Preventive Effects of a Traditional Chinese Medicine (Sho-saiko-to) on Septic Shock Symptoms; Approached from Heme Metabolic Disorders in Endotoxemia

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Sho-saiko-to, one of the most frequently prescribed Kampo medicines, is used to treat chronic hepatitis and has shown confirmed clinical efficacy. The present study was performed with respect to heme metabolism to study the preventive effects of Sho-saiko-to against endotoxemia. Endotoxin was injected intraperitoneally at a dose of 6 mg/kg into Sho-saiko-to (500 mg/kg/d, p.o.)-pretreated rats, and its administration clearly prevented the endotoxin-induced hypoferremia. In rats pretreated with Sho-saiko-to, the activity of hepatic δ-aminolevulinate synthetase and cytochrome P-450 level 18 h after endotoxin injection were significantly increased as compared to rats treated with endotoxin alone. Similarly, Sho-saiko-to significantly depressed the endotoxin-induced increase in heme oxygenase activity in liver microsomes. These findings suggested that the extent of shock syndrome caused by endotoxin may be due, at least in part, to changes in heme metabolic disturbance during endotoxemia. Sho-saiko-to may therefore protect rats against lethality caused by endotoxin through its ability to regulate the heme metabolism in septic shock.

Key words Kampo medicine; Sho-saiko-to; septic shock, endotoxin; heme metabolic disturbance; preventive effect

Despite the remarkable progress in clinical medicine, sepsis and shock continue to be major clinical problems in intensive care units. Sepsis is the leading cause of death in critically ill patients in the U.S.A. developing in 750000 people annually, and more than 210000 of them die. 1) Septic shock may be associated with a toxic state initiated by the stimulation of monocytes by bacterial toxins such as endotoxins, which are released into the bloodstream. Macrophages stimulated by microorganisms or their toxins show induction of a variety of biologically active mediators known as cytokines, and tumor necrosis factor (TNF-α) is recognized as an important mediator in the development of endotoxicity. In addition, TNF-α is considered to be a major early mediator in the systemic inflammatory response syndrome observed during gram-negative sepsis. 2) Kampo medicines involve a system of drug therapy developed from clinical experience accumulated over some thousands of years in China. Sho-saiko-to has been used to treat various inflammatory diseases including hepatitis and is currently one of the most important prescriptions in Kampo medicine in Japan. 3) We reported previously 4) that Sho-saiko-to improves endotoxin shock based on our series of studies on metabolic pharmacologic effects. We therefore suggested that Sho-saiko-to may protect animals from the severe shock syndrome induced by endotoxin. In addition, we suggested recently that Sho-saiko-to suppresses cytotoxicity or TNF-α production in macrophages treated with endotoxin and that it may be useful in improving septic shock symptoms. 5)

Heme oxygenase (HO) is the rate-limiting enzyme in the oxidative degradation of heme into bilirubin, iron, and carbon monoxide. HO-1 known as one of the HO-isoenzymes was reported to be induced in the liver and lung after administration of endotoxin in rodents, and it is believed to confer protection against endotoxin-induced oxidative injury. 6) At the vascular level, there is cooperation between nitric oxide (NO) and HO-1 in the control of vascular tone. 7) HO-1 and NO expression thus seem to be co-regulated. On the other hand, Wink et al. 8) suggested that NO may play a role in regulation of cytochrome P-450 (c.p. P-450) level in vivo. Recently, we suggested that endotoxin-induced NO production is involved in heme metabolic disturbances caused by endotoxin challenge. 9) Interestingly, we earlier reported 10) that Sho-saiko-to shows a suppressive effect on NO generation in macrophages stimulated with endotoxin or endotoxin plus recombinant human TNF-α (rhTNF-α). Therefore, based on the current information regarding responses to endotoxin, we designed the following experiments to investigate whether Sho-saiko-to is involved in attenuation of the heme metabolic disturbance caused by endotoxin challenge.

