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Ameliorating effects of compounds derived from *Salvia miltiorrhiza* root extract on microcirculatory disturbance and target organ injury by ischemia and reperfusion

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Abstract

Ischemia and reperfusion (I/R) exerts multiple insults in microcirculation, frequently accompanied by endothelial cell injury, enhanced adhesion of leukocytes, macromolecular efflux, production of oxygen free radicals, and mast cell degranulation. Since the microcirculatory disturbance results in injury of organ involved, protection of organ after I/R is of great importance in clinic. *Salvia miltiorrhiza* root has long been used in Asian countries for clinical treatment of various microcirculatory disturbance-related diseases. This herbal drug contains many active water-soluble compounds, including protocatechuic aldehyde (PAl), 3,4-dihydroxyphenyl lactic acid (DLA) and salvianolic acid B (SalB). These compounds, as well as water-soluble fraction of *S. miltiorrhiza* root extract (SMRE), have an ability to scavenge peroxides and are able to inhibit the expression of adhesion molecules in vascular endothelium and leukocytes. Moreover, lipophilic compounds of SMRE also prevent the development of vascular damage; NADPH oxidase and platelet aggregation are inhibited by tanshinone IIA and tanshinone IIB, respectively, and the mast cell degranulation is blunted by cryptotanshinone and 15,16-dihydrotanshinone I. Thus, the water-soluble and lipophilic compounds of SMRE appear to improve the I/R-induced vascular damage multifactorially and synergically. This review will summarize the ameliorating effect of compounds derived from SMRE on microcirculatory disturbance and target organ injury after I/R and will provide a new perspective on remedy with multiple drugs.

Keywords: *Salvia miltiorrhiza* root extract; Ischemia and reperfusion; Organ injury; Antioxidation; Adhesion molecules; Mast cell degranulation; Platelet aggregation

Abbreviations: 15,16-DTsI, 15,16-dihydrotanshinone I; ALT, alanine aminotransferase; AP-1, activator protein-1; ARF, acute renal failure; AST, aminotransferase activities; CA, caffeic acid; CASMC, coronary artery smooth muscle cells; CTs, cryptotanshinone; CYP, cytochrome P450; DLA, 3,4-dihydroxyphenyl lactic acid; DPPH, 1.1-diphenyl-2-picrylhydrazyl; eNOS, endothelial nitric oxide synthase; ERK, extracellular-signal-regulated kinase; ET-1, endothelin-1; HAEC, human aortic endothelial cells; HASMC, human aortic smooth muscle cells; HO-1, hmooxygenase-1; HSC, hepatic stellate cells; HUVEC, human umbilical vein endothelial cells; I/R, ischemia and reperfusion; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; iNOS, inducible nitric oxide synthase; ITsII, isotanshinone II; ITsI, isotanshinone I; LDQs, lipophilic diterpenoid quinines; LPS, lipopolysaccharide; LsAA, lithospermic acid A; LsAB, lithospermic acid B; LsA, lithospermic acid; MDA, malondialdehyde; MLB, magnesium lithospermate B; NF-κB, nuclear factor-κB; NO, nitric oxide; $^{\bullet}O^{-}$, superoxide anion; PA, protocatechuic acid; PAl, protocatechuic aldehyde; PPAR-α, peroxisome proliferator-activated receptor alpha; RA, rosmarinic acid; ROS, reactive oxygen species; SalA, salvianolic acid A; SalB, salvianolic acid B; SMRE, *Salvia miltiorrhiza* root extract; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TsC, tanshindiol C; TsIIA, tanshinone IIA; TsIIB, tanshinone IIB; TsI, tanshinone I; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells; WSC, water-soluble compounds; WSF, water-soluble fraction.

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1. Introduction

Ischemia and reperfusion (I/R) occurs in a wide range of situations, including trauma, vascular reflow after contraction, precutaneous transluminal coronary angioplasty, thrombolysis treatment, organ transplantation, and hypovolemic shock with resuscitation. I/R exerts multiple insults in microcirculation, frequently accompanied by endothelial cell injury, enhanced adhesion of leukocytes, macromolecular efflux, production of oxygen free radicals and mast cell degranulation (Han et al., 2001). Thus, much effort has been made to attenuate the microcirculatory disturbance by ablating one of the insults in the pathogenetic process. Preliminary results demonstrated that pretreatment with antibodies against adhesion molecules inhibits I/R-induced leukocyte adhesion to postcapillary venules (Kurose et al., 1994a), pretreatment with antioxidants inhibits I/R-induced oxygen free radical production, leukocyte adhesion to postcapillary venules and mast cell degranulation (Han et al., 2001) and pretreatment with mast cell degranulation inhibitors attenuated I/R-induced microcirculatory injury (Kurose et al., 1997). However, the outcomes of these attempts have been far from satisfactory to date. This is deemed to attribute to the observation that the microcirculatory disturbance is a complicated pathologic process consisting of multiple and coordinated events, and once this process has been initiated, it can be interrupted only by a remedy of multiple compositions targeting respectively at different insults causing the microcirculatory disturbance.

For several decades, *Salvia miltiorrhiza* root (Labiatae, Laminaceae; Fig. 1) has been widely used in clinics in China,

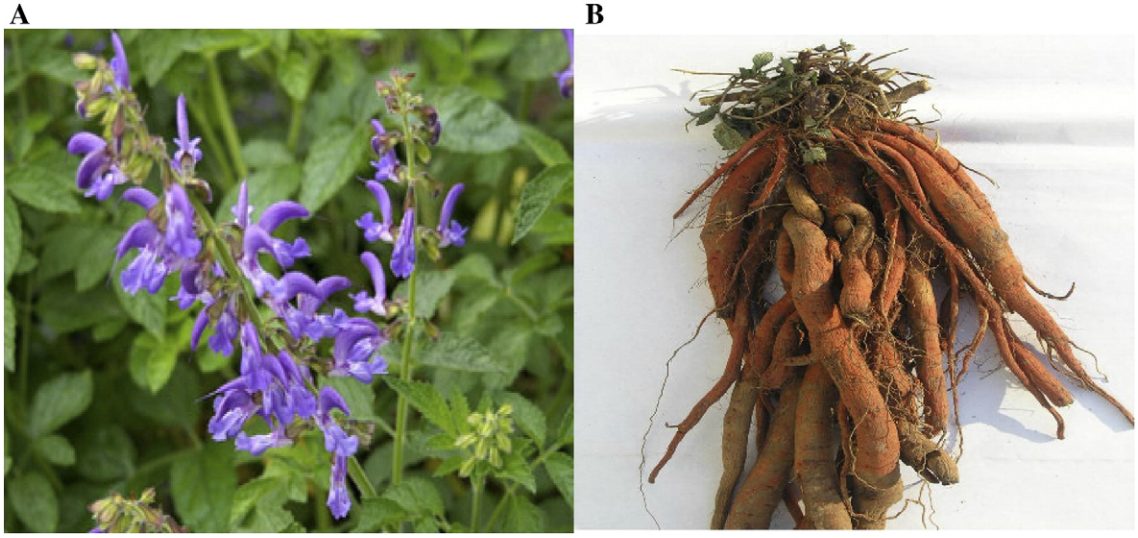

Fig. 1. Morphology of *S. miltiorrhiza*. (**A**) Portion above ground. (**B**) Roots for pharmaceutical use.

Korea, Japan and other Asian countries for the treatment of various microcirculatory disturbance-related diseases, such as cardiovascular disease, cerebrovascular disease, liver dysfunction, renal deficiency and diabetic vascular complication. Chemical constituents from *S. miltiorrhiza* root extract (SMRE) are classified into 2 major categories: water-soluble compounds (WSC) and lipophilic diterpenoid quinines (LDQ), the compounds of both have been mostly identified and purified (Gu et al., 2004; Hu et al., 2005; Shi et al., 2005; Liu et al., 2006; Lv & Yao, 2006; Yang et al., 2006; Zhou et al., 2006a). The WSCs of SMRE are mainly phenolic acid compounds, including single phenolic acids and polyphenolic acids. Single phenolic acids include protocatechuic aldehyde (PAl), protocatechuic acid (PA), caffeic acid (CA), and 3,4-dihydroxyphenyl lactic acid (DLA, also called danshensu), whereas polyphenolic acids include rosmarinic acid (RA), lithospermic acid (LsA), salvianolic acid A (SalA), salvianolic acid B (SalB) (Fig. 2), and other salvianolic acids. The major LDQs of SMRE are tanshinone I (TsI), tanshinone IIA (TsIIA), tanshinone IIB (TsIIB), cryptotanshinone (CTs), tanshindiol C (TsC), 15,16-dihydrotanshinone I (15,16-DTsI), isotanshinone I (ITsI), isotanshinone II (ITsII; Fig. 3) and other tanshinones. The in vivo metabolic pathways of some constitutes of the WSCs have been elucidated (Zhang et al., 2005; Wu et al., 2006).

