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Protective effects of *Salvia miltiorrhizae* on the hearts of rats with severe acute pancreatits or obstructive jaundice*

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Abstract: Objective: To investigate the therapeutic effects and mechanisms of *Salvia miltiorrhizae* (Danshen) in the treatment of severe acute pancreatitis (SAP)- or obstructive jaundice (OJ)-induced heart injury. Methods: A total of 288 rats were used for SAP- (n=108) and OJ-associated (n=180) experiments. The rats were randomly divided into sham-operated, model control, and *Salvia miltiorrhizae*-treated groups. According to the difference of time points after operation, SAP rats in each group were subdivided into 3, 6 and 12 h subgroups (n=12), whereas OJ rats were subdivided into 7, 14, 21, and 28 d subgroups (n=15). At the corresponding time points after operation, the mortality rates of the rats, the contents of endotoxin and phospholipase A_2 (PLA_2) in blood, and pathological changes of the hearts were investigated. Results: The numbers of dead SAP and OJ rats in the treated groups declined as compared with those in the model control group, but not significantly (P>0.05). The contents of endotoxin (at 6 and 12 h in SAP rats and on 7, 14, 21, and 28 d in OJ rats, respectively) and PLA_2 (at 6 and 12 h in SAP rats and on 28 d in OJ rats, respectively) in the treated group were significantly lower than those in the model control group (P<0.01 and P<0.001, respectively). Besides, myocardial pathological injuries were mitigated in SAP and OJ rats. Conclusion: In this study, we found that *Salvia miltiorrhizae* improved myocardial pathological changes, reduced the content of PLA_2 in blood, and decreased the mortality rates of SAP and OJ rats, exerting protective effects on the hearts of the rats.

Key words: *Salvia miltiorrhizae*, Traditional Chinese medicine, Severe acute pancreatitis (SAP), Obstructive jaundice (OJ), Heart
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INTRODUCTION

Since severe acute pancreatitis (SAP) and obstructive jaundice (OJ) are common diseases, the study on their pathogenesis and treatment has been a hot spot in clinic for a long time (Fuentes-Orozco *et al*., 2008; Gencay *et al*., 2008; Morioka *et al*., 2008; Muñoz-Castañeda *et al*., 2008; Tsuyuguchi *et al*., 2008). SAP is a systemic disease resulted from pancreatic self-digestion and characterized by pancreatic necrosis. It can induce vascular leakage, shock, systemic inflammatory response syndrome (SIRS), and even multiple organ dysfunction (MODS) (Rau *et al*., 2006; Zhang Q. *et al*., 2001; Zhang X.P. *et al*., 2007c). Although SAP has many complications and a higher mortality rate, its underlying mechanisms have not been fully clarified yet (Garcea *et al*., 2006; Granger and Remick, 2005; Lytras *et al*., 2008; Yousaf *et al*., 2003). As studies on SIRS and MODS deepen, the SAP with the complication of heart injury has attracted more attention. Among all extrapancreatic

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organ injuries, heart injury is relatively seldom studied though cardiac decompensation is one of the complications that are responsible for the high mortality rate of SAP (Zhang *et al*., 2006a). The SAP-induced heart injury includes changes in heart function, such as cardiac arrhythmia, cardiogenic shock, toxic myocarditis, pericarditis, myocardial infarction (Zhang and Chen, 2006; Zhang *et al*., 2007d). The heart injury in OJ is also relatively seldom studied (Assimakopoulos *et al*., 2008; Wang *et al*., 2002).

At present, effective drugs for heart injury in SAP and OJ are lacking, and have been investigated by few studies (Celebi *et al*., 2008; Zhang *et al*., 2006b). The development and utilization of traditional Chinese medicine show good prospects in the therapy of SAP, since it has advantages of low cost, more extensive pharmacological effects, and fewer side effects. *Salvia miltiorrhizae* (Danshen) injection is one of the traditional Chinese medicines for SAP. The raw material of *Salvia miltiorrhizae* injection is the root of wild *Salvia miltiorrhizae*. Its main active ingredients include water-soluble substances such as tanshinol and salvianic acid, as well as fat-soluble substances such as tanshinone and dihydrotanshinone. These water-soluble substances play a dominant role in the pharmaceutical effects of *Salvia miltiorrhizae* injection, which can improve microcirculation, scavenge oxygen free radicals, regulate the metabolism of inflammatory lipid mediators, reduce the levels of endotoxin, protect intestinal mucosa, and decrease the levels of inflammatory mediators (Zhang *et al*., 2006b; Zhang and Liu, 2006; Zhang and Li, 2005). Some scholars believe that *Salvia miltiorrhizae* can also enhance macrophage function, inhibit platelet aggregation, reduce blood viscosity, prevent microvascular coagulation, and relieve vascular spasm and occlusion (Han, 1999; Yang, 1997).