MATERIALS AND METHODS

Materials Male Wistar rats, 7—8 weeks, each weighing 200 to 250 g, were purchased from Japan SLC Inc. (Hamamatsu, Japan) and maintained at the Tohoku Pharmaceutical University Experimental Animal Center for at least one week before the experiment. They were housed in air-conditioned rooms at 23 ± 1 °C with a 12 h light/dark cycle. Salmonella typhimurium lipopolysaccharide (endotoxin, Westphal preparation obtained from Difco Laboratories, Detroit, MI, U.S.A.) was used throughout this study. The traditional Chinese preparation Sho-saiko-to was obtained from Tsumura Co. (Tokyo, Japan). Tsumura Sho-saiko-to (crude powder extract, TJ-9) contains spray-dried aqueous extracts of seven crude drugs in the following amounts: Bupleuri Radix 7.0 g, Pinelliae Tuber 5.0 g, Scutellariae Radix 3.0 g, Zizyphi Fructus 3.0 g, Ginseng Radix 3.0 g, Glycyrrhizae Radix 2.0 g, and Zingiberis Rhizoma 1.0 g. Sho-saiko-to was suspended in distilled water and given to rats at a dose of 500 mg/kg through a stomach catheter once per day for 5 d. On the 6th day, rats were divided into four groups of five rats each. Rats in Group A (control) were injected i.p. with sterile saline

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alone. Those in Group B (endotoxin alone) were injected with 6 mg/kg of endotoxin. Those in Group C (Sho-saiko-to alone) were injected i.p. with sterile saline, and Sho-saiko-to pretreated rats in Group D (endotoxin/Sho-saiko-to) were injected i.p. with 6 mg/kg of endotoxin. Animals were killed by decapitation at defined time points under light anesthesia with ethyl ether.

Biochemical Determinations  Serum iron level was estimated colorimetrically using Fe-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Livers were excised with saline and homogenized in 3 vol. of 0.1 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose (20% w/v). Microsomes were prepared by the method of Kato and Takayanagi. Microsomal cyt. P-450 content was determined from the carbon monoxide-difference spectrum (450—490 nm) using an extinction coefficient of 91 mM⁻¹ cm⁻¹ as described by Omura and Sato. The activity of HO in microsomes was measured spectrophotometrically as the level of bilirubin formation. The activity of δ-ALA synthetase was assayed by the method of Marver et al. using total liver homogenate as the enzyme source. Protein contents were determined by the method of Lowry et al.

Statistical Analysis  Data are expressed as the mean±S.E. Statistical significance was evaluated using Student’s t-test. Differences with p values of less than 0.05 were regarded as significant.

RESULTS

Preventive Effects of Sho-saiko-to on Serum Iron Levels and δ-ALA Synthetase Activity in Endotoxin-Poisoned Rats  Heme metabolism may be linked to iron level, therefore this experiment examined the effect of administration of Sho-saiko-to on serum iron level and δ-ALA synthetase activity in the liver of rats 18 h after endotoxin (6 mg/kg, i.p.) injection. As shown in Fig. 1a, hypoferremia was found 18 h after administration of endotoxin to rats, but Sho-saiko-to showed a clear preventive effect against endotoxin-induced hypoferremia. Similarly, as shown in Fig. 1b, activity of δ-ALA synthetase in the liver of rats given endotoxin alone was markedly lower than that in the control, while the depression of activity was markedly inhibited by pretreatment with Sho-saiko-to.

Effects of Sho-saiko-to Challenge on HO Activity and Cyt. P-450 Level in Liver Microsomes of Rats Given Endotoxin  As noted above, Sho-saiko-to protected against hypoferremia and decrease of δ-ALA synthetase activity in liver in endotoxin-poisoned rats. HO and δ-ALA synthetase are the rate-limiting enzymes in heme catabolism and synthesis, respectively. Therefore, we next examined the effects of Sho-saiko-to on HO activity and cyt. P-450 level in liver
microsomes 18 h after administration of endotoxin. As shown in Fig. 2a, HO activity was markedly increased in endotoxin-treated rats, while it showed a significant decrease in the liver of endotoxin/Sho-sai-to-toasted rats. In contrast, the content of cyt. P-450 in the endotoxin injected rat liver was markedly lower than that in the control, while the level in those treated with endotoxin/Sho-sai-to was higher than that in the animals given endotoxin alone (Fig. 2b). These findings suggested that the protective effect of Sho-sai-to against endotoxin-induced severe shock syndrome may involve heme metabolic regulation.

