

Tanshinones as Possible Therapeutic Agents for Cardiovascular, Cerebral, Hepatic, and Bone Diseases

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ABSTRACT

The root of *Salvia miltiorrhiza* (Tan shen) has been used as a traditional drug for circulatory disorders including ischemic diseases such as angina pectoris. Effective components protecting the myocardium against ischemia-induced metabolic disturbances; tanshinone IIA, tanshinone I, tanshinone V, tanshinone VI, and dihydrotanshinone were isolated. Among them, we found tanshinone VI as the most effective and possible agent with cardioprotection against ischemia/reperfusion injury in Langendorff manner by using rat isolated hearts. We have shown that the possible mechanisms underlying an enhancement of post-hypoxic contractile recovery of perfused hearts may be exerted via an enhancement of energy restoration and an attenuation of Ca²⁺ overload in the reoxygenated myocardium. We also found another beneficial effect of tanshinone VI on cardiac remodeling in primary cultured cardiomyocytes and cardiac fibroblasts. It is generally accepted that cardiac remodeling such as cardiac hypertrophy and fibrosis enhances the development and progression of heart failure following various cardiac diseases including acute myocardial infarction. Treatment of cardiomyocytes and cardiac fibroblasts with tanshinone VI attenuated humoral factor-induced cellular hypertrophy and fibro-

sis under *in vitro* experimental conditions. Thus, tanshinone VI may be a possible agent for attenuating the development of cardiac remodeling such as cardiac hypertrophy and fibrosis, which may lead to cardiac contractile dysfunction. Furthermore, inhibition of tanshinone VI to bone resorption through the prevention of osteoclast differentiation by inhibiting receptor activator of NFκB ligand (RANKL) expression and NFκB induction, was exhibited. Pharmacological suppression of NFκB and RANKL induced by tanshinone VI *in vivo* may be an effective approach to improve bone loss suppressing osteoclastic bone resorption in rheumatoid arthritis. Anti-inflammatory effect of tanshinone I, having a similar abietane structure to tanshinone VI, is shown on neuroprotection against cerebral ischemia-reperfusion injury in the gerbil hippocampus, and apoptosis in activated hepatic satellite cells (HSCs). Neuroprotection of tanshinone IIA against cerebral ischemia/reperfusion injury through inhibition of macrophage migration inhibitory factor and tanshinone IIA-mediated HSC-T6 apoptosis through Akt inhibition and ERK-Bax-caspase-3/9 signaling pathways are presented. Tanshinone IIA decreased the lipopolysaccharide-induced activation of rat hepatic satellite cells. Possible therapeutic effects of tanshinones VI, I, and IIA on cardiac contractile failure, cerebral infarction, bone resorption, and apoptosis of hepatic satellite cells are discussed.

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Key words: Tanshinone VI; Cardiac contractile force; Osteoclastogenesis; Tanshinone I; Neuroprotection; Inhibition of activated HSCs; Tanshinone IIA; Bone resorption; HSCs; Liver injury

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INTRODUCTION

Tan shen extract has conceived to improve systemic circulation, in particular coronary circulation^[1]. In the series of our studies on the effect of Tan shen, several ingredients having an abietane diterpene structure with an ortho-diketone group, showed beneficial effects on post-hypoxic recovery of cardiac contractile force. Among the active components, tanshinone VI, which was the most effective agent isolated first time, was focused on cardiac function and metabolism in the hypoxic and post-hypoxic rat hearts^[2]. Natural compounds that suppress osteoclast differentiation may have therapeutic value in treating osteoporosis and other bone erosive diseases such as rheumatoid arthritis or metagenesis associated with bone loss. Recently, Nicolin V. and his colleagues investigated anti-osteoporotic compounds from Tan shen and analyzed the effect of tanshinone VI on osteoclasts differentiation^[3]. Recent studies on tanshinone VI shows that (i) tanshinone VI against post-hypoxic recovery of cardiac contractive force in isolated rat hearts, (ii) insulin-like growth factor-1-induced hypertrophy of cardiomyocytes isolated from neonatal hearts via phosphorylation of ERK and Akt, and (iii) inhibition of bone resorption by using a physiologic three-dimensional osteoblast/bone marrow model of cell co-culture. Taken together, neuroprotective activity and induction of apoptosis in activated hepatic satellite cells of tanshinone I, and IIA against cerebral ischemia/perfusion injury in rats and anti-inflammatory effects of tanshinone IIA on osteoporosis and apoptosis of HSC-T6 cells in liver injury are presented. The present review summarizes anti-inflammatory efficacy of tanshinones on the pharmacological activities and possible mechanisms of action for tanshinones as therapeutic agents in post-hypoxic recovery of cardiac contractile force, neuroprotection against cerebral injury, regulation of osteoclastogenesis, and protection of hepatic diseases. Figure 1 shows tanshinones isolated from *Salvia miltiorrhiza* roots.

PROTECTIVE EFFECTS OF TANSHINONE VI IN PERFUSED RAT HEARTS AGAINST HYPOXIA/REOXYGENATION INJURY

When abietane pigments such as tanshinone I, cryptotanshinone, and tanshinone VI, which are isolated from the Tan shen extract, were treated to perfused hearts under hypoxic conditions, the contractile recovery during reoxygenation were significantly enhanced. Especially, treatment of hypoxic hearts with tanshinone VI exerted the most effective recovery on the post-hypoxic contractile force. The protective effects of tanshinone VI against hypoxia/reoxygenation injury were associated with a decrease in the release of ATP metabolites such as adenosine, inosine, and hypoxanthine from hypoxic hearts. This finding suggests that treatment of hypoxic hearts with tanshinone VI enhances to restore myocardial high-energy phosphates under reoxygenated conditions, because ATP metabolites are the substrates for ATP reproduction in the myocardium during reoxygenation. We also found that treatment with 42 nmol/L tanshinone VI attenuated an increase in myocardial Ca^{2+} content, which is so-called " Ca^{2+} overload", during hypoxia/reoxygenation. The attenuation of Ca^{2+} overload in the hypoxic/reoxygenated hearts was associated with a reduction of creatine kinase release, an indicator for the cardiac cell necrosis, from the reoxygenated myocardium. Taken together, tanshinone VI is a possible agent for myocardial protection against hypoxia/reoxygenation injury via an improvement of myocardial energy production and a prevention of

myocardial Ca^{2+} overload during hypoxia/reoxygenation^[4,5].

Synthesis of water-soluble tanshinone derivatives; sodium tanshinone VI 1-phenolate, 1'-O-hydrogen succinyltanshinone VI 1-O-hydrogen succinate, and disodium 1'-O-succinyltanshinone VI 1-O-succinate, were carried out and then effects of these agents on post-hypoxic contractile dysfunction of perfused rat hearts were examined. The efficacies of water soluble tanshinone VI derivatives on cardiac performance and energy metabolism during hypoxia/reoxygenation were similar to that of tanshinone VI^[6].

These our studies suggest two possible mechanisms for the recovery of post-hypoxic cardiac contractility, i.e. enhancement of the myocardial energy store restoration after oxygen deficiency and prevention of hypoxia/reoxygenation-induced calcium overload in the cardiac tissue.

