Review

Salvia miltiorrhiza Bunge: Bioactive Compounds and Bioactivities

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Article history: Received 16 July 2015, Received in revised form 18 August 2015, Accepted 21 August 2015, Published 28 August 2015.

Abstract: The rhizome of Salvia miltiorrhiza Bunge is a famous traditional Chinese medicine, and widely used for the treatment of cardiovascular and cerebrovascular diseases. The chemical components of S. miltiorrhiza have been widely studied, and tanshinone I, tanshinone IIA, tanshinone IIB, cryptotanshinone, salvianolic acid A and salvianolic acid B are its main bioactive components. These compounds possess many bioactivities, such as cardiovascular and cerebrovascular protection, anticancer, antioxidant, neuroprotective, anti-inflammation, and antibacterial effects, and may serve as a potential specific medicine in the treatment of some chronic diseases. This review summarizes current knowledge about bioactive compounds and bioactivities of S. miltiorrhiza, and special attention was paid to the mechanisms of action. In addition, extraction and separation methods of main components of S. miltiorrhiza were discussed.

Keywords: Salvia miltiorrhiza; tanshinone; salvianolic acid; antioxidant; cardioprotection; cerebroprotection; anticancer.
1. Introduction

The rhizome of Salvia miltiorrhiza Bunge (Danshen in Chinese) is a famous traditional Chinese medicine for multiple therapeutic remedies. In virtue of its good performance and few side effects as confirmed in the long-time clinical use, S. miltiorrhiza was widely adopted in traditional Chinese medicinal preparations for the treatment of heart disease, cerebrovascular disease, dysmenorrhea, chronic renal failure, neuroasthenic insomnia, and cancer (Wu et al., 2010). The global trend of favoring healthcare with medicines or neutraceuticals derived from natural products has prompted an increasing interest recently on the development of herbal medicines (Chen et al., 2007). The pharmaceutical actions of traditional Chinese medicine were attributed to particular compounds. With the development of extraction, separation and analytical technology, the chemical components of S. miltiorrhiza were revealed. The bioactivities of crude extract and some bioactive components of S. miltiorrhiza have been widely studied. This review summarizes current knowledge about bioactive compounds and bioactivities of S. miltiorrhiza, and special attention was paid to the mechanisms of action. In addition, extraction and separation methods of main components of S. miltiorrhiza were discussed.

2. Chemical Components

The liposoluble compositions of S. miltiorrhiza are almost conjugate quinine or ketone compounds, such as tanshinone I, tanshinone IIA, tanshinone IIB, tanshinone V, tanshinone VI, cryptotanshinone, hydroxytanshinone, methyl tanshinonate, methylene tanshiquinone, przewaquinone A, przewaquinone B, przewaquinone E, and miltirone (Li and Wang, 2009). The chemical structures of some liposoluble compositions of S. miltiorrhiza are given in Table 1.

The hydrosoluble compositions are phenolic acids, such as salvianolic acid A, salvianolic acid B, salvianolic acid C, salvianolic acid D, salvianolic acid E, salvianolic acid G, rosmarinic acid, ursolic acid, and protocatechualdehyde (Cui et al., 2011).

The essential oil from the air-dried leaves of S. miltiorrhiza was obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Hexadecanoic acid (17.0%), germacrene D (9.1%), phytol (8.9%), β-caryophyllene (7.1%) and methyl linolenate (5.3%) were the main components (73.3%) (Li and Wang, 2009). Leaf of S. miltiorrhiza was rich in health-promoting phenolics and was a novel resource of natural antioxidants. The acetone and methanol extracts of leaves of S. miltiorrhiza were evaluated by various in vitro antioxidant assays. HPLC and correlation analysis show that salvianolic acid B and rosmarinic acid constitute the most abundant phenolic compounds. They are the major contributors to antioxidant activities. The results
suggested that *S. miltiorrhiza* leaves could be considered as a new potential source of natural phenolic antioxidants for food, pharmaceutical, cosmetics or nutraceutical industries (Zhang et al., 2010). In another study, three essential oils of the fresh roots as well as the callus and hairy root cultures of *S. miltiorrhiza* obtained by hydrodistillation were investigated by GC and GC-MS. Ethyl hexadecanoate was found to be the main component in the root oil, and 2, 5-hexanedione to be the main component in the oils of callus and hairy root cultures (Lou et al., 2014).

Table 1. The chemical structures of several liposoluble compositions of *S. miltiorrhiza*

<table>
<thead>
<tr>
<th>Structures</th>
<th>Names</th>
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<tbody>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>tanshinone I</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>tanshinone II A</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>tanshinone II B</td>
</tr>
<tr>
<td>C&lt;sub&gt;5&lt;/sub&gt; – OH</td>
<td>hydroxytanshinone</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – COOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>methyltanshinonate</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>cryptotanshinone</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>isotanshinone I</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>isotanshinone II</td>
</tr>
<tr>
<td>C&lt;sub&gt;8&lt;/sub&gt; – CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>isocryptotanshinone</td>
</tr>
</tbody>
</table>

Several components were isolated from the crude ethanol extract of the cultured hairy roots of
S. miltiorrhiza by bioassay-guided fractionation. They were identified by means of physicochemical and spectrometric analysis as tanshinone IIA, tanshinone I, cryptotanshinone, dihydrotanshinone I, rosmarinic acid, caffeic acid, and danshensu (Zhao et al., 2011). In addition, 22 trace elements, including Ca, Mg, Fe, Zn, V, Mn, Cr, Cu, Rb, Sr, Ba, Pb, B, As, Se, Li, Co, Ni, Mo, Cd, Sn and Hg, were determined in the roots of S. miltiorrhiza by ICP-MS/ICP-AES (He et al. 2010). The samples were obtained from four planting regions and grew in the same place, and it was found that the contents of traces elements of S. miltiorrhiza varied from different places.

3. Extractions, Separation and Analytical Methods

3.1. Extraction Methods

Water was suitable for extracting hydrosoluble compositions salvianolic acids with the extracting time of 40-50 min, while alcohol is suitable for extracting liposoluble compositions tanshinones with the extracting time of 20-30 min. In the study of Wan et al. (2009), methanol is the best solvent to extract tanshinones, including cryptotanshinone, tanshinone I, and tanshinone IIA. For organic solvents, the involvement of toxic and volatile organic solvents causes significant environmental problems. Tian et al. (2009) used the ionic liquid-modified silica sorbents developed by the surface chemical modification of the commercial silica using synthesized ionic liquids, and extraction of cryptotanshinone, tanshinone I and tanshinone IIA from S. miltiorrhiza were obtained. In addition, non-ionic surfactant-assisted extraction was proposed as an alternative, effective, cheap and green extraction method (Bi et al., 2011).