**DISCUSSION**

We reported recently that hypoferremia was found 6—24 h after administration of endotoxin to rats, but after 2 d the level had returned to almost the normal range. These results were consistent with those described previously. The mechanism of the hypoferremia has been shown to involve altered processing of heme-derived iron within the reticuloendothelial system, which limits the supply of iron to the extracellular plasma transferring pool. In addition, the hypoferremia occurring in endotoxin-poisoned animals may result from an increase in iron uptake in hepatocytes. In the present study, when rats given Sho-sai-to were injected with endotoxin, the serum iron level was markedly higher in comparison to that in rats treated with endotoxin alone (Fig. 1a). Oxygen-derived free radicals, generated during reperfusion after ischemia or hypoxia or by activated neutrophils, are known to act as mediators of tissue injury. Free radical injury occurs via lipid peroxidation in a variety of disease processes including shock. We reported previously that endotoxin injection resulted in liver lipid peroxide formation and membrane damage in experimental animals, causing decreased levels of free radical scavengers or quenchers. In a previous study, we also observed that Sho-sai-to appears to protect the liver cell plasma membrane from injury by free radicals, which occurs in the state of tissue ischemia during endotoxemia. Iron not only participates in numerous biological processes but also plays a central role in oxidative stress as the major catalyst for hydroxyl radical formation via Fenton reaction. Miyahara and Tatsumi suggested that Sho-sai-to markedly inhibited iron-induced lipid peroxidation in microsomes and mitochondria in the rat liver, and also identified ginsenoside Rf and baicalin as being active. It is, therefore, of interest that the preventive effects of Sho-sai-to on endotoxin-induced oxidative injury in tissue are caused, at least in part, by inhibition of hypoferremia as described above.

Heme metabolism may be linked with iron levels. HO and \( \delta \)-ALA synthetase activities are the rate-limiting enzymes in heme catabolism and heme synthesis, respectively. In the present study, significantly decreases were observed in the hepatic \( \delta \)-ALA synthetase activity and cyt. P-450 levels in endotoxin-poisoned rats, but HO activity in the poisoned rats was higher than that in the control rats (Figs. 1b, 2a, b). It is, therefore, of interest that the degradation of hepatic cyt. P-450 heme is closely related to stimulation of HO activity and regulation of \( \delta \)-ALA synthetase activity in the liver in endotoxemia. As shown in Fig. 1b and Fig. 2a, \( \delta \)-ALA synthetase and HO activities in the liver showed significant recovery in endotoxin/Sho-sai-to-toasted rats. We also observed that the administration of Sho-sai-to prevented the depression of cyt. P-450 content in liver caused by endotoxin (Fig. 2b). Induction of cyt. P-450-linked monooxygenase activity in rat liver microsomes by Sho-sai-to was reported by Ohnishi et al. In addition, Kojima et al. reported increased cyt. P-450<sub>16</sub> mRNA expression in association with Sho-sai-to administration, suggesting that this may represent the molecular mechanism of the hemopreventive effect of Sho-sai-to.

Interestingly, NO has been used for many years as a spin-label probe to study the roles of heme groups in catalytic centers of cyt. P-450 enzymes. Consequently, cyt. P-450 dependent reactions were shown to be inhibited when microsomal preparations were exposed to NO. Similarly, Katsenke et al. reported that the degree of cyt. P-450 inhibition by endotoxin was directly correlated with plasma nitrite level, and that the NO synthase inhibitor \( \delta \)-nitro-L-arginine methyl ester significantly prevented the inhibition of cyt. P-450 function by endotoxin. On the other hand, Takahashi et al. reported that HO-1 may represent an important response to NO or NO-related oxidative stress. Their findings strongly supported the suggestion that NO formation may play a significant role in cyt. P-450 inactivation or HO induction in liver caused by endotoxin. In addition, we recently reported that the extent of endotoxin-induced NO formation may be due, at least in part, to changes in heme metabolic regulation induced by endotoxin. Many studies have linked the production of NO to endotoxin-induced hypotension, vascular hyporesponsiveness and death, suggesting that excess production of NO plays an important role in the development of septic shock. In previous studies, we suggested that the Sho-sai-to suppresses NO generation in macrophage J774A.1 cells stimulated with endotoxin or rhTNF-\( \alpha \), and that it may be useful in improving endotoxin-induced shock symptoms. Judging from the protective effect of NO production in endotoxemia after challenge by Sho-sai-to in our own previous experiment as described above, we conjectured that this Kampo medicine may regulate the heme metabolism through its ability to inhibit NO induction in septic shock. Thus, it is possible that the preventive effects of Sho-sai-to against lethality caused by endotoxin may be at least partially due to change in heme metabolism regulation in septic shock.

**REFERENCES**