Endotoxin and phospholipase A_2 (PLA_2) are closely related with SAP- and OJ-induced heart injury via a complicated mechanism in which myocardial metabolism is affected and myocardial structure is destroyed. Endotoxin plays a crucial role in the development of heart injury in SAP and OJ (Zhang *et al*., 2006a; Zhang and Chen, 2006). When SAP occurs, endotoxin can activate cardiovascular endothelial cells, promote the release of a large number of cytokines by these cells, induce myocardial energy metabolism disorders, myocardial lipid peroxidation and increased production of oxygen free radicals, and thereby impair the function and structure of cardiovascular endothelial cells and myocardial cells (Geisler *et al*., 2005; Zhang *et al*., 2007e). When OJ occurs, endotoxin can directly or indirectly impair myocardial mitochondria, sarcoplasmic reticulum and contractile proteins, cause microcirculation disturbance, affect myocardial lipid metabolism, induce the release of vasoactive substances, influence renin-angiotensin-aldosterone axis, and alter cardiac hemodynamics. The synergistic effects of these factors can ultimately result in myocardial necrosis and heart failure.

Within a certain range, the levels of PLA_2 can sensitively reflect the extent of pancreatic injury and are therefore used as an important parameter in the diagnosis of SAP (Motoyoshi *et al*., 2006). PLA_2 is an important factor to mediate pancreatic and extrapancreatic injury. When SAP occurs, pancreatic lysosomes can release a large amount of PLA_2. Once being activated, PLA_2 can act upon myocardial mitochondria, affect the levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), alter myocardial ultrastructure (de Windt *et al*., 2001), and induce mitochondrial dysfunction. Mitochondrial damage can suppress the production of adenosine triphosphate (ATP), induce metabolic disorders (Zhang and Chen, 2006), and decrease myocardial cell function (Zhang *et al*., 2007e; Ju *et al*., 2003). In OJ, cytoplasmic free calcium overload is induced due to the presence of myocardial ischemia and hypoxia, reduction in ATP synthesis, ATP-dependent calcium pump disorders, and membrane lipid peroxidation damage. As a result, PLA_2 is activated. The activated PLA_2 can promote membrane phospholipid hydrolysis, induce membrane damage and myocardial dysfunction, and lead to heart failure (Wang *et al*., 2002). Some clinical studies have shown that *Salvia miltiorrhizae* has some favorable effects in the auxiliary treatment of SAP and OJ (Zhang *et al*., 2006b; Peng *et al*., 2001).

In the present study, we examined the pathological changes in the hearts of SAP and OJ rats and investigated the protective effects of *Salvia miltiorrhizae* on the hearts of SAP and OJ rats and the associated mechanisms by tissue microarray technology.

MATERIALS AND METHODS

Animals

A total of 288 healthy male Sprague-Dawley (SD) rats of clean grade, weighing 270~330 g, were provided by the Laboratory of Animal Research Center of Zhejiang Chinese Traditional Medical University, China. Sodium taurocholate and sodium pentobarbital were purchased from Sigma Corp., USA. *Salvia miltiorrhizae* injection (each 10-ml vial contains active components equivalent to 15 g of the original medicine) was purchased from Chiatai Qingchunbao Pharmaceutical Co., Ltd., China. Endotoxin enzyme-linked immunosorbent assay (ELISA) kit was purchased from Associates of Cape Cord, USA, and the calculation unit for content is EU/ml. The serum secretory PLA_2 enzyme assay ELISA kit was purchased from RδD System Ins, USA, and the calculation unit for content is U/ml.