Extraction at room temperature was a traditional method. According to Pan et al. (2002), S. miltiorrhiza samples was mixed with an appropriate solvent, and the suspensions were left at the room temperature of about 24-27 °C with periodical mixing by shaking during the extraction time. One of the conventional extraction methods of S. miltiorrhiza is alcohol heating reflux extraction, but it is unsatisfactory because of the high temperature, long heating time and losing of effective components. In the study of Pan et al. (2002), heating reflux extraction was performed with S. miltiorrhiza, and solvent in a flask and the suspensions were made to boil. Mechanical stirrer was used at the same time, and the water-bath temperature can be set according to the experimental design. The extraction time of 45-60 min was optimal to obtain high extraction rate of tanshinones. Furthermore, Soxhlet extraction (SHE) was carried out for S. miltiorrhiza. The solvent remained in the boiling state during the time, and the optimal time of extraction was 90 min (Pan et al., 2002). In the study of Yang et al. (2007), SHE was carried out by extracting powdery S. miltiorrhiza sample with 20% acetonitrile in methanol (v/v) for 4 h. In addition, ultrasonic extraction with an ultrasonic bath was applied to the extraction of S. miltiorrhiza (Pan et al., 2002). S. miltiorrhiza was mixed with an appropriate solvent. The
suspensions were sonicated with continuous power for a pre-setting time. The optimum ultrasonic extraction time was 75 min. Microwave-assisted extraction (MAE) method was also used to extract tanshinones (tanshinone IIA, cryptotanshinone and tanshinone I) with analysis by HPLC. It can be conducted at room temperature, and the percentage extraction can reach high in a short time. The percentage of tanshinones was even higher than the earlier methods, and it was more effective and convenient (Pan et al., 2001 & 2002). In their study, extraction at room temperature (ERT), heating reflux extraction, ultrasonic extraction and Soxhlet extraction were compared with MAE. As to the extracting time, MAE only needed 2 min, whereas ERT, heat reflux extraction, ultrasonic extraction and SHE needed 24 h, 45, 75 and 90 min, respectively, and the MAE achieved the highest extraction yield of all. In a conclusion, MAE was more effective than the conventional methods. According to the study of Guo et al. (2012), ultrasonic extraction technology was combined with ammonium sulfate/ethanol aqueous two-phase system (ATPS) for the separation of salvianolic acid B from S. miltiorrhiza, demonstrating that it is a very efficient tool for the extraction and purification.

Microwave assisted micellar extraction technique (MAME) has been optimized for the extraction of tanshinones from S. miltiorrhiza, in which non-ionic surfactant Genapol X-080 solution was employed as extract agent. MAME has the higher extraction yield of tanshinones and shorter extraction time than static extraction or ultrasonic assisted extraction techniques. In addition, MAME has similar extraction efficiency with the heating reflux method (Shi et al., 2009).

Yang et al. (2007) reported for the first time on the joint use of dynamic continuous ultrasound-assisted extraction with high intensity ultrasonic probe (CUAE-HIUP) and solid-phase extraction (SPE) in the extraction of S. miltiorrhiza. They isolated sodium danshensu (SDan) and four tanshinones (dihydrotanshione I, tanshinone I, cryptotanshinoneand tanshinone IIA) from the samples, and then injected the extract into a HPLC system to determine the five analyses. The CUAE-HIUP was compared with 3 conventional extraction techniques, such as ERT, Soxhlet extraction, microwave-assisted extraction (MAE). The extracting times of the 4 methods are 3 min (CUAE-HIUP), 24 h (RTE), 4h (SHE) and 2 min (MAE), and CUAE-HIUP achieved the highest extraction yield (98.9%) and used the least amount of solvent.

Supercritical carbon dioxide (SC-CO₂) extraction has been used to extract and purify the effective compositions of S. miltiorrhiza. This method need lower operating temperature, and has higher extraction efficiency and selectivity. With no organic solvents, it turns out to be very clean and safety in the extraction of traditional Chinese medicine. Dean et al. (1998) used methanol-modified supercritical carbon dioxide to extract tanshinone IIA, and the yields were similar compared with Phytosol solvent extraction (PSE). Li et al. (2002) applied ethanol in the study as co-solvent with SC-CO₂ fluid, and yields were the same as methanol. Dong et al. (2006) obtained liposoluble compositions by SC-CO₂ extraction, which were determined by HPLC-diode array detection using isocratic elution.
Overlapping peaks of the chromatogram were resolved by heuristic evolving latent projection (HELP) and fixed size moving window evolving factor (WFA) analysis, which proved satisfactory.

Chen et al. (2011) showed that the extraction process of salvianic acid B by decompressing inner ebullition can be finished in 40 s when the operation pressure is 0.084 MPa, the concentration of ethanol is 50%, the immersing time is 20 min, water consumption is 10 times to raw material, and the extraction temperature is in the range of 40~60 °C. The extraction rate by decompressing inner ebullition is 135 times faster than that by ultrasound-assisted extraction. In another study, Liu et al. (2013a) applied ionic liquid-based ultrahigh pressure-assisted extraction (IL-UPE) in extracting five tanshinones from S. miltiorrhiza, and they optimized the solvents, ionic liquids with different cations and anions, ionic liquids concentration, extraction pressure, extraction time and solid/liquid ratio. In addition, Shi et al. (2013) established a dispersive liquid-liquid microextraction based on solidification of a floating organic droplet (DLLME-SFO) for the extraction of S. miltiorrhiza, and then used the HPLC-UV detection method to determine the tanshinones.

### 3.2. Separation and Analytical Methods

High-performance liquid chromatography is widely applied as a method of separating different components and analyzing the extracts. High-performance liquid chromatography with a coulometric electrode array detection (HPLC-CEAD) system was used to determine the four hydrosoluble components, salvianolic acid A, salvianolic acid B, protocatechuic acid, and protocatechuic aldehyde, and was applied in fingerprinting S. miltiorrhiza (Ma et al., 2006 & 2007). Zhang et al. (2008) used HPLC-CEAD to determine the tanshiones (cryptotanshinone, tanshinone I, and tanshinone IIA) in S. miltiorrhiza, and the method was stable, sensitive, and reproducible. Luo et al. (2008) established a RP-HPLC for the simultaneous determination of salvianolic acid A, salvianolic acid B and protocatechuic aldehyde in water extract of S. miltiorrhiza, and the method recovery, reproducibility and stability of the samples were accorded with the regulation, turning out that it is a rapid and accurate method to manipulate the quality of S. miltiorrhiza. With HPLC, Lee et al. (2010a) isolated salvianolic acid B fraction represented a 75.0% recovery, with a purity exceeding 99.2%.

Flow injection-capillary electrophoresis system was used to separate and determine three water-soluble components, protocatechuic aldehyde (PAH), β-(3, 4-dihydroxyphenyl) lactic acid (DSS) and protocatechuic acid (PA). The study was carried out by using an unmodified fused-silica capillary and direct ultraviolet detection, and the method turned out to be applied successfully to monitor these three components in S. miltiorrhiza (Liu et al., 2007).

