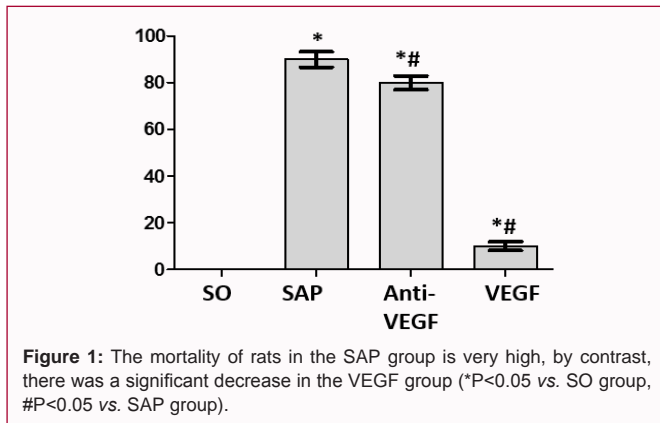


Figure 1: The mortality of rats in the SAP group is very high, by contrast, there was a significant decrease in the VEGF group (*P<0.05 vs. SO group, #P<0.05 vs. SAP group).

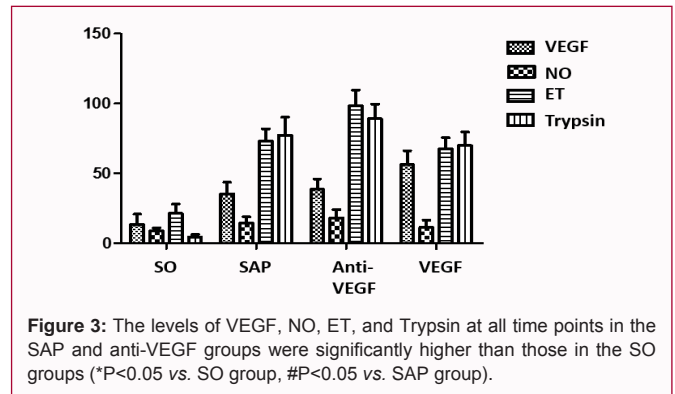


Figure 3: The levels of VEGF, NO, ET, and Trypsin at all time points in the SAP and anti-VEGF groups were significantly higher than those in the SO groups (*P<0.05 vs. SO group, #P<0.05 vs. SAP group).

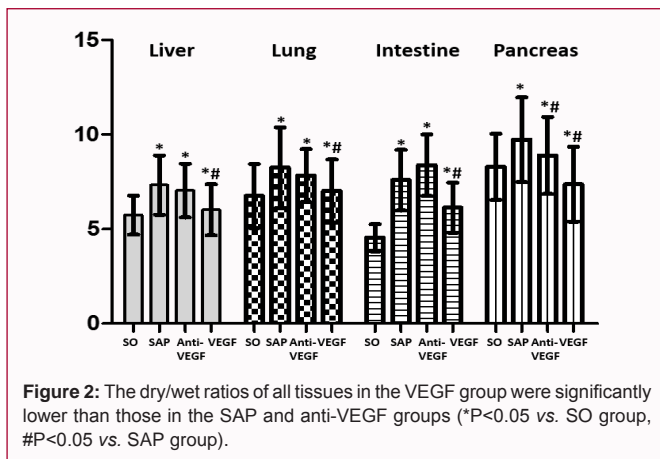


Figure 2: The dry/wet ratios of all tissues in the VEGF group were significantly lower than those in the SAP and anti-VEGF groups (*P<0.05 vs. SO group, #P<0.05 vs. SAP group).

Table 1: Results of mortality after 24 h in each group.

