World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v3.i2.81

World J Gastroenterol 1997 June 15; 3(2): 81-83
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL RESEARCH

Protective effect of rhubarb on the intestinal mucosal barrier

De-Chang Chen, Xin-Yi Yang, Xiang-Yu Zhang, Xie-Yun Chen

De-Chang Chen, Xin-Yi Yang, Xiang-Yu Zhang, Xie-Yun Chen, Emergency and Critical Care Center, Shanghai Chang Zheng Hospital, Shanghai 200003, China

De-Chang Chen, born in 1963 in Shanghai and graduated from the Second Military Medical University in 1986. De Chang is an attending doctor, holds a Master's degree in critical care medicine, and is engaged in studies on gut failure in critical illness and has published 15 papers

Author contributions: All authors contributed equally to the work.

Supported by The National Natural Science Foundation of China, No. 39370666.

Original title: *China National Journal of New Gastroenterology* (1995-1997) renamed *World Journal of Gastroenterology* (1998-).

Correspondence to: Dr. De-Chang Chen, Emergency and Critical Care Center, Shanghai Chang Zheng Hospital, Shanghai 200003, China
Telephone: +86-21-63275997-432

Received: November 30, 1996
Revised: January 31, 1997
Accepted: March 1, 1997
Published online: June 15, 1997

Abstract

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 lipoperoxide (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 EU/mL ± 0.069 EU/mL *vs* rhubarb group 0.345 EU/mL ± 0.055 EU/mL), and decreased tissue SOD and tissue LPO levels (SOD serum, intestine and liver levels: endotoxin group 122.92 NU/mL ± 43.19 NU/mL, 292.24 NU/mL ± 88.76 NU/mL, 272.70 NU/mL ± 85.79 NU/mL *vs* rhubarb group 312.23 NU/mL ± 54.93 NU/mL, 391.09 NU/mg ± 98.16 NU/mg, 542.86 NU/mg ± 119.93 NU/mg; LPO content in the intestine and liver: endotoxin group 8.57 μmol/L ± 2.58 μmol/L, 86.97 μmol/L ± 46.54 μmol/L *vs* rhubarb group 3.05 μmol/L ± 1.13 μmol/L, 13.18 μmol/L ± 19.64 μmol/L). 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

Chen DC, Yang XY, Zhang XY, Chen XY. Protective effect of rhubarb on the intestinal mucosal barrier. *World J Gastroenterol* 1997; 3(2): 81-83 Available from: URL: http://www.wjgnet.com/1007-9327/full/v3/i2/81.htm DOI: http://dx.doi.org/10.3748/wjg.v3.i2.81

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 endotoxins[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 show 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.

Table 1 The effect of rhubarb on plasma endotoxin (EU/mL, $\bar{x} \pm s$)

Group	*n*	Pre shock	24 h after resuscitation
Shock	16	0.163 ± 0.042	0.557 ± 0.069[b]
Rhubarb	14	0.173 ± 0.039	0.345 ± 0.055[d]
Placebo	5	0.209 ± 0.034	0.625 ± 0.049
Sham shock	5	0.198 ± 0.034	0.166 ± 0.043

The data were expressed as endotoxin unit (EU) per milliliter of plasma [b]$P < 0.01$ *vs* sham-shocked rats. [d]$P < 0.01$ versus the shocked rats and the placebo treated rats.

Table 2 The effect of rhubarb on the content of tissue superoxidedismutase (NU/mL or mg, $\bar{x} \pm s$)

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[b]	391.09 ± 98.16[a]	542.86 ± 119.93[b]
Placebo	4	149.71 ± 19.45	257.16 ± 73.78	213.86 ± 22.53

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

Table 3 The effect of rhubarb on the content of tissue lipoperoxide (μmol/L, $\bar{x} \pm s$)

Group	*n*	Small intestine	Liver
Endotoxin	17	8.57 ± 2.58	86.97 ± 46.54
Rhubarb	8	3.05 ± 1.13[a]	13.18 ± 19.64[a]
Placebo	4	5.97 ± 1.18	74.88 ± 16.42

The data were expressed as nanomole per milliliter of 5% tissue hemogenate. [a]$P < 0.01$ *vs* endotoxin and placebo group.

Endotoxin model ICR mice (20-30 g) were intraperitoneally administered 0.4 mg endotoxin, then were fasted overnight with free access to water. Mice were sacrificed 24 h after endotoxin administration. Livers, small intestine and plasma were collected, and the livers and small intestine were weighed, homogenized and diluted into 5% homogenate. Plasma was centrifuged at 3000 r/min for 10 min, and the supernatant was collected for superoxidedismutase (SOD) and lipoperoxide (LPO) measurements.