Tian et al. (2000 & 2002) successfully used high-speed counter-current chromatography (HSCCC) for isolating and purifying tanshinones from the roots of S. miltiorrhiza by stepwise elution.
Tanshinone IIA, tanshinone I, dihydrotanshinone I and cryptotanshinone are all at purities of over 95% in a single run. In the study of Gu et al. (2003, 2004a & b, 2006), high-performance liquid chromatography, thin-layer chromatography (TLC) and high-performance capillary electrophoresis (HPCE) were compared with HSCCC in the separation and purification of S. miltiorrhiza. S. miltiorrhiza samples from 3 different locations were separated and purified by HSCCC in a step-wise elution. This study demonstrated that the contents of each components of S. miltiorrhiza varied greatly in different samples, which confirmed that the locations and climates have a great impact on the S. miltiorrhiza quality. Compared with HPLC, HSCCC separated one more component, and the precision was satisfied as well. Compared with thin-layer chromatography (TLC) scan, HSCCC separated four more components, which was as effective as TLC scan in showing the concentration distribution of all kinds of constituents. Compared with non-aqueous capillary electrophoresis (NACE), a kind of HPCE, HSCCC performed better in analysis of liposoluble compositions (tanshinones), separating five more components. In a conclusion, the fingerprint of HSCCC contains more chemical information than that of any other method above, which proved that HSCCC could be a feasible and cost effective method in the development of the fingerprint of traditional Chinese medicine. 3, 4-Dihydroxyphenyllactic acid, salvianolic acid B and protocatechualdehyde were separated and purified in one step by HSCCC with organic/aqueous solvent system, supplying an efficient method to purify hydrosoluble compounds directly from crude samples of S. miltiorrhiza. HSCCC was used to isolate and purify tanshinone I and tanshinone IIA from the extract of the rhizome of S. miltiorrhiza (Wu et al., 2010). As a result, they obtained over 98% pure tanshinone IIA and over 94% pure tanshinone I.

Meng et al. (2014) successfully constructed a novel multi-channel multi-dimensional chromatography and applied 2D and 3D CCC to resolve the bioactive tanshinones from complex extracts. This new method decreased analysis time, reduced solvent consumption and increased resolution and peak capacity by coupling of multiple columns with the same or different separation mechanisms.

4. Bioactivities

4.1. Effect on Cardiovascular and Cerebrovascular Diseases

The effects of magnesium lithospermate B, sodium rosmarinate and magnesium lithospermate were compared on intracellular Ca\(^{2+}\) concentrations in cultured rat thoracic aorta vascular smooth muscle cells (VSMCs). They all attenuated intracellular Ca\(^{2+}\) concentrations increase in VSMCs induced by 20 \(\mu\)M ATP in the absence of extracellular Ca\(^{2+}\). Magnesium lithospermate B also suppressed the increase induced by 1 \(\mu\)M thapsigargin, but not the other two drugs. Magnesium lithospermate B and sodium rosmarinate attenuated intracellular Ca\(^{2+}\) concentrations increase induced...
by 60 mM KCl in the presence of extracellular Ca\(^{2+}\), but not magnesium lithospermate. It can be concluded that the three drugs studied can regulate Ca\(^{2+}\) homeostasis in VSMCs via different pathways, which was related to the mechanisms of the vasodilator action of *S. miltiorrhiza* (Chen et al., 2009). The cardiovascular effects of the water-soluble extract of *S. miltiorrhiza* (SME) and its magnesium tanshinoate B-enriched form (containing 70% of MTB (MTB70)) were compared and the results indicated that MTB70 caused a bigger reduction in blood pressure than SME. MTB is one of the major components responsible for the cardiovascular effects of *S. miltiorrhiza*, and that the beneficial cardiovascular effect of the extract is more significant when blood pressure was elevated (Leung et al., 2010).

Tanshinone IIA failed to attenuate two-kidney two-clip-induced blood pressure elevation in rats but prominently attenuated the interstitial fibrosis caused by the hypertension. The gene expression of MMP-9 and TIMP-1 were suppressed by high-dose tanshinone IIA, just like Valsartan, a usual prescription medicine for the treatment of hypertension and congestive heart failure. Tanshinone IIA was also found attenuating cardiac dysfunction at both high and low dose. Taken together, tanshinone IIA can help prevent cardiac fibrosis and collagen metabolism in rats with renovascular hypertension (Fang et al., 2010). The increased NAD(P)H oxidase activity and expression as well as O\(_2^-\) production in two-kidney two-clip-induced hypertensive rats was inhibited by tanshinone IIA in the further study, which indicated that the heart protection effect of tanshinone IIA was related to its antioxidant effect (Wang et al., 2011a).

Two extracts of *S. miltiorrhiza* (extract 1: using 50% ethanol; extract 2: using 96% ethanol) were administrated to rats for 7 days. Then systolic arterial blood pressure of conscious rats and bioelectric heart activity of unconscious rats were measured after placing rats for 60 mm in the controlled acute hypobaric hypoxia (500 mm Hg). Contents of malonyldialdehyde, lipid peroxidase concentration, and activity of superoxide dismutase and glutathione peroxidase were analyzed and assayed, which were oxidative stress parameters in the blood of rats. The results turned out that the lowering of systolic arterial blood pressure shown in hypoxia affected control rats was augmented by extract 1, and was reversed by extract 2 on the contrary. Hypoxia-induced tachycardia and levels of oxidative stress parameters were normalized by both the two extracts (Buchwald et al., 2012).

The effects and possible mechanisms of the pharmacodynamic interaction between paeanol and salvianolic acid A on cerebrovascular malfunctioning were investigated on rats. The rats with experimental diabetes were administrated with paeanol, salvianolic acid A, and paeanol + salvianolic acid A for eight weeks. Cerebral arteries of the rats showed decreased vascular reactivity to acetylcholine (ACh) which was normalized in all the three treated groups. Phenylephrine (PE)-induced contraction response decreased, and phenylephrine and CaCl\(_2\)-induced vasoconstrictions were partially
inhibited in the treated groups. Diabetes-induced vascular damage was prevented by salvianolic acid A treatments. Combination of paeanol and salvianolic acid A proved to be the most efficient treatment regimen, reducing oxidative stress and regulating intracellular Ca\(^{2+}\) mechanisms (Hu et al., 2012).

The effects of salvianolate on reactive oxygen species (ROS) production in mouse cardiomyocytes in vitro were investigated. Primary ventricular cardiomyocytes were treated with H\(_2\)O\(_2\) (1.25 mmol/L), after which ROS and iNOS production were markedly increased, the levels of NO, total antioxidant capacity and transforming growth factor beta 1 (TGF \(\beta\)1) in the culture medium were decreased, and TGF \(\beta\)1 and Smad2/3 expression in the cells were significantly increased. The H\(_2\)O\(_2\)-induced alterations in the culture medium, and the increases of TGF \(\beta\)1 and Smad2/3 expression in the cells were reversed by addition of salvianolate. The results suggested that ROS and iNOS production were inhibited, and TAOC and NO levels were increased by salvianolate via downregulation of Smad2/3 and TGF \(\beta\)1 expression (Fei et al., 2013).