Group	Number of cases (n)	Mortality (%)
SO	10	0
SAP	10	90 ± 3.32 [*]
VEGF	10	10 ± 1.85 [#]
Anti-VEGF	10	80 ± 2.94 [#]

into the pancreaticobiliary duct, and the arterial clamp was then removed. For the rats in the SO group, the abdomen was opened and the duodenum was turned, and the abdominal wall was then sutured. After establishment of the SAP model, VEGF (10 µg/kg) and VEGF antibodies (10 µg/kg) were immediately injected through the dorsal vein of the penis in the VEGF and anti-VEGF groups, respectively. Rat specimens were collected at 6 hr, 12 hr, and 24 hr after model establishment.

Comparison of mortality of rats at 24 hr

The mortality of rats was calculated using the ratio between the number of deaths of rats within 24 hr and the total number of rats in each group. This study only compared mortality at 24 hr after model establishment.

Measurement of the water content (dry/wet ratio) in tissues of the liver, lung, and small intestine of rats

Rats were sacrificed and dissected, and 1 cm³ of tissues in the same locations of the liver, lung, pancreas, and small intestine were collected. The wet weight was measured. Tissues were baked at 70° for 72 hr, and the dry weight was measured. The water content (%) = (wet weight-dry weight)/wet weight.

Detection of serum VEGF, nitric oxide (NO), endothelin (ET), and trypsin using ELISA

Before the rats were sacrificed and dissected, 4 mL venous blood was collected from the portal vein and centrifuged for 2 min to collect serum samples. Some specimens were stored at -80°C for ELISA detection. The ELISA reagent kits provided by Beijing Jingmei Biotechnology were used to detect the levels of VEGF, NO, ET, and trypsin in plasma. The detection was performed strictly according to the procedures in the reagent kit instructions.

Observation of ultra structure of microcirculation in lung and pancreatic tissues

A volume of 1 mm³ of each aforementioned tissue was fixed in 25 mL/L glutaraldehyde at 4°C for 8 hr. Tissues were dehydrated in gradient alcohol, immersed in epoxy resin Epon 812, and used for ultra-thin sections in an LKB-V ultra-thin microtome (50 nm to 70 nm). Changes in the ultra structure of the microcirculation in tissues

[13,14]. Because of the physiological functions of VEGF, we considered that using VEGF as a therapeutic drug for SAP could improve microcirculation disturbance and block the cascade reaction to further improve the prognosis of SAP patients [15]. Thus, this study used VEGF as a therapeutic drug and observed its treatment effects on microcirculation disturbance in SAP rats, in order to provide an animal experiment basis for seeking novel clinical treatments.

Materials and Methods

Materials

A total of 120 healthy adult male Sprague-Dawley (SD) rats with a body weight of 250 g to 300 g were provided by the Experimental Animal Center of the College of Medicine of Xi’an Jiaotong University. Sodium taurocholate was provided by Sigma; VEGF and anti-VEGF antibodies were provided by Abcam. Enzyme-Linked Immunosorbent Assay (ELISA) reagent kits were provided by Beijing Jingmei Biotechnology. This study was approved by the Experimental Animal Ethics Committee of Xi’an Jiaotong University.

Experimental animals and model establishment

A total of 120 healthy, adult male SD rats were randomly divided into either the Sham Operation (SO) group, SAP group, VEGF treatment group, or VEGF antibody (anti-VEGF) treatment group. Each group had 10 rats. Rats were fasted for 12 hr for food and for 4 hr for water and then anesthetized by intraperitoneal injection of 25 g/L pentobarbital sodium (1.2 mg/kg). A median incision in the upper abdomen was made at a length of approximately 2 cm. The pancreaticobiliary duct was located, and its proximal and distal ends were occluded using an arterial clamp. The SAP model was established by a retrograde injection of 40 g/L sodium taurocholate

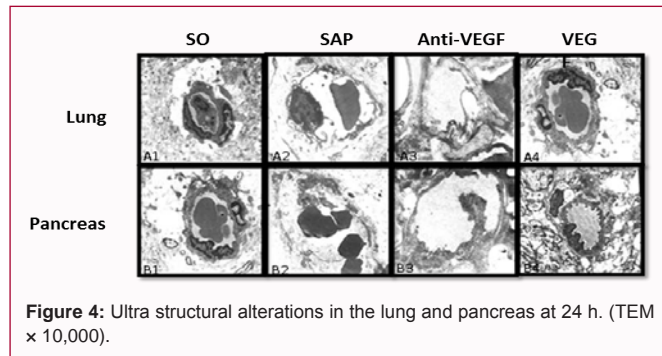


Figure 4: Ultra structural alterations in the lung and pancreas at 24 h. (TEM $\times 10,000$).

were observed under an electron microscope.