EFFECTS OF TANSHINONE VI ON HYPERTROPHY OF CULTURED CARDIOMYOCYTES ISOLATED FROM NEONATAL RAT HEARTS

The possible effects of tanshinone VI on hypertrophy and fibrosis in cultured cardiomyocytes and cardiac fibroblasts prepared from neonatal rats were examined. Tanshinone VI had no significant effects on protein synthesis of cardiomyocytes in the absence of stimulatory factors in the culture medium. The protein synthesis in the cells was markedly increased by the presence of endothelin-1 (ET-1), phenylephrine (PE), or insulin-like growth factor-1 (IGF-1), suggesting that hypertrophy of cardiomyocytes was induced under *in vitro* experimental conditions. The ET-1-, PE-, or IGF-1 induced increase in protein synthesis was attenuated by treatment with tanshinone VI. Furthermore, the effects of tanshinone VI on IGF-1-induced changes in biochemical parameters during the development of hypertrophy in cardiomyocytes were examined. IGF-1 induced an increase in phosphorylated extracellular signal-regulated kinase 1/2 (ERK), whereas IGF-1 did not change ERK protein content in cardiomyocytes. When the cells were incubated with tanshinone VI, IGF-1-induced increase in protein synthesis of cardiomyocytes was attenuated, associated with a reduction of ERK phosphorylation.

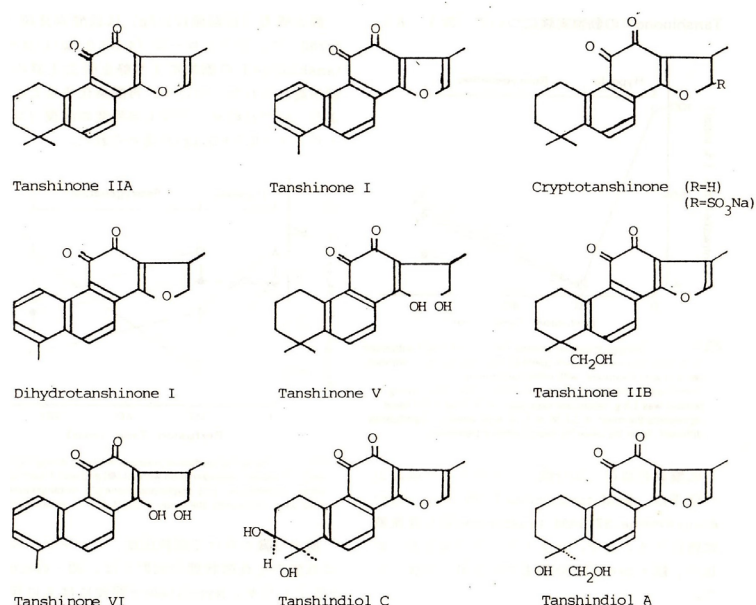


Figure 1 Structure of Tanshinones isolated from *Salvia miltiorrhiza* root

These findings suggest that tanshinone VI is a possible agent for the suppression of IGF-1-induced hypertrophy of cardiomyocytes via an attenuation of ERK activation^[7].

Effects of collagen synthesis in cardiac fibroblasts, which was evaluated by [³H]-proline incorporation into acid-insoluble fraction of the fibroblasts, were examined. Fetal bovine serum (FBS) or IGF-1 increased collagen synthesis in a concentration-dependent manner. The increase in collagen synthesis by the presence of FBS or IGF-1 was also inhibited by tanshinone VI, whereas tanshinone VI did not affect collagen synthesis of cardiac fibroblasts in the absence of any humoral factors in the medium.

In this study, we found interesting crosstalk between cardiomyocytes and cardiac fibroblasts. Fibroblast-conditioned medium (FB-CM) increased protein synthesis in cardiomyocytes in a concentration-dependent manner, suggesting that cardiac fibroblasts release humoral factors and induce hypertrophy of the cardiomyocytes. Tanshinone VI attenuated the FB-CM-induced increase in protein synthesis by cardiomyocytes. These findings show that tanshinone VI may attenuate the humoral factor-induced hypertrophy of cardiomyocytes and fibrosis of cardiac fibroblasts.

Our findings suggest that tanshinone VI may improve the development of cardiac remodeling such as hypertrophy and fibrosis in the myocardium under pathophysiological conditions^[8]. We have shown the first report that tanshinone VI is a possible therapeutic agent for cardiac remodeling during the development of heart failure^[9].

ANTI-INFLAMMATORY EFFECTS OF TANSHINONE I ON NEURO-PROTECTION

Zhou S. and his colleagues investigated whether the neuroprotective effect of tanshinone I is associated with inhibition of peroxynitrite-caused DNA damage, a critical event leading to peroxynitrite-induced cytotoxicity. The results showed that tanshinone I can significantly inhibit peroxynitrite-induced DNA damage both in ϕ X-174 plasmid DNA and rat primary astrocytes. EPR spectroscopy indicates that tanshinone I potently diminished the DMPO-hydroxyl radical adduct signal from peroxynitrite. These results demonstrate for the first time that tanshinone I can protect against peroxynitrite-induced DNA damage, hydroxyl radical formation and cytotoxicity, which might have implications for tanshinone I-mediated neuroprotection^[10].

Park JH. and his colleagues examined the neuroprotective effect of tanshinone I against ischemic damage and its neuroprotective mechanism in the gerbil hippocampal CA1 region (CA1) induced by 5 min of transient global cerebral ischemia. Pre-treatment with tanshinone I protected pyramidal neurons from ischemic damage in the stratum pyramidale (SP) of the CA1 after ischemia-reperfusion. The pre-treatment with tanshinone I increased the immunoreactivities and protein levels of interleukine (IL)-4 and IL-13, anti-inflammatory cytokines in the tanshinone I-treated-sham-operated-groups compared with those in the vehicle-treated-sham-operative-groups; however, the treatment did not increase the immunoreactivities and protein levels of pro-inflammatory cytokines, such as IL-2 and tumor necrosis factor- α . On the other hand, in the tanshinone I-treated-ischemia-operated-groups, the immunoreactivities and protein levels of all the cytokines were maintained in the SP of the CA1 after transient cerebral ischemia. In addition, the authors examined that IL-4 injection into the lateral ventricle did not protect pyramidal neurons from ischemic damage. In conclusion, these findings indicate that the pre-treatment with tanshinone I can protect against ischemia-induced neuronal death in the CA1 via the

increase or maintenance of endogenous inflammatory cytokines, and exogenous IL-4 does not protect against ischemic damage^[11].

Brain infarction and neuronal damage were examined using 2,3,5-triphenyltetrazolium chloride staining, hematoxylin and eosin histochemistry, and Fluoro-Jade B histofluorescence. Pretreatment with tanshinone I (10 mg/kg) was associated with a significant reduction infarct volume 1 day after hypoxia-ischemia was induced. In addition, tanshinone I protected against hypoxia-ischemia-induced neuronal death in the ipsilateral region. The findings by Lee JC. and his colleagues' suggested that tanshinone I has strong potential for neuroprotection against hypoxic-ischemic damage^[12]. These neuroprotective effects of tanshinone I in the gerbil hippocampal CA1 region and mouse model of cerebral hypoxia-ischemia may be used in research into new anti-stroke medications.

