Protective effect of rhubarb on the intestinal mucosal barrier

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AIM: To investigate the mechanism of rhubarb protection of the gut barrier.

METHODS: The gut barrier damage models caused by hemorrhagic shock and intraperitoneal endotoxin were used to study the protective effect of rhubarb on the intestinal mucosal barrier. Rats were randomly divided into four groups, as follows: treatment (rhubarb) group; Positive control group; Negative control group; Placebo treatment group. Plasma endotoxin, tissue superoxidedismutase (SOD) and lipoxygenase (LPO) concentrations were measured and histological analysis was performed. Rhubarb was observed to have a protective effect on the gut.

RESULTS: Rhubarb decreased intestinal permeability, attenuated endotoxin absorption (endotoxin serum levels: shock group 0.557 μmol/L ± 0.019 μmol/L vs rhubarb group 0.345 μmol/L ± 0.055 μmol/L), and decreased tissue SOD and tissue LPO levels (SOD serum, EU/mL ± 0.069 EU/mL vs rhubarb group 3.05 EU/mL ± 0.345 EU/mL). Gut histopathology revealed that rhubarb promoted goblet cell proliferation, increased mucus secretion and protected intestinal mucosa in the hemorrhagic shock model.

CONCLUSION: Rhubarb may protect the gut barrier by decreasing intestinal permeability, scavenging oxygen free radicals, and promoting goblet cell proliferation within the intestinal mucosa.

Key words: Rhubarb; Shock; Hemorrhagic; Endotoxin; Intestinal mucosa; Free radicals

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INTRODUCTION

Gut-derived infection is the primary route of intensive care unit (ICU)-acquired infection[1] and is an initial pathophysiological response in multiple organ dysfunction syndrome (MODS) to trauma, shock and infection. Prevention of gut barrier damage can prevent fatal complications after severe trauma. Recent studies have shown that rhubarb has therapeutic effect on intestinal mucosal barrier damage caused by hemorrhagic shock and intraperitoneal endotoxin[2,3]. The aim of this study was to examine the mechanism of rhubarb protection of the gut barrier.

MATERIALS AND METHODS

Animal models
Hemorrhagic shock model The hemorrhagic shock model was generated as described previously, with some modifications[4]. Briefly, Sprague-Dawley rats (350 g-450 g) were fasted overnight, then anesthetized with intraperitoneal 50 mg/kg sodium pentobarbital. The right carotid arteries were cannulated under sterile conditions, and the arterial blood pressure was measured. The animals were bled 30 min later, and the mean arterial blood pressure was reduced to 5.32 kPa and maintained for 60 min by withdrawing or reinfusing shed blood as needed. The shed blood was kept at 37 ℃. The animals were resuscitated at the end of the shock period by reinfusing all of the shed blood. Catheters were sealed with heparin caps. The animals were given 2 mL saline at 2, 8, and 12 h after shock resuscitation via the heparin cap. Sham shock rats were anesthetized and their carotid arteries were cannulated, but no blood was withdrawn or infused. They received 2 mL saline at 2, 8, and 12 h after the operation. Blood samples were withdrawn from the carotid artery before shock and 24 h after shock resuscitation for endotoxin measurement.
ICR mice (20-30 g) were intraperitoneally injected with endotoxin (EU/mL, x ± s). Plasma endotoxin was measured using the limulus assay according to the manufacturer's instructions. The samples were centrifuged at 3000 r/min for 10 min to isolate the plasma. Each sample was diluted 1:4 with pyrogen-free water and heated at 100 °C for 6 min to remove any inhibitory or activating substances that can interfere with the limulus assay. The samples were stored at -40 °C until use. Plasma endotoxin was measured using the limulus assay according to the manufacturer's instructions. The results are expressed as nitrate unit per milliliter or microgram. *P < 0.05, **P < 0.01, vs endotoxin and placebo group.