The biological actions of the compounds isolated from SMRE have been clarified over the last few years, and several lines of evidence have been accumulated indicating the diversity of the potentials of SMRE in attenuating microcirculatory disturbance, including antioxidation (Zhao et al., 1996; Wu et al., 1998; Liu et al., 2001; Soung et al., 2003; Lin et al., 2006b), inhibition of expression of adhesion molecules (Chen et al., 2001c; Ren et al., 2002; Ding et al., 2005a; Ling et al., 2005; Sieveking et al., 2005; Zhou et al., 2005), inhibition of platelet aggregation (Wu et al., 1996), inhibition of mast cell degranulation (Ryu et al., 1999; Choi & Kim, 2004), inhibition of apoptosis (Nakazawa et al., 2005; Lee et al., 2006b), amelioration of the injury of target organs, such as the heart (Sun et al., 2005; Chang et al., 2006; Zhang et al., 2006), brain (Lo et al., 2003; Koo et al., 2004), liver (Xing et al., 2005; Lee et al., 2006a, 2006b; Wan et al., 2006), kidneys (Kang et al., 2004; Chen & Wang, 2006) and lungs (Chen et al., 2003). Therefore, SMRE has emerged as a candidate for improving microcirculatory disturbance by acting on multiple targets. Some reviews already exist concerning the major ingredients of SMRE and preclinical results (Ji et al., 2000; Wu et al., 2004; Jiang et al., 2005; Wang et al., 2006c). This review will focus on the ameliorating effects of compounds derived from SMRE on microcirculatory disturbance and target organ injury induced by I/R.

2. Effects of compounds derived from SMRE on pathogenesis of microcirculatory disturbance induced by ischemia and reperfusion

I/R leads to several injurious responses in microcirculation, such as an enhanced oxygen free radical production from endothelial cells (Granger, 1988), increased expression levels of L-selectin in leukocytes (Redlin et al., 2001; Wei et al., 2005) and E-selectin in endothelial cells (Russell et al., 2000; Cassie et al., 2004), causing the rolling of leukocytes (Kurose et al., 1994a; Arndt et al., 1995), and increased expression levels of CD11b/CD18 in leukocytes and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells (Harmon et al., 2004; Lan et al., 2004). These molecules enable leukocytes adhere to postcapillary venules (Kurose et al., 1994b), resulting in the release of oxygen free radicals (Salas et al., 1999), which subsequently damage the vascular endothelium (Han et al., 2001; Sievert, 2003). In addition, postcapillary venules also respond to I/R in the forms of mast cell degranulation (Kurose et al., 1998; Han et al., 2001, 2006) and vasoactive substance release (Frangogiannis et al., 1998), which have been implicated in the enhancement of leukocyte adhesion to postcapillary venules and albumin efflux (Kurose et al., 1997). Endothelial cell injury, leukocyte adhesion, platelet aggregation, release of oxygen radicals from the endothelium or leukocytes and mast cell degranulation after reperfusion are considered to be closely related and interplay in the process of microvasculatory injury induced by I/R. Thus, ameliorating I/R-induced microcirculatory disturbance may be achieved by various means, including scavenging peroxides, inhibiting adhesion molecule expression in leukocyte, platelet and vascular endothelium, impeding adhesion of leukocyte and platelet to vascular endothelium, alleviating mast cell degranulation and depressing release of inflammatory mediators.

2.1. Effects on endothelial cells

Increasing accumulation of evidence derived from experiments using endothelial cells demonstrates the antioxidizing ability of LDQs of SMRE. In this regard, TsIIA was found to decrease nitric oxide (NO) level and superoxide dismutase (SOD) activity in human umbilical vein endothelial cells (HUVEC; Lin et al., 2006b). Additionally, the function of antiperoxides or anti-LPO is observed in other LDQs, as well as WSCs of SMRE in other cell types. For example, SalA inhibits LPO of the brain by I/R and scavenges hydroxyl radicals in vitro (Du & Zhang, 1997). SalB was reported to scavenge 1,1-diphenyl-2-picrylhydrazyl potentially (DPPH), inhibit LPO, and eliminate reactive oxygen species (ROS) accumulated in primary rat hepatocytes and hepatic stellate cells (HSC; Lin et al., 2006c), inhibit ROS production and calcium influx in PC12 cells induced by β-amyloid peptide Aβ (25–35) (Lin et al., 2006a), scavenge hydrogen peroxide in a dose-dependent manner in vitro and inhibit tumor necrosis factor-α (TNF-α)-induced ROS production and NADPH oxidase activity in human aortic smooth muscle cells (HASMC; Zhang & Wang, 2006). LsAB is found to exert an antinitration effect by scavenging peroxynitrite, which has a structure of hydroxyl groups and double bonds (Soung et al., 2003). On the other hand, tanshinones, LDQs of SMRE, were shown to scavenge lipid free radicals generated from LPO of the myocardial mitochondrial membrane in I/R-induced injury of the rat heart (Zhao et al., 1996). DLA was reported to scavenge superoxide anion ($^{\bullet}O_2^-$) from the xanthine–xanthine oxidase system and protect the myocardial mitochondrial membrane from LPO in rat heart by I/R (Zhao et al., 1996).

The ability of DLA to scavenge peroxide is associated with occurrence of the structure 3,4-dihdroxyl in the

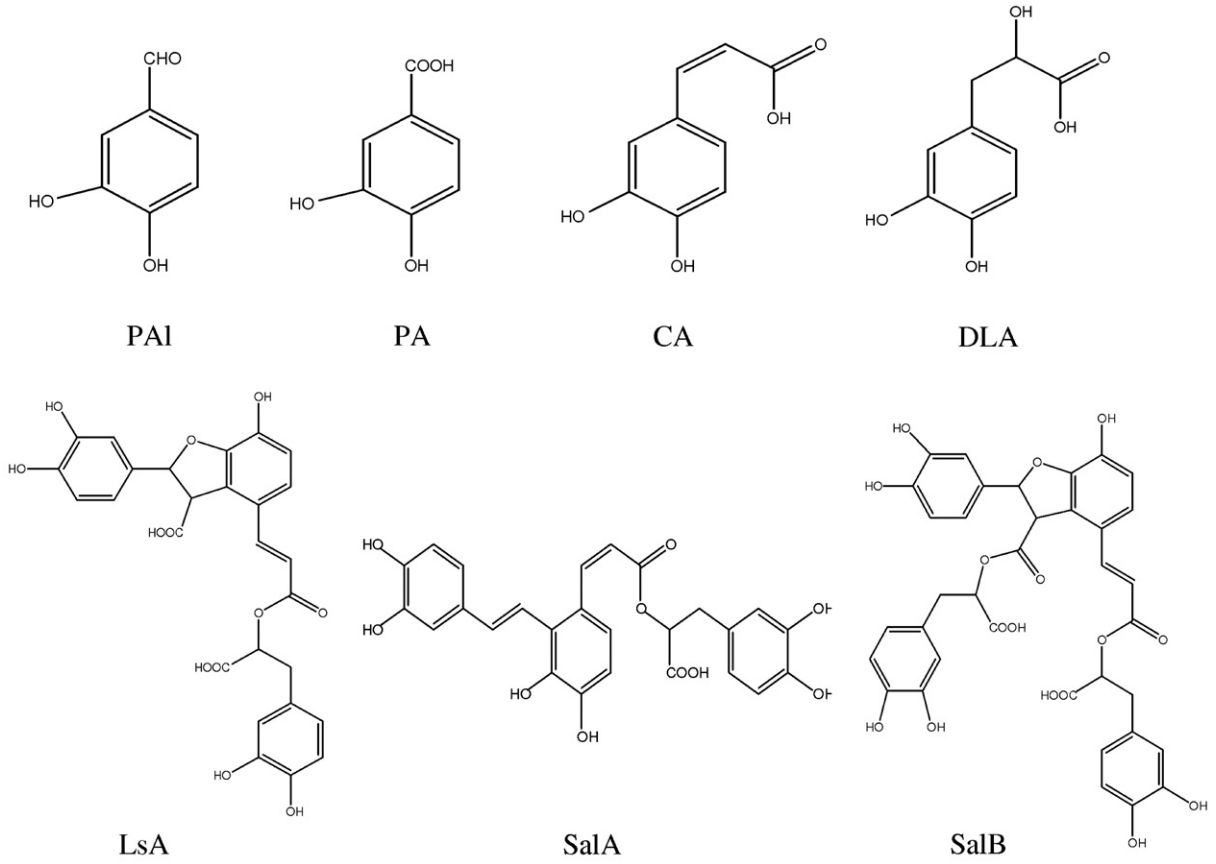

Fig. 2. Major water-soluble compounds derived of SMRE. Pal, protocatechuic aldehyde; PA, protocatechui acid; CA, caffeic acid; DLA, 3, 4-dihydroxyphenyl lactic acid (named as danshensu); LsA, lithospermic acid; SalA, salvianolic acid A; SalB, salvianolic acid B.

