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Effects of resveratrol on calcium regulation in rats with severe acute pancreatitis

Liancai Wang, Qingyong Ma *, Xiangli Chen, Huanchen Sha, Zhenhua Ma

Department of Hepatobiliary Surgery, First Hospital of Xi'an Jiaotong University, 1 Jiankang Road, Xi'an, 710061, Shaanxi Province, China

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Abstract

Intracellular calcium overload plays a key role in severe acute pancreatitis. Resveratrol can decrease the severity of pancreatitis; however, the mechanism of action of resveratrol has not been determined. The aim of our study was to examine the relationship between calcium overload and the effects of resveratrol in severe acute pancreatitis. Animals were randomly divided into 3 groups: control group (sham operation), model group (0.1 ml/100 g of 3.5% sodium taurocholate used to induce severe acute pancreatitis), and treated group (treated with resveratrol, 10 mg/kg). In model group, the severity of pancreatitis was aggravated; this was evaluated by pancreatic weight/body weight and lung weight/body weight ratios, serum amylase activities, and pancreatic histopathological scoring; the Ca^{2+}–Mg^{2+}–ATPase and Ca^{2+}–ATPase activities decreased while PLA_2 activity and $[Ca^{2+}]_i$ increased gradually with time. Compared to the control group, in the model group, these changes were observed in the pancreatic tissue at the 3 h time point and in the lung tissue at the 6 h time point. Resveratrol ameliorated the changes in the laboratory parameters and significantly reduced the pathological damage in the tissues at the corresponding time points. In conclusion, intracellular calcium overload leads to tissue damage in severe acute pancreatitis, and the beneficial effects of resveratrol appear to be mediated by reducing the intracellular calcium overload; this not only limits pancreatic cellular injury but also secondary lung injury.

Keywords: Severe acute pancreatitis; Resveratrol; Calcium overload

1. Introduction

Severe acute pancreatitis is a life-threatening cause of acute abdomen and requires urgent treatment. Studies have found that pancreatic microcirculation disturbance (Benz et al., 2002; Foitzik et al., 2002), leukocyte overactivation (Bhatia et al., 2000), apoptosis of pancreatic acinar cells (Bhatia, 2004), and bacterial translocation from the gastrointestinal tract (Cicalese et al., 2001) play important roles in severe acute pancreatitis.

Different theories have been proposed to explain the etiopathogenesis, aggravation, and restoration of severe acute pancreatitis from various points of view. However, the initial triggering agent in the pathophysiology of severe acute pancreatitis remains unknown.

In recent years, an improved theory of calcium overload has been given increasing importance. Under physiological conditions, calcium remains stable (Qi et al., 2002), whereas in pathological conditions, some factors can influence intracellular calcium regulation and cause an increase in intracellular calcium content, resulting in calcium overload (Gao and Fang, 2004). Studies indicate that calcium overload is not only one of the etiological factors of severe acute pancreatitis but also aggravates severe acute pancreatitis by affecting other organs (Li and Zhang, 2001). The integrity of the cytomembrane and endocytoplasmic/sarcoplasmic reticulum membrane is important in intracellular calcium regulation; the Ca^{2+}–Mg^{2+}–ATPase activity of the cytomembrane and the Ca^{2+}–ATPase activity of the endocytoplasmic/sarcoplasmic reticulum membrane (Pariente et al., 1999) are also involved in this regulation. We propose that in severe acute pancreatitis, the infernal circle between the decreased activities of Ca^{2+}–Mg^{2+}–ATPase and Ca^{2+}–ATPase and the increased activity of phospholipase A_2 (PLA_2) disturbs the calcium regulatory activity of the pancreatic acinar cells and causes severe injury to the pancreas and other organs and cells.

* Corresponding author. Tel.: +86 44 29 85323899; fax: +86 44 29 85323473.
E-mail address: Springcoming369@163.com (Q. Ma).

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Resveratrol is one of the polyphenol compounds extracted from plants such as the giant knotweed rhizome; it has many beneficial effects, such as antiinflammatory and antioxidative activity and inhibition of platelet aggregation. Most studies on resveratrol focus on carcinoma and cardiovascular diseases. Some studies on the electrophysiology of resveratrol found that resveratrol could affect several ionic channels, including Na^+, K^+, and Ca^{2+} channels. Further, it was observed that resveratrol had different effects on the calcium in various tissues or cells; it increased $[Ca^{2+}]_i$ in endothelial (Buluc and Demirel-Yilmaz, 2006) and vascular smooth muscle cells (Campos-Toimil et al., 2005) and reduced $[Ca^{2+}]_i$ in ventricular myocytes (Zhang et al., 2006; Liu et al., 2005), pig papillary muscle cells (Zhao et al., 2004), and thrombin-stimulated human platelets (Dobrydneva et al., 1999).

