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• *BRIEF REPORTS* •

Chinese medicine compound Changtong oral liquid on postoperative intestinal adhesions

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Abstract

AIM: The aim of this study was to observe the effect of a Chinese medicine compound Changtong oral liquid (CT) on tissue plasminogen activity (t-PA), plasminogen activator inhibitor (PAI), TGF-β1 and hydroxyproline (OHP).

METHODS: Two sets of animal experiments were performed in the present study. Forty New Zealand rabbits and 48 Sprague-Dawley (SD) rats were assigned randomly to one of the five groups: sham adhesion, adhesion with saline, adhesion with low dosage of the CT, adhesion with middle dosage of the CT and adhesion with high dosage of the CT. t-PA and PAI activity in plasma, OHP and TGF-β1 expression in adhesion were investigated. Analysis of variance was used to test differences among groups.

RESULTS: CT treatment increased plasma t-PA activity in rabbits but decreased TGF-β1 activity in rats. The data were expressed from low to high dose respectively as follows: t-PA, 46.1±8.6 μkat/L, 59.6±10.1 μkat/L, 64.0±11.5 μkat/L; TGF-β1 28±7.23%, 31±3.05%, 30±4.04%. There were significant differences compared with saline-treated animals (t-PA 26.4±5.1 μkat/L, TGF-β1 54±5.51%). OHP content in cecum of rabbits from middle and high but not low dose of CT lowered significantly as compared with saline-treated rabbits, 0.3641±0.1373, 0.3348±0.0321, 0.2757±0.0497 mg/g *vs* 0.4183±0.0883 mg/g of protein, $P>0.05$, $P<0.05$, $P<0.05$ respectively. The rabbit plasma PAI activity and OHP content in abdominal wall had no difference in all groups.

CONCLUSION: CT treatment significantly enhanced t-PA activity in rabbits, but decreased TGF-β1 content in rats, OHP content in cecum of rabbits, and failed to affect the activity of PAI and OHP content in abdominal wall in rabbits, compared with saline group. The result suggests that CT could effectively prevent adhesions without interfering wound healing.

Key words: Chinese medicine compound; Postoperative intestinal adhesions

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INTRODUCTION

Adhesions result from normal peritoneal wound healing response and develop in the first 5-7 d after injury. Adhesion formation and adhesion-free re-epithelialization are alternative pathways, both of which begin with coagulation which initiates a cascade of events resulting in the buildup of fibrin gel matrix. When unremoved, the fibrin gel matrix serves as the progenitor to adhesions by forming a band or bridge when two peritoneal surfaces coated with it are apposed. The band or bridge becomes the basis for the forming of an adhesion.

Intra-abdominal adhesion formation and reformation after surgery is a significant cause of morbidity. Postoperative adhesions, account for 40% of all cases of intestinal obstruction and 60-70% of these involve the small bowel[1]. Currently, there is no ideal method of preventing adhesion formation. In terms of surgical technique, gentle tissue handling, the no-touch technique, meticulous hemostasis, copious irrigation, prevention of infection, avoidance of powdered gloves which can evoke a foreign body response in the peritoneum and prevention of extensive thermal injury have all been described as means of adhesion prevention. Multiple adjuvants to post-surgical adhesion prevention have also been evaluated. They include agents that prevent inflammation such as both steroidal and non-steroidal anti-inflammatory medications, agents that degrade fibrin such as recombinant tissue plasminogen activator and barrier methods involving the application of an absorbable material/solution/gel intraperitoneally to prevent the peritoneal surfaces from adhering together[3]. Many Chinese medicines had been used to prevent adhesion. These include drugs for promoting blood circulation and removing blood stasis, such as safflower, *Radix Salviae Miltiorrhizae*, *Radix Angelicae Sinensis*, *Radix et Rhizoma rhei* and drugs for regulating Qi,

such as *Magnolia officinalis* Rehd. *Saussurea lappa* C. B. Clarke. Some Chinese medicine compound had also been used to prevent adhesion, such as Decoction of Simo, Minor decoction for purgation. Most of these drugs can be anti-inflammatory, promoting intestine peristalsis. Till recently, there was some lack of knowledge about how the Chinese medicine compound prevents adhesion at cellular and molecular level.

Changtong oral liquid (CT) is a Chinese medicine compound, which is included in *Radix Salviae Miltiorrhizae*, *Radix et Rhizoma rhei*, *etc.* In rat and rabbit models, we observed the effect that it prevents postoperative intestinal adhesion formation according to macroscopic adhesion grading[2,12].