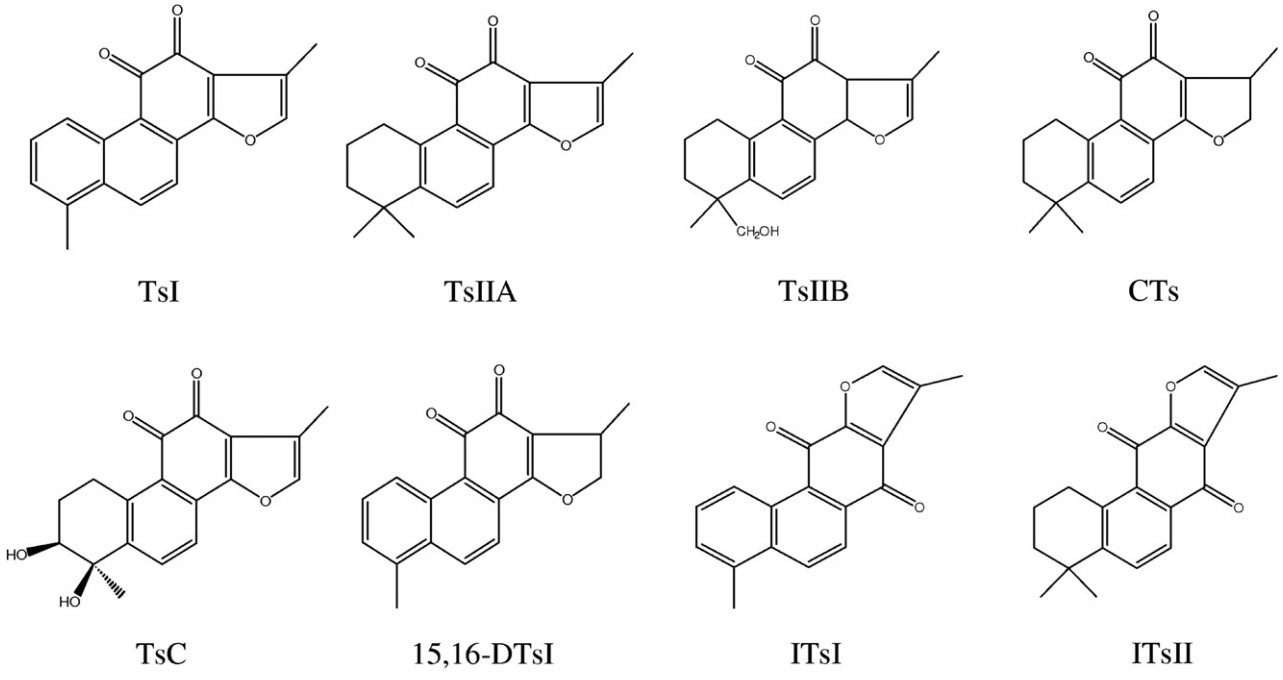

Fig. 3. Major lipophilic compounds of SMRE. TsI, tanshinone I; TsIIA, tanshinone IIA; TsIIB, tanshinone IIB; CTs, cryptotanshinone; TsC, tanshindiol C; 15, 16-DTsI, 15, 16-dihydrotanshinone I; ITsI, isotanshinone I; ITsII, isotanshinone II.

molecule, as evidenced by the fact that modification or deletion of dihdroxyl eliminates the peroxide scavenging potential of DLA (Chen et al., 1999). DLA exists in WSC of SMRE as a common structure, suggesting that DLA is indispensable for the capacity of WSC to scavenge peroxide.

I/R stimulate the production of peroxides from vascular endothelium through both xanthine and xanthine oxidase and NADPH oxidase, which is a crucial insult in I/R-induced microcirculatory disturbance. Scavenging peroxides not only alleviates DNA damage and LPO and reduces injury to endothelium and basement membrane, but also diminishes the degradation of inhibitor κB, the activation of nuclear factor-κB (NF-κB) and the nuclear translocation of P50 and P65, resulting in inhibition of expression of various adhesion molecules and inflammatory mediators, such as E-selectin, ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), TNF-α, interleukin-1β (IL-1β) and interleukin-6 (IL-6). Thus, interference with NF-κB pathway is reasoned to be one of the strategies to ameliorate I/R-induced microcirculatory disturbance.

The results regarding the effects of SMRE and its main compounds on E-selectin expression on vascular endothelial cells have been to date conflicting. Chen et al. (2001c) reported that water-soluble fraction (WSF) from SMRE and SalB inhibit the activation of NF-κB and do not affect the expression of endothelial cell E-selectin induced by TNF-α. Whereas, Ren et al. (2002) showed that WSF inhibit the expression of the adhesion molecule E-selectin.

On the other hand, conclusions concerning the effects of WSF and its main components on the expression of ICAM-1 and VCAM-1 on vascular endothelial cells almost agree with each other. To mention a few, WSF was reported to inhibit the following: the expression of adhesion molecules ICAM-1 and VCAM-1 on HUVECs induced by TNF-α (Ren et al., 2002) and the TNF-α-induced translocation of NF-κB from the cytoplasm to the nucleus (Ding et al., 2005a). PAl and SalB were reported to be able to inhibit TNF-α-induced expression of ICAM-1 and VCAM-1 on endothelial cells. PAl inhibits the expression of ICAM-1 and VCAM-1 and mRNA expression of ICAM-1 and VCAM-1 on HUVECs, as well as NF-κB and activator protein-1 (AP-1) DNA binding activities induced by TNF-α in a dose-dependent manner (Zhou et al., 2005). SalB was shown to attenuate VCAM-1 and ICAM-1 expression and to inhibit the activation of NF-κB in endothelial cells induced by TNF-α (Chen et al., 2001c).

Cardiotonic pills are Chinese medicines containing WSF as the major ingredients in addition to panax notoginseng and borneol, and one pill of cardiotonic pills (25 mg) includes 9 mg of WSF from SMRE, 1.76 mg of panax notoginseng, 0.5 mg of borneol and 13.74 mg of polyethylene glycol (Horie et al., 2005). It is currently used for the treatment of cardiovascular diseases related to microcirculatory disturbance. Cardiotonic pills were demonstrated to inhibit the TNF-α-induced expression of ICAM-1 and VCAM-1 on HUVECs in a dose-dependent manner and to inhibit the platelet-derived growth factor BB-induced DNA synthesis and cell proliferation in vascular smooth muscle cells, but not to affect the expression of adhesion molecules (Ling et al., 2005).

The secretion level of endothelin-1 (ET-1) increases after I/R, which binds to the ET-A receptor and subsequently decreases blood flow (Hassan et al., 1997; Wolfard et al., 1999; Fernandez et al., 2002). L-arginine-derived NO is enhanced by I/R and binds with $^{\bullet}O_2^-$ to generate peroxynitrite, which is an important mediator of cytotoxicity.