ANTI-INFLAMMATORY EFFECTS OF TANSHINONE IIA AGAINST CEREBRAL ISCHEMIA/REPERFUSION INJURY

Wu GB. and his colleagues investigated the effects of tanshinone IIA on isolated rat coronary arteriole and underlying mechanisms. Coronary arterioles were carefully dissected, cannulated and pressurized. Tanshinone IIA-elicited vascular inner diameter change was recorded by a computerized diameter tracking system. To investigate the mechanisms governing the vasodilative effects of tanshinone IIA, the roles of endothelium, endothelium-derived vasoactive factors and potassium channels were assessed respectively. Endothelium denudation, inhibition of nitric oxide synthesis, inhibition of the cytochrome P450 epoxygenase, and blockade of the large conductance Ca^{2+} -activated K^{+} channel (BKca) significantly decreased the vasodilation elicited by tanshinone IIA. The results indicated that tanshinone IIA induces an endothelium-dependent vasodilation in coronary arterioles; nitric oxide and cytochrome P450 metabolites contribute to the vasodilation; activation of BKca channels plays an important role in the vasodilation^[13].

The study of Fan GW. and his colleagues' was undertaken to characterize the estrogenic activity of tanshinone IIA and demonstrate a functional role of this activity in RAW264.7 cells. In transient transfection assay, tanshinone IIA (10 μM) increases ERE-luciferase activity in an estrogen receptor (ER) subtype-dependent manner when either ER α or ER β were co-expressed in Hela cells. In LPS-induced RAW264.7 cells, tanshinone IIA exerts anti-inflammatory effects by inhibition of iNOS gene expression and NO production, as well as inhibition of inflammatory cytokine expression such as IL-1 β , IL-6, and TNF- α via ER-dependent pathway. Therefore, it could serve as a potential selective estrogen receptor modulator to treat inflammation-associated neurodegenerative and cardiovascular diseases without increasing the risk of breast cancer^[14].

In an *in vivo* study, rat myocardial infarction (MI) model was induced by permanent left anterior descending coronary artery ligation. After the operation rats were divided into three groups (sham, MI and tanshinone IIA). Tanshinone IIA was administered intra-gastrically at a dose of 60 mg/kg body wt./day. One week later, rats were sacrificed and the hemodynamic, pathological and molecular biological indices were examined. In an *in vitro* study, the inflammatory model was established by TNF- α stimuli on cardiomyocyte and cardiac fibroblasts. Tanshinone IIA attenuates the MI pathological changes and improves heart function, and reduces expression of MCP-1, TGF- β 1 and macrophage infiltration. Furthermore, tanshinone IIA could also decrease the expression of TNF- α and activation of nuclear transcription factor-kappa B. *In vitro*,

tanshinone IIA could reduce MCP-1 and TGF- β_1 secretion of cardiac fibroblasts^[15]. The study of Ren ZH. and his colleague's demonstrated that the cardioprotective effects of tanshinone IIA might be attributed to its capacity for inhibiting inflammatory responses.

Ischemia/reperfusion (I/R) injury is associated with systemic inflammatory response. Macrophage migration inhibitory factor (MIF) has been implicated in many inflammatory process. Tanshinone IIA (TSA) is one of the active ingredient in Dan shen. Chen Y. and his colleagues demonstrated that TSA has protective effects against focal cerebral I/R injury. Rats were subjected to middle cerebral artery occlusion for 2 hours. This was followed by reperfusion. Neurological deficits, brain water content, and infarct volume were measured, and it was found that neurological dysfunction, brain edema, and brain infarction were significantly attenuated by TSA 6 hours after reperfusion. And myeloperoxidase (MPO) activity was measured at 6 and 24 hours, and it was found that neutrophil infiltration was significantly higher in the vehicle+I/R group than in the TSA+I/R group. ELISA demonstrated that TSA could inhibit MIF expression and the release of TNF- α and IL-6 induced by I/R injury. Western blot analysis and immunofluorescence staining showed that MIF expression was significantly lower in the TSA+I/R group than in the vehicle+I/R group. MIF was found almost all located in neurons and hardly any located in astrocytes in the cerebral cortex. Western blot analysis and EMSA demonstrated that NF- κ B expression and activity were significantly increased in the vehicle+I/R group. However, these changes were attenuated by TSA. In conclusion, the results suggest that TSA helps alleviate the proinflammatory responses associated with I/R-induced injury and that this neuroprotective effects may occur through down-regulation of MIF expression in neurons^[16].

Tang Q. and his colleagues compared the potential neuroprotective effects of tanshinone IIA monotherapy, tetramethylpyrazine (TMP) monotherapy, and tanshinone IIA + TMP combination therapy in adult rat subjected to cerebral ischemic injury using the permanent middle cerebral artery occlusion (MCAO) model and in primary cortical neuron culture exposed to oxygen-glucose deprivation (OGD) model. Apoptosis factors (Bax, Bcl-2, caspase-3, and trmp-7) were analyzed using western blot and immunohistochemistry. The results suggest that tanshinone IIA + TMP combination therapy was more effective than TMP monotherapy but not than tanshinone IIA monotherapy. Tanshinone IIA monotherapy is more effective than TMP monotherapy in protecting the neuron against hypoxia/ischemia both *in vitro* and *in vivo*. Interestingly, tanshinone IIA significantly increased the phosphorylation of Akt in primary cortical neuronal culture exposed to OGD, which was abolished by PI3K inhibitor LY294002. The PI3K/AKT signaling pathway may be involved in the neuroprotective mechanism of tanshinone IIA on primary cortical neurons^[17]. Shi LL. and his colleagues determined whether tanshinone IIA protects neurons against A β (25-35)-induced cytotoxicity and detected the association of this protective effect with calpain and the p35/Cdk5 pathway. The results showed that tanshinone IIA protected neurons against the neurotoxicity of A β (25-35), increased the viability of neurons, decreased expression of phosphorylated tau in neurons induced by A β (25-35), improved the impairment of the cell ultra-structure (such as nuclear condensation and fragmentation, and neurofibril collapse). Further more, the authors found that tanshinone IIA possessed neuroprotective action and the protection may involve in calpain and the p35/Cdk5 pathway. These data suggested that tanshinone IIA possesses protective effects against neurotoxicity and tau hyperphosphorylation induced by A β (25-35) at 10 μ M^[18].

