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World J Gastroenterol 2005;11(18):2830-2833
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Gastric mucosal injury due to hemorrhagic reperfusion and efficacy of *Salvia miltiorrhizae* extract F and cimetidine

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Supported by the National Natural Science Foundation of China, No. 3870890
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Received: 2004-03-18 Accepted: 2004-04-27

Abstract

AIM: To observe the gastric mucosal injury caused by hemorrhagic shock and reperfusion and to compare the effect between *Salvia miltiorrhizae* extract F (SEF) and cimetidine (CI) on it.

METHODS: A model of hemorrhage/reperfusion injury was produced by Itoh method. Wistar rats were randomly divided into three groups: 0.9% sodium chloride treatment group (NS group), SEF treatment group (SEF group), and CI treatment group (CI group). Saline, SEF and CI were injected respectively. The index of gastric mucosal lesions (IGML) was expressed as the percentage of lesion area in the gastric mucosa. The degree of gastric mucosal lesions was categorized into grades 0, 1, 2, 3. Atom absorption method was used to measure the intracellular calcium content. Radioimmunoassay was used to measure the concentrations of prostaglandins.

RESULTS: IGML (%) and grade 3 (%) were 23.18±6.82, 58.44±9.07 in NS group, 4.42±1.39, 20.32±6.95 in SEF group and 3.74±1.56, 23.12±5.09 in CI group, and the above parameters in SEF group and CI group decreased significantly (IGML: SEF *vs* NS, $t = 6.712$, $P = 0.000<0.01$; CI *vs* NS, $t = 6.943$, $P = 0.000<0.01$; grade 3: SEF *vs* NS, $t = 8.386$, $P = 0.000$; CI *vs* NS, $t = 8.411$, $P = 0.000$), but the grade 0 and grade 1 damage in SEF group (22.05±5.96, 34.12±8.12) and CI group (18.54±4.82, 30.15±7.12) were markedly higher than those in NS group (3.01±1.01, 8.35±1.95; grade 0: SEF *vs* NS, $t = 8.434$, $P = 0.000<0.01$; CI *vs* NS, $t = 7.950$, $P = 0.000<0.01$; grade 1: SEF *vs* NS, $t = 8.422$, $P = 0.000<0.01$; CI *vs* NS, $t = 8.448$, $P = 0.000<0.01$). The intracellular calcium content (μg/mg) in SEF group (0.104±0.015) and CI group (0.102±0.010) was markedly lower than that in NS group (0.131±0.019, SEF *vs* NS, $t = 2.463$, $P = 0.038<0.05$; CI *vs* NS, $t = 3.056$, $P = 0.017<0.05$). The levels (pg/mg) of PGE_2, 6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$/TXB_2 were 540±183, 714±124, 17.38±5.93 in NS group and 581±168, 737±102, 19.04±8.03 in CI group, 760±192, 1 248±158, 33.42±9.24 in SEF group, and the above parameters in SEF group markedly raised (PGE_2: SEF *vs* NS, $t = 2.282$, $P = 0.046<0.05$; SEF *vs* CI, $t = 2.265$, $P = 0.047<0.05$; 6-keto-$PGF_{1\alpha}$: SEF *vs* NS, $t = 6.583$, $P = 0.000<0.000$; SEF *vs* CI, $t = 6.708$, $P = 0.000<0.01$; 6-keto-$PGF_{1\alpha}$/TXB_2: SEF *vs* NS, $t = 3.963$, $P = 0.003<0.001$; SEF *vs* CI, $t = 3.243$, $P = 0.009<0.01$), whereas TXB_2 level in SEF group (45.37±7.54) was obviously lower than that in NS group (58.28±6.74, $t = 3.086$, $P = 0.014<0.05$) and CI group (54.32±6.89, $t = 2.265$, $P = 0.047<0.05$). No significant difference was shown between NS group and CI group (PGE_2: $t = 0.414$, $P = 0.688>0.05$; 6-keto-$PGF_{1\alpha}$: $t = 0.310$, $P = 0.763>0.05$; TXB_2: $t = 1.099$, $P = 0.298>0.05$; 6-keto-$PGF_{1\alpha}$/TXB_2: $t = 0.372$, $P = 0.718>0.05$).