The effect of SMRE on ET-1 or NO has attracted much attention over the past few years. The data derived from experiments on animals suggested that WSF from SMRE decreased inducible NO synthase (iNOS) level but increased endothelial NO synthase (eNOS) expression level in the small pulmonary arteries of rats (Chen et al., 2003). The LDQs of SMRE, including TsIIA and CTs, were reported to exert effects on ET-1 or NO. TsIIA was reported to inhibit markedly the production of NO and suppress the expression of iNOS in a dose-dependent manner in activated RAW 264.7 cells (Jang et al., 2003). Zhou et al. provided evidence that CTs inhibit basal and TNF-α-stimulated ET-1 secretion in a concentration-dependent manner and also increase basal and TNF-α-attenuated NO production. CTs induce a concentration-dependent increase in eNOS expression level without significantly changing neuronal NO synthase expression in HUVECs regardless of the presence or absence of TNF-α. The decrease in ET-1 expressing levels in response to CTs is not reversed by the NO synthase inhibitor N^G-nitro-L-arginine methyl ester, whereas TNF-α-induced NF-κB activity is significantly decreased by CTs, suggesting that CTs inhibit ET-1 production, at least in part, through a mechanism that involves NF-κB without affecting NO production (Zhou et al., 2006b). Clinically, intravenous administration of a SMRE injection at 200 mg/kg body weight to children with congenital heart defects and pulmonary hypertension before cardiac surgery decreased ET-1 production during cardiac surgery (Xia et al., 2003).

It has been shown that I/R-induced apoptosis is mediated by multiple factors, including intracellular Ca^{2+} overload and peroxide that initiate the imbalance of Bc12 and Bax proportion and activation of death protein caspase-3 (Takuma et al., 1999; Zingarelli et al., 2002; Glanemann et al., 2004; Lopez-Neblina et al., 2005). WSF from SMRE, DLA, PA and PAl were reported to protect against endothelial cell damage caused by homocystein (Chan et al., 2004) and to abolish oxysterol-induced endothelial cell apoptosis in vitro and in vivo (Nakazawa et al., 2005). On the other hand, it is reported that SMRE exhibits a potent cytotoxicity, inducing cell death; thus, the potential of SMRE in the treatment of cancer was suggested. For example, TsIIA was reported to mediate cytotoxicity in human endothelial cells that may occur through the activation of quinine oxidoreductase, which induces a Ca^{2+} imbalance (increase in intracellular Ca^{2+} concentration), release of cytochrome *c*, thus causing a loss of mitochondrial membrane potential and resulting in the subsequent activation of caspases (Yang et al., 2005).

Physiologically, the venular wall is almost impermeable to macromolecules, such as albumin. I/R induces a surge in the concentrations of oxygen free radicals, cytokines, and protease generated from vascular endothelial cells and leukocytes, which attack and damage vascular endothelial cells and basement

membrane from inside (Granger, 1988; Zimmerman et al., 1988; Granger et al., 1993; Hastie et al., 1997; Chen et al., 2001b; Han et al., 2001). Moreover, mast cells around venules and capillaries degranulate in response to I/R, releasing proinflammatory mediators, such as platelet-activating factor, TNF-α, IL-1β, prostaglandins, and vasoactive substances like histamine (Tsinkalovsky & Laerum, 1994; Frangogiannis et al., 1998; Kimura et al., 1998; Rocha et al., 2003), which increase the permeability of venules or capillaries and promote albumin leakage (Kurose et al., 1994a, 1994b, 1997; Han et al., 2001). Furthermore, vascular endothelial growth factor (VEGF) and mitogen-activation protein kinase were reported to participate in the process of endothelial cell injury through the phosphorylation of the VEGF receptor and different mitogen-activation protein kinase subgroups (e.g., p38, extracellular signal-regulated kinase (ERK) 1/2, and C-jun N-terminal kinase), and the inhibition of these pathways or expression of VEGF might offer a new treatment strategy for endothelial damage (Kusaka et al., 2004). The results regarding the effects of SMRE and its main compounds on VEGF are still controversial. Ding et al. (2005a) reported that DLA and SalB inhibit TNF-α-enhanced endothelial permeability, whereas PAl does not. They presented evidence that DLA and SalB inhibit VEGF expression and ERK activation in TNF-α-stimulated HUVECs. On the other hand, Lay et al. (2003) reported that extract of SMRE and SalB enhance angiogenic processes on endothelial cells through the up-regulation of VEGF and VEGF receptor genes.

Our in vivo investigation revealed that a SMRE injection ameliorates lipopolysaccharide (LPS)-induced injury to endothelium of mesenteric venule of rat, reducing peroxide generation from and leukocyte adhesion to venular walls, as well as impeding albumin leakage (Han et al., 2007). However, no direct evidence is available to date regarding the protective effect of SMRE and its major compounds on I/R-induced injury of vascular endothelium.

2.2. Effects on leukocytes

Much evidence demonstrates that I/R enhances the expression of adhesion molecules of various types on leukocytes. Leukocyte adhesion to endothelial cells leads to an explosive release of peroxides (Zimmerman et al., 1988; Panes & Granger, 1996; Peake & Suzuki, 2004; Masztalerz et al., 2006). As a consequence of the explosive peroxide release and concomitant damage of DNA (Salvemini & Cuzzocrea, 2002; Morihira et al., 2006), endothelial cells, the vascular basement membrane and perivascular cells are injured (Zimmerman et al., 1988; Ar'Rajab et al., 1996; Reiter et al., 2004). Inhibiting the expression of L-selectin and CD11b/CD18 on leukocytes, leukocyte rolling and leukocyte adhesion to the vascular wall is considered as a promising measure to improve microcirculatory disturbance.

The protective effect of SMRE against leukocyte adhesion is suggested by several lines of evidence. To this end, PAl was shown to inhibit U937 (a human monocytic cell line) cell adhesion to HUVECs (Zhou et al., 2005). In addition, SalB and WSF from SMRE were found to inhibit the binding of U937 cells to human aortic endothelial cells (HAEC) stimulated by TNF-α (Chen et al., 2001c). The WSF were reported to inhibit the adhesion of *N*-formyl-methionyl-leucyl-phenylalanine-activated neutrophils to HUVECs (Ren et al., 2002), and inhibit the adhesion of HL-60 cells to endothelial cells induced by TNF-α (Ding et al., 2005b). A compound herb preparation containing SMRE was reported to suppress ICAM-1 expression and monocyte adhesion to endothelial cells (Sieveking et al., 2005). Our in vivo research also demonstrated that cardiotonic pills inhibit leukocyte adhesion to the liver induced by intestinal I/R (Horie et al., 2005). We have reported that DLA, SalB and a SMRE injection are able to ameliorate the expression of adhesion molecules CD11b and CD18 and the production of peroxides in leukocyte, and to inhibit leukocyte adhesion to mesenteric venular wall in rat challenged by LPS (Guo et al., 2007; Han et al., 2007). However, no study has been reported to elucidate the inhibitory effect of SMRE or its main ingredients on the expression of L-selectin and leukocyte rolling along the vascular wall induced by I/R stimulation either in vivo or in vitro.

2.3. Effects on platelets

Platelets, when challenged by I/R or other stimuli, increase the expression level of the adhesion molecule CD31 leading to its adhesion to vascular endothelial cells and forming a soft thrombus. Moreover, the formation of hard thrombi is initiated by the enhanced adhesion of platelets and promoted transformation of fibrinogen to fibrin. This notion is also supported by the clinical finding that P-selectin and CD31 expression levels increase in the serum of acute myocardial infarction patients (Gurbel et al., 1998; Serebruany et al., 1998).

Several studies have been conducted to clarify the effect of SMRE on platelet aggregation. In the early eighties of last century, Lee et al. (1987) reported that isotanshinnone IIB (ITsIIB) inhibits ADP- and collagen-induced platelet aggregation in vitro, and this finding was confirmed by succeeding works showing that WSF from SMRE inhibits platelet aggregation (Wang et al., 1989). This inhibitory effect was proposed to be related to several events, such as the inhibition of Ca^{2+} influx in platelets (Wu et al., 1996), an increase in the number of fibroblast cells in the G_0/G_1 phase and the attenuation of collagen secretion (Liu et al., 1992). Oleoyl neocryptotanshinone II and oleoyl danshenxinkun A were also reported to inhibit platelet aggregation induced by arachidonic acid in rabbits (Lin et al., 2001).

Our research proved that SMRE-containing cardiotonic pills can inhibit the formation of a thrombus in the rat mesentery induced by photochemical reaction. This function is related to their protective effect against the expression of the leukocyte adhesion molecules CD11 and CD18, as well as the platelet adhesion molecule CD31 (Wang et al., 2006b).