ANTI-INFLAMMATORY EFFECTS OF TANSHINONE IIA ON OSTEOPOROSIS

Kim HK. and his colleagues examined the effect of *Salvia miltiorrhiza* (SM) on osteoclastogenesis and osteoblast differentiation, which are two important markers of the bone physiology. Through a rapid, sensitive and specific isocratic liquid chromatography/tandem mass spectrometry method for the simultaneous quantitative determination of four diterpenoids; tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone in SM, the authors tried to correlate the amount of tanshinone compounds in SM into the antiosteoclast activity. The SM fraction with a low concentration of tanshinone IIA (1 μ g/mL) had no effect on the alkaline phosphatase activity (osteoblast differentiation), but completely inhibited osteoclastogenesis. Although the tanshinone compound itself showed similar effects, the concentrations of commercially available tanshinone(diterpenoids: tanshinone I, tanshinone IIA, cryptotanshinone, and dihydrotanshinone) needed for antiosteoclast activity was almost 1000 times more than that of tanshinone in SM fraction. This suggests that there are other unknown compounds in the SM extract that have a synergistic effect with tanshinone. These results also suggest that tanshinone can be a good marker compound to explain the antiosteoporotic function of SM^[19]. Tanshinone IIA (TSA) inhibited osteoclast differentiation in cocultures of bone marrow cells and calvarial osteoblasts. TSA regulated the expression of receptor activator of NF- κ B ligand and osteoprotegerin in osteoblasts treated with lipopolysaccharide (LPS). Also, TSA inhibited prostaglandin E₂ (PGE₂) synthesis by inhibiting cyclooxygenase-2 (Cox-2) expression induced by LPS. Furthermore, TSA greatly suppressed bone loss in the mouse models of bone loss. The findings suggest that TSA inhibits osteoclast formation by inhibiting COX-2/PGE₂ signaling and by suppressing bone erosion *in vivo*. The study of Kwak HB. and his colleagues' suggested that TSA may be of therapeutic value as an anti-bone-resorptive drug in the treatment of bone-related disease^[20]. Bone is a dynamic tissue that is regulated by the activity of bone-resorbing osteoclasts and bone-forming osteoclasts. Excessive osteoclast formation causes diseases such as osteoporosis and rheumatoid arthritis. Natural substances may be useful as therapeutic drugs to prevent many diseases in humans because they avoid the many side effects of treatment with chemical compounds. Kim HH. and his colleagues indicated that tanshinone IIA (TSA) inhibits the receptor activator of NF- κ B ligand (RANKL)-mediated osteoclast differentiation of osteoclast precursors. TSA suppressed the expression levels of c-Fos and NFATc1 induced by RANKL. However, retrovirus-mediated overexpression of c-Fos induced the expression of NFATc1 despite the presence of TSA and reversed the inhibitory effect of TSA on osteoclast differentiation. Also, the introduction of osteoclast precursors with the NFATc1 retrovirus led to osteoclast differentiation in the presence of TSA. The results suggest that TSA may have a role as a therapeutic drug in the treatment of bone disease such as osteoporosis^[21]. Tanshinone IIA suppressed the expression levels of c-Fos and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) induced by receptor activator of NF κ B ligand (RANKL). However, retrovirus-mediated overexpression of c-Fos induced the expression of NFATc1 despite the presence of tanshinone IIA and reversed the inhibitory effect of tanshinone IIA on osteoclast differentiation. Also, the introduction of osteoclast precursors with the NFATc1 retrovirus led to osteoclast differentiation in the presence of tanshinone IIA. The studies of Kwak HB. and his colleagues' suggested that tanshinone IIA may

have a role as a therapeutic drug in the treatment of bone disease such as osteoporosis^[22]. Cui Y. and his colleagues examined the *Salvia miltiorrhiza* (SM)-induced anti-osteoporotic effect and its possible mechanisms with various doses of SM. Sprague-Dawley female rats aged 12 weeks, divided into six groups: sham-operated control (SHAM), ovariectomized (OVX) rats supplemented with SM (1, 3, 10 and 30 mg/kg) orally for 8 weeks. At the end of the experiment, blood samples were collected and biochemistry analysis was performed. Specimens from both tibia and liver were processed for light microscopic examination. Dual energy X-ray absorptiometry (DEXA) and μ -computed tomography (CT) analyses of the tibia were also performed. SM treatment significantly ameliorated the decrease in bone mineral density and trabecular bone mass according to DEXA and trabecular bone architecture analysis of trabecular bone structure parameters by μ -CT scanning. In serum biochemical analysis, SM decreased the released TRAP-5b, an osteoclast activation marker and oxidative stress parameters including MDA and NO induced by OVX. In conclusion, the preventive effect of SM was presumably due to its anti-oxidative stress partly via modulation of osteoclast maturation and number. In current study, SM appears to be a promising osteoporosis therapeutic natural product^[23]. Jie L. and his colleagues investigated whether tanshinone IIA promotes apoptosis in rheumatoid arthritis (RA)-affected fibroblast-like synoviocytes (FLS). The viability of an immortalized FLS cell line derived from RA patients was assessed by 3-(4,5-methylthiazole-2-yl)-5,3-carboxymethoxy phenyl-1-2,4- sulfophenyl-2H-tetrazolium assay after tanshinone IIA treatment. Apoptosis was measured by terminal deoxynucleotidyl transferase (dUTP) nick-end labelling (TUNEL) assay and flow cytometry. Cell cycle was evaluated by flow cytometry. The results support the conclusion that tanshinone IIA treatment likely induces apoptosis of RA-FLS through blockade of the cell cycle in the G2/M phase and a mitochondrial pathway. These data suggest that tanshinone IIA may have therapeutic potential for RA^[24].

INHIBITORY EFFECT OF TANSHINONE VI ON OSTEOCLASTOGENESIS

In ongoing investigation into anti-osteoporotic compounds from natural products, Nicolin V. and his colleagues have analyzed the effect of tanshinone VI (TS-VI; 10 ng/mL) on osteoclasts differentiation, using a physiologic three-dimensional osteoblast/bone marrow model of cell co-culture. TS-VI greatly inhibits osteoclast differentiation and suppresses bone resorption through distribution of the actin ring; subsequently, the authors intended to examine the precise inhibitory mechanism of TS-VI on osteoclast differentiating factor. This study shows, for the first time, that TS-VI prevents osteoclast differentiation by inhibiting receptor activation of ligand (RANKL) expression and receptor activator for nuclear factor κ B (NF κ B) induction. Based on these results, Nicolin V. and his colleagues hypothesized that pharmacological suppression of NF κ B and RANKL induced by TS-VI *in vivo* may be an effective approach to improve bone loss suppressing osteoclastic bone resorption^[3]. Natural compounds, such as tanshinone IIA and VI, that suppress osteoclast differentiation may have therapeutic value in treating osteoporosis and other bone erosive diseases, such as rheumatoid arthritis or metastasis associated with bone loss.