The potential cerebra protective and antioxidant effects against global cerebral ischemia/reperfusion (I/R) of the polysaccharides (DSP) from the roots of S. miltiorrhiza were investigated in rat model. The neurological deficit scores, percentage of infarction and brain edema, and the generation of mitochondrial reactive oxygen species (ROS) were significantly decreased by pretreatment with DSP. In addition, mitochondria superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were increased and malondialdehyde (MDA) production was reduced in cerebral ischemia brain (Tu et al., 2013). In another study, the effects against global cerebral ischemia/reperfusion (I/R) of a water-soluble polysaccharide (SMP1) isolated from the roots of S. miltiorrhiza were investigated in rats in vivo. Left anterior descending coronary artery occlusion (LAD) was treated with 30 min of ischemia followed by 4 h of reperfusion. Myocardial superoxide dismutase (SOD), Na\(^+\)-K\(^+\)-ATPase and Ca\(^{2+}\)-Mg\(^{2+}\)-ATPase activities were significantly decreased and myocardial malondialdehyde (MDA) level and serum activities of creatine kinase (CK) and lactate dehydrogenase (LDH) in I/R rats were increased. Infarct size and high apoptosis index of cardiac cell were increased in IR rats. After administration of SMP1, all these biochemical parameters in the I/R rats were normalized remarkably. The infarct sizes were found significantly decreased by SMP1. The results indicated that SMP1 had a protective effect against myocardial I/R injury in rats via amelioration of oxidative stress and inhibition of myocardial apoptosis (Song et al., 2013).

4.2. Anticancer

It was showed that cryptotanshinone inhibited cancer cell proliferation by arresting cells in G\(_1\) - G\(_0\) phase of the cell cycle, which was associated with the inhibition of cyclin D1 expression and retinoblastoma protein phosphorylation. Cryptotanshinone was also found inhibiting the signaling
pathway of the mammalian target of rapamycin (mTOR). As a result in vitro model (tube formation), cryptotanshinone inhibited murine lymphatic endothelial cells tube formation partly by inhibiting VEGFR-3-mediated ERK1/2 phosphorylation and partly by inhibiting expression of the small GTPases (Chen et al., 2010; Luo et al., 2011). Cryptotanshinone was identified as a potent stimulator of endoplasmic reticulum (ER) stress which can activate apoptotic pathways in damaged cells, resulting in apoptosis in many cancer cell lines, including HepG2 hepatoma and MCF7 breast carcinoma (Park et al., 2012). Cryptotanshinone was found to induce reactive oxygen species (ROS) in a concentration- and time-dependent manner, activating p38 mitogen-activated protein kinase (MAPK) and c-jun N-terminal kinase (JNK) and inhibiting extracellular signal-regulated kinases 1/2 (Erk1/2), which led to caspase-independent cell death in tumor cells (Rh30, DU145, and MCF-7) (Chen et al., 2012a).

The anti-angiogenic effects of tanshinone IIA were confirmed both in vivo and in vitro. Results from gelatin zymography showed that the extracellular matrix metalloproteinase-2 (MMP-2) activity was reduced dose-dependently by tanshinone IIA. And the dose-dependent decrease of MMP-2 and increase of tissue inhibitor of metalloproteinase-2 (TIMP-2) secretion from cytosol of vascular endothelial cells were simultaneously observed after tanshinone IIA treatment, according to western blot analysis and ELISA (Tsai et al., 2011). In another study, a polysaccharide (SMP-W1) was purified and characterized from S. miltiorrhiza, and its anticancer potential was investigated. The results indicated that SMP-W1 possessed strong in vivo and in vitro anti-tumor activity and improved the immune response in tumor-bearing mice, suggesting SMP-W1 could be developed as an anticancer agent with immunomodulatory activity (Liu et al., 2013b). In addition, a study in vitro confirmed that tanshinone VI inhibited the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Tanshinone VI also had a remarkable anti-angiogenesis effect evaluated with an epithelial cell tube formation assay. The consequent inhibition of metastases formation and angiogenesis without toxicity made tanshinone VI a good candidate for its therapeutic use in humans (Nicolin et al., 2013).

4.2.1. Effects on leukemia

A study revealed that tanshinone IIA inhibited the growth of THP-1 cells and caused significant apoptosis, the suppression was both in time- and dose-dependent manner (Liu et al., 2009). Tanshinone IIA-induced apoptosis on THP-1 cells was mainly related to the disruption of mitochondrial membrane potential (Δψm) and activation of caspase-3 as well as down-regulation of anti-apoptotic protein Bcl-2, survivin and up-regulation of pro-apoptotic protein Bax. Another study showed that tanshinone I could inhibit the growth of three kinds of leukemia cells (U937, THP-1 and SHI 1) and cause apoptosis in a time- and dose-dependent manner, which was highly correlated with activation of caspase-3 and decreasing of hTERT mRNA expression and telomerase activity as well as
down-regulation of survivin expression (Liu et al., 2010a). Tanshinone I also could inhibit the growth of leukemia cells (human K562 and HL-60 cells) and cause apoptosis in a time- and dose-dependent manner, which was mainly related to the disruption of Δψm, the upregulation of Bax expression, and the activation of caspase-3. This process was highly correlated with the inactivation of PI3K/Akt/survivin signaling pathways (Liu et al., 2010b). Tanshinone IIA was confirmed to have different cytotoxic activities on five types of leukemia cells, with the highest toxicity on U-937 cells. Tanshinone IIA-induced apoptosis might result from the activation of PXR, which suppressed the activity of NF-κ B and led to the down-regulation of CCL2 expression (Liu et al., 2012). It was confirmed that tanshinone IIA acted synergistically on the induction of MR2 cell apoptosis with arsenic trioxide (ATO) which was an effective medicine in the treatment of leukemia. The study indicated that tanshinone IIA may be beneficial in the treatment of all-trans retinoic acid (ATRA) -resistant acute promyelocytic leukemia (APL) and in combination with ATO for APL therapy in the clinic (Li et al., 2012). Effect of tanshinone IIA and cryptotanshinone on the Janus activated kinase (JAK)/signal transducer and activator of transcription (STAT) signaling during apoptotic process were investigated (Jung et al., 2013). The study indicated that the anticancer activity of tanshinone IIA and cryptotanshinone was mediated by JAK/STAT3/5 and SHP1/2 signaling, and tanshinone IIA had the potential for combination therapy with imatinib in K562 chronic myeloid leukemia (CML) cells. In another study, tanshinone IIA induced apoptosis by increasing the sub-G1 DNA contents and DNA fragmentation in KBM-5 CML cell line, and it was suggested that tanshinone IIA induced mitochondria-dependent apoptosis via activation of JNK in KBM 5 cells as a potent anti-cancer agent for CML therapy (Yun et al., 2013). Autophagic cell death was induced by tanshinone IIA via activation of AMPK and ERK and inhibition of mTOR and p70 S6K in KBM-5 cells as a potent natural compound for leukemia treatment (Yun et al., 2014). Cryptotanshinone induced apoptosis of HL-60 cell lines by mitochondria pathway, suggesting that CPT may serve as a potential therapy for leukemia (Ni et al., 2014).