Statistical analyses

Data analyses were performed using SPSS 19.0. The comparison within the same group was performed using a one-way analysis of variance. The mortality of rats was examined using the χ^2 test. The results are expressed as the mean \pm standard deviation ($\bar{x} \pm s$). $P < 0.05$ indicates the difference had statistical significance.

Results

The mortality of rats at 24 hr

The mortality of rats at 24 hr was 0.0% (0/8) in the SO group, 90% (9/10) in the SAP group, 10.0% (1/10) in the VEGF group, and 80% (8/10) in the anti-VEGF group (Figure 1). The mortality of rats in the VEGF group was significantly lower than that in the SAP group ($P < 0.05$) (Table 1).

The dry/wet ratios of tissues in liver, lung, small intestine, and pancreas

The dry/wet ratios of all tissues in the anti-VEGF group were the highest among all groups. The dry/wet ratios of all tissues in the VEGF group was significantly lower than those in the SAP and anti-VEGF groups ($P < 0.05$) (Figure 2). These results suggest that VEGF plays an obvious role in the reduction of tissue edema in SAP rats (Table 2).

ELISA detection results of serum VEGF, NO, ET, and trypsin

The levels of VEGF, NO, ET, and trypsin at all time points in the SAP and anti-VEGF groups were significantly higher than those in the SO group (Figure 3). In the VEGF group, the VEGF level was the highest among all groups and the NO and ET levels were significantly lower than those in the SAP and anti-VEGF groups ($P < 0.05$). The

trypsin levels in the three groups other than the SO group did not have significant differences (Table 3).

Changes in the ultra structure of the microcirculation in lung and pancreatic tissues

No tissues in the SO group had obvious pathological changes under a Transmission Electron Microscope (TEM). The typical pathological changes, such as vascular endothelial cell apoptosis, mitochondrial swelling, cell edema, capillary congestion, thrombosis, destruction or disappearance of vascular endothelial integrity in the microcirculation, extra vascular bleeding, and edema, were observed in the SAP and anti-VEGF groups under a TEM and became gradually aggravated over time. However, those pathological changes in the VEGF group were significantly attenuated (Figure 4).

Discussion

We designed this study according to the hypothesis that protease is the culprit in SAP [16,17]. The results indicated that the serum trypsin levels in pancreatitis in all groups significantly increased after the establishment of the SAP model, suggesting that trypsin indeed participated in the SAP pathological damage process. To further validate that the destructive function of trypsin on the vascular wall was the core link in the SAP pathophysiological process, VEGF was applied, which has obvious vascular repair functions [18]. The results showed that over time, the mortality of rats in the VEGF group was significantly lower than that in the SAP group. The mortality of rats at 24 hr, the gold standard for treatment effects, was significantly lower in the VEGF group, indicating that the treatment effect of VEGF was ideal. To further investigate its mechanisms of action, the water content of rat organs was measured using the dry/wet ratio of tissues in these organs. The results indicated that the water content of all tissues was significantly higher in SAP, suggesting that obvious vascular leakage was indeed present in SAP. To further confirm the reason for vascular leakage, a detailed observation of the ultra structure of tissues in the pancreas and lung was performed. The results showed destruction of the vascular wall integrity in the microcirculation in SAP. The major presentations were endothelial lysis, defect of the vascular wall, endothelial cell apoptosis, and even necrosis. In addition, the phenomenon of leakage of the visible components of blood, such as erythrocytes, to extra vascular tissue spaces was also observed. This was a very special pathological change. Any type of inflammation is centered on the vascular response, and the general presentation is the increase in vascular wall permeability to cause the extravasation of plasma components into the interstitial spaces.

