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Effect of resveratrol on microcirculation disorder and lung injury following severe acute pancreatitis in rats

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Abstract

AIM: To investigate the mechanism of resveratrol underlying the microcirculation disorder and lung injury following severe acute pancreatitis (SAP).

METHODS: Twenty-four rats were divided into 3 groups (SAP, sham and resveratrol groups) randomly. SAP model was established by injecting 4% sodium taurocholate 1 mL/kg through puncturing pancreatic ducts. Sham (control) group (8 rats) was established by turning over the duodenum. Resveratrol was given at 0.1 mg/kg b.m. intraperitoneally. Rats were sacrificed 9 h after SAP was induced. Blood samples were obtained for hemorrheological examination. Lung tissues were used for pathological observation, and examination of microvascular permeability, dry/wet ratio and myeloperoxidase (MPO) activity. Gene expression of intercellular adhesion molecule-1 (ICAM-1) was detected by RT-PCR.

RESULTS: Compared with SAP group, resveratrol relieved the edema and infiltration of leukocytes in the lungs. Resveratrol improved markers of hemorrheology: high VTB (5.77 ± 1.18 mPas vs 9.49 ± 1.34 mPas), low VTB (16.12 ± 3.20 mPas vs 30.91 ± 7.28 mPas), PV (4.69 ± 1.68 mPas vs 8.00 ± 1.34 mPas), BSR (1.25 ± 0.42 mm/h vs 0.03 ± 0.03 mm/h), VPC ($54.67 \pm 3.08\%$ vs $62.17 \pm 3.39\%$), fibrinogen (203.2 ± 87.8 g/L vs 51.3 ± 19.1 g/L), original hemolysis (0.45 ± 0.02 vs 0.49 ± 0.02), and complete hemolysis (0.41 ± 0.02 vs 0.43 ± 0.02) ($P < 0.05$). Resveratrol decreased the OD ratio of ICAM-1 gene (0.800 ± 0.03 vs 1.188 ± 0.10), dry/wet ratio (0.74 ± 0.02 vs 0.77 ± 0.03), microvascular permeability (0.079 ± 0.006 vs 0.112 ± 0.004) and MPO activity (4.42 ± 0.32 vs 5.03 ± 0.51) significantly ($P < 0.05$).

CONCLUSION: Resveratrol can improve the microcirculation disorder of the lung by decreasing leukocyte-endothelial interaction, reducing blood viscosity, improving the decrease of blood flow, and stabilizing erythrocytes in SAP rats. It may be a potential candidate to treat SAP and its severe complications (ALI).

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Key words: Severe acute pancreatitis; Resveratrol; Lung injury; Microcirculation disorder

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INTRODUCTION

Many factors are contributive to the progression of pancreatic microcirculatory disturbance. Whether the disturbance of pancreatic microcirculation is an initiating factor or a consequence of progressive pancreatitis is still debatable.

Microcirculatory disorders in severe acute pancreatitis (SAP) are not confined to the pancreas but can also be found in the colon, liver, and lungs, and they not only affect capillary blood flow but also involve prolonged changes of capillary permeability and leukocyte-endothelial interaction. Hypoperfusion has a major role in the pathogenesis of multiple organ failure, which is the main cause of death in SAP. Prevention and treatment of microcirculatory disorders can actively improve the outcome of patients with SAP. ICAM-1 is correlated with capillary permeability, blood flow and leukocyte rolling. Inhibiting ICAM-1 is a target to improve microcirculation^[1].

Lung injury, a severe complication of SAP, has a high case-fatality rate. Lung injury manifested clinically as adult respiratory distress syndrome (ARDS) is a common cause of morbidity and mortality following acute pancreatitis (AP)^[2,3]. Systemic inflammatory response (SIRS) is an important factor that has a strong influence on the incidence of acute lung injury (ALI), and ALI is an important determinant of prognosis and severity of patients with AP^[4,5]. Further progression of SIRS can be prevented and the incidence of MODS as well as hospital mortality decrease^[6].

Resveratrol (Res) is an effective component of some Chinese traditional medicines and has various pharmacological effects including antiinflammatory properties, modulation of lipid metabolism and prevention of cancer^[7-9]. Its antiinflammatory effect is related to inhibiting oxidation, leukocyte priming and expression of inflammatory mediators. Recently it has been found to prevent and cure cardiovascular diseases and improve significantly microcirculatory disorders by protecting the blood vessel endothelium and inhibiting platelet aggregation^[10-12]. Therefore we speculate that Res might exhibit the beneficial effect on SAP complicated by ALI. Up till now, most studies have been *in vitro* and the present experiment was to investigate its *in vivo* role in SAP complicated by ALI.