4.2.2. Effects on liver cancer

Tanshinone IIA was confirmed to inhibit invasion and metastasis of hepatocellular carcinoma (HCC) and the inhibitory effect became stronger with increases of time and dose. The inhibitory effect of invasion and metastasis of HCC cells in vitro and in vivo was mediated by reducing the expression of the metalloproteinases MMP2 and MMP9 and by blocking NF-κ B activation (Xu et al., 2009). In another study, cytotoxic effects of several tanshinones from S. miltiorrhiza on doxorubicin-resistant human liver cancer cells were demonstrated, indicating that cryptotanshinone suppressed doxorubicin efflux, a process mediated by P-glycoprotein, in a Pgp-overexpressed HepG2 subclone (R-HepG2 cells), and tanshinone IIA provided the best synergism with doxorubicin despite its moderate cytostatic
and pro-apoptotic effects (Lee et al., 2010b). In addition, anticancer effects of tanshinone IIA exhibited dose- and time- dependent action on human hepatoma BEL-7402 cells through apoptosis and G₀/G₁ arrest. The cells were treated with tanshinone and their intracellular calcium was increased, mitochondrial membrane potential was decreased and MT 1A mRNA expression was induced, which indicated that tanshinone IIA-induced cancer cell apoptosis acted via activation of calcium-dependent apoptosis signaling pathways and upregulation of MT 1A expression (Dai et al., 2012).

4.2.3. Effects on prostate cancer

Cryptotanshinone was identified as a potent signal transducer and activator of transcription 3 (STAT3) inhibitor. Shin et al. (2009) first reported that cryptotanshinone had antitumor activity through the inhibition of STAT3. STAT3 Tyr705 phosphorylation was inhibited by cryptotanshinone rapidly in DU145 prostate cancer cells. In another study, it was demonstrated that tanshinone IIA significantly decreased the viable cell number of LNCaP (phosphate and tensin homolog (PTEN) mutant, high AKT, wild type p53) prostate cancer cells more sensitively than against the PC-3 (PTEN null, high AKT, p53 null) prostate cancer cells. The findings suggested that tanshinone IIA-induced apoptosis involves mitochondria intrinsic caspase activation cascade and an inhibition of the PI3K/AKT survival pathway (Won et al., 2010). Fas/APO-1/CD95 was a member of the tumor necrosis factor (TNF) receptor superfamily, however, prostate cancer displayed resistance to Fas-induced apoptosis. It was demonstrated that the apoptosis inhibitory protein, Bcl-2, was highly expressed in response to Fas in DU145 prostate cancer cells, thereby conferring resistance to apoptosis. Cryptotanshinone can suppress Bcl-2 expression and augment Fas sensitivity in DU145 cells. Cryptotanshinone significantly blocked activation of JNK and p38 MAPK which acted upstream of Bcl-2 expression in Fas-treated DU145 cells. Cryptotanshinone sensitized several tumor cells to a broad range of anti-cancer agents (Park et al., 2010).

5,16-Dihydrotanshinone I (DHTS) significantly inhibited the proliferation of human prostate DU145 carcinoma cells and induced apoptosis via induction of ER stress and/or inhibition of proteasome activity, and may have therapeutic potential for prostate cancer patients (Chuang et al., 2011). The inhibitory efficacy of cryptotanshinone and tanshinone I against LNCaP xenograft model were tested and the tanshinones showed 10-30 folds stronger inhibitory potency than Casodex (racemic), an antiandrogen compound that is usually used in the treatment of prostate cancer. All the 3 tested tanshinones were devoid of androgen receptor agonist activity under castrate condition. Tanshinone IIA inhibited androgen receptor nuclear translocation, decreased protein and mRNA abundance of androgen receptor and its target prostate-specific antigen, and stimulated proteosomal degradation of androgen receptor (Zhang et al., 2012a). Prostate cancer cell death caused by tanshinone IIA was in a dose-dependent manner, and cell cycle arrest at G₀/G₁ phase was noted in
LNCaP cells, which was correlated with increase levels of CDK inhibitors and decrease of the checkpoint proteins. Tanshinone IIA induced ER stress in prostate cancer cells and suppressed LNCaP xenograft tumor growth, which caused 86.4% reduction in tumor volume after 13 days of treatment (Chiu et al., 2013).

4.2.4. Effects on gastric cancer

The cytotoxic effects of tanshinone IIA on gastric cancer were described by Chen et al. (2012). The study indicated that tanshinone IIA can induce gastric cancer cell growth inhibition and apoptosis in a time- and concentration- dependent manner. Tanshinone IIA can both cause cell cycle arrest in the G2/M phase and trigger the intrinsic apoptotic signaling pathway. The results suggested that tanshinone IIA may serve as an effective adjunctive agent in the treatment of gastric cancer. In another study, tanshinone IIA exerted powerful inhibitory effects on cell proliferation which was time- and dose-dependent. Tanshione IIA induced apoptosis of SGC7901 cells, reduced the number of cells in S phase and increased those in G0/G1 phase. Tanshinone IIA significantly increased the sensitivity of SGC7901 gastric cancer cells to ADR and Fu. Tanshinone IIA markedly decreased migratory and invasive abilities of SGC7901 cells. The results showed that tanshinone IIA can reverse the malignant phenotype of SGC7901 gastric cancer cells, indicating that it may be a promising therapeutic agent (Xu et al., 2013).

A neutral polysaccharide fraction (SMPA) prepared from the roots of S. miltiorrhiza was tested for its immune enhancing function in gastric cancer rats by intragastric administration. The results showed that splenocyte proliferation was significantly stimulated, anti-inflammatory cytokines production was promoted, pro-inflammatory cytokine secretion was inhibited, the killing activity of natural killer cells and cytotoxic T lymphocytes were augmented, and phagocytotic function of macrophages was increased in gastric cancer rats. Moreover, SMPA administration evidently elevated total intracellular granzyme-B and IFN-γ levels produced by splenocytes in gastric cancer rats. Taken together, these results suggested that SMPA could act as an effective immunomodulator and might be explored as a potential supplemental source for gastric cancer therapy (Wang et al., 2014).