A better understanding of the mechanism of CT at the cellular and molecular level would undoubtedly help develop more effective treatment strategies. Many peritoneal fluid factors are associated with established peritoneal adhesion, such as tissue plasminogen activity (t-PA), plasminogen activator inhibitor (PAI), TGF-β[1], metalloproteinases[4], interleukin-10[5]. In this section, we study how CT adjusts the cellular events t-PA, PAI, TGF-β1 and hydroxyproline (OHP)[3], which is involved in the peritoneal healing.

MATERIALS AND METHODS

Animals

Specific pathogen-free Sprague-Dawley (SD) rats of both sexes, weighing 200-240 g, and New Zealand rabbits of both sexes, weighing 1.8-2.2 kg, were obtained from the animal center of The First Military Medical University. They were housed under barrier-sustained conditions and kept at 25 ℃ with 12-h light/dark cycles. The rats had free access to water and chow. All animals received care in compliance with the guidelines of China Ministry of Health

Reagents

TGF-β1 antibody (1∶100) was purchased from Santa Cruz Biotechnology Inc. (USA). S-P kit was purchased from Maixing Biotechnology Co., Ltd (Xiamen, China), t-PA kit was purchased from Department of Molecular Genetics, Fudan Medical College, PAI was purchased from Sun Biotechnology Co., Ltd (Shanghai, China).

Drug

CT was manufactured by Nanfang Hospital. Danshengsu [D(+)-β-(3,4- dihydroxyphenyl)lactic acid] contents is 0.4 g/L.

Experiment model

The model utilized in this experiment is postoperative intestinal adhesion on animals.

The SD rats and New Zealand rabbits were subjected to a laparotomy (trauma).

They were anesthetized with intraperitoneal sodium pentobarbital, and their cecum were isolated using aseptic techniques, exposed to air for 5 min, scratched chorion 10 times by scalpel, a drop of absolute alcohol dropped on the injury, clamped segmental artery by tweezers for 2 min, then put cecum in original site. A laparotomy was then performed on each animal and the abdominal cavity was closed with running 3-0 silk surgical sutures.

Experimental protocol

Forty New Zealand rabbits and 48 SD rats were assigned randomly to one of the five groups: sham adhesion, adhesion with saline, adhesion with low dosage of the CT, adhesion with intermediate dosage of the CT and adhesion with high dosage of the CT. Each animal was orally administered after operation.

Assay for TGF-β1, t-PA, PAI and OHP

A blood sample of 2 mL was taken from the rabbits on the 3rd d after the operation. Chromogenic assay was used to identify the activity of t-PA and PAI in plasma. The animals were killed on the 7th d, a tissue sample of 1 g was taken from the abdominal incision line and adhesive segment. The expression of OHP was examined by spectrophotometric method. The expression of TGF-β1 was examined by immunohistochemistry method.

Statistical analysis

All measurements are expressed as the mean±SD and were analyzed by one-way analysis of variance with the Student-Newman-Keuls multiple comparisons or *t*-test when comparing the differences between the means of four or two groups at the same time point. Probabilities less than 0.05 were considered to be statistically significant.

RESULTS

Effect of CT on t-PA, PAI

The t-PA content was significantly increased in three groups of the CT when compared with saline group ($P<0.001$). The t-PA content was significantly decreased in saline group when compared with sham adhesion group ($P<0.05$). There was no significant difference among the three groups of the CT. But the PAI content did not have variations in each group (Table 1).

Table 1 Effect of the CT on the t-PA, PAI in plasma of rabbits with adhesion (mean±SD)

Group	Dosage (g/kg)	Number	t-PA (μkat/L)	PAI (μkat/L)
Sham	-	8	38.3±11.4	12.5±0.8
Saline	-	8	26.4±5.1[b]	12.5±2.4
CT	2.15	8	46.1±8.6[a]	13.3±1.5
CT	4.30	8	59.6±10.1[a]	10.8±1.8
CT	8.60	8	64.0±11.5[a]	13.5±1.9

[a]$P<0.05$ *vs* sham adhesion group, [b]$P<0.001$ *vs* saline group.

Effect of CT on TGF-β1

The TGF-β1 content was significantly decreased in three groups of the CT when compared with saline group ($P<0.001$). The TGF-β1 content was significantly increased in saline group when compared with sham adhesion group ($P<0.001$). There were no significant differences among the three groups of the CT (Table 2).

Effect of CT on OHP

The OHP content in cecum was significantly decreased in

groups of middle and high dosage of the CT when compared with saline group ($P<0.05$ or 0.01). The OHP content in cecum was significantly increased in saline groups when compared with sham adhesion group ($P<0.001$). The OHP content in abdominal wall had no significant difference in groups of the CT when compared with saline group (Table 3).