In our previous studies, we found that resveratrol has some beneficial effects on severe acute pancreatitis (Ma and Ma, 2005). Studies indicate that pancreatic intracellular calcium overload plays an important role in severe acute pancreatitis. Therefore, we hypothesize that the beneficial effects of resveratrol on severe acute pancreatitis are at least partly related to reducing the pancreatic intracellular calcium overload.

2. Materials and methods

The experimental protocol followed the Principles of Laboratory Animal Care formulated by the National Institutes of Health, USA, and was approved by the ethics committee of the University of Szeged.

2.1. Animals

Male Sprague–Dawley (SD) rats weighing approximately 250–280 g were housed at a constant room temperature (25 °C) with light–dark cycles of 12 h; they were allowed free access to water and standard laboratory chow. The rats were randomly divided into 3 groups and starved for 12 h before beginning the experiment.

2.2. Induction of severe acute pancreatitis

After anesthesia, the pancreatic bile duct was occluded with 2 microvascular clamps; one clamp was placed on the duct at the hilum of the liver and the other close to the duodenum to prevent reflux. Sodium taurocholate (3.5%, Sigma T-0705; 0.1 ml/100 g body weight) was then injected into the pancreatic bile duct for 60 s.

2.3. Experimental protocol

We randomly divided 72 male SD rats into 3 groups, namely, control group (sham operation), model group (induction of severe acute pancreatitis), and treated group (treatment with resveratrol). In control group, the pancreas was slightly flipped. In model group, 3.5% sodium taurocholate (0.1 ml/100 g) was administered through the pancreatic bile duct. In treated group, resveratrol (5 mg/ml, 10 mg/kg) was administered through the vena dorsalis penis 10 min after the induction of severe acute pancreatitis. Eight rats from each group were sacrificed by postcava exsanguinations 3, 6, and 12 h postoperatively; their pancreatic and lung tissues were excised for isolating cells and for estimating $[Ca^{2+}]_i$ and the activities of Ca^{2+}–Mg^{2+}–ATPase, Ca^{2+}–ATPase, and PLA_2 and also for histopathological study. The severity of pancreatitis was assessed by pancreatic weight/body weight and lung weight/body weight ratios, serum amylase activities, and pancreatic histopathological scoring.

2.4. Assays

The pancreatic weight/body weight and lung weight/body weight ratios were evaluated as an estimate of the degree of edema. For serum assays, blood samples were centrifuged for 20 min at 2500 ×*g*. The serum amylase activities were determined by using an auto analyzer.

2.4.1. Activities of Ca^{2+}–Mg^{2+}–ATPase and Ca^{2+}–ATPase

The samples were homogenized in a 9-fold excess (w/v) of ice-cold physiological saline in an ultrasonic disintegrator. The homogenates were centrifuged at 1000 ×*g* for 10 min, and supernatants were obtained whose protein concentration was assessed using a Coomassie Blue kit. The ATPase activities were assayed by the quantization of phosphonium ions; the assay was performed in accordance with the ATPase detection protocol developed by the Nanjing Jiancheng Bioengineering Institute.

2.4.2. Activity of PLA_2

One gram of each tissue sample was homogenized in 4 ml solution (0.1 mol/L glycine, 3.57 mmol/L boric acid and 6.03 mmol/L sodium deoxycholate) and incubated in a 60 °C waterbath for 30 min and cooled at 4 °C. The PLA_2 activity was determined by modified microtitrimetry (Chen and Wu, 1989) and calculated by the amount of chlorhydric acid used (PLA_2 (U)$=(N\times V\times 10^6\times 2.5)/t$; N and V represent the concentration

Table 1
Pancreatic histological alterations in control group, model group and treated group (data are expressed as mean±S.E.M.)

Time points (h)	*n*	Control group	Model group	Treated group
3	8	0.28±0.11	9.24±0.62[a]	5.28±0.65[a,b]
6	8	0.22±0.17	11.36±0.54[a]	5.60±0.42[a,b]
12	8	0.11±0.05	13.56±0.64[a]	6.00±0.72[a,b]

[a]Significant difference with control group at the same time point ($P<0.05$).
[b]Significant difference with model group at the same time point ($P<0.05$).

Table 2
Lung histological alterations in control group, model group and treated group (data are expressed as mean±S.E.M.)

Time points (h)	*n*	Control group	Model group	Treated group
3	8	0.57±0.29	1.01±0.62	0.92±0.58
6	8	0.51±0.33	7.86±0.53[a]	4.31±0.39[a,b]
12	8	0.68±0.35	14.5±0.73[a]	8.04±0.40[a,b]

[a]Significant difference with control group at the same time point ($P<0.05$).
[b]Significant difference with model group at the same time point ($P<0.05$).