Animal grouping

A hundred and eight rats were used for SAP-associated experiments and randomly divided into sham-operated, model control, and *Salvia miltiorrhizae*-treated groups (n=36). They were further randomly subdivided into 3, 6, and 12 h subgroups (n=12). Another 180 rats were utilized for OJ-associated experiments and randomly divided into sham-operated, model control, and *Salvia miltiorrhizae*-treated groups (n=60), and they were further randomly subdivided into 7, 14, 21, and 28 d subgroups (n=15). All groups were separated according to time duration after operation.

Preparation of SAP models and associated therapeutic regimen

The improved Aho (1980)'s method was used to prepare SAP rat models. The rats were anesthetized with an intraperitoneal injection of 25 mg/ml sodium pentobarbital (0.2 ml/100 g body weight), and the thigh skin was cut open to expose femoral vein and a transfusion passage was established, through which continuous infusion was maintained using a micro-infusion pump (1 ml/(h·100 g body weight)). Subsequently, a median abdominal wall incision was made to expose the duodenal papilla, and a No. 5 syringe needle was used to prick a small hole in the mesenteric avascular area. The epidural catheter was first inserted into the duodenal cavity via the hole, and then placed into the bile-pancreatic duct toward the direction of the papilla. The catheter head was temporarily clamped using a microvascular clamp, and another microvascular clamp was used to occlude the common bile duct at the confluence of hepatic ducts to prevent a backflow of injected drugs into the liver. 35 mg/ml sodium taurocholate (0.1 ml/100 g body weight) was transfused at a flow rate of 0.2 ml/min using a microinjection pump (produced by Zhejiang University, China). After completing the transfusion, microvascular forceps and epidural catheter were maintained for further 4 min and then removed. After suturing the hole in the lateral wall of the duodenum, the abdominal cavity was closed conventionally. The sham-operated group was performed just by moving the pancreas and duodenum after opening the abdominal cavity. Fifteen minutes after successful operation, a single dose of *Salvia miltiorrhizae* injection (0.4 ml/100 g body weight) (Yang *et al*., 2005; Zheng *et al*., 2007) was given via the femoral vein to rats in the treated group, whereas equal volume of physiological saline solution was used in the sham-operated and the model control groups. Continuous infusion of physiological saline solution using a microinjection pump was then maintained until the end of the 3-, 6-, and 12-h observation periods in the corresponding groups.

Preparation of OJ models and associated therapeutic regimen

After rats were anesthetized with an intraperitoneal injection of 25 mg/ml sodium pentobarbital (0.2 ml/100 g body weight), the abdominal cavity was opened to identify and dissociate the common bile duct along the hepatoduodenal ligament. For rats in the model control and the treated groups, the proximal end of the common bile duct was double-ligated with surgical threads, the common bile duct was cut off, and a layered suture of the abdominal wall was performed to close the abdominal cavity. For rats in the sham-operated group, the common bile duct was only dissociated but not ligated, and a layered suture of the abdominal wall was also performed to close the abdominal cavity. An intraperitoneal injection of *Salvia miltiorrhizae* at a dose of 0.2 ml/(d·100 g body weight) (Li *et al*., 2002) was given to rats in the treated group, whereas equal volume of physiological saline solution

was used in the sham-operated and the model control groups. Injection was maintained until the end of the 7-, 14-, 21-, and 28-d observation periods in the corresponding groups.

Test items and indexes

At the corresponding time points after operation, SAP and OJ rats were anesthetized with 25 mg/ml sodium pentobarbital and euthanized. Blood samples and heart specimens were collected. Light and electronic microscopic analyses were performed for the heart pathological changes of the rats. The contents of endotoxin in plasma and PLA_2 in serum were determined according to the manufacturer's instructions.

Statistical analysis

Normal data were expressed as mean±*SD*, whereas non-normal data were expressed as medians (interquartile range). Analysis of variance (ANOVA) and pairwise comparisons were used for normal data, whereas non-normal data were subjected to non-parametric test, among which Kruskal-Wallis *H* test was used for pairwise comparisons and Mann-Whitney *U* test for multiple comparisons. Yates' chi-square test (χ^2) was used for intergroup comparisons of mortality rates. Software SPSS 15.0 was used for all data analyses.

RESULTS

Mortality rate in SAP rats

In the model control group, 1 and 5 rats died at 3 and 12 h after the operation, respectively; 3 in the treated group died at 12 h; and no rats died in the remaining groups. There was no marked difference in mortality rate between the time points at 3 and 6 h, whereas at 12 h the mortality rate in the model control group was significantly higher than that in the sham-operated group (P=0.037).