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 ℃ for 6 min to remove any inhibitory or activating factors that can interfere with the limulus assay. The samples were stored at -40 ℃ 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 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 Thiobarbituric acid chromatography was used to measure LPO levels[5].

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 $\bar{x} \pm s$. 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 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 groups compared to the rhubarb group, suggesting that rhubarb can markedly decrease tissue LPO formation (Table 3).

According to our data, rhubarb may function in scavenging oxygen free radicals. We performed histological analysis of three mice from each group. Ileal mucosal epithelial cells were necrotic and disordered in the endotoxin and placebo groups, while the ileum only showed mild submucosal edema with no evidence of hemorrhage or necrosis in the rhubarb 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 failure, gut barrier function is lost, the GI tract becomes a reservoir for endotoxins and pathogenic bacteria that can translocate into 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[2]. 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 hemorrhagic shock- and endotoxin-induced gut barrier, including clinical research, animal models, ultrastructural analysis, and biochemistry. It is currently thought that intestinal mucosal hypoperfusion, oxygen free radicals and cytokines are three primary pathogenic factors[6-8] 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[9]. The current 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 scavenging prevented hemorrhagic shock-induced gut mucosa damage[10]. As discussed above, oxygen free radicals play an important role in gut mucosa injury, which is consistent with previous studies[11]. 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 secrete 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[12,13]. Rhubarb can also promote bile excretion, which can bind endotoxin to inhibit endotoxin absorption. Some studies have demonstrated that rhubarb increased the PGI_2/TXA_2 ratio in tissues. Other possible mechanisms of rhubarb function in the gut require further studies.

REFERENCES

1 **Marshall JC**, Christou NV, Meakins JL. The gastrointestinal tract. The "undrained abscess" of multiple organ failure. *Ann Surg* 1993; **218**: 111-119 [PMID: 8342990 DOI: 10.1097/00000658-199308000-00001]

2 **Chen DC**, Jin BW. The effect of rhubarb on gut derived infection. *Zhongguo Jijiu Yixue* 1993; **13**: 7-9

3 **Chen DC**, Jin BW, Chen JD. The effect of rhubarb on gut derived infection caused by endotoxin. *Zhonghua Yixue Zazhi* 1994; **3**: 84-86

4 **Nakayama S**, Kramer GC, Carlsen RC, Holcroft JW. Infusion of very hypertonic saline to bled rats: membrane potentials and fluid shifts. *J Surg Res* 1985; **38**: 180-186 [PMID: 3968876 DOI: 10.1016/0022-4804(85)90025-3]

5 **Chen SZ**. Comparison of three methodology of lipoperoxide TAB color reaction. *J Clin Lab* 1984; **2**: 8-10

6 **Morris SE**. Decreased mesenteric blood flow independently promotes bacterial translocation in chronically instrumented sheep. *Surg Forum* 1989; **40**: 88-94

7 **Parks DA**, Bulkley GB, Granger DN, Hamilton SR, McCord JM. Ischemic injury in the cat small intestine: role of superoxide radicals. *Gastroenterology* 1982; **82**: 9-15 [PMID: 6273253]

8 **van Lanschot JJ**, Mealy K, Wilmore DW. The effects of tumor necrosis factor on intestinal structure and metabolism. *Ann Surg* 1990; **212**: 663-670 [PMID: 2256757 DOI: 10.1097/00000658-199012000-00003]

9 **Zheng QC**. The effect of oxygen free radicals on acute ischemic injury of small intestine. *Zhonghua Waike Zazhi* 1989; **6**: 68-71

10 **Ma L**. Studies on the mechanism of pathogensis of gut derived infection caused by endotoxin. *Zhonghua Zhengxing Waike Zazhi* 1996; **6**: 164-166

11 **Chen DC**, Jin BW. The effect of the vitamin C on protection of gut barrier. *Zhongguo Jijiu Yixue* 1994; **14**: 8-10

12 **Sheng DC**, Feng H. The effect of rhubarb on fever caused by endotoxin and the concentration of cAMP of spinal fluid. *Zhonghua Bingli Shengli Zazhi* 1989; **5**: 77-79

13 **Chen CM**, Wang WF. Selection of Chinese traditional herb resistant to anaerobic bacteria *in vitro*. *Dier Junyi Daxue Xuebao* 1989; **16**: 399-400

S- Editor: Filipodia **L- Editor:** Jennifer **E- Editor:** Liu WX

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327