4.2.5. Effects on colon carcinoma

Migration and invasion of colon carcinoma cells was inhibited by tanshinone II A in a dose- and time- dependent manner. Metastasis of colon carcinoma SW480 cells in vivo was also significantly inhibited. The effect resulted from changes in the levels of urokinase plasminogen activator, matrix metalloproteinases (MMP)-2, MMP-9, matrix metalloproteinase protein (TIMP)-1, TIMP-2 and apparent inhibition of the NF-κ B signal transduction pathway (Shan et al., 2009). In another study, the
proliferation of human benign (SW480) and malignant (SW620) colorectal cancer cells were significantly inhibited by 15, 16-Dihydrotanshinone I (DHTS), as shown in MTT and FWC analysis. Activating transcription factor (ATF)-3, a basic leucine zipper-type transcription factor, was found to be predominantly up-regulated in DHTS-treated SW480 and SW620 cells. Overexpression of ATF3 resulted in a significant augmentation of DHTS-induced apoptosis of SW480 cells, but resistance to DHTS-induced apoptosis of SW620 cells, which meant that the effect of ATF3 varied with the degree of malignancy of colorectal cancer (Suk et al., 2013).

4.2.6. Effects on breast cancer

Tanshinone IIA was compared with tamoxifen to determine the anticancer activity on human breast cancer cell lines (estrogen receptor -positive and -negative) in vitro and in vivo. Breast cancer cell proliferation was significantly inhibited by tanshinone IIA in a dose- and time- dependent manner and apoptotic cell populations increased, while tamoxifen inhibited only estrogen receptor-positive breast cancer cells prominently and had no effect on estrogen receptor-negative cells. And the inhibition effect of tamoxifen was significant weaker than that of tanshinone IIA. The expression of P53 and Bcl-2 was decreased by tanshinone IIA in both estrogen receptor -positive and -negative xenografted nude mice (Lu et al., 2009). In addition, hypoxia-induced chemoresistance (DOX resistance) in breast cancer cells was reversed with tanshinone IIA. In hypoxic conditions, tanshinone IIA significantly decreased cell viability and proliferation but not apoptosis. Tanshinone IIA ameliorated hypoxia-induced DOX resistance and epithelial-mesenchymal transition in breast cancer cell lines, which may be attributed to the downregulation of HIF-1 α expression (Fu et al., 2014).

4.2.7. Effects on lung cancer

The effects of cryptotanshinone, tanshinone I and tanshinone IIA on the proliferation inhibition of lung cancer cell lines were evaluated, and tanshinone I was the most potent via cell cycle arrest and apoptosis induction. Aurora A gene knockdown by siRNA dramatically eliminated the T1 activity in vitro, which suggested that Aurora A gene is an important functional target for tanshinone I (Li et al., 2013). In another study, anticancer effect of cryptotanshinone was assessed in the A549 lung cancer cell line and xenograft models of human lung tumors. Growth inhibition, cell cycle arrest and apoptosis factors, were identified in vitro, and inhibition of tumor formation, improvement of body condition and pathological apoptotic effects were detected in vivo. Cryptotanshinone was a potential drug for the treatment and prevention of human lung cancer (Chen et al., 2014).

4.2.8. Effect on glioma

Tanshinone IIA inhibited the cellular growth and induced cell apoptosis. The activity of signal
transducer and activator of transcription 3 (STAT3), which is usually constitutively activated in a variety of malignancies, was significantly reduced by tanshinone IIA parallel with a significant attenuation of expression of Bcl-XL and cyclin D1, the downstream targets of STATS. Tanshinone IIA may act as an effective adjunctive agent in the treatment of glioma for its targeting of constitutive STAT3 signaling (Tang et al., 2010). In another study, effect of cryptotanshinone on the proliferation of human glioma cell lines (T98G and U87) was evaluated, and cryptotanshinone significantly suppressed glioma cell proliferation. Cryptotanshinone inhibited the phosphorylation of STAT3 Tyr705. Overexpression of constitutively active mutant STAT3C reversed the inhibitory effect of cryptotanshinone, while knockdown STAT3 gene showed a similar inhibitory effect as cryptotanshinone treatment, suggesting that the effect of cryptotanshinone was related to inhibiting STAT3 signaling (Lu et al., 2013).

4.2.9. Effect on cervical cancer

The effects of dihydrotanshinone I and irradiation were investigated both in vitro and in vivo. After treatment with irradiation, dihydrotanshinone I, and dihydrotanshinone I + irradiation, the apoptosis of HeLa cells was 5.8, 13.3 and 22.5%, respectively. Dihydrotanshinone I significantly reduced the survival of irradiated HeLa cell. The HPV E6 gene expression was down-regulated by combination treatment with irradiation and dihydrotanshinone I. A significant decrease in tumor growth was induced by combination treatment with dihydrotanshinone I and irradiation, without signs of general or organ toxicity (Ye et al., 2012). In addition, tanshinone IIA exhibited strong growth inhibition against cervical cancer cells in dose- and time- dependent manners. G2/M arrest was initiated after a 24 h exposure to tanshinone IIA according to the flow cytometric analysis. A comprehensive proteomic analysis was performed to survey global protein changes induced by tanshinone IIA treatment on HeLa cells. The proteins (vimentin, maspin, α- and β-tubulin, and GRP75) contributed to the cytotoxicity of tanshinone IIA (Pan et al., 2010).

4.2.10. Effect on other neoplasms

Tanshinone IIA may be an efficacious anti-osteosarcoma drug as it could induce cell apoptosis and inhibit proliferation, migration, and invasion in vitro. The effect of tanshinone IIA on inhibiting proliferation and inducing apoptosis was in a time- and dose- dependent manner. Caspase activation mediated tanshinone IIA activity. Antiapoptotic Bcl-2, MMP-2, and MMP-9 levels were reduced, while proapoptotic Bax levels were increased (Zhang et al., 2012b). In addition, Celecoxib was a selective inhibitor of cyclooxygenase-2 (COX-2), an important mediator of inflammation which was closely associated with head and neck squamous cell carcinoma (HNSCC) development. However, the cardiac toxicity of celecoxib limited long-term use of it. Zhao et al. (2010) took a study that the dose of
celecoxib can be lowered if combined with salvianolic acid B. The results revealed that combination of Sal-B with low-dose celecoxib resulted in a more significant anticancer effect in HNSCC than either agent alone. The combination treatment may be a potential therapeutic schedule in targeting inflammatory-associated tumor development. In another study, antiproliferative effect of tanshinone IIA on human oral cancer KB cells was explored. Tanshinone IIA induced growth inhibition on KB cells according to the observation of cell morphology. Tanshinone IIA resulted in a cell cycle arrest in G2/M phase. Caspases with the cleaved poly (ADP-ribose) polymerase was activated, which indicated that the apoptosis induced by tanshinone IIA in KB cells was mediated through the mitochondria-dependent caspase pathway (Tseng et al., 2014).

