Effect of resveratrol on pancreatic oxygen free radicals in rats with severe acute pancreatitis

Zhen-Dong Li, Qing-Yong Ma, Chang-An Wang

ABSTRACT

AIM: To investigate the therapeutic effects of resveratrol (RESV) as a free radical scavenger on experimental severe acute pancreatitis (SAP).

METHODS: Seventy-two male Sprague–Dawley rats were divided randomly into sham operation group, SAP group, and resveratrol-treated group. Pancreatitis was induced by intraductal administration of 0.1 mL/kg 4% sodium taurocholate. RESV was given intravenously at a dose of 20 mg/kg body weight. All animals were killed at 3, 6, 12 h after induction of the model. Serum amylase, pancreatic superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) were determined. Pathologic changes of the pancreas were observed under optical microscope.

RESULTS: The serum amylase, pancreatic MPO and the score of pathologic damage increased after the induction of pancreatitis, early (3, 6 h) SAP samples were characterized by decreased pancreatic SOD and increased pancreatic MDA. Resveratrol exhibited a protective effect against lipid peroxidation in cell membrane caused by oxygen free radicals in the early stage of SAP. This attenuation of the redox state reflected by lower serum amylase, less severe pancreatic lesions, normal pancreatic MDA levels, as well as diminished neutrophil infiltration in pancreas.

CONCLUSION: RESV may exert its therapeutic effect on SAP by lowering pancreatic oxidative free radicals and reducing pancreatic tissue infiltration of neutrophils.

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Key words: Severe acute pancreatitis; Resveratrol; Oxygen free radical; Neutrophil
Pancreatic histopathologic scoring in rats (mean ± SE)

| Time (h) | Sham operation | SAP | RESV-treated |
|---------|----------------|-----|--------------|
| 3       | 0.283 ± 0.112  | 9.286 ± 0.624  | 5.283 ± 0.646² |
| 6       | 0.219 ± 0.171  | 11.357 ± 0.533³ | 5.986 ± 0.417² |
| 12      | 0.112 ± 0.051  | 13.559 ± 0.636³ | 6.003 ± 0.717² |

*P < 0.01 vs Sham operation; *P < 0.01 vs SAP group.

**Histopathologic analysis**

Tissue samples of the pancreas were fixed in 40 g/L paraformaldehyde and embedded with paraffin. Five-micrometer thick sections were stained with hematoxylin/eosin and examined and graded as previously described. The total surface of the slides was scored by one blinded pathologist for four different variables (edema, acinar necrosis, hemorrhage and fat necrosis, inflammation and perivascular infiltrate) to determine the severity of pancreatic injury.

**Detection of serum amylase and measurement of pancreatic SOD, MDA, and MPO**

Amylase activity in serum was determined using an automatic biochemistry analyzer (Hitachi 7170). Pancreas was homogenized in physiological saline or 5 g/L HTAB using ultrasonication. The SOD content was measured using the xanthine oxidase technique based on the spectrophotometric monitoring of SOD-mediated reduction of DTNB at 550 nm. The concentration of MDA was quantified by thiobarbituric acid reaction and MPO contents were determined as described by Bhatia et al.

**Statistical analysis**

Results were expressed as mean ± SE. Statistical analysis was done using the SPSS10.0 software package. One-way analysis of variance was used to establish whether the difference among the three groups was statistically significant. *P < 0.05 was considered statistically significant.

**RESULTS**

**Histopathology**

There was no or a small amount of clear ascitic fluid in sham operation group. More than 8 mL turbid hemorrhagic ascites could be seen in all rats of SAP group. No obvious change or slight edema could be seen in sham operation group. Pancreas in SAP group displayed disparate edema with punctiform or lamellar hemorrhage and necrosis. Saponified spots could be seen at pancreas, epiploon, mesentery, peritoneum, and perinephric fat. Pancreatic tissue was normal in sham operation group. In SAP group, pancreatic tissue displayed interstitial edema, widened lobula interspace, inflammatory cell infiltration, hemorrhage and necrosis. Microthromb could be found inside the small vessels around focal necrosis of the pancreas. In contrast, in RESV-treated group, the ascitic fluid diminished significantly and turbidity was lower than that in SAP group. Saponified spots, pancreatic edema, necrosis, inflammatory cell infiltration decreased significantly in RESV-treated group (Table 1).