ANTI-ANDROGENIC EFFECT OF TANSHINONE IIA

Cui L. and his colleagues investigated the skeletal effects of total

tanshinones in ovariectomized (OVX) rats by analyzing cancellous bone histomorphometry of four lumbar vertebrae (LV4) and proximal tibial metaphyses (PTM). Four-month-old Sprague-Dawley female rats were sham-operated and treated with vehicle or ovariectomized and treated with either vehicle, total tanshinones (200 mg/kg/d, equivalent to 35 μ g/kg/d of tanshinone IIA and 16 mg/kg/d of cryptotanshinone) or 17 α -ethynylestradiol (30 g/kg/d as positive treatment group) starting one day post-surgery for 10 weeks. Double *in vivo* fluorochromone labeling was administered to all rats. The undecalcified longitudinal LV4 and PTM sections were cut and stained with Goldner's Trichrome (4- μ m thickness) or unstained (8- μ m thickness) for the bone histomorphometric analysis. A significant decrease in trabecular bone volume (BV/TV) and trabecular number (Tb.N) and a significant increase in osteoclast surface (OCS/BS) and mineralizing surface (MS/BS) were found in both LV and PTM of vehicle-treated OVX rats compared with sham controls. Tanshinones completely prevented the decreases in BV/TV and Tb.N and the increase in OCS/BS in the LV4, and partially prevented the decreases in BV/TV and Tb.N in the PTM of OVX rats. In addition, tanshinones increased trabecular thickness (Tb.Th) whereas it did not alter MS/BS. Moreover, tanshinones had no effect on uterine weight and body weight of OVX rats. Estrogen treatment increased BV/TV and Tb.N and decreased OCS/BS, but, also markedly decreased MS/BS and increased uterine weight in OVX rats. In conclusion, the current study demonstrated that the adequate supply of tanshinones prevented OVX-induced cancellous bone loss in rats through inhibition of elevated bone resorption^[25].

Expression of metastatic suppressor, mammary serine protease inhibitor (maspin), is lost in advanced prostate cancer. Clinically relevant mutations in androgen receptor (AR) convert antiandrogens into AR agonists, promoting prostate tumor growth. Liu W. and his colleagues discovered that tanshinone IIA is a potent antagonist of mutated ARs and induces maspin expression through AR. Tanshinone IIA suppressed AR expression and induced apoptosis in LNCaP cells. Syntheses of tanshinone-IIA derivatives revealed that 4,4-dimethyl group at ring A is important for tanshinone IIA's antiandrogenic and maspin induction activities^[26].

INHIBITORY EFFECT OF TANSHINONE IIA ON RAT HEPATIC SATELLITE CELLS AND INDUCTION OF APOPTOSIS BY TANSHINONE I VIA CYTOCHROME C RELEASE IN ACTIVATED HEPATIC SATELLITE CELLS

Hepatic satellite cells (HSCs) play central roles in hepatic fibrosis, and apoptosis is suggested to eliminate activated HSCs in fibrosis. Treatment of rat HSCs transformed by simian virus 40 (T-HSC/CI-6) with tanshinone I resulted in the induction of typical DNA-fragmentation and -ladder formation in a concentration- and time-dependent manner. Kim JY. and his colleagues demonstrated that tanshinone I induces apoptosis of T-HSC/CI-6 and that tanshinone I-induced apoptosis involves caspase activation through cytochrome c release and loss of mitochondrial membrane potential^[27].

Yin HQ. and his colleagues demonstrated that the standardized fraction of *Salvia miltiorrhiza* was able to protect RAW264.7 cells from ethanol- and lipopolysaccharide(LPS)-induced production of superoxide radical, activation of NADPH oxidase and subsequently death of the cells. Among four main components, tanshinone IIA was the most potent in protecting cells from LPS- and ethano-induced cytotoxicity. LPS or ethanol induced the expression of CD14, iNOS,

and SCD1 and decreased RXR- α , which was completely reversed by tanshinone IIA. In H4IIEC3 cells, 10 μ M tanshinone IIA effectively blocked ethanol-induced fat accumulation as evidenced by Nile Red binding assay. These results indicated that tanshinone IIA may have potential to inhibit alcoholic liver disease by reducing LPS- and ethanol-induced Kupper cell sensitization, inhibiting synthesis of reactive oxygen/nitrogen species, inhibiting fatty acid synthesis and stimulating fatty acid oxidation^[28].

Protective effects of tanshinone IIA (TSN) antioxidant-induced liver injury was examined by enzyme-linked immunosorbent assay and histochemistry of several cytokines. TSN was found to significantly reduce plasma alanine aminotransferase and aspartate aminotransferase levels in mice with concanavalin A-induced immune-mediated liver injury. TSN increases T-lymphocyte subset of CD3+, CD4+ and CD8+ ratios. TSN significantly reduces inflammatory cytokines, including interleukin-2, interleukin-4, interferon- γ and tumor necrosis factor, while elevates anti-inflammatory cytokine, IL-10. Qin XY. and his colleagues suggested that TSN may provide a potential drug candidate for injury therapeutics^[29]. TSN improves blood circulation and treats chronic hepatitis and hepatic fibrosis. Activation of hepatic satellite cells (HSC) is the predominant event in liver fibrosis. The therapeutic goal in liver fibrosis is the reversal of fibrosis and selective clearance of activated HSCs. Che XH. and his colleagues used rat HSCs transformed by Simian virus 40 (t-HSC/C1-6) to overcome the limitations inherent in studying subcultures of HSCs. Treatment of t-HSC/C1-6 cells with TSN inhibited cell viability in a dose- and time-dependent manner. TSN induced apoptosis as demonstrated by DNA fragmentation, poly (ADP-ribose) polymerase and caspase-3 cleavage, increased Bax/Bcl-2 protein ratio, and depolarization of mitochondrial membranes to facilitate cytochrome c release into the cytosol. Furthermore, this compound markedly induced S phase cell cycle arrest, and down-regulated cyclins A and E, and cdk2. Thus, Che XH. and his colleagues indicated that TSN induces apoptosis and S phase cell cycle arrest in rat HSCs *in vitro*^[30]. Pan TL. and Wang PW. examined that *Salvia miltiorrhiza* ethanol extract (SMEE) remarkably ameliorates liver fibrogenesis in dimethylnitrosamine-administrated rat model. Tanshinone IIA significantly inhibited rat hepatic satellite cells (HSC) viability and led to cell apoptosis. Proteome tools elucidated that increased prohibitin (a member of the Band-7 family of protein) is involved in cell cycle arrest under tanshinone IIA is the treatment while knockdown of prohibitin could attenuate tanshinone IIA-induced apoptosis. In addition, tanshinone IIA mediated translocation of C-Raf which interacted with prohibitin activating mitogen-activated protein kinase (MAPK) and inhibiting AKT signaling in HSC. MAPK antagonist suppressed ERK phosphorylation which was necessary for tanshinone IIA-induced expression of Bax and cytochrome c. PD98059 (MAPK inhibitor) also abolished tanshinone IIA-modulated cleavage of poly ADP ribose polymerase. The findings suggested that tanshinone IIA could contribute to apoptosis of HSC by promoting ERK-Bax-caspase pathways through C-Raf/prohibitin complex^[31]. Since apoptosis of activated hepatic satellite cells (HSCs) is well-accepted anti-fibrotic strategy, Parajuli DR. and his colleagues investigated the direct effect of PF2401-SF, prepared from ethanol extract of *Salvia miltiorrhiza*, on t-HSC/C1-6 cells *in vitro* and on CCl₄-induced liver injury *in vivo*. They evaluated the activation and cleavage of hallmarks of apoptosis, namely, caspase 3, 8, and 9, and PARP. Upregulation of the pro-apoptotic Bax protein and downregulation of the anti-apoptotic Bcl2 protein were also analysed. Furthermore, in the PF2401-SF treated rats, apoptosis induction of activated HSCs was demonstrated

by reduced distribution of α -SMA-positive cells and the presence of high number of TUNEL-positive cells *in vivo*. The findings suggest that PF2401-SF can mediate HSCs apoptosis induction, and may be a potential herb medicine for the treatment of liver fibrosis^[32].