Table 2: Results of mortality after 24 hr in each group and the dry/wet ratio of tissues in the liver, lung, small intestine, and pancreas.

Group	Number of cases (n)	Dry/wet ratio of liver (%)	Dry/wet ratio of lung (%)	Dry/wet ratio of small intestine (%)	Dry/wet ratio of pancreas (%)
SO	10	5.74 \pm 1.03	6.75 \pm 1.69	4.54 \pm 0.72	8.29 \pm 1.75
SAP	10	7.33 \pm 1.57 [*]	8.25 \pm 2.13 [*]	7.59 \pm 1.61 [*]	9.73 \pm 2.24 [*]
VEGF	10	6.02 \pm 1.35 [#]	7.02 \pm 1.66 [#]	6.13 \pm 1.33 [#]	7.37 \pm 1.98 [#]
Anti-VEGF	10	7.04 \pm 1.42 [*]	7.83 \pm 1.40 [*]	8.38 \pm 1.62 [*]	8.89 \pm 2.04 [#]

Table 3: Results of VEGF, NO, ET and trypsin in serum at 24 hr.

Group	Number of cases (n)	VEGF (ng/mL)	NO (ng/mL)	ET (ng/mL)	Trypsin (mg/L)
SO	10	13.34 \pm 7.53	8.74 \pm 2.17	21.37 \pm 6.65	4.54 \pm 1.72
SAP	10	35.27 \pm 8.42 [*]	14.33 \pm 4.57 [*]	73.12 \pm 8.78 [*]	77.32 \pm 13.05 [*]
VEGF	10	56.26 \pm 9.87 [#]	11.34 \pm 5.16 [#]	67.54 \pm 8.03 [#]	69.89 \pm 9.74 [*]
Anti-VEGF	10	38.54 \pm 7.52 [#]	18.04 \pm 6.05 [#]	98.36 \pm 11.38 [#]	89.24 \pm 10.43 [*]

However, the presentations of SAP are vascular wall destruction and tissue bleeding, which strongly confirms our previous hypothesis that activated trypsin affects vascular wall substrates to destroy the vascular wall integrity, thus inducing this special pathological phenomenon. Previous reports suggested that early stages of SAP are accompanied by the increase in plasma levels of ET and NO levels, and altered systolic and diastolic function of local pancreatic blood vessels. Plasma levels of NO are likely to be increased due to increased activity of NO synthase [19]. Increased levels of ET upregulate intracellular calcium levels and promote activation of inflammatory cells [20]. To confirm the role of inflammatory factors in vascular leakage in SAP, the levels of serum NO and ET was detected. The levels of serum NO and ET significantly increased in SAP; however, their levels did not particularly change in the VEGF group when the VEGF treatment effect was very obvious. These results suggest that inflammatory factors do not play a very critical role in the SAP pathophysiological process.

Furthermore, we also showed the phenomenon of the increase in serum VEGF in SAP. It has been reported that VEGF is involved in the process of organ dysfunction in SAP [21,22]. However, the conclusion was only based on the phenomenon of the increase in VEGF in SAP. Our study showed that, with the increase in the VEGF level, pathological damage of organs in all groups was significantly attenuated; in particular, the vascular wall integrity in microcirculation was significantly improved. These results suggested that VEGF indeed had the effect of a significant improvement of microcirculation injury in SAP. The increase in VEGF in SAP might be completely due to the increase in the self-protective responses in the body.

Overall, our application of VEGF as a therapeutic drug had unexpected excellent results. Its specific mechanism might be the rapid repair of the damaged vascular intima and maintenance of the vascular endothelial integrity to prevent systemic multiple organ dysfunction induced by obvious vascular leakage in SAP. Whether there are other mechanisms still remains to be further studied.

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