CONCLUSION: Both SEF and CI could inhibit reperfusion-induced injury in gastric mucosa, but with different mechanisms. SEF could not only enhance the protective effect of gastric mucosa, but also abate the injury factors, while CI can only abate the injury factors.

Key words: Hemorrhagic shock; Reperfusion injury; Gastric mucosa; Radix *Salvia miltiorrhizae*; Cimetidine

Zhang LH, Yao CB, Gao MQ, Li HQ. Gastric mucosal injury due to hemorrhagic reperfusion and efficacy of *Salvia miltiorrhizae* extract F and cimetidine. *World J Gastroenterol* 2005; 11(18): 2830-2833
http://www.wjgnet.com/1007-9327/11/2830.asp

INTRODUCTION

Under certain circumstances, reperfusion after hemorrhage can lead to multiple organ injury called reperfusion injury. Gastric mucosa mainly manifestsas stress ulcer, hemorrhage, necrosis, and perforation[1-3]. In the present study, we observed the resistance of *Salvia miltiorrhizae* extract F (SEF) and H_2 receptor antagonist cimetidine (CI) to mucosal injury in gastric corpus caused by hemorrhagic shock reperfusion and probed into their mechanisms to provide a theoretic basis for exploring effective drugs

against reperfusion injury of gastric mucosa.

MATERIALS AND METHODS

Drugs

SEF was extracted from *S. miltiorrhizae* provided by Chemical Assay Center of China Medical University. CI was produced in Guangdong Xiaolan Pharmaceutical Factory (batch no. 900603).

Animal models

A hemorrhagic shock/reperfusion model was duplicated using modified Itoh method[4]. Healthy male Wistar rats, weighing 260-300 g, were fasted for 24 h before experiments. The rats were then anesthetized intraperitoneally with 5 mg/100 g of 20% urethane. Tracheostomy was performed and a PE-250 tube was inserted into the trachea to ensure an open airway. The blood pressure was monitored through a polyethylene tube placed in the right carotid artery. A femoral artery was cannulated to withdraw and reinfuse the shed blood, caudal vein was punched for injection of fluid or medication. The abdomen was opened and gastric lumen was washed gently with warm saline till pH 6.0. Normal saline, SEF or CI was then administered (0.03 mL/min), and 25 min later 0.1 mol/L HCl [1 mL/(min·100 g)] was instilled into the stomach via a gastric tube. Five minutes after intragastric HCl induction, blood was withdrawn from the femoral artery. The mean arterial blood pressure was reduced to 2.67-4.00 kPa and maintained for 20 min. The shed blood was then reinfused, and 20 min later the rats were killed and the model of hemorrhagic shock/reperfusion injury was established.

Grouping

Rats were randomly divided into three groups: NS group (treated with normal saline), SEF group, and CI group. NS, SEF (1 g/100 g), or CI (6.5 mg/100 g) was injected respectively.

Index of gastric mucosal lesion

Index of gastric mucosal lesion (IGML) was expressed as a percentage of lesion area in corpus[5].

Depth of gastric mucosal lesion (DGML)

Mucosa taken from anterior gastric corpus was divided into the following grades under a light microscope[6]: grade 0: normal gastric mucosa; grade 1: surface mucosa cells were damaged; grade 2: in addition to extensive luminal damage, cells lining the gastric pits were also disrupted and exfoliated; grade 3: cell destruction extended into the gastric gland. Samples analyzed by scanning electron microscopy (SEM) were evaluated as follows[7]: grade 0: normal gastric mucosa; grade 1: surface cells were flattened with irregular shape, and gaps between individual cells were present; grade 2: the basal lamina was exposed and largely devoid of surface mucous cells, but still showed continuity; grade 3: most of the basal lamina were disrupted, and only parts of it were still intact, regular surface was no longer present. According to the ratio of damaged length to the whole slide, grading between light microscopy and SEM had a close correlation ($r = 0.846$, $P<0.01$).

Intracellular calcium concentration

Atom absorption spectrometry was used to measure the intracellular calcium content.