Light and electron microscopic analyses in SAP rats

In the sham-operated group, the myocardial fibers under light microscopy were normal, and the ultramicrostructure of the heart under electron microscopy was also normal. In the model control group, no obvious gross pathological changes were observed. Under light microscopy, granular condensation and dissolution of the sarcoplasm were seen in extremely few myocardial fibers, and mild inflammatory cell infiltration appeared in the myocardium and epicardium. Under electron microscopy, intercalated disk widening, mitochondrial swelling, and the rupture of mitochondrial cristae were seen. Myocardial cell apoptosis and the disappearance of mitochondrial cristae were also observed (Fig.1).

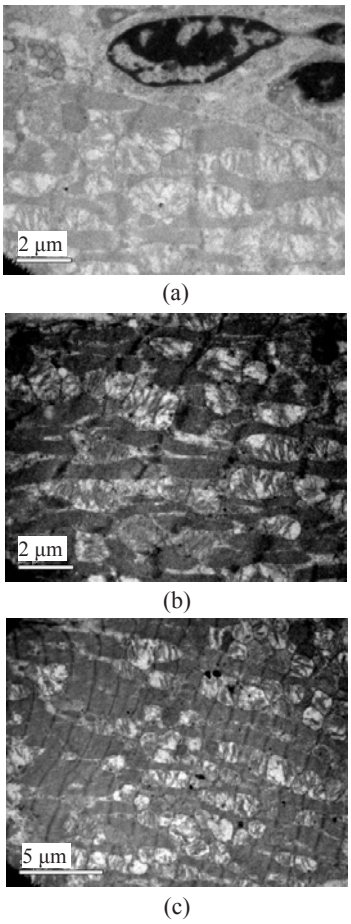
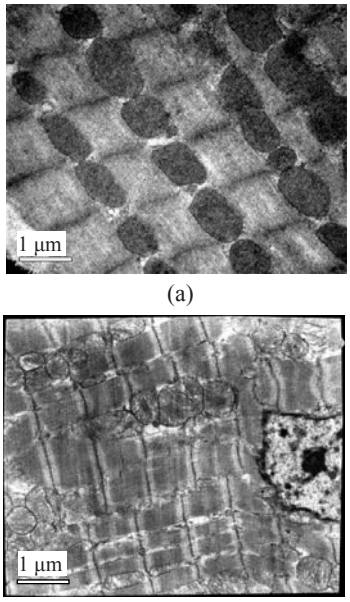


Fig.1 Electron microscopic analyses of the model control SAP group at 12 h after operation

(a) Apoptosis of myocardial cells, dissolution of mitochondrial cristae as well as swelling and vacuolation of some mitochondria; (b) Disordered arrangement of myofilaments and sarcomeres; (c) Mictochondrial swelling and vacuolation as well as disordered arrangement of myofilaments and sarcomeres

In the *Salvia miltiorrhizae*-treated group, under light microscopy, granular condensation and dissolution of the sarcoplasm were seen in extremely few cardiac fibers, and mild inflammatory cell infiltration

was observed in the myocardium and epicardium. There was no marked difference between the treated group and the model control group. Under electron microscopy, myofilament space was widened and myofilaments were arranged disorderly. Myocardial cells were roughly normal and contained abundant mitochondria. The ultramicrostructure changes were significantly more improved in the treated group than those in the model control group (Fig.2).



(a)

(b)

Fig.2 Electron microscopic analyses of the *Salvia miltiorrhizae*-treated SAP group at 12 h after operation
(a) Ordered arrangement of myofilaments, sarcomeres and mitochondria; (b) Disordered arrangement of myocardial fibers, clear sarcomeres, abundant mitochondria, and intact structure

Plasma endotoxin in SAP rats

At all time points after operation, the contents of plasma endotoxin in the model control group were significantly higher than those in the sham-operated group (P<0.001); at 3 and 12 h, the endotoxin contents in the treated group were significantly higher than those in the sham-operated group (P<0.01); at 6 and 12 h, the endotoxin contents in the treated group were significantly lower than those in the model control group (P<0.01) (Table 1).