**DISCUSSION**

The gastrointestinal (GI) tract is traditionally considered a nutritional organ, as its main function is to transport and process nutritional substrates. It was recently shown that the GI tract is a key organ affected by pathophysiological processes in critical illnesses. Upon gastrointestinal functional failure, gut barrier function is lost, the GI tract becomes a route for bacterial translocation and endotoxin absorption by the systemic organs and circulation, inducing systemic inflammatory response syndrome (SIRS). Our study shows that hemorrhagic shock can increase intestinal permeability, resulting in endotoxin absorption within the GI tract. Rhubarb may decrease the shock-induced increase in intestinal permeability and attenuate endotoxin absorption by the GI tract. Our previous work has also shown that rhubarb inhibited bacterial translocation[21]. As a traditional Chinese herb, rhubarb can be used to protect gastrointestinal function, particularly gut barrier. Our findings suggest that rhubarb can be used to prevent and treat gastrointestinal failure due to critical illness. Many studies in various fields have examined the mechanism of Pathophysiology of Nanjing Railway Medical College. Measurements were acquired according to the manufacturer’s protocol. SOD concentrations were expressed as nitrate units per milliliter.

**LPO measurements** Thioarbituric acid chromatography was used to measure LPO levels.[20]

**Morphological analysis** The proximal ileum was isolated after the animal was sacrificed. Three samples per group were analyzed by light microscopy. Tissues were fixed in 5% formaldehyde. The tissues were then dehydrated to 95% ethanol and embedded in paraffin. Semi-thin (2-3 μm) sections were cut and stained with 1% eosin.

**Statistical analysis** The results are expressed as x ± s. Variance analysis was used to compare quantitative data. P values less than 0.05 were considered statistically significant.

**RESULTS**

**Hemorrhagic shock model**

Hemorrhagic shock can increase intestinal permeability. The concentration of endotoxin in the plasma of shocked rats markedly increased 24 h after shock resuscitation, while it did not change 24 h after operation in sham shocked rats. Rhubarb administration decreased intestinal permeability of rats subjected to hemorrhagic shock, as they had lower plasma endotoxin levels than rats in the shock group or placebo group (Table 1).

We performed histological analysis on three rats from each group to determine the effect of rhubarb on ileal mucosa structure. The villi of animals in the shock group and placebo group showed submucosal edema and mucosal necrosis at villar tips. We also observed inflammatory cell infiltration and hemorrhage in the submucosa. However, villi in the rhubarb group showed mild submucosal edema but no mucosal necrosis. We also observed increased goblet cell proliferation in the intestinal villi.

**Endotoxin model**

Tissue SOD concentrations were much lower in the endotoxin group and placebo group compared to the rhubarb group, and these differences were statistically significant. These data suggest that rhubarb attenuates tissue SOD consumption (Table 2).

The tissue LPO concentration was much higher in the control group compared to the rhubarb group, which indicates that the rhubarb group has a lower LPO concentration than the control group. We also observed increased goblet cell proliferation in the intestinal villi.

**Experimental design**

**Hemorrhagic shock model** Rats were randomly divided into the following four groups: Shock group (n = 26), sham shock group (n = 5), rhubarb group, and placebo group (n = 8). The rhubarb powder (50 mg/kg; Rhubarb Lab, Xiang Shan Traditional Chinese Medicine Hospital, Shanghai) was administered through an oral gastric tube before shock, and 4 and 12 h after shock resuscitation. The placebo group was given 1 mL saline at the same time points.

**Endotoxin model**

The mice were divided into the following four groups: Endotoxin group (n = 17), endotoxin given intraperitoneally, control group (n = 5, intraperitoneal saline), rhubarb group (n = 8, 10 mg rhubarb administered orally 4 and 12 h after endotoxin administration), and placebo group (n = 4, treated with saline in place of rhubarb).

**Endotoxin measurements** Blood was withdrawn from the carotid artery, and 20 U heparin sodium was added to each sample. The samples were centrifuged at 3000 r/min for 10 min to isolate the plasma. Each sample was diluted 1:4 with pyrogen-free water and heated at 100 °C for 6 min to remove any inhibitory or activating factors that can interfere with the limulus assay. The samples were stored at -40 °C until use. Plasma endotoxin was measured using the limulus assay according to the manufacturer’s instructions. The limulus kits were provided by the Shanghai Clinical Medical Laboratory Center.