4.3. Antioxidant Effect

Aqueous extract of *S. miltiorrhiza* containing 3, 4-dihydroxybenzoic acid, 3, 4-dihydroxyphenyl lactic acid and salvianolic acid B was used for the treatment of the rat aortic smooth muscle A10 cells. Growth of the homocysteine stimulated rat A10 cells was inhibited dramatically, and the concentration of intracellular reactive oxygen species (ROS) was significantly decreased. Protein kinase C β-1 (PKC β-1) and phosphorylated mitogen-activated protein kinase (p-MAPK) expression were markedly down-regulated, suggesting that the inhibitory effect of the polyphenol-rich *S. miltiorrhiza* on the homocysteine-induced growth of rat A10 cells was realized via the PKC/p44/42 MAPK-dependent pathway (Hung et al., 2009). In addition, methanolic extract of *S. miltiorrhiza* was thought to have antioxidant effect. Different fractions from methanolic extract of *S. miltiorrhiza* were assayed in vitro. Ethyl acetate (EtOAc) fraction showed the highest 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. The EtOAc fraction have the best result of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity and ferric reducing antioxidant power (FRAP) compared to the other fractions. The EtOAc fraction contained the highest level of the total phenolic contents (Kim et al., 2010).

The antioxidant effect of leaf of *S. miltiorrhiza* was evaluated, with acetone and methanol as two different extractants. The results showed that total phenolic contents of acetone and methanol extracts were 39.0 ± 1.13 and 54.3 ± 1.1 mg gallic acid equivalents/g, respectively. In DPPH radical scavenging assay, EC50 of methanol extracts was 7.0 ± 0.28 μg/mL, while in superoxide radical quenching assay, it was 246.5 ± 10.35 μg/mL. Methanol extracts can significantly inhibited linoleic acid oxidation (93.2%), which was equivalent to butylated hydroxytoluene. HPLC and correlation analysis show that the major antioxidant components were salvianolic acid B and rosmarinic acid, which were also the most abundant phenolic compounds (Zhang et al., 2010). In another study, the EA.hy926 cells were incubated with different concentrations of tanshinone IIA (5, 10 and 20 μg/μL) or
DMEM, and then treated with H₂O₂, after which loss of cell viability and excessive cell apoptosis were observed. While cell apoptosis was attenuated in different concentrations of tanshinone IIA pretreated cells, and the elevation of ROS evoked by H₂O₂ was also markedly inhibited by the treatment of tanshinone IIA. The expressions of pro-apoptotic proteins were significantly decreased, however, the expression of anti-apoptotic protein Bcl-2 was significantly increased by tanshinone IIA, leading to obvious reduction of Bax/Bcl-2 ratio in EA.hy926 cells induced by H₂O₂ (Jia et al., 2012).

The composition and pharmacological effects of water-extracts of *S. miltiorrhiza* obtained from heat reflux water extraction and microwave-assisted extraction with water at different temperatures were compared, turning out that the third-round microwave-assisted extraction with water (100 °C) extract had the highest phenolic acids and tanshinones, with the strongest antioxidant activity in DPPH assay and FRAP assay. Salvianolic acid was the most significant marker in the antioxidant and vasodilation effects (Zhou et al., 2012). In another study, the root, callus and hairy root cultures of *S. miltiorrhiza* obtained by hydrodistillation were investigated by GC and GC-MS. The antioxidant effect of callus and hairy root oils were better than that of root oil, indicating that the essential oils of *S. miltiorrhiza* could be a potential source of antioxidant agents (Lou et al., 2014). In addition, the antioxidant effect of a pair of new phenolic acid stereoisomers, (R)-norsalvianolic acid L and (S)-norsalvianolic acid L were assessed by the DPPH and ABTS assays in vitro. The IC₅₀ values of (R)-norsalvianolic acid L were 6.9 and 9.7 μM respectively, which was close to the control salvianolic acid B (7.8 and 7.1 μM respectively), while the IC₅₀ values of (S)-norsalvianolic acid L were 27.1 and 25.3 μM, respectively (Liu et al., 2014a).

The antioxidant effect of tanshinones was related to activating nuclear factor (erythroid-derived 2) - like 2 (Nrf2). The structure-activity relationships of tanshinones for Nrf2 activation was elucidated, and electron affinity (EA) and energy level of the lowest unoccupied molecular orbital (E-LUMO) were calculated, which was helpful to find new multifunctional antioxidants (Sun et al., 2014). In addition, a likely mechanism of the activities of traditional Chinese medicine was interacting with redox balance and prevention of oxidative stress (Matkowski et al., 2013). Some components of *S. miltiorrhiza* were promising antioxidants, such as salvianolic acid B, tanshinone IIA, rosmarinic acid. The underlying mechanism of antioxidant effect of *S. miltiorrhiza* would be elucidated gradually.

4.4. Neuroprotective Effects

Alzheimer's disease was related to the deposition of amyloids in neurons and a cholinergic neurotransmission deficit in the brain. Learning and memory could be improved by cryptotanshinone in several pharmacological models of Alzheimer's disease. Amyloid plaque deposition was significantly attenuated in the brain of amyloid precursor protein (APP)/PS1 (a gene associated with
Alzheimer's disease) transgenic mice (APP/PS1 mice). Cryptotanshinone was confirmed to strongly improve spatial learning and memory. The effects of the cryptotanshinone on APP processing was investigated in rat cortical neuronal cells which overexpressed Swedish mutant human APP695. P-amyloid protein (Aβ) was decreased, while the N-terminal APP cleavage product, sAPP α was significantly increased, and α-secretase activity was increased (Mei et al., 2009). In the study of Wong et al. (2010), cryptotanshinone was found to be an inhibitor of both human acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). And AChE inhibition was in a reversible manner. The task learning ability of rats treated with scopolamine was significantly reversed by cryptotanshinone. Compared to the control rats, the rats fed with cryptotanshinone were able to develop a spatial searching strategy (Wong et al., 2010). The neuroprotective effect of tanshinone IIA against amyloid β-peptide (Aβ25-35)-induced cell death was investigated in cultured cortical neurons. A significant viability loss, cell apoptosis, and Bcl-2/Bax ratio reduction were caused by the exposure of Aβ25-35. Aβ25-35 was also found to increase levels of malondialdehyde production, the intracellular ROS elevation, the activity of caspase-3, and decrease activities of superoxide dismutase and glutathione peroxides and mitochondrial membrane potential (MMP) in the cultured cortical neurons. Those Aβ25-35-induced events could be suppressed by pretreatment of tanshinone IIA, which indicating that the neuroprotective effect of tanshinone IIA against Aβ was associated with its antioxidative potential (Liu et al., 2010c). The role of cryptotanshinone on non-amyloidogenic processing of amyloid-β protein precursor (Aβ PP) and its regulation by protein kinase C (PKC) were investigated. Intracellular and secreted levels of Aβ40 and Aβ12 in N2a mouse neuroblastoma cells were strongly reduced by cryptotanshinone stably expressing human Swedish Aβ PP (N2a-SwedAβ PP). The maturation of “a disintegrin and metalloproteinase-10” (ADAM10), a α-secretase candidate was increased. Treatment of neuroblastoma cells with cryptotanshinone induced the co-translocation of ADAM10 and PKC-α to the cell membrane, the site at which Aβ PP was cleaved, and this translocation was significantly reduced by GO6976, which suggesting that CTS-induced sAβ PP α secretion is regulated by a PKC-α and ADAM10 cascade in neuroblastoma cells and may be involved in the lowering of Aβ production (Durairajan et al., 2011).