**Serum amylase**

Compared to sham operation group, the serum amylase in SAP group increased at all time points (*P < 0.01), decreased significantly in RESV-treated group when compared to SAP group at the corresponding time points (*P < 0.01, Figure 1).
Compared to sham operation group, pancreatic SOD descended and MDA increased in SAP group at 3 and 6 h ($P < 0.01$), but there was no difference between the two groups at 12 h in SOD and MDA. In contrast, pancreatic SOD increased and MDA descended in RESV-treated group at 3 and 6 h when compared to SAP group ($P < 0.01$), but there was no difference in SOD and MDA at 12 h between two groups (Figures 2A and 2B).

**MPO in pancreatic tissue**

Compared to sham operation group, the pancreatic MPO in SAP group increased at all time points ($P < 0.01$), but decreased significantly in RESV-treated group when compared to SAP group at the corresponding time points ($P < 0.01$, Figure 3).

**DISCUSSION**

The pathogenesis and therapeutics of SAP are constantly emphasized in general surgery. Sanfey et al.[11] have suggested a possible involvement of oxygen free radicals (OFRs) in acute pancreatitis. In 1995, Kishimoto et al.[12] detected pancreatic OFRs in acute pancreatitis using the technique of chemiluminescence probe and high sensitive photon counting and found that OFRs emerge 2-3 h after the induction of acute pancreatitis, demonstrating that there exists peroxidation in acute pancreatitis. OFRs can attack polyunsaturated fatty acid's aldehyde group inside the biomembrane, initiating lipid peroxidation and accordingly forming lipid peroxidation products, as such MDA, which result in the loss of membrane stability and release of acinar cell enzyme precursors, and activate phospholipase A1 which can decompose lecithinum inside cellular membrane, further causing tissue damage. SOD is an internal antioxidase. OFRs in vivo are augmented when acute pancreatitis develops, which results in the consumption of antioxidant, SOD activity decrease. Therefore, it is difficult to prevent damage to the pancreas and other organs by lipid peroxidation. Detection of pancreatic SOD and MDA can reflect the peroxidation of pancreatic acinar cells and indirectly reflect the damage due to OFRs.

A number of antioxidant therapies can improve pancreatitis induced by the administration of cerulein[13] and infusion of taurocholate[14]. Lasztity et al.[15] found that when enteral formula enriched with n-3 polyunsaturated fatty acids is used in the treatment of acute pancreatitis, the erythrocyte SOD activity is elevated significantly. Leonard et al.[8] showed that RESV can scavenge OFRs as measured by spin trapping competitions using sodium formate as a second free radical scavenger, and is effective in inhibiting lipid peroxidation of cellular membranes. In the present study, when compared to sham operation group, 3 h after the induction of SAP, pancreatic SOD decreased significantly, reaching perigee at 6 h, and returned to the level of sham operation group at 12 h. In contrast, 3 h after the induction of SAP, pancreatic MDA increased significantly, reaching to apogee at 6 h, and returned to the level of sham operation group at 12 h. Simultaneously, the serum amylase and pancreatic histopathologic score increased gradually. The results indicate that overproduction of OFRs occurs in early SAP and is a significant factor for aggravating pathogenetic condition. This is coincident with the research by Reinheckel et al.[16]. When compared to SAP group, pancreatic SOD in RESV-treated group increased significantly at 3 and 6 h. 14

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h (P < 0.01), whereas pancreatic MDA in RESV-treated group decreased significantly at 3 and 6 h (P < 0.01). On the other hand, compared to SAP group, both serum amylase and pancreatic histopathologic score in RESV-treated group decreased at all three time points (P < 0.01) indicating that RESV can depress earlier OFR production and lipid peroxidation of cellular membrane, diminish enzyme precursor release and necrosis of acinar cells, thus ameliorating pancreatic pathological lesions.

Neutrophils are the other major cellular source of OFRs during acute pancreatitis[17,18], and can directly release several inflammatory cytokines, evoking systemic inflammatory reactive syndrome (SIRS), since OFRs can exert a chemoattractant effect, thereby promoting accumulation of leukocytes in the inflamed gland[19]. Decreased acinar OFR production after RESV treatment may contribute to the reduced neutrophil infiltration, further ameliorating SIRS in SAP. In our study neutrophil sequestration within the pancreas was estimated by measuring tissue MPO activity. When compared to SAP group, the pancreatic MPO decreased at all the time points in RESV-treated group (P < 0.01). Moreover, studies showed that RESV can suppress the activation of NF-κB. Thus, RESV treatment might lead to the suppression of NF-κB activation and the subsequent prevention of several inflammatory mediator genes from being actively expressed[20-22]. This mechanism may also help to reduce the sequestration of neutrophils in the pancreas and the associated OFR generation, thus effectively attenuating pancreatic damage.