**Measurements**

**SOD measurements** SOD kits were provided by the Department

| Table 1 | The effect of rhubarb on plasma endotoxin (EU/mL, x ± s) |
|---------|----------------------------------------------------------|
| Group   | n  | Pre shock | 24 h after resuscitation |
|---------|----|-----------|--------------------------|
| Shock   | 16 | 0.183 ± 0.042 | 0.057 ± 0.097 |
| Rhubarb | 14 | 0.173 ± 0.039 | 0.343 ± 0.051 |
| Placebo | 8  | 0.209 ± 0.034 | 0.625 ± 0.049 |
| Sham shock | 5  | 0.198 ± 0.034 | 0.166 ± 0.043 |

| Table 2 | The effect of rhubarb on the content of tissue superoxidizedimutase (NU/mL or mg, x ± s) |
|---------|-------------------------------------------------|
| Group   | n  | Plasma | Small intestine | Liver |
|---------|----|--------|-----------------|------|
| Endotoxin | 17 | 122.92 ± 43.19 | 292.24 ± 88.76 | 272.70 ± 85.79 |
| Rhubarb    | 8  | 312.23 ± 54.93 | 391.09 ± 98.16 | 542.86 ± 119.93 |
| Placebo   | 4  | 149.71 ± 19.45 | 257.16 ± 73.78 | 213.86 ± 22.53 |

| Table 3 | The effect of rhubarb on the content of tissue lipoperoxide (μmol/L, x ± s) |
|---------|-------------------------------------------------|
| Group   | n  | Small intestine | Liver |
|---------|----|-----------------|------|
| Endotoxin | 17 | 8.57 ± 2.58 | 86.97 ± 46.54 |
| Rhubarb    | 8  | 3.05 ± 1.13 | 12.38 ± 19.64 |
| Placebo   | 4  | 5.97 ± 1.18 | 74.88 ± 16.42 |

The data were expressed as nitrate unit per milliliter or microgram. *P < 0.05, **P < 0.01, vs endotoxin and placebo group.

The data were expressed as nanomole per milliliter or microgram. *P < 0.05, **P < 0.01, vs endotoxin and placebo group.
of hemorrhagic shock- and endotoxin-induced gut barrier, includ-
ing clinical research, animal models, ultrastructural analysis, and
biochemistry. It is currently thought that intestinal mucosal hypoper-
fusion, oxygen free radicals and cytokines are three primary patho-
genic factors that induce gut barrier damage, especially oxygen
free radical injury. Because the intestinal mucosa contains a large
amount of xanthine oxidase, oxygen free radicals are overproduced
upon ischemia reperfusion, leading to gut mucosa injury. The cur-
rent study revealed that rhubarb alleviated intraperitoneal endotoxin-
induced gut mucosa injury and scavenged oxygen free radicals. Our
previous study also demonstrated that oxygen free radical scaveng-
ing prevented hemorrhagic shock-induced gut mucosa damage. As
discussed above, oxygen free radicals play an important role
in gut mucosa injury, which is consistent with previous studies.
Importantly, rhubarb can act as an oxygen free radical scavenger to
protect the gut mucosa in patients suffering from critical illnesses.

Morphological analysis revealed that rhubarb promoted goblet
cell proliferation within the intestinal mucosa, which secretes large
amounts of mucus. The mucus can prevent endotoxin absorption, inhibit bacteria adherence to intestinal epithelial cells and bacterial
translocation. Several studies have shown that rhubarb can promote
endotoxin excretion within the gut, prevent bacterial overproduction
and maintain the balance of the bacteria flora. Rhubarb can also
promote bile excretion, which can bind endotoxin to inhibit endo-
toxin absorption. Some studies have demonstrated that rhubarb
increased the PGI2/TXA2 ratio in tissues. Other possible mechanisms
of rhubarb function in the gut require further studies.

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