Serum PLA_2 in SAP rats

At all time points, the contents of serum PLA_2 in the model control group and the treated group were significantly higher than those in the sham-operated group (P<0.001); at 6 and 12 h, the PLA_2 contents in the treated group were significantly lower than those in the model control group (P<0.01) (Table 1).

Table 1 Comparison of endotoxin and PLA_2 levels among the different SAP groups

Groups	Endotoxin content (EU/ml)	PLA_2 content (U/ml)
Sham-operated group		
3 h	0.3±0.5	31.5±10.1
6 h	0.3±0.2	40.4±12.1
12 h	0.3±0.1	46.0±12.7
Model control group		
3 h	$0.5\pm0.1^{**}$	$97.0\pm33.4^{**}$
6 h	$0.5\pm0.2^{**}$	$117.7\pm35.3^{**}$
12 h	$0.6\pm0.1^{**}$	$126.3\pm15.7^{**}$
Treated group		
3 h	$0.4\pm0.1^{*}$	$95.1\pm22.9^{**}$
6 h	$0.4\pm0.1^{\#\#}$	$87.2\pm24.9^{**\#}$
12 h	$0.4\pm0.1^{*\#\#}$	$87.5\pm27.8^{**\#}$

All data are expressed as mean±SD; $^{*}P$<0.01 and $^{**}P$<0.001 vs the sham-operated group; $^{\#}P$<0.01 and $^{\#\#}P$<0.001 vs the model control group

Mortality rate in OJ rats

In the model control group, 2, 4, 4, and 7 rats died on 7, 14, 21, and 28 d, respectively; 3, 2 and 4 rats died in the treated group on 14, 21, and 28 d, respectively. The mortality rates on 7 d showed no marked difference among the three experimental groups; On 14 and 21 d, the mortality rates in the sham-operated group were significantly lower than those in the model control group (P=0.032); On 28 d, the mortality rate in the sham-operated group was significantly lower than that in both the model control group (P=0.006) and the treated group (P=0.032).

Light and electron microscopic analyses in OJ rats

In the sham-operated group, under light microscopy, at all time points after operation, no significant difference in myocardial alterations was noted; the striations of some myocardial fibers were obscure, and sarcoplasmic condensation and dissolution were seen in few myocardial cells. On 7 d, the striations of some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in some myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 14 d, the striations of some

myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in few myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 21 and 28 d, the striations of some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in some myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. The ultramicrostructure of the heart under electron microscopy was normal.

In the model control group, under light microscopy, at all time points after operation, no significant difference in myocardial alterations was noted; sarcoplasmic condensation and dissolution present in few myocardial cells were aggravated with the prolongation of time. On 7 d, some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in few myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 14 d, some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in few peripheral myocardial cells; focal dissolution, necrosis, and hemorrhage were seen in extremely few myocardial fibers; sub-epicardial and myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 21 d, the striations of some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in some myocardial cells; myocardial capillaries showed mild congestion; mild adipose tissue infiltration and inflammatory cell infiltration were seen. On 28 d, some myocardial cells showed turbidity and swelling; the striations of myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in few myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. Under electron microscopy, mitochondrial swelling, vacuolation of some mitochondria, disappearance of mitochondrial cristae, increased number of apoptotic myocardial cells, and disordered arrangement of myofilaments and sarcomeres as well as myofilament disruption in some rats were observed (Fig.3).

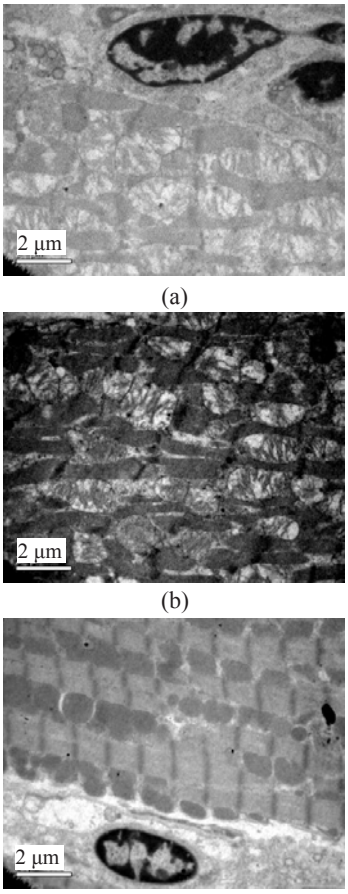
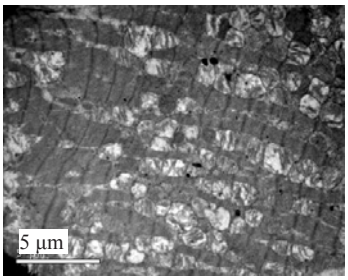