The possible neuroprotective effects of tanshione IIA were evaluated on H2O2-induced oxidative stress in rats. Primary rat cortical neurons were protected by pretreatment with tanshione IIA against H2O2-induced cytotoxicity. The elevation of Ca2+ (i) evoked by H2O2 was significantly reduced. The increase in Bax/Bcl-2 ratio induced by H2O2 was prevented by pretreatment with tanshione IIA. H2O2-induced hippocampal LTP impairment was reversed by preincubation of tanshione IIA for 20 min prior to H2O2 exposure, which meant tanshione IIA acted as a novel promising therapeutic agent for oxidative stress injury in neurodegenerative diseases (Wang et al., 2011b). In another study, effects of S. miltiorrhiza aqueous/ethanol extracts, total polyphenols, total tanshinones and 3 phenolic
compounds against toxicity mediated by Aβ25-35 were tested with PC-12 cells. RSM aqueous/ethanol extracts and total polyphenols revised Aβ25-35-induced cytotoxicity, and salvinianolic acid A and B could protect PC-12 cells by blocking Aβ25-35-induced Ca2+-intake, lactate dehydrogenase release, cell viability decrease and apoptosis. *S. miltiorrhiza* ethanol extract, total tanshinones, tanshinone I and dihydrotanshinone I remarkably inhibited acetylcholinesterase *in vitro* (Zhou et al., 2011). In addition, *S. miltiorrhiza* extract (SME) had neuroprotection against Aβ25-35-induced apoptosis in SH-SY5Y cells and the underlying mechanisms were investigated. The increased intracellular reactive oxygen species levels was suppressed by SME, the protein expression of cleaved caspase-3 was decreased, and cytosolic cytochrome c and Bax/Bcl-2 ratio were decreased either, which suggested that SME provided substantial neuroprotection against Aβ25-35-induced neurotoxicity in SH-SY5Y cells, at least in part, via inhibiting oxidative stress and attenuating the mitochondria-dependent apoptotic pathway (Yu et al., 2014).

4.5. Anti-inflammation Effect

*S. miltiorrhiza* extract was purified and called PF2401-SF which showed protection against liver injury *in vivo*, at a greater potency than an ethanol extract. PF2401-SF was enriched with tanshinone I (11.5%), tanshinone IIA (41.0%), and cryptotanshinone (19.1%). The potential anti-inflammatory effects of PF2401-SF were investigated *in vitro* and *in vivo*. PF2401-SF showed anti-inflammatory potency on lipopolysaccharide (LPS)-induced nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells. Heme oxygenase (HO)-1 expression was induced by PF2401-SF through extracellular signal-regulated kinases (ERK1/2) phosphorylation. Inflammation on carrageenan- or dextran-induced acute arthritis in rats was significantly reduced after treatment with PF2401-SF. PF2401-SF may be a potential candidate for the treatment of various inflammatory diseases (Jiang et al., 2013).

It was investigated whether tanshinone IIA could inhibit hepatic stellate cells (HSCs) activation. The results turned out that tanshinone IIA had no cytotoxicity against HSCs. Tanshinone IIA suppressed LPS stimulated NF-κ B luciferase activities, nuclear translocation of NF-κ B-p65, and phosphorylations of ERK, JNK and p38. In addition, LPS-induced HSCs chemotaxis was significantly inhibited by tanshinone IIA, in both wound-healing and trans-well invasion assays. Moreover, attenuated LPS-induced mRNA expressions of CCL2, CCL3, CCL5, IL-1β, TNF-α, IL-6, ICAM-1, iNOS, and α-SMA in HSC-T6 cells were attenuated by tanshinone IIA. It could be concluded that tanshinone IIA decreased LPS-induced HSC activation (Liu and Huang, 2014).

4.6. Antibacterial Effect
The extracts of *S. miltiorrhiza* were tested for antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in clinic. The chloroform (CHCl₃) and n-hexane (HEX) extracts (minimum inhibitory concentration (MIC), 0.0078-0.3125 µg/mL; minimum bactericidal concentration (MBC), 0.019-0.625 µg/mL) were found to have the strongest antibacterial activity against MRSA. Additionally, when the CHCl₃ and FLEX extracts were co-administered with ampicillin or oxacillin, a synergistic effect against MRSA was observed. Furthermore, a time-kill study evaluating the effects of the extracts against MRSA indicated that treatment with the CHCl₃ extract in combination with ampicillin or oxacillin produced rapid bactericidal activity. These results suggested that *S. miltiorrhiza* extracts may have potent antimicrobial activity and can be a suitable agent for treating MRSA infections (Cha, 2009).

Cryptotanshinone demonstrated strong antibacterial activity against clinic isolated methicillin and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA). The combination effects of cryptotanshinone with antibiotics were synergistic against most of tested clinic isolated MRSA, MSSA, and VRSA except additive, MRSA 4 and 16 in oxacillin, MRSA 6, 12, and 15 in ampicillin, and MRSA 6, 11, and 15 in vancomycin (FIC index < 0.75-1.0). Furthermore, a time-kill study showed that the growth of the tested bacteria was completely attenuated after 2-6 h of treatment with the 1/2 MIC of CT, regardless of whether it was administered alone or with ampicillin, oxacillin, or vancomycin. The results suggested that cryptotanshinone could be employed as a natural antibacterial agent against multidrug-resistant pathogens infection (Cha et al., 2014).

5. Conclusions

Accumulated evidence demonstrated that tanshinone I, tanshinone IIA, cryptotanshinone and salvianolic acid A were main bioactive components of *S. miltiorrhiza*. The extraction, separation and analysis methods had been developed. The efficiency of the methods varied with the specific component to be extracted. When choosing the appropriate extract method, the purpose, efficiency and economy must be taken into consideration. *S. miltiorrhiza* showed a variety of bioactivities and many effects on health, such as the effect on cardiovascular and cerebrovascular diseases, and anticancer, antioxidant, neuroprotective, anti-inflammation and antibacterial effects. The treatment on cardiovascular and cerebrovascular diseases was main use of *S. miltiorrhiza*, and the mechanism was explored intensively. The anticancer effect also attracted so much attention, especially for leukemia. The antioxidant of *S. miltiorrhiza* was explored, and the major antioxidant components were salvianolic acid B and rosmarinic acid. *S. miltiorrhiza* was also useful for Alzheimer's disease and other related neurodegenerative disease. In the future, more studies on the anti-inflammation and
antibacterial effect of S. miltiorrhiza should be carried out, and the underlying mechanisms of bioactivities need to get further scientific explanation.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81372976), Key Project of Guangdong Provincial Science and Technology Program (No. 2014B020205002), and the Hundred-Talents Scheme of Sun Yat-Sen University.

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