Liu YW. and Huang YT. examined whether tanshinone IIA could inhibit HSC activation. The cell line of rat HSC-T6 was stimulated with lipopolysaccharide (LPS or endotoxin) which is an important activator for Kupffer cells and HSCs in liver injury. Cytotoxicity was assessed by MTT assay. HSC-T6 cells were pre-treated with tanshinone IIA (1, 3 and 10 μ M), then induced by LPS (100 ng/mL). NF- κ B activity was evaluated by the luciferase reporter gene assay. Western blotting analysis was performed to measure NF- κ B-p65, and phosphorylations of MAPKs (ERK, JNK, p38). Cell chemotaxis was assessed by both wound-healing assay and trans-well invasion assay. Quantitative real-time PCR was used to detect gene expression in HSC-T6 cells. All concentrations of drugs showed no cytotoxicity against HSC-T6 cells. LPS stimulated NF- κ B luciferase activities, nuclear translocation of NF- κ B-p65, and phosphorylations of ERK, JNK and p38, all of which were suppressed by tanshinone IIA. In addition, tanshinone IIA significantly inhibited LPS-induced HSCs chemotaxis, in both wound-healing and trans-well invasion assays. Moreover, tanshinone IIA attenuated LPS-induced mRNA expressions of CCL2, CCL3, CCL5, IL-1 β , TNF- α , IL-6, ICAM-1, iNOS and α -SMA in HSC-T6 cells. The present results demonstrated that tanshinone IIA decreased LPS-induced HSC activation^[33].

Liver fibrosis and its end-stage disease cirrhosis are a major health burden affecting millions of people worldwide. Anti-inflammation via inhibition of NF- κ B pathway in HSC is one therapeutic approach to hepatic fibrosis. Tanshinone IIA inhibited LPS-induced HSC activation which might hold potential for treating hepatic fibrosis, and the effect of tanshinone IIA on the hepatotoxicity is a part of ongoing project to develop a potential pre-clinical medicine for liver fibrosis.

CONCLUSION AND FUTURE PERSPECTIVES

Salvia miltiorrhiza (Danshen) is a very useful herb in the treatment of cardiovascular disorders. It has been studied in clinical trials having some limitations in China. The clinical trials present evidence of the efficacy of tanshinones, the lipophilic components isolated from Danshen, for stroke and heart attack. Today investigations on the biological activities of Danshen and its applications are still undergoing. Dantonic Dripping Pill, which includes active constituents of *Salvia miltiorrhiza* and *Panax notoginseng*, is the first traditional Chinese medicine approved by U.S. FDA for phase II and III clinical trials. So far phase II clinical trials have already been completed in the U.S. FDA to evaluate its safety and efficacy in patients with chronic stable angina pectoris, and phase III study is ongoing. (<http://clinicaltrials.gov/>, No. NCT00797953 and NCT01659580) Hu T. and Cho CH. reported that Danshen and its active components tanshinones are promising candidates to be developed as novel therapeutic agents for treating a broad spectrum of disease including those in the gastrointestinal tract and liver^[34]. Tanshinone groups possess broad range anti-cancer potential through anti-proliferation, induction of differentiation and inhibition of adhesion. Xu Y. and his colleagues investigated that sodium tanshinone IIA sulfonate (STS) was shown to be capable of protecting C57BL/6 mice from immune-mediated liver injury *in vivo*, and the protection was associated with its suppressive effect on the production of important inflammatory mediators through modulating NF- κ B and IFN- γ /STAT 1 signaling pathways^[35]. A

randomized, controlled, multicentre clinical trial study, supported by several National Natural Science Foundation of China, started to evaluate beneficial effect of water soluble STS on pulmonary hypertension (ClinicalTrials.gov: NCT01637675). Wang J. and his colleagues investigated that STS exerted protective effects on hypoxic pulmonary hypertension in animals, including lowering pulmonary artery pressure and decreasing the pulmonary artery thickness and right ventricular hypertrophy. These effects, at least in part were found to be achieved through the regulation of intracellular Ca²⁺ homeostasis in pulmonary arterial smooth cells^[36]. Wang J. and his colleagues showed that the modulation of tanshinone IIA on pulmonary vasoreactivity under both acute and chronic hypoxia condition may provide a new insight for curing hypoxic pulmonary hypertension^[37]. These findings are substantially agreeable to our earlier study suggesting two possible mechanisms of tanshinone VI for the recovery of post-hypoxic cardiac contractility, i.e. enhancement of the myocardial energy store restoration after oxygen deficiency and prevention of hypoxia- and reoxygenation-induced calcium overload in the cardiac tissue^[2].

A prospective randomized open-label blinded end point designed trial started on sodium tanshinone IIA sulfonate (STS). On the background of statin therapy, a daily 80 mg dose of STS intravenously for 14 days reduced level of circulating inflammatory markers of hs-CRP, sCD40L and MCP-1 in unstable angina/acute non-ST segment elevation myocardial infarction patients. The study by Li S. and his colleagues' suggested that STS could be used as a complementary treatment in coronary heart disease patients with enhanced inflammatory reaction^[38].

Tanshinone VI having a similar structure to tanshinone IIA, was isolated from *Salvia miltiorrhiza* root first time. The present study of tanshinone VI suggested that tanshinone VI is beneficial for the recovery of cardiac contractility after a certain period of oxygen-deficiency, possibly through mechanisms involving improvement of myocardial energy production upon oxygen-replenishment and/or inhibition of calcium accumulation in the cardiac cell. Furthermore, tanshinone VI-reduced cellular hypertrophy and fibrosis might be attributed to a reduction of phosphorylation in extracellular signal-regulated kinase, leading to a decrease in free radical production or a scavenge of free radicals. Review article of Zhang Y. and his colleagues' indicated that tanshinones (Tan IIA and VI) may exert their inhibitory actions through modulating of inflammatory and immune responses, or regulating specific pathway such as androgen receptor (AR). It is noteworthy that the mechanisms for tanshinones to inhibit AR transcriptional activity are distinct from classical AR antagonists. Classical AR antagonists such as flutamide suppresses ligand-binding activation of AR via competition with androgen ligands (e.g., testosterone and dihydrotestosterone) to bind the hormone binding pocket within the C-terminal ligand binding domain of AR. Tanshinones were suggested as potential modulators of androgen synthesis and AR signaling pathway. The findings may provide a scientific basis of developing new tanshinone-based therapeutic agents for prostate cancer via suppressing AR pathway^[39].