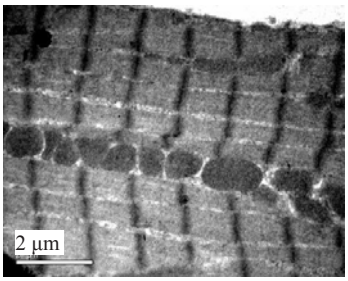
Fig.3 Electron microscopic analyses of the model control OJ group on 28 d after operation
(a) Apoptosis of myocardial cells, dissolution of mitochondrial cristae as well as swelling and vacuolation of some mitochondria; (b) Disordered arrangement of myofilaments and sarcomeres; (c) Apoptosis of myocardial cells and disappearance of mitochondrial cristae

In the treated group, under light microscopy, at all time points after operation, no significant difference in myocardial alterations was noted. On 7 d, the cytoplasm of few myocardial cells was obscure; sarcoplasmic condensation and dissolution were seen in few myocardial cells; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 14 d, the structure of some sub-epicardial myocardial fibers was unclear; sarcoplasmic condensation and dissolution were seen in few myocardial cells; myocardial interstitium was widened; myocardial capillaries showed mild congestion; mild inflammatory cell infiltration was seen. On 21 d, the striations of some myocardial fibers were obscure; sarcoplasmic condensation and dissolution were seen in few myocardial cells; the structure of few myocardial cells was unclear; myocardial capillaries showed mild congestion; mild adipose tissue

infiltration and inflammatory cell infiltration were seen. On 28 d, the structure of some sub-epicardial myocardial fibers was unclear; sarcoplasmic condensation and dissolution were seen in some myocardial cells; myocardial interstitium was widened; myocardial capillaries showed mild congestion; mild adipose tissue infiltration and inflammatory cell infiltration were seen. Under light microscopy, myocardial pathological alterations on 14, 21, and 28 d after operation in the treated group showed varying degrees of mitigation as compared with those in the model control group. Under electron microscopy, in the treated group, mitochondrial swelling was lessened, the number of apoptotic myocardial cells decreased, the cristae of few mitochondria disappeared, and few myofilaments and sarcomeres were arranged disorderly. The overall pathological alterations in the treated group were improved as compared with those in the model control group (Fig.4).



(a)



(b)

Fig.4 Electron microscopic analyses of the *Salvia miltiorrhizae*-treated OJ group on 28 d after operation
(a) Mictochondrial swelling and disordered arrangement of myofilaments and sarcomeres; (b) Normal structure of myocardium

Plasma endotoxin in OJ rats

At all time points after operation, the contents of plasma endotoxin in the sham-operated group were significantly lower than those in the model control group and the treated group, and the contents of plasma endotoxin in the treated group were significantly lower than those in the model control group (P<0.01) (Table 2).

Table 2 Comparison of endotoxin and PLA_2 levels among the different OJ groups

	Endotoxin content (EU/ml)	PLA_2 content (U/ml)
Sham-operated group		
7 d	0.2±0.3	208.1±26.6
14 d	0.2±0.4	198.5±46.3
21 d	0.2±0.05	207.2±38.6
28 d	0.3±0.04	203.0±31.0
Model control group		
7 d	0.5±0.1*	570.3±239.6
14 d	0.6±0.1*	653.5±208.9*
21 d	0.7±0.1*	701.0±151.7*
28 d	0.8±0.1*	738.6±60.2*
Treated group		
7 d	0.3±0.1$^{*\#}$	514.4±104.3
14 d	0.5±0.1$^{*\#}$	577.7±129.5*
21 d	0.5±0.1$^{*\#}$	593.2±146.3*
28 d	0.5±0.1$^{*\#}$	645.6±95.1$^{*\#}$

All data are expressed as mean±SD; $^{*}P$<0.01 and $^{**}P$<0.001 vs the sham-operated group; $^{\#}P$<0.01 and $^{\#\#}P$<0.001 vs the model control group

Serum PLA_2 in OJ rats

On 14, 21, and 28 d, the contents of serum PLA_2 in the sham-operated group were significantly lower than those in the model control group and the treated group (P<0.01); on 28 d, the PLA_2 content in the treated group was significantly lower than that in the model control group (P<0.01) (Table 2).