2.4. Inhibition of mast cell degranulation

The degranulation of perivascularly localized mast cells is one of the distinct events following I/R, which results in a

cascade of sequel, such as the release of proinflammatory mediators (Tsinkalovsky & Laerum, 1994; Frangogiannis et al., 1998; Kimura et al., 1998; Rocha et al., 2003), increases in the expression levels of the adhesion molecules ICAM-1 and VCAM-1 on endothelial cells and CD11b/CD18 on leukocytes (Frangogiannis et al., 1998), the enhancement of leukocyte adhesion to the vascular wall and the increase in the permeability of venules or capillaries resulting in albumin leakage (Kurose et al., 1994b, 1997). Thus, the inhibition of mast cell degranulation is considered as one of the most important strategies for ameliorating microcirculatory disturbance, and LDQs of SMRE has proved promising in this respect. For example, 15,16-DTsI was found to inhibit mast cell degranulation and Fcε R1 (high-affinity IgE receptor)-mediated tyrosine phosphorylation of ERK and phospholipase Cγ2 (Choi & Kim, 2004). It was reported that 15,16-DTsI and CTs inhibit mast cell degranulation and release of β-hexosaminidase from cultured mast cells (Ryu et al., 1999). Our study demonstrated that pretreatment with DLA, SalB or SMRE injection alleviates LPS-induced mast cell degranulation in mesentery of rat (Guo et al., 2007; Han et al., 2007). However, it is still unclear whether WSCs of SMRE are effective for inhibition of mast cell degranulation if it is administrated when microcirculatory disturbance has been initiated. Also unclear is the effect of WSCs on the mast cell degranulation induced by I/R.

2.5. Inhibition of cytokine production

I/R induces the release of multiple cytokines, including IL-1β, TNF-α and IL-6 from endothelial cells, leukocytes and mast cells (Engles et al., 1997; Raijmakers et al., 1997; Sutter et al., 1997; Mizutani et al., 2000; Rocha et al., 2003; Huda et al., 2004; Meldrum et al., 2005; Ustunsoy et al., 2006). These cytokines further promote the expression of adhesion molecules on endothelial cells or leukocytes (Ren et al., 2002; Gallova et al., 2004; Ding et al., 2005b), enhance leukocyte adhesion to the vascular wall, exacerbate microcirculatory disturbance and cause damage of endothelial cells and perivascular cells (Meldrum & Donnahoo, 1999; Pomerantz et al., 2001; Kim et al., 2002).

The effects of SMRE on cytokine production have been the focus of much attention. An experiment showed that injection of WSCs suppresses an anti-cardiolipin antibody induced by β2-glycoprotein I, which may be due to the restoration of high Th/Ts (T helper/inducer cells to T suppressor/cytotoxic cells) ratio and intorleukin-2 (IL-2) activity (Chen et al., 2001a). Various LDQs of SMRE, including TsIIA, TsI, DTs, CTs, tanshiones and 15,16-DTsI, were reported to inhibit the production of inflammatory factors. For example, TsIIA inhibits the production of IL-1β and TNF-α in RAW264.7 cells (Jang et al., 2003). TsI, DTs and CTs inhibit intorleukin-12 (IL-12) production in LPS-activated macrophages and also interferon-γ production in keyhole-limpet-hemocyanin-primed lymph node cells and also inhibit TNF-α-induced NF-κB activity (Zhou et al., 2006b). Tanshiones inhibit the expression of the IL-12, p40 gene at the mRNA level, the promoter activation of the IL-12 p40 gene, and NF-κB binding to the κB site in mouse macrophages by LPS stimulation (Kang et al., 2000). 15,16-DTsI was reported to inhibit IL-1β, TNF-α, and the TNF-α converting enzyme in LPS-stimulated BV-2 cells (Lee et al., 2006a).

In summary, the WSCs and LDQs of SMRE attenuate the I/R-induced microcirculatory disturbance by acting at various pathological manifestations, with DLA, SalA, SalB and WSF scavenging peroxides, tanshiones and TsIIA decreasing the activity of NADPH oxidase, tanshiones inhibiting LPO, PAl, SalB, WSF and cardiotonic pills inhibiting the expression of the adhesion molecule ICAM-1 on the endothelium, CTs and 15,16-DTsI suppressing mast cell degranulation, and TsIIB and WSF inhibiting platelet aggregation.

3. Improving effects of compounds derived from SMREM on organ injury induced by ischemia and reperfusion

3.1. Protection of heart

Cardiac muscles have abundant capillaries to meet the demands for oxygen and nutrients and to eliminate wastes generated in various metabolisms, which are particularly important for the heart as it beats all the time. I/R induces a cascade of reactions in the heart, namely, the activation of NF-κB and AP-1 via peroxides, resulting in the expression of ICAM-1 on endothelial cells (Fan et al., 2002) and the release of TNF-α, which induces leukocyte adhesion to the endothelium and the subsequent production of peroxides that affect endothelial cells from inside. Endothelial cells and the basement membrane are injured by peroxides and proteinases, resulting in the efflux of albumin. The peroxides and proinflammatory mediators released from adherent and emigrated leukocytes also induce Ca^{2+} overload in the cardiac muscle, initiating the apoptotic program and resulting in apoptosis, consequently. Moreover, cardiac muscle cell death may result from other insults generated following I/R, such as proteinases released by leukocytes and hypoxia in a non-flow region formed by endothelial cell injury or swelling. Apparently, ablating one or more of the deleterious factors mentioned above would be a potential strategy to impede the I/R-induced cascade that would otherwise lead to cardiac muscle cell death or apoptosis.

Notes to Table 1:
AP-1, activator protein-1; CASMCs, coronary artery smooth muscle cells; DPPH, 1.1-diphenyl-2-picrylhydrazyl; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ERK, extracellular-signal-regulated kinase; HAECs, human aortic endothelial cells; HASMCs, human aortic smooth muscle cells; HCs, hepatocytes; HSCs, hepatic stellate cells; HUVECs, Human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; iNOS, inducible nitric oxide synthase; LPO, lipid peroxidation; MDA, malondialdehyd; NF-κB, nuclear factor kappaB; NO, nitric oxide; ROS, Reactive oxygen species; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1. VEGF, vascular endothelial growth factor; VSMCs, vascular smooth muscle cells. The action of stimulation is highlighted by grey color while inhibition not.

Table 1
Compounds derived from SMRE and in vitro ameliorating effects

Compounds	Cells/tissues	Effects	References
PAl	HUVECs	U937cell adhesion	Zhou et al., 2005
		Expression of ICAM-1	
		ICAM-1 mRNA	
		VCAM-1	
		VCAM-1 mRNA.	
		NF-κB and AP-1 DNA-binding activity	
DLA	HUVECs	ERK activation	Ding et al., 2005a
		Expression of VEGF	
	Neutrophils	Expression of CD11b, CD18	Guo et al., 2007
		Production of $^{\bullet}O_2^-$, H_2O_2	
	Xanthine–xanthine oxidase	$^{\bullet}O_2^-$	Zhao et al., 1996
	VSMCs	Ca^{2+} influx	Lam et al., 2007
SalA	Mice brain	MDA	Du & Zhang, 1997
SalB	HAECs	Activation of NF-κB	Chen et al., 2001c
	HUVEs	Expression of ICAM-1	Zhou et al., 2005
		VCAM-1	
		Expression of VEGF	Ding et al., 2005a
		ERK activation	
		VEGF, VEGF receptor genes	Lay et al., 2003
	HASMCs	ROS	Zhang & Wang, 2006
		NADPH oxidase activity	
	Porcine CASMCs	Ca^{2+}-activated K^+ channels	Lam et al., 2006
	Neutrophils	Expression of CD11b, CD18	Guo et al., 2007
		Production of $^{\bullet}O_2^-$	
	U937	Adhesion to HAECs	Chen et al., 2001c
	Rat HCs	DPPH, LPO, ROS	Lin et al., 2006b
	RatHSCs	DPPH, LPO, ROS	Lin et al., 2006c
TsI	Macrophages	IL-12 production	Zhou et al., 2006b
	Lymph node cells	IFN-γ production	Zhou et al., 2006b
		NF-κB activity	
TsIIA	HUVECs	NO level, SOD activity	Lin et al., 2006b
	RAW264.7	IL-1β and TNF-α production	Jang et al., 2003
		NO production	Jiang et al., 2003
		Expression of iNOS	
ITsIIB	Platelet	Platelet aggregation	Lee et al., 1987
CTs	HUVECs	ET-1, NO, eNOS, nNOS	Zhou et al., 2006b
		NF-κB activity	
		ET-1 production	
	Mast cell	Degranulation	Ryu et al., 1999
	Macrophages	IL-12 production	Zhou et al., 2006b
Tansiones	Macrophages	NF-κB binding to κB site	Kang et al., 2000
		Expression of IL-12, P40 gene	
15,16-DTsI	Mast cell	Degranulation, Fcε R1, ERK phospholipase Cγ2	Choi & Kim, 2004
WSF of SMRE	HAECs	NF-κB nuclear translocation	Chen et al., 2001c
		HL-60 cells adhesion	
	HUVECs	Expression of ICAM-1	Ding et al., 2005b
		Expression of VCAM-1	
		NF-κB activity	Chen et al., 2001c
	Cardiomyocytes	Anoxia/reoxygenation-induced intracellular Ca^{2+} concentration	Cao et al., 2003
	VSMCs	Ca^{2+} influx	Lam et al., 2007
	Platelet	Aggregation	Wang et al., 1989
		Ca^{2+} influx	Wu et al., 1996
	Fibroblast cell	G_0/G_1 phase	Liu et al., 1992
	Collagen	Secretion	Liu et al., 1992
Cardiotonic pills	HUVECs	Expression of ICAM-1, VCAM-1	Ling et al., 2005
	VSMCs	Proliferation	Ling et al., 2005
	Neutrophils	CD11b, CD18	Wang et al., 2006b
	Platelet	CD31	Wang et al., 2006b
SMRE injection	Neutrophils	Production of $^{\bullet}O_2^-$, H_2O_2	Han et al., 2007
	HUVECs	Expression of ICAM-1	Han et al., 2007
	Neutrophils	Expression of CD11b, CD18	Han et al., 2007