Table 2 Effect of the CT on the expression of TGF-β1 of rats with adhesion (mean±SD)

Group	Number	Dosage (g/kg)	Rate of positive reaction (%)
Sham	10		12±2.08
Saline	10		54±5.51[b]
CT	10	4.3	28±7.23[d]
CT	9	8.6	31±3.05[d]
CT	9	17.2	30±4.04[d]

[b]$P<0.001$ *vs* saline group. [d]$P<0.001$ *vs* sham adhesion group.

Table 3 Effect of the CT on OHP in rabbits with adhesions (mean±SD)

Group	Dosage (g/kg)	Number	OHP (mg/g of protein)	
			Cecum	Abdominal wall
Sham	-	8	0.2056±0.0983	0.2373±0.1189
Saline	-	8	0.4183±0.0883[d]	0.2527±0.1202
CT	2.15	8	0.3641±0.1373	0.2302±0.1577
CT	4.30	8	0.3348±0.0321[a]	0.2784±0.2325
CT	8.60	8	0.2757±0.0497[b]	0.1957±0.3721

[a]$P<0.05$, [b]$P<0.01$ *vs* saline group, [d]$P<0.001$ *vs* sham adhesion group.

DISCUSSION

The peritoneal leukocytes, mesothelial cells, and macrophages are important cellular components of peritoneal healing[1,6]. After injury to the peritoneum, there is increased vascular permeability in vessels supplying the damaged area, followed by an exudation of inflammatory cells, ultimately leading to the formation of a fibrin matrix. The fibrin matrix is gradually organized and replaced by tissue containing fibroblasts, macrophages, and giant cells. This fibrin matrix connects two injured peritoneal surfaces forming fibrin bands. These fibrin bands can be broken down by fibrinolysis into smaller molecules as fibrin degradation products. Under conditions of aberrant peritoneal healing, ischemia results in a reduction in fibrinolytic activity and thus persistence of the fibrin bands. The organization of the fibrin bands over time results in the persistence of the adhesions. The cell population changes with the maturity of the adhesion tissue, with the initial cell type at d 1-3 being mainly polymorphonuclear leukocytes and on d 5-7 being mainly fibroblasts. Adhesion tissue also contains nerve fibers and small vascular channels of endothelial cells. The role of the fibrinolysis in adhesion formation is to breakdown fibrin clots that are formed during the healing process. In the healthy mesothelial layer, t-PA and PAI protein were present, compared with the submesothelial layer, where only PAI-1 and not t-PA were present. Inflamed peritoneum, t-PA expression in the mesothelium was substantially reduced, whereas PAI-1 expression in the submesothelial layer was intensified. Expression of t-PA in the mesothelium but not the submesothelium suggested that t-PA was responsible for the clearance of fibrin in the peritoneal cavity proper[7]. CT can significantly increase the t-PA content in rabbit models. Maybe it is one of the reasons that CT prevents postoperative intestinal adhesion formation.

In peritoneal healing and adhesion formation, latent TGF-β is activated by plasmin in its active form, TGF-β not only interacts with the fibrinolytic system and extracellular matrix but also with many other cellular mediators involved in the process of adhesion formation[8]. It is healthily found in platelets, macrophages, and wound fluid. It is a key factor in healthy wound healing and is also a potent inducer of tissue fibrosis in peritoneal wound healing. During the acute phase of the inflammatory response, peritoneal macrophages and/or mesothelial cells produce TGF-β. It can contribute to the synthesis of the extracellular matrix by stimulating fibroblastic cell production of collagen and fibronectin. TGF-β overexpression by the parietal peritoneum and the serosal surfaces of the pelvic organs as well as increased concentrations of TGF-β in the peritoneal fluid have been associated with an increased incidence of adhesion formation in both humans and animals. Krause[9] utilized heterozygous mice to study that healthy TGF-β levels can modulate the injury response that regulates the extent of adhesion formation. The TGF-β1 content was significantly decreased in CT groups, but the TGF-β1 content was significantly increased in saline group when compared with sham adhesion group. Maybe it is another reason that CT prevents postoperative intestinal adhesion.

OHP content was considered to play a role in wound healing. Baykal[3] and Ozogul[10] used adhesion grading and tissue OHP levels to value adhesion severity, they found that there was linear correlation between adhesion degree and tissue OHP levels ($r = 0.86$, $P<0.001$; $r = 0.73$, $P = 0.00000$). Avsar[11] found that OHP levels in the tissue were significantly lower in groups, which has low adhesion formation rate in rats. Ozogul[10] considered that OHP levels showed significant correlation with adhesion severity. In our study, although CT decreases cecum OHP level, there was no significant difference in abdominal wall OHP content between the groups. CT plays a role in the prevention of adhesion formation without affecting wound healing.

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