Prostaglandin level

Prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$ (6-keto, a metabolite of PGI_2), TXB_2 (a metabolite of TXA_2) kits were provided by Biochemistry Laboratory of General Hospital of the PLA and assayed with radioimmunoassay.

Statistical analysis

Data were represented as mean±SD and analyzed by t test in SPSS10.0. $P<0.05$ was considered statistically significant.

RESULTS

Comparison of IGML among different groups

IGMLs (%) were 4.42±1.39 and 3.74±1.56 in SEF ($n = 7$) and CI ($n = 6$) groups respectively. They were significantly lower than 23.18±6.82 in NS ($n = 9$) group (SEF *vs* NS, $t = 6.712$, $P = 0.000<0.01$; CI *vs* NS, $t = 6.943$, $P = 0.000<0.01$). There was no significant difference between SEF and CI groups ($t = 0.855$, $P = 0.413>0.05$).

Comparison of DGML among different groups

As shown in Table 1, DGMLs in normal gastric mucosa (grade 0) and mildly injured mucosa (grade 1) in SEF and CI groups were much higher than those in NS group ($P<0.01$), and DGML in severely injured mucosa (grade 3) was much lower than that in NS group ($P<0.01$). There was no obvious difference between SEF and CI groups (grade 0: $t = 1.326$, $P = 0.214>0.05$; grade 1: $t = 0.952$, $P = 0.354>0.05$; grade 3: $t = 0.799$, $P = 0.443>0.05$).

Table 1 Comparison of depth of gastric mucosal lesion among different groups (%, mean±SD)

Group	n	Damage grade			
		0	1	2	3
NS	7	3.01±1.01	8.35±1.95	31.32±4.49	58.44±9.07
SEF	6	22.05±5.96[b]	34.12±8.12[b]	25.96±10.04	20.32±6.95[b]
		$t = 8.434$,	$t = 8.422$,		$t = 8.386$,
		$P = 0.000$	$P = 0.000$		$P = 0.000$
CI	6	18.54±4.82[b]	30.15±7.12[b]	26.59±8.32	23.12±5.09[b]
		$t = 7.950$,	$t = 8.448$,		$t = 8.411$,
		$P = 0.000$	$P = 0.000$		$P = 0.000$

[b]$P<0.01$ *vs* NS group.

Comparison of intracellular calcium concentration among different groups

Intracellular calcium concentrations (μg/mg) were 0.1044±0.0147 and 0.1020±0.0103 in SEF ($n = 6$) and CI ($n = 6$) groups, respectively, much lower than 0.1308±0.0194 in NS ($n = 7$) group (SEF *vs* NS, $t = 2.463$, $P = 0.038<0.05$; CI *vs* NS, $t = 3.056$, $P = 0.017<0.05$). There was no significant difference between SEF and CI groups ($t = 0.433$, $P = 0.674>0.05$).

Comparison of prostaglandin concentration among different groups

The levels of PGE_2, 6-keto-$PGF_{1\alpha}$ and 6-keto-$PGF_{1\alpha}$/TXB_2 in SEF group were much higher than those in NS and CI groups ($P<0.01$, $P<0.05$) and TXB_2 was much lower than that in NS and CI groups ($P<0.05$). In CI group, PGE_2 and the ratio of 6-keto-$PGF_{1\alpha}$/TXB_2 were higher than those in NS group, while TXB_2 was lower than that in NS group. There was no significant difference between these two groups (PGE_2: $t = 0.414$, $P = 0.688>0.05$; 6-keto-$PGF_{1\alpha}$: $t = 0.310$, $P = 0.763>0.05$; TXB_2: $t = 1.099$, $P = 0.298>0.05$; 6-keto-$PGF_{1\alpha}$/TXB_2: $t = 0.372$, $P = 0.718>0.05$, Table 2).