SMRE has been widely used in China for the treatment of cardiovascular diseases (Feldman et al., 2000), and a number of studies have been carried out in attempts to identify the biological actions and the mechanism underlying such actions. In vitro investigations revealed the beneficial effects of SMRE and its major components on I/R-induced heart injury, including the inhibition of the production of peroxides, the expression of ICAM-1, and the release of proinflammatory mediators. Some experimental research on animals demonstrated that both WSCs and LDQs of SMRE can improve myocardiac infarction induced by I/R. Treatment with WSF of SMRE increases survival rate, decreases the ratio of infarct size to left ventricular size (Ji et al., 2003; Sun et al., 2005), and protects the mitochondrial membrane from I/R injury and LPO (Zhao et al., 1996). LsAB were reported to reduce myocardial damage in the postischemic rabbit heart (Fung et al., 1993). The effect of SMRE on Ca^{2+} flux may be involved in the protective effects against I/R-induced heart injury, as suggested by the observations that (1) DLA and WSF inhibit Ca^{2+} influx in vascular smooth muscle cells (VSMC; Lam et al., 2007), and WSF increase the utilization of extracellular Ca^{2+} (Lei & Chiou, 1986a), (2) SalB activates the opening of Ca^{2+}-activated K^+ channels of porcine coronary artery smooth muscle cells (CASMC) through the activation of guanylate cyclase without the involvement of NO synthase activation (Lam et al., 2006), (3) WSF attenuates anoxia and reoxygenation-induced changes in contraction and intracellular Ca^{2+} concentration in rat cardiac ventricular myocytes (Cao et al., 2003), (4) sodium TsIIA sulfonate prevents the increase in intracellular Ca^{2+}concentration mediated by angiotensin II in myocytes (Takahashi et al., 2002). It appears that the effects exerted by WSF from SMRE on I/R-induced heart injury involve multiple factors, as indicated by the findings that the WSF improves blood flow in coronary arteries (Lei & Chiou, 1986b; Sugiyama et al., 2002), enhances coronary collateral circulation in the ischemic dog heart (Liu & Lu, 1999), enhances angiogenic processes in SVR cells through the up-regulation of VEGF and VEGF receptor genes (Lay et al., 2003) and augments the expression of VEGF mRNA in nonischemic parts in acute myocardial infarction rat hearts (Ji et al., 2003). Perfusion with TsI, CTs and tanshinone VI restored cardiac contractile force in an isolated heart under hypoxia and reoxygenation, which is associated with ATP metabolism in the myocardium (Yagi et al., 1989). DLA was reported to scavenge $^{\bullet}O_2^-$ generated from the xanthine–xanthine oxidase system and protect myocardial mitochondrial membrane from LPO in I/R-induced injury rat heart (Zhao et al., 1996). To our knowledge, no in vivo study has been reported so far regarding the ameliorating effects of SMRE and its major ingredients on cardiac microcirculatory disturbance induced by I/R.

3.2. Protection of brain

Brain cells possess active and fast operating metabolism. The microcirculation within the brain is extremely well developed and complex, and gaps between capillaries are less than 100 μm. After arterial occlusion, the up-regulated expressions of cytokines including IL-1 and IL-6 increase the expression of E-selectin and ICAM-1 on the vascular endothelium, which in turn promote leukocyte adherence to and accumulation on the vascular endothelium. Integrins then structurally modify the basal lamina and extracellular matrix. These inflammatory signals promote leukocyte transmigration across the endothelium and mediate inflammatory reactions (Huang et al., 2006). Injury of endothelial cells and increased vascular permeability lead to tissue edema, which exacerbates neuronal hypoxia. Hypoxia or peroxides enhance the production of glutamate in glial cells, resulting in the opening of voltage-operated Ca^{2+} channel and an intracellular Ca^{2+} overload. Peroxides also activate NF-κB and initiate the apoptotic program leading to neuronal apoptosis. Amelioration of the I/R-induced microcirculatory disturbance is a reasonable strategy for preventing the subsequent neuronal death or apoptosis, and SMRE has been proved to be a promising candidate in this regard. Augmented prostaglandin E2 accumulation has been demonstrated in the lesion sites of rodent transient ischemia models. Microsomal prostaglandin E synthase 1 may be a crucial determinant of ischemic brain injury and a valuable target for the treatment of human stroke (Ikeda-Matsuo et al., 2006).

Clinically, SMRE has also been used for the treatment of cerebrovascular disease in China (Wu et al., 2004; Wang et al., 2006a). In vitro studies showed that SMRE and its major ingredients have protective effects against the production of peroxides, the expression of ICAM-1, and the release of proinflammatory mediators. Several in vivo investigations have been conducted to identify the actions of SMRE and to better understand the mechanisms underlying such actions. Lo et al. (2003) reported that pretreatment with an intraperitoneal injection of WSF from SMRE decreases the area of cerebral infarct and the level of a peroxide-dependent illuminant, luminol-CL, in peripheral blood of SD rats that are subject to occlusion of both the common carotid arteries and the right middle cerebral artery for 90 min to induce ischemia, followed by a 24-h reperfusion. It was reported that TsIIA and TsIIB penetrate the blood–brain barrier and that pretreatment with TsIIA and TsIIB decreases the volume of brain infarct caused by middle cerebral artery occlusion in adult mice (Lam et al., 2003). The ability of TsIIA, as well as DTs, CTs and TsI to penetrate the blood–brain barrier was also reported by others who further revealed that these substances inhibit acetylcholinesterase (Ren et al., 2004). Other actions of WSC of SMRE have been explored. Pyda et al. (2006) found that treatment with SalB decreases ROS production and increased intracellular Ca^{2+} concentration in PC12 cells induced by Aβ(25–35). The results obtained by Koo et al. (2004) demonstrated that SMRE inhibits $^{\bullet}O_2^-$ production by rat microglias and inhibits $^{\bullet}O_2^-$ production by microglias stimulated with phorbol myristate acetate or opsonized zymosan. WSF from SMRE was observed to enhance high-K^+ stimulated dopamine release (Koo et al., 2004). However, the in vivo effects of its effective derivatives on I/R-induced cerebral microcirculatory disturbance remain to be clarified. Recirculation must be instituted within a narrow time window. Whether these agents are purely neuroprotective or are acting at the microvascular or neurovascular unit must be clarified.