Narducci P. and his colleagues demonstrated that bone erosion depends mainly on the synergistic action of the cytokines, where the receptor activator of nuclear factor κB ligand (RANKL), produced by osteoblasts, fibroblasts, and T cells, and receptor activator of nuclear factor κB (RANK) are mainly expressed on pre-osteoclasts, possibly of the macrophage lineage. TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF protein family along with RANK and RANKL, with which it shares homology, could be a key factor in this process^[40]. Tanshinone VI which prevents osteoclast differentiation

by inhibiting RANKL expression and NFκB induction^[3], and its water soluble derivative^[6] may have therapeutic value in treating osteoporosis and other bone erosive diseases such as rheumatoid arthritis or metastasis associated with bone loss. Tanshinone VI and IIA may exert their inhibitory actions against excessive osteoclast formation through modulating of inflammatory and immune responses.

Since the activated hepatic satellite cells (HSC) is the predominant event in the progression of liver fibrosis, selective clearance of HSC should be a potential strategy in therapy. The inhibitory effect of tanshinone IIA on apoptosis of HSC-T6 cells through Akt inhibition and ERK-Bax-caspase-3/9 signaling pathway was presented as a potential medicine for the treatment of liver fibrosis. Anti-inflammation via inhibition of NFκB pathways in hepatic satellite cells is one therapeutic approach to hepatic fibrosis. Tanshinone IIA (TS), is a lipophilic abietane type diterpene with diketone group, was tested to inhibition of HSC activation. Tanshinone IIA decreased LPS-induced HSC activation, without cytotoxicity to HSC-T6 cells at the working concentrations of TS. The suppressive effect of a water-soluble sulfonate derivative (STS) of tanshinone IIA (TS) on concanavalin A induced hepatitis in mice, an experimental model of immune-mediated liver injury, was exhibited and the mechanism of anti-inflammatory effects of STS may be attributed to its modulation of crucial inflammatory signaling pathways, including NFκB and IFN-γ/STAT1. Several placebo-controlled, randomized clinical trials are required to confirm efficacy and establish the safety of long-term STS for these new therapeutic indications. Present review summarized recent studies on the pharmacological activities and possible mechanisms of action for tanshinones as therapeutic agents in hypoxia/reoxygenation injury of heart and cerebral/neuro-protection, osteoporosis and hepatic satellite cells in liver injury with emphasis of tanshinone IIA and VI. Investigations of structure-activity relationship and molecular mechanisms of tanshinones (tanshinone VI, I, and IIA) have produced some insights into the chemical and biochemical basis for bioactivities of these tanshinones and will be a key to pave the way for the clinical study.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

- 1 Perry LM, *Salvia miltiorrhiza* Bunge. In: Medical Plants of East and Southeast Asia (Ed. Perry LM.), pp.192-193. The MIT Press, Cambridge, MA. 1980.
- 2 Takeo S, Tanonaka K, Hirai K, Kawaguchi K, Ogawa M, Yagi A, Fujimoto K. Beneficial effect of Tan-Shen, an extract from the root of *Salvia*, on post-hypoxic recovery of cardiac contractile force. *Biochem.Pharmacol.* 1990;40: 1137-1143.
- 3 Nicolini V, Dal Piaz F, Nori SL, Narducci P, De Tommasi N. Inhibition of bone resorption by tanshinone VI isolated from *Salvia miltiorrhiza* Bunge. *Eur J Histochem.* 2010; 54: e21-e23.
- 4 Yagi A, Fujimoto K, Tanonaka K, Hirai K, Takeo S. Possible active components of Tan-shen (*Salvia miltiorrhiza*) for protection of the myocardium against ischemia-induced derangements. *Planta Med.* 1989;55:51-54. Japanese Patent:2633609
- 5 Yagi A, Fujimoto K, Niwa T, Tanonaka K, Takeo S. Effect of abietane-type pigments from *Salvia miltiorrhiza* on post-hypoxic recovery of cardiac contractile force in rats. *Planta Med.* 1991;57:288-289.