DISCUSSION

With the increase in the incidence of SAP and OJ, heart injuries in SAP and OJ have attracted more attention. Some researchers have proposed new concepts, such as "pancreatic-cardiac syndrome" and "jaundiced heart", to describe these diseases (Wang *et al*., 2002; Guo *et al*., 2001). At present, the drugs that show specific protective effects on heart injury in SAP or OJ are lacking. Moreover, the pharmacological effects of conventional myocardial protective drugs, such as dopamine (an adrenergic receptor stimulant), amrinone (a phosphodiesterase inhibitor), metaprolol (a β-blocker), spironolactone (an aldosterone receptor

antagonist), are limited. In contrast, *Salvia miltiorrhizae* exerts therapeutic effectiveness on SAP and OJ in many aspects, since it possesses a variety of pharmacological effects that can work together (Zhang and Liu, 2006; Zhang and Li, 2005).

Through comparing myocardial pathological alterations, we found that varying degrees of myocardial damage were present in SAP and OJ rats in the model control group. After treatment with *Salvia miltiorrhizae*, myocardial pathological alterations under electron microscopy in SAP rats were obviously mitigated, whereas those under light and electron microscopies in OJ rats were also improved, suggesting that *Salvia miltiorrhizae* can improve myocardial pathological alterations and prevent heart injury in SAP and OJ rats. Additionally, we found that the mortality rates of SAP and OJ rats in the model control group were almost two times high as those in the treated group, suggesting that *Salvia miltiorrhizae* injection can indeed reduce the mortality rate of rats. However, no statistically significant difference in the mortality rate of rats was observed, which may be due to the limited sample size. The results of this study show that the contents of plasma endotoxin in SAP and OJ rats in the model control group were significantly higher than those in the treated group, indicating that SAP and OJ can increase the contents of plasma endotoxin in rats. We believe that SAP and OJ can lead to an increase in the contents of endotoxin not only through causing intestinal mucosal barrier dysfunction and dissolving of bacterial cell wall (Zhang *et al*., 2007b), but also through inducing hepatic injury decreasing in phagocytic clearance function of Kupffer cells (Zhang *et al*., 2007a).

After treatment with *Salvia miltiorrhizae*, the contents of plasma endotoxin in SAP and OJ rats in the treated group were significantly lower than those in the model control group, indicating that *Salvia miltiorrhizae* can effectively lower the levels of plasma endotoxin. We speculate that this effect may be associated with hepatic injury and intestinal mucosal barrier dysfunction in SAP and OJ, which has been proved in our previous studies (Zhang *et al*., 2008a; 2008b). On one hand, *Salvia miltiorrhizae* has protective effects on the liver, since it can enhance the phagocytic clearance function of Kupffer cells, decrease the levels of inflammatory mediators, and scavenge free radicals. On the other hand, *Salvia miltiorrhizae* can protect intestinal mucosa barrier and inhibit the invasion of intestinal flora into blood. The synergistic action of these two factors can eventually antagonize the effects of endotoxin.

The results of this study show that the contents of PLA_2 in SAP and OJ rats in the model control group were significantly higher than those in the sham-operated group. After the treatment with *Salvia miltiorrhizae*, the contents of PLA_2 in the treated group were significantly lower than those in the model control group, indicating that *Salvia miltiorrhizae* can effectively reduce the contents of PLA_2. These results suggest that *Salvia miltiorrhizae* effectively inhibited the release of PLA_2 from pancreatic lysosomes in SAP rats as well as effectively enhanced calcium efflux and mitigated cytoplasmic free calcium overload in OJ rats.

We conclude that *Salvia miltiorrhizae* exerted protective effects on the hearts of SAP and OJ rats through lowering the levels of endotoxin and PLA_2 as well as improving myocardial damage. Besides, *Salvia miltiorrhizae* also improved the survival rates of SAP and OJ rats. Therefore, the application of *Salvia miltiorrhizae* for treating SAP and OJ deserves further attention and in-depth studies.

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