Table 2 Comparison of PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2 concentrations in gastric mucosa among different groups (mean±SD)

Group	*n*	PGE_2 (pg/mg)	6-keto-$PGF_1\alpha$ (pg/mg)	TXB_2 (pg/mg)	6-keto-$PGF_1\alpha$/TXB_2
NS	6	540±183	714±124	58.28±6.74	17.38±5.93
SEF	6	760±192[a]	1 248±158[b]	45.37±7.54[a]	33.42±9.24[b]
		$t = 2.282$,	$t = 6.583$,	$t = 3.086$,	$t = 3.963$,
		$P = 0.046$	$P = 0.000$	$P = 0.014$	$P = 0.003$
CI	6	581±168[c]	734±102[d]	54.32±6.89[c]	19.04±8.03[d]
		$t = 2.265$,	$t = 6.708$,	$t = 2.265$,	$t = 3.243$,
		$P = 0.047$	$P = 0.000$	$P = 0.047$	$P = 0.009$

[a]$P<0.05$, [b]$P<0.01$ *vs* NS group; [c]$P<0.05$, [d]$P<0.01$ *vs* SEF group.

DISCUSSION

More and more researches have focused on gastric mucosa reperfusion injury. Though scholars both at home and abroad have done many studies on it, there is no satisfactory therapeutic method or drug yet. The present study showed that both SEF and CI could obviously reduce injury area and depth of gastric mucosa caused by hemorrhagic shock and reperfusion, and protect gastric mucosa. Although the inhibitory rate of these drugs on gastric mucosa injury was similar, SEF had advantages in preventing severe injury, making injured gastric mucosa easier to recover. Furthermore, clinical observations showed *Salvia* water decoction (used to produce SEF) had no rebinding after stopping medication, and fewer side-effects. Some patients had reddened faces, which showed spontaneous recovery. With highly purified drugs, we would get better therapeutic effects and fewer side-effects.

Reperfusion injury of gastric mucosa is a kind of acute gastric mucosa lesion. Gastric mucosa is affected by both injury factors (such as gastric acid and pepsin)[8,9] and protective factors (such as prostaglandin and gastric mucus)[10,11]. Normally, the protective factors surpass injury factors so that human gastric corpus could perform digestive function, prevent stomach and duodenum from injury. According to the equilibrium theory by Sun and Shen, the increase of injury factors and/or abating of protective factors could cause gastric mucosa injury. This study showed that SEF could increase the concentration of prostaglandin in gastric mucosa and reduce intracellular calcium content, while CI could lower intracellular calcium content besides being a H_2 receptor antagonist. The mechanisms of these two drugs are different. SEF could increase both protective factors and weakened injury factors in gastric mucosa, while CI could merely weaken injury factors.

It is generally believed that under stress a large amount of adrenal glucocorticoid secretion can increase gastric acid secretion, decrease mucus and increase histamine release from mast cells. Histamine combines with H_2 receptors on parietal cells to stimulate the latter to secrete gastric acid. CI is a H_2 receptor antagonist, it competes with histamine to combine with H_2 receptors to diminish gastric acid secretion caused by histamine. SEF could increase the level of PGE_2 and 6-keto-$PGF_{1\alpha}$ in gastric mucosa and reduce the content of TXB_2. PGE_2 could obviously inhibit basal gastric acid secretion and stimulate gastric acid secretion caused by histamine, pentagastrin and food in dogs and humans. Prostaglandin has cell protective effects. PGE_2 could obviously increase gastric mucus content, thicken gastric mucosa gel layer. PGE_2, PGI_2 could dilate blood vessels, increase blood flow and carbohydrate secretion, enhance resistance of gastric mucosa against injury. In addition, prostaglandin could extend life span of epithelia and thicken mucosa layer[12-16]. This study showed that both SEF and CI could inhibit intracellular calcium overload. Increase of intracellular calcium content could activate xanthine dehydrogenase and cause many xanthine oxidases accumulated in body. Thus, after reperfusion, gastric mucosa gained oxygen again and produced superoxide anion explosively, and caused gastric mucosa injury. Moreover, inhibition of intracellular calcium overload could enhance energy metabolism, reduce membrane phospholipid decomposition[17-20]. So, the resistant mechanisms of these two drugs to reperfusion injury after gastric mucosa ischemia have similarities as well as differences. Whether combination of these two drugs could enhance therapeutic effects still needs further exploration.

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Science Editor Chen WW, Zhu LH and Wang XL **Language Editor** Elsevier HK