Table 2
Compounds derived from SMRE and their in vivo targeting insults in organs by I/R

Compounds	Organ/animals	Insults by I/R	References
DLA	Rat heart	LPO of myocardial mitochondrial membrane after I/R	Zhao et al., 1996
LsAB	Rabbit	I/R-induced myocardial damage	Fung et al., 1993
	Rat kidneys	Aquaporin-2, Na^+, K^+-ATPase improves creatinine urinary sodium excretion urinary osmolality solute-free reabsorption in I/R-induced ARF rats	Kang et al., 2004
SalA	Mice	Memory	Du & Zhang, 1997
	Mice brain	MDA	Du & Zhang, 1997
TsIIA	Mice brain	I/R-induced brain infarct size	Lam et al., 2003
TsIIB	Mice brain	I/R-induced brain infarct size	Lam et al., 2003
Tanshinones	Rabbits heart	I/R-induced lipid free radicals from myocardial mitochondrial membranes	Zhao et al., 1996
WSF of SMRE	Rat small pulmonary arteries	iNOS, eNOS ultrastructure injury HO-1	Chen et al., 2003
	Rat heart	I/R-induced left ventricular infarct size heart mitochondrial membrane injury and LPO	Ji et al., 2003; Sun et al., 2005
		Survival rate	
		VEGF, VEGF receptor genes	Lay et al., 2003
		VEGF mRNA	Ji et al., 2003
		Vasodilatation of coronary arteries	Ji et al., 2003
	Guinea-pig heart	Coronary arteries blood flow	Lei & Chiou, 1986a, 1986b
	Dog heart	Coronary collateral circulation	
		relaxed coronary arteries	Liu & Lu, 1999
	Rat brain	I/R-induced cerebral infarct size	Lo et al., 2003
		Peroxide	
	Rat liver	I/R-induced ALT, AST Plasma endotoxin, MDA	Xing et al., 2005
		SOD	
		PPAR-α	Kwon et al., 2005
	Rat lungs	Tunica media thickening in small pulmonary arteries, restore ultrastructure injury	Chen et al., 2003
		HO-1, iNOS, eNOS	
SMRE injection	Children	ET-1	Xia et al., 2003
	Rat liver	ALT, MDA, ultrastructural damage	Zhang et al., 2003
Cardiotonic pills	Rat liver	I/R-induced leukocytes adhesion to liver, ALT plasma endotoxin and TNF-α	Horie et al., 2005

ALT, alanine aminotransferase; ARF, acute renal failure rats; AST, aminotransferase activities; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1;HO-1, hmooxygenase-1; iNOS, inducible nitric oxide synthase; I/R, ischemia and reperfusion; LPO, lipid peroxidation; MDA, malondialdehyd; PPAR-α, peroxisome proliferator-activated receptor alpha; VEGF, vascular endothelial growth factor. The action of stimulation is highlighted by grey color while inhibition not.

3.3. Protection of liver

The hepatic microvasculature consists of 2 afferent types of vessel, namely, the portal venules reaching the terminal portal venule and the hepatic arterioles reaching to the terminal hepatic arteriole with a network of sinusoids running between the liver cell cords and the terminal hepatic venule (Oda et al., 2006). After I/R, the liver manifests numerous reactions, including the up-regulation of the expression of ICAM-1 mRNA and protein (Meyer et al., 1998), an increase in the number of leukocytes adhering to hepatic portal venules and sinusoids, a decrease in the number of perfused sinusoids (Horie et al., 1996), and increases in serum alanine aminotransferase (ALT) activity, and plasma endotoxin and TNF-α levels (Horie et al., 2005). It was reported that I/R-induced peroxide and cytokine production leads to hepatocyte apoptosis through several different signaling pathways (Sakon et al., 2002).

Accumulated evidences indicate the effectiveness of SMRE for improving I/R-induced liver injury. Pretreatment with a SMRE injection markedly decreases serum ALT and malondialdehyde (MDA) levels and attenuates pathohistological changes (e,g. swelling, degeneration, focal necrosis and infiltration of leukocytes) and ultrastructural damage (mitochondria swelling, rupture and even breakdown) induced by I/R in the rat liver (Zhang et al., 2003). The results of the study by Xing et al. (2005) confirmed that pretreatment with WSF from SMRE reduces ALT and aminotransferase activities (AST) and the levels of plasma endotoxin and MDA, and increases liver SOD activity in SD rats after I/R. We have observed that cardiotonic pills can inhibit I/R-induced hepatic damage, inhibit leukocyte adhesion to hepatic sinusoids and decrease LPS and TNF-α levels in serum after gut I/R (Horie et al., 2005).

Both in vivo and in vitro studies have been carried out to evaluate the role of SMRE in the attenuation of liver damage induced by injuries other than I/R. The results demonstrated that pretreatment with WSF from SMRE inhibits bile duct ligation and scission-induced rat hepatic injury, significantly decreases the number of apoptotic cells with a decrease in Bax protein level and an increase in Bcl-2 protein level, and induces a cytoplasmic sequestration of p53 (Oh et al., 2002). WSF was reported to decrease the histological grades of fibrosis, to ameliorate the portal hypertensive state, and to enhance the vascular sensitivity of mesenteric arteries to phenylephrine in bile duct ligation and scission rats (Huang et al., 2001). In an experimental model in which oxidative stress is induced, it has been reported that SalB scavenges free radical DPPH, inhibits

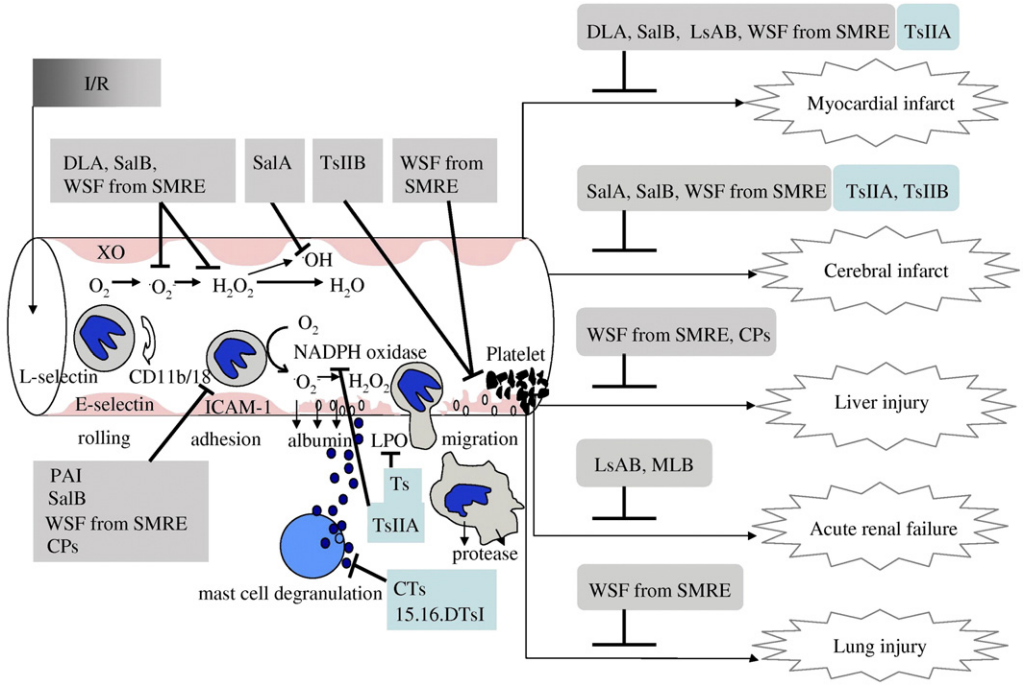

Fig. 4. Mechanism by which the compounds of SMRE attenuate microcirculatory disturbance and organ injury induced by ischemia and reperfusion. I/R, ischemia and reperfusion; XO, xanthine oxidase; L-selectin, leukocyte selectin; E-selectin, endothelial selectin; CD11b/CD18, neutrophil adhesion molecule CD11b and CD18; LPO, lipid peroxidation; ⊥, denotes inhibition. Left side of the figure illustrates the various insults induced by I/R in microvessels and the tagerts that different compounds or preparations of SMRE act at. DLA, SalB, SalA, WSF from SMRE are capable of scavenging various peroxides, SalB, PAI, WSF from SMRE and cardiotonic pills (CPs) inhibit the expression of adhesion molecules in both leukocyte and endothelium, while CTs, 15,16-DTsI impedes degranulation of mast cells located perivascularly. TsIIA exerts protective effect by inhibition of NADPH oxidase, tanshiones (Ts) by inhibition of LPO. TsIIB and WSF from SMRE are able to alleviate platelet aggregation. The right side of the figure depicts the identified potential of SMRE and its derived preparations in amelioration of organ injury induced by I/R. DLA, SalB, LsAB, WSF from SMRE and TsIIA are of benefit to myocardial infarct by I/R, SalA, SalB, WSF from SMRE, TsIIA and TsIIB to cerebral infarct, WSF and CPs to liver injury, LsAB and MLB to ARF, while WSF to lung injury.