- 6 Yagi A, Okamura N, Tanonaka K, Takeo S. Effects of tanshinone VI derivatives on post-hypoxic contractile dysfunction of perfused rat hearts. *Planta Med.* 1994;60:405-409.
- 7 Kawahara Y, Tanonaka K, Arino T, Maki T, Ishihara N, Takagi N, Yagi A, Takeo S. Effects of tanshinone VI on insulin-like growth factor-1-induced hypertrophy of isolated cardiomyocytes from neonatal rats. *Exp Clin Cardiol.* 2004;9:165-168.
- 8 Maki T, Kawahara Y, Tanonaka K, Yagi A, Takeo S. Effects of tanshinone VI on the hypertrophy of cardiac myocytes and fibrosis of cardiac fibroblasts of neonatal rats. *Planta Med.* 2002;68:1103-1107
- 9 Arino T, Tanonaka K, Kawahara Y, Maki T, Takagi N, Yagi A, Takeo S. Effects of tanshinone VI on phosphorylation of ERK and Akt in isolated cardiomyocytes and cardiac fibroblasts. *Eur J Pharmacol.* 2008;580:298-305.
- 10 Zhou S, Chen W, Su H, Zheng X. Protective properties of tanshinone I against oxidative DNA damage and cytotoxicity. *Food Chem Toxicol.* 2013;62:407-412.
- 11 Park JH, Park OK, Cho JH, Chen BH, Kim IH, Ahn JH, Lee JC, Yan BC, Yoo KY, Lee CH, Hwang IK, Kwon SH, Lee YL, Won MH, Choi JH. Anti-inflammatory effect of tanshinone I in neuroprotection against cerebral ischemia-reperfusion injury in the gerbil hippocampus. *Neurochem Res.* 2014;39:1300-1312.
- 12 Lee JC, Park JH, Park OK, Kim IH, Yan BC, Ahn JH, Kwon SH, Choi JH, Kim JD, Won MH. Neuroprotective effect of tanshinone I from Danshen extract in a mouse model of hypoxia-ischemia. *Anat Cell Biol.* 2013; 46:183-190.
- 13 Wu GB, Zhou EX, Qing DX. Tanshinone IIA elicited vasodilation in rat coronary arteriole: roles of nitric oxide and potassium channels. *Eur J Pharmacol.* 2009;617:102-107.
- 14 Fan GW, Gao XM, Wang H, Zhu Y, Zhang J, Hu LM, Su YF, Kang LY, Zhang BL. The anti-inflammatory activities of Tanshinone IIA, an active component of TCM, are mediated by estrogen receptor activation and inhibition of iNOS. *J Steroid Biochem and Mol Biol.* 2009;113:275-280.
- 15 Ren ZH, Tong YH, Xu W, Ma J, Chen Y. Tanshinone IIA attenuates inflammatory responses of rats with myocardial infarction by reducing MCP-1 expression. *Phytomedicine* 2010;17:212-218.
- 16 Chen Y, Wu X, Yu S, Lin X, Wu J, Li L, Zhao J, Zhao Y. Neuroprotection of tanshinone IIA against cerebral ischemia/reperfusion injury through inhibition of macrophage migration inhibitory factor in rats. *PLoS one* 2012; 7:e40165.
- 17 Tang Q, Han R, Xiao H, Shen J, Luo Q, Li J. Neuroprotective effects of tanshinone IIA and/or tetramethylpyrazine in cerebral ischemic injury in vivo and *in vitro*. *Brain Res.* 2012; 1488:81-91.
- 18 Shi LL, Yang WN, Chen XL, Zhang JS, Yang PB, Hu XD, Han H, Qian YH, Liu Y. The protective effects of tanshinone IIA on neurotoxicity induced by β -amyloid protein through calpain and the p35/Cdk5 pathway in primary cortical neurons. *Neurochem Int.* 2012;61:227- 235.
- 19 Kim HK, Woo ER, Lee HW, Park HR, Kim HN, Jung YK, Choi JY, Chae SW, Kim HR, Chae HJ. The correlation of *Salvia miltiorrhiza* extract-induced regulation of osteoclastogenesis with the amount of components tanshinone I, tanshinone IIA, cryptotanshinone, and dihydro-tanshinone. *Immunopharmacol Immunotoxicol.* 2008;30:347-364.
- 20 Kwak HB, Sun HM, Ha H, Kim HN, Lee JH, Kim HH, Shin HI, Lee ZH. Tanshinone IIA suppresses inflammatory bone loss by inhibiting the synthesis of prostaglandin E2 in osteoblasts. *Eur J Pharmacol.* 2008; 601:30-37.
- 21 Kim HH, Kim JH, Kwak HB, Huang H, Han SH, Ha H, Lee SW, Woo ER, Lee ZH. Inhibition of osteoclast differentiation and bone resorption by tanshinone IIA isolated from *Salvia miltiorrhiza* Bunge. *Biochem Pharmacol.* 2004;67:1647-1656.
- 22 Kwak HB, Yang D, Ha H, Lee JH, Kim HN, Woo ER, Lee S, Kim HH, Lee ZH. Tanshinone IIA inhibits osteoclast differentiation through down-regulation of c-Fos and NFATc1. *Exp Mol Med.* 2006;38:256-264.
- 23 Cui Y, Bhandary B, Marahatta A, Lee GH, Li B, Kim DS, Chae SW. Characterization of *Salvia miltiorrhiza* ethanol extract as an anti-osteoporotic agent. *BMC Compl Altern Med.* 2011;11:120-130.
- 24 Jie L, Du H, Huang O, Wei S, Huang R, Sun W. Tanshinone IIA induces apoptosis in fibroblast-like synoviocytes in rheumatoid arthritis via blockade of the cell cycle in the G2/M phase and a mitochondrial pathway. *Biological & Pharmaceutical Bulletin* 2014;37:1366-1372.
- 25 Cui L, Wu T, Liu YY, Deng YF, AI CM, Chen HQ. Tanshinone prevents cancellous bone loss induced by ovariectomy in rats. *Acta Pharmacol Sin.* 2004;25:678-684.
- 26 Liu W, Zhou J, Geng G, Shi Q, Sauriol F, Wu JH. Antiandrogenic maspin induction, and antiprostata cancer activities of tanshinone IIA and its novel derivatives with modification in ring A. *J Med Chem.* 2012;55:971-975.
- 27 Kim JY, Kim KM, Nan JX, Zhao YZ, Park PH, Lee SJ, Sohn DH. Induction of apoptosis by tanshinone I via cytochrome c release in activated hepatic stellate cells. *Pharmacol Toxicol* 2003;92:195-200.
- 28 Yin HQ, Kim YS, Choi YJ, Kim YC, Sohn DH, Ryu SY, Lee BH. Effects of tanshinone IIA on the hepatotoxicity and gene expression involved in alcoholic liver disease. *Arch Pharm Res.* 2008;31:659-665.
- 29 Qin XY, Li T, Yan L, Liu QS, Tian Y. Tanshinone IIA protects against immune-mediated liver injury through activation of T-cell subsets and regulation of cytokines. *Immunopharmacol Immunotoxicol.* 2010;32:51-55.
- 30 Che XH, Park EJ, Zhao YZ, Kim WH, Sohn DH. Tanshinone IIA induces apoptosis and S phase cell cycle arrest in activated rat hepatic stellate cells. *Basic Clinical Pharmacol Toxicol.* 2010;106:30-37.
- 31 Pan T-L, Wang P-W. Explore the molecular mechanism of apoptosis induced by tanshinone IIA on activated rat hepatic stellate cells. Evidence-based Complementary and Alternative Medicine 2012; 2012: Article ID 734987, 15 pages.
- 32 Parajuli DR, Park EJ, Che XH, Jiang WY, Kim YC, Sohn DH, Lee SH. PF2401-SF, standardized fraction of *Salvia miltiorrhiza*, induces apoptosis of activated hepatic stellate cells *in vitro* and *in vivo*. *Molecules* 2013;18:2122-2134.
- 33 Liu YW, Huang YT. Inhibitory effect of tanshinone IIA on rat hepatic stellate cells. *PLoS One.* 2014;9:e103229.
- 34 Hu T, Cho CH. Potential applications of tanshinones in gastrointestinal and hepatic diseases. *Biomol Res Ther.* 2013;2: 110.
- 35 Xu Y, Feng D, Wang Y, Lin S, Xu L. Sodium tanshinone IIA sulfonate protects mice from Con A-induced hepatitis via inhibiting NF κ B and IFN- γ /STAT1 pathways. *J Clin Immunol.* 2008; 28:512-519.
- 36 ClinicalTrials.gov; NCT01637675 Efficacy and safety study of sodium tanshinone IIA sulfonate on pulmonary hypertension. Wang J, Lu W, Wang W, Zhang N, Wu H, Liu C, Chen X, Chen Y, Chen Y, Jiang Q, Xu L, Tian L, Ran P, Zhong N. Promising therapeutic effects of sodium tanshinone IIA sulfonate towards pulmonary arterial hypertension in patients. *J Thorac Dis.* 2013;5:169-172.
- 37 Wang J, Dong MQ, Liu ML, Xu DQ, Luo Y, Zhang B, Liu LL, Xu M, Zhao PT, Gao YQ, Li ZC. Tanshinone IIA modulates pulmonary vascular response to agonist and hypoxia primarily via inhibiting Ca²⁺ influx and release in normal and hypoxic pulmonary hypertension rats. *Eur J Pharmacol.* 2010 640:129-138.

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- 38 Li S, Shang Q, Chen K, Xu H. Sodium tanshinone IIA sulfate reduces elevated serum high sensitive C-reactive protein in patients with coronary heart disease: a prospective randomized open-label blinded end point trial. *J Am Coll Cardiol.* 2014;64(16_S). Clinical Trial Registraton number:ChiCTR-TRC-12002361.
- 39 Zhang Y, Jiang P, Ye M, Kim SH, Jiang C, Lu J. Tanshinones: Sources, Pharmacokinetics and Anti-cancer activities. *Int.J. Mol. Sci.* 2012;13:13621-13666.
- 40 Narducci P, Bareggi R, Nicolini V. Interaction of human recombinant tumor necrosis factor-related apoptosis-inducing ligand and osteoprotegerin could contribute to enhancement of the erosive processes induced by human synovial cells. *J Rheumatol.* 2009;36:1837-1839.

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