In conclusion, overproduction of OFRs takes place in early SAP, and is a significant factor for aggravating pathogenetic condition. RESV can ameliorate pathological lesions in the pancreas by lowering pancreatic OFRs and reducing pancreatic tissue infiltration of neutrophils. It may have certain therapeutical effects on acute pancreatitis.

REFERENCES

1 Kollár P, Hotolová H. [Biological effects of resveratrol and other constituents of wine]. Ceská Slov Farm 2003; 52: 272-281
2 Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res 2000; 47: 549-555
3 Jang DS, Kang BS, Ryu SY, Chang IM, Min KR, Kim Y. Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. Biochem Pharmacol 1999; 57: 705-712
4 Docherty JJ, Fu MM, Stiffler BS, Limperos RJ, Pokabla CM, DeLucia AL. Resveratrol inhibition of herpes simplex virus replication. Antiviral Res 1999; 43: 145-155
5 Cheong H, Ryu SY, Kim KM. Anti-allergic action of resveratrol and related hydroxystilbenes. Planta Med 1999; 65: 266-268
6 Fontecave M, Lepovire M, Elleingand E, Gerez C, Guittet O. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. FEBS Lett 1998; 421: 277-279
7 Jang M, Cai L, Udani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Knighton AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 1997; 275: 218-220
8 Leonard SS, Xia C, Jiang BH, Stinefelt B, Klandorf H, Harris GK, Shi X. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochem Biophys Res Commun 2003; 309: 1017-1026
9 Schmidt J, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL. A better model of acute pancreatitis for evaluating therapy. Ann Surg 1999; 219: 44-56
10 Bhatia M, Brady M, Zagoni K, Christman SE, Cameron F, Neoptolemos JP, Slavin J. Treatment with neutralising antibody against cytokine induced neutrophil chemoattractant (CINC) protects rats against acute pancreatitis associated lung injury. Gut 2000; 47: 838-844
11 Sanfey H, Bulikley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. Ann Surg 1984; 200: 405-413
12 Kishimoto W, Nakao A, Nakano M, Takahashi A, Inaba H, Takagi H. Detection of superoxide free radicals in rats with acute pancreatitis. Pancreas 1995; 11: 122-126
13 Demols A, Van Laethem JL, Quertinmont E, Legros F, Louis H, Le Moine O, Devière J. N-acetylcysteine decreases severity of acute pancreatitis in mice. Pancreas 2000; 20: 161-169
14 Rau B, Poch B, Gansauge F, Bauer A, Nüssler AK, Nevalainen T, Schoenberg MH, Beger HG. Pathophysiologic role of oxygen free radicals in acute pancreatitis: initiating event or mediator of tissue damage? Ann Surg 2000; 231: 352-360
15 Lasztiity N, Hamvás J, Biró L, Németh E, Marosvölgyi T, Décsi T, Pap A, Antal M. Effect of enterally administered n-3 polysaturated fatty acids in acute pancreatitis–a prospective randomized clinical trial. Clin Nute 2005; 24: 198-205
16 Reinheckel T, Nedelev B, Prase J, Augustin W, Schulz HU, Lippert H, Halangk W. Occurrence of oxidatively modified proteins: an early event in experimental acute pancreatitis. Free Radic Biol Med 1998; 24: 393-400
17 Poeh B, Gansauge F, Rau B, Wittel U, Gansauge S, Nüssler AK, Schoenberg M, Beger HG. The role of polymorphonuclear leukocytes and oxygen-derived free radicals in Experimental acute pancreatitis: mediators of local destruction and activators of inflammation. FEBS Lett 1999; 461: 268-272
18 Wisner J, Green D, Ferrell L, Renner I. Evidence for a role of oxygen derived free radicals in the pathogenesis of caerulein induced acute pancreatitis in rats. Gut 1988; 29: 1516-1523
19 Tsai SH, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NFkappaB in macrophages by resveratrol. Br J Pharmacol 1999; 126: 673-680
20 Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol 2000; 164: 6509-6519
21 Holmes-McNary M, Baldwin AS. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 2000; 60: 3477-3483

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