MATERIALS AND METHODS

Experimental animals and grouping

Healthy male SD rats weighing 250-300 g were provided by the Experimental Animal Center of Medical School of Xi'an Jiaotong University. All animals were randomly divided into 3 groups with 8 rats in each group, including SAP group, Res group and control (sham operation) group.

Preparation of animal models

Rats were starved but allowed free access to water for 12 h

before the experiments, then anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and fixed. The biliopancreatic duct was cannulated through the duodenum and the hepatic duct was closed by means of a clip placed at the hilum of the liver. SAP model was established by injecting 4% sodium taurocholate 1 mL/kg through puncturing pancreatic ducts. After the SAP model was successfully established in Res group (8 rats), Res was given at 0.1 mg/kg b.m. intraperitoneally. Control group (8 rats) was established by turning over the duodenum. Animals from all groups were injected subcutaneously with saline solution 5 mL in back. All rats were sacrificed 9 h after modeling. Venous blood was drawn for hemorrheologic examination. Tissue samples of lung were taken for pathological observation, examination of water content, capillary permeability, activity of MPO and intercellular adhesion molecule-1 (ICAM-1) RT-PCR.

Measurement of water content in lung tissue

Wet lung tissue was weighed, then heated for 72 h at 70 °C, the dried tissue was weighed. The water content = (wet weight - dry weight)/ wet weight.

Detection of lung capillary permeability

The change of lung capillary permeability was detected by injecting Evans blue into hind leg veins with a spectrophotometer at 625 nm. The concentration of Evans blue taken from lung tissue was expressed as milligrams of stain per gram of the wet weight of lung.

Assay of MPO activation

Lung samples were homogenized in hexadecyltrimethylammonium bromide solution. Samples were sonicated and centrifuged, supernatants were collected. The change in absorbance at 460 nm over 1 min was recorded at 30 s, 90 s.

Hemorrheological examination

Blood sedimentation (BS) was detected with 0.2 mL venous blood, after centrifugation haematocrit was read. The content of fibrinogen (CB) was recorded after incubated in water bath and centrifugation. The viscosity of total blood (VTB) and plasma (PV) was checked up in another 1-2 mL venous blood.

RT-PCR examination of intercellular adhesion molecule-1 (ICMA-1)

Total RNA was extracted with from the lung Trizol reagents (LIFE TECHNOLOGIES), quantified based on the measured absorbance at 260 nm. Synthetic oligonucleotide primers (TaKaRa) based on the cDNA sequences of rat ICAM-1 and β -actin were prepared: ICAM-1, 5'-AAGCTGGC CGTGGCTC TCTTG-3', 5'-AGCCCTCTTCAAAACTTCTC-3'; β -actin, 5'-GATGCTGACCCCTGGAGAGCA-3', 5'-CAGGGACTTCCC ATCCACCT-3'. RT-PCR was performed as described by the RT-PCR kit (Promega). PCR conditions were as follows: at 42 °C for 15 min, at 99 °C for 5 min for 1 cycle, at 94 °C for 1 min, at 55 °C for 1 min and at 72 °C for 2 min for 35 cycles, and a final extension at 72 °C for 10 min. PCR products were electrophoresed

on a 0.8% agarose gel containing ethidium bromide. The gel was scanned on a white/ultraviolet transilluminator UVP.

Statistical analysis

Data were expressed as mean \pm SD. ANOVA was used to analyze the data with the SPSS10.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Pathological observation of lung tissue

Alveolar septal thickening, interstitial edema, infiltration of numerous leukocytes and thrombus formation in microvessels were observed in the lungs of SAP group. But in the Res group, histological changes, such as interstitial edema and infiltration of inflammatory cells, were significantly reduced.

Water content, capillary permeability and activation of MPO in lungs (Table 1)

Compared with the control group, water content and capillary permeability in lung tissues of both SAP group and Res group were obviously increased ($P < 0.01$). Compared with SAP group all indices in Res group had a significant decrease ($P < 0.05$).

Table 1 Water content, capillary permeability and activation of MPO in lung tissues (mean \pm SD)

	Control	SAP	Res
Water content	0.69 \pm 0.03	0.77 \pm 0.03	0.74 \pm 0.02 ^b
Capillary permeability (mg/g)	0.048 \pm 0.005	0.112 \pm 0.004	0.079 \pm 0.006 ^b
Activation of MPO (U/g)	1.02 \pm 0.03	5.03 \pm 0.51	4.42 \pm 0.32 ^b

^b $P < 0.01$ vs control.

Hematological examination (Table 2)

Compared with control group, all parameters of hemorrheology in ASP group and Res group were in a lower level ($P < 0.05$). Compared with SAP group each parameter of hemorrheology in Res group had a favorable improvement ($P < 0.05$).