LPO, eliminates ROS accumulation in primary rat hepatocytes and HSCs, and inhibits α-smooth muscle actin and collagen synthesis and deposition in HSCs, without showing direct cytotoxicity on both primary rat hepatocytes and HSCs (Lin et al., 2006c).

It was reported that WSF increases hepatic glutathione concentration and inhibits the elevation of hepatic iNOS protein content and nitrite concentration in rats (Lee et al., 2003). Recent evidence has shown that SMRE-containing traditional Chinese medicine restores mRNA levels of peroxisome proliferator-activated receptor α (PPAR-α) and microsomal triglyceride transfer protein (an essential factor for the secretion of triglycerides from the liver; Kwon et al., 2005). Activation of PPAR-α results in not only the activation of enzymes involved in lipid metabolism but also the inhibition of NF-κB activation, which may be implicated in the mechanism underlying the protective effect of SMRE and its major ingredients against adhesion molecule expression, the release of proinflammatory mediators, and the inhibition of apoptosis.

Some studies have been conducted to explore the role of SMRE in the pharmacokinetics in the liver. Available evidence reveals that TsIIA inhibits the activity of cytochrome P450 2A (CYP2A; Ueng et al., 2003) and that pretreatment with a WSF from SMRE increases liver microsomal protein content, CYP enzyme levels, and erythromycin *N*-demethylase activity (Jinping et al., 2003). Treatment with SMRE attenuates the CCl_4-induced increase in hepatic microsomal CYP2E1 level (Lee et al., 2003). An oral administration of TsIIA causes a dose-dependent increase in liver microsomal 7-methoxyresorufin *O*-demethylation activity in arylhydrocarbon-responsive C57Bl/6l mice, but not in nonresponsive DBA/2J mice. Induction of CYP1A2 by TsIIA depends on the arylhydrocarbon-responsiveness and occurs at the pretranslational level (Ueng et al., 2004). An oral administration of ethyl acetate fraction of SMRE in C57B/6J mice increases liver microsomal 7-methoxyresorufin *O*-demethylation activity, enhances metabolism of drugs such as tolbutamide, nifedipine and warfarin, and increases the protein level of CYP1A and CYP3A. TsIIA, but not the WSF was found to induce CYP1A, CYP2C and CYP3A (Kuo et al., 2006).

3.4. Protection of kidneys

I/R-induced renal injury is an important cause of acute renal failure (ARF) after renal transplantion (Hoffmann et al., 2002), major surgery (Meldrum & Donnahoo, 1999), trauma, and septic as well as hemorrhagic shock. I/R induces a series of alterations in kidney cells, including degradation of inhibitor κB (Ishibashi et al., 1999), activation of NF-κB, expression of adhesion molecules, release of proinflammatory mediators, and adhesion of leukocytes, which markedly contribute to renal microcirculatory disturbance and apoptosis (Meldrum & Donnahoo, 1999; Daemen et al., 2002; Bando et al., 2004).

Recent studies have demonstrated the beneficial effects of SMRE to the improvement of renal microcirculation. For example, an intravenous administration of magnesium lithospermate B (MLB) enhances renal cortical microperfusion (Chen & Wang, 2006). In addition, LsAB ameliorates the expression of aquaporin 2 and Na^+, K^+-ATPase, and improves creatinine clearance, urinary sodium excretion, urinary osmolality, and solute-free reabsorption in I/R-induced ARF rats (Kang et al., 2004).

3.5. Protection of lungs

The lungs respond to I/R injury via a group of reactions similar to those of other organs, such as oxidant production (Minamiya et al., 1998), accumulation of peroxides (Reignier et al., 1997), enhanced leukocyte adherence (Reignier et al., 1997), release of proinflammatory mediators (Raijmakers et al., 1997), and increase in vascular permeability (Minamiya et al., 1998), which lead to pneumonic damage. Thus, it is predictable that SMRE exhibits ameliorating effect also in the lungs subjected to I/R. WSF from SMRE also reduces hypoxia-induced tunica media thickening in the small pulmonary arteries in rats, restores ultrastructure injury, decreases hemooxygenase-1 (HO-1) and iNOS levels, and increases eNOS expression level (Chen et al., 2003). Moreover, SMRE protects multiple organ injuries caused by high-energy-induced abdominal wounds through anti-free radical effects (Fu et al., 1992). Nevertheless, studies about the effects of its major ingredients on I/R-induced lung injury are not sufficient, and more works are required.

Taken together, WSCs and LDQs of SMRE protect the heart, brain, liver, kidneys and lungs from I/R-induced injury via a spectrum of actions, such as the inhibition of peroxide production, leukocyte adhesion to the vascular endothelium, and apoptosis. DLA, SalB, LsAB, WSF from SMRE and TsIIA attenuate cardiac muscle injury. SalA, SalB, WSF, TsIIA and TsIIB protect the brain from damage. WSF and cardiotonic pills ameliorate liver injury. LsAB and MLB protect the kidneys, and WSF protects the lungs from injury.

4. Conclusion

(1) The DLA, SalA, SalB and WSF of SMRE exhibit an antioxidative effect, whereas PA1, SalA and SalB inhibit the expression of adhesion molecules. Because both SalA and SalB possess the same structure as that of DLA, it is reasonable to assume that among the WSCs, DLA is one of the major agents effective for the antioxidation and inhibiting expression of adhesion molecules, by which actions DLA, PAl, SalA and SalB protect or improve microcirculatory disturbance induced by I/R.

(2) As to the LDQs of SMRE, TsIIA shows an antioxidative effect, tanshiones inhibits LPO, TsIIB exerts the effect of anti-aggregation of platelets, whereas CTs and 15, 16-DTsI inhibit mast cell degranulation, all of which are diterpenoids. Thus, it is proposed that diterpenoids of SMRE protect or improve I/R-induced microcirculatory disturbance via several actions, such as the inhibition of LPO, platelet aggregation, and mast cell degranulation. However, no data exist supporting the inhibitory effect of the LDQs on the expression of adhesion molecules; therefore, it is not suggested that leukocyte adhesion to endothelium is inhibited by the administration a diterpenoid of LDQs, only.

Taking together the notions above, the most effective way to use SMRE is to administer the WSCs and LDQs concomitantly, so that the former scavenges peroxides and inhibits leukocyte adhesion, thus ameliorating the impact on the vessel from inside, and the latter inhibits mast cell degranulation, thus alleviating damage of vessel from outside, leading to the improvement of I/R-induced microcirculatory disturbance by acting at multiple targets.

(3) No study has been published yet regarding the in vivo dynamics and intravital observations of the effects of WSCs or LDQs on the microcirculatory disturbance induced by I/R in the heart, brain, liver, kidneys and lungs. Further studies are required to clarify these issues with respect to these organs, including the changes in the diameter of venules, RBCs' velocity, leukocyte rolling, adhesion and emigration, peroxide production from leukocytes and vascular walls, albumin efflux, and mast cell degranulation.

(4) Studies of the protective effect of WSCs and LDQs from I/R-induced injury in the heart and brain were performed more frequently than those in the liver, kidneys or lungs. Nevertheless, all organs affected by I/R may share a common pathology, viz., microcirculatory disturbance, and it is thus predicted that the major WSCs and LDQs may prevent multiorgan injuries induced by I/R, taking into account the observation that WSF from SMRE, SMRE compound preparation, and LsAB protect the liver, kidneys and lungs from reperfusion injury.

The identified ameliorating effect of SMRE and its major compounds on I/R induced microcirculatory disturbance and organ injury is summarized in Tables 1 and 2 and Fig. 4. The I/R-induced microcirculatory disturbance and the subsequent target-organ injuries are complicated pathological processes involving multiple insults, thus an interference acting at multitargets is required to interrupt these processes. Previous in vitro studies suggest that the WSCs and LDQs of SMRE are capable of attenuating I/R-induced microcirculatory disturbance and protection of multiple-organ injury by exerting various actions, such as inhibition of the production of peroxides, alleviating the expression of adhesion molecules in leukocytes, endothelial cells and platelets, and the adhesion of leukocyte to vascular endothelium and platelet aggregation, and preventing mast cell from degranulation. Further systematic in vivo research is appealed to explore the improving effect of WSCs and LDQs of SMRE on I/R-induced microcirculatory disturbance in vital organs, including heart, brain, liver and kidney, and to develop the novel medication that contens SMRE-derived compounds. To this end, preclinical and clinical trials are imperative.

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