RT-PCR examination of ICMA-1

The OD ratio of genetic expression of ICAM-1 in control group, SAP group and Res group was 287 \pm 0.03, 1.188 \pm 0.10 and 0.800 \pm 0.03, respectively. SAP group and Res group had a notable increase in OD ratio compared with control group ($P < 0.05$), and Res group had a marked decrease compared with SAP group ($P < 0.05$).

DISCUSSION

Polyphenolic phytoalexin resveratrol has been found in more than 70 plant species as a natural potent compound, which can chelate copper ion, inhibit lipid peroxidation and platelet

Table 2 Hemorrheological examination (mean \pm SD)

Group	High VTB (mPa.s)	Low VTB (mPa.s)	PV (mPa.s)	BSR (mm/h)	VPC (%)	Fibrinogen (g/L)	Original hemolysis	Complete hemolysis
Control	5.65 \pm 1.04	14.57 \pm 4.00	3.13 \pm 0.61	9.17 \pm 0.98	44.92 \pm 2.73	452.3 \pm 29.9	0.44 \pm 0.00	0.36 \pm 0.00
SAP	9.49 \pm 1.34	30.91 \pm 7.28	8.00 \pm 1.34	0.03 \pm 0.03	62.17 \pm 3.39	51.3 \pm 19.1	0.49 \pm 0.02	0.43 \pm 0.02
Res	5.77 \pm 1.18 ^a	16.12 \pm 3.20 ^a	4.69 \pm 1.68 ^a	1.25 \pm 0.42 ^a	54.67 \pm 3.08 ^a	203.2 \pm 87.8 ^a	0.45 \pm 0.02 ^a	0.41 \pm 0.02 ^a

VTB: viscosity of total blood; PV: plasma viscosity; BSR: blood sedimentation rate. VPC: volume packed cells. ^a $P < 0.05$ vs control group and SAP group.

aggregation and scavenge free radicals. Recently it has been shown to have extensive effects including protection against cardiovascular diseases and cancer. Some researches have also demonstrated that Res could exert its beneficial effects on ischemia-reperfusion injury, shock and MODS by inhibiting oxidation, leukocyte priming and expression of inflammatory mediators as well as by protecting microcirculation^[11,12-17]. Although its pharmacological role has been comprehensively investigated, there are no reports about the effect of Res on SAP complicated by ALI.

As a factor in initiation, promotion, progression of different diseases, microcirculatory disorder has been paid more and more attention. After observing microcirculation changes in different organs including the lung in rat SAP model, Foitzik *et al*^[18] found that the injury of microcirculation was present in almost all the organs, which involved prolonged pathological changes such as leukocyte rolling, capillary permeability and leukocyte-endothelial interaction. So enhancing microcirculation may be a potential treatment of SAP and its severe complication ALI.

The mechanism of Res underlying microcirculatory disorder is to protect the microvessel endothelium, dilate blood vessels and inhibit platelet aggregation. Studies have indicated that in pathological conditions such as inflammation and shock, Res can suppress leukocyte adhesion to endothelium and subsequently reduce infiltration of leukocytes into inflammatory sites as well as influence the kinetic behaviors of calcium channels to dilate vessels and improve microcirculatory disorders^[12,19,20]. The results of *in vitro* studies have also shown that Res can significantly reduce the capillary permeability as an inhibitor of adhesion molecule expression. Res was found to suppress thromboxane formation and adenosine diphosphate, collagen or thrombin-induced platelet aggregation by inhibiting the activity of calcium channels to block rapidly calcium ion influx into the activated platelets^[21].

In this study, Res decreased remarkably the capillary permeability and tissue water content. We also found that there was a dramatic decline in blood rheology of resveratrol-treated rats, including whole blood and plasma viscosity, hematocrit (HCT) and erythrocyte osmotic fragility compared to the SAP group. The results indicated that Res could reduce capillary permeability and whole blood and plasma viscosity (PV), leukocyte rolling, thus increasing the blood flow in different organs. The reduction of erythrocyte osmotic fragility showed that Res might stabilize erythrocytes to decrease thrombus formation by the erythrocyte fragments. We also detected the expression of ICAM-gene, which was significantly lower in the Res-treated group than in the SAP group. ICAM-1 could mediate leukocyte adhesion and migration; the expression of ICAM-1 preceded the fulminant leukocyte infiltration of the organs. Anti-inflammatory effect of Res was involved in the reduction of ICAM-1 expression. The assay of MPO showed that Res suppressed leukocyte adhesion to endothelium and reduced infiltration of leukocytes into inflammatory sites.

In summary, Res can inhibit the expression of inflammatory mediators of the lung and pancreas and enhance microcirculation in SAP. It may be a potential candidate to treat SAP and its severe complication (ALI), but its pharmacodynamics, pharmacokinetics, side-effect, administration route and dose need to be further studied.

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