Review Article

Molecular Mechanisms of Cardioprotective Actions of Tanshinones

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Received 22 October 2015; Accepted 17 January 2016

Academic Editor: Hani El-Nezami

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Tanshinones are lipophilic compounds derived from *Salvia miltiorrhiza* (*Danshen*) that has been widely used to treat coronary heart diseases in China. The cardioprotective actions of tanshinones have been extensively studied in various models of myocardial infarction, cardiac ischemia reperfusion injury, cardiac hypertrophy, atherosclerosis, hypoxia, and cardiomyopathy. This review outlines the recent development in understanding the molecular mechanisms and signaling pathways involved in the cardioprotective actions of tanshinones, in particular on mitochondrial apoptosis, calcium, nitric oxide, ROS, TNF- α , PKC, PI3K/Akt, IKK/NF- κ B, and TGF- β 1/Smad mechanisms, which highlights the potential of these compounds as therapeutic agents for treating cardiovascular diseases.

1. Introduction

Danshen, also called red sage root, is dried roots of *Salvia miltiorrhiza* that is cultivated widely in many Asian countries including China, Korea, and Japan. *Danshen* and *Danshen* related products have long been used as traditional Chinese medicine to treat cardiovascular dysfunctions such as angina pectoris, stroke, and hypertension [1–4]. For example, a recent systematic review indicates that *Danshen* dripping pill is more effective than isosorbide dinitrate in treating angina pectoris [5]. Although with its long history of medicinal use, the mechanism of actions of *Danshen* has only been intensively investigated in recent years, with the progress in elucidating its phytochemistry and active compounds. The identified chemical constituents of *Danshen* can be classified into lipophilic and hydrophilic components. The main lipophilic constituents are tanshinones which are diterpene quinones. There are more than 40 tanshinones that have been isolated [6]. The main tanshinones include tanshinone I (TI), tanshinone IIA (TIIA), tanshinone IIB (TIIB), tanshinone VI (TVI), cryptotanshinone (CT), and dihydrotanshinone (DHT) as illustrated in Figure 1.

Tanshinones have been demonstrated with various pharmacological activities including antioxidant, anti-inflammatory, antibacterial, antineoplastic, immunomodulatory, cardioprotective, and neuroprotective actions and have been used in treating various conditions, including cardiovascular diseases, cancer, and diabetes [7–14]. One of the promising therapeutic actions of tanshinones is their cardioprotective actions [15]. For example, TIIA has been shown to dilate coronary vessels [16] and regulate vascular endothelial functions [17] and inhibit myocardial infarction [18], ischemia reperfusion (I/R) injury [19, 20], and cardiomyopathy [21]. It also reduced atherosclerosis [22]. CT has also been shown to reduce atherosclerosis and I/R induced cardiac injuries [23, 24], potentially via vasodilatation [25] and anti-inflammatory actions [26]. Similarly, TVI has been shown with antihypertrophy activity in cardiomyocytes [27], and DHT has been reported with vasorelaxation and antiplatelet actions [28, 29]. Tables 1 and 2 summarize cardioprotective actions of major tanshinones *in vitro* and *in vivo*. This short review outlines the recent development in understanding the molecular mechanisms of cardioprotective actions of tanshinones, with a focus on their regulation of various signaling pathways.

FIGURE 1: Chemical structures of major tanshinones.

2. Effects of Tanshinones on Atherosclerosis

Atherosclerosis is a vascular endothelial dysfunction caused by inflammation promoted by low-density lipoproteins (LDL) [30]. Various atherogenic stimuli, including diabetes and oxidative stress, can induce endothelial dysfunction, leading to atherosclerosis [31]. Current approaches for reducing atherosclerosis have been focused on anti-inflammatory, antioxidant, and vasodilator mechanisms [32]. Studies have demonstrated the antiatherosclerosis actions of various tanshinones and their associations with various signaling pathways. For example, a tanshinone mixture was shown to inhibit proliferation of vascular smooth muscle cells (VSMCs) *in vitro*, which is associated with reduction of extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, cyclin D1, and enhancing p21^{waf1/cip1} (cyclin-dependent kinase inhibitor 1) expressions [33]. This is supported by the finding of inhibition of intimal hyperplasia by tanshinone in the ligated mouse carotid artery *in vivo* [34]. Treatment with total tanshinone reduced the development of intimal thickening of injured vessels and proliferating cell nuclear antigen- (PCNA-) positive vascular smooth muscle cells in mice, indicating that tanshinone may potentially inhibit VSMCs proliferation from arterial injury and reduce occurrence of atherosclerosis [34].

TIIA has been shown to increase nitric oxide (NO) generation and inhibiting platelet aggregation/leukocyte adhesion *in vitro* [73]. It also attenuated H_2O_2 induced endothelial dysfunction by enhancing superoxide dismutase (SOD) activity [38] and reducing caspase 3 activity in human umbilical vein endothelial cell (HUVECs) [39]. The antiatherosclerosis action of TIIA was associated with its anti-inflammatory activities by inhibition of cluster of differentiation 40 (CD40)

and p53 protein expression [38, 39], and inhibition of tumor necrosis factor- α (TNF- α) induced expressions of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and fractalkine, possibly through inhibition of IKK/NF- κ B signaling pathway [37, 74]. TIIA was found to reduce oxidized LDL protein production and superoxide production by enhancing Cu/Zn SOD in rat [70] and also decreased cholesterol content and oxLDL level in apolipoprotein E deficient (ApoE^{-/-}) mice [22]. It also inhibited the expression of inflammatory regulator CD40 and matrix metalloproteinase-2 (MMP-2) in high fat diet induced atherosclerosis in rabbits [71]. A recent study in ovariectomized ApoE^{-/-} mice found that TIIA had a phytoestrogen-like activity reducing aortic lipid deposition and serum levels of malonyl dialdehyde (MDA), nuclear factor-kappa B (NF- κ B), and lipids, involving inhibiting p-ERK 1/2 [75]. Further study is required to elucidate the role of NO synthesis and redox signaling (specifically on regulation of mitochondrial anti/prooxidative stress) in the actions of TIIA.

Similarly, CT was found to attenuate TNF- α induced endothelial dysfunction in HUVECs by reducing endothelin-1 accumulation, enhancing eNOS/NO, and inhibiting of NF- κ B pathways [26]. In human aortic smooth muscle cells (HASMCs), CT inhibited TNF- α induced activation of NF- κ B and activator protein 1 (AP1, a transcriptional factor that regulates genes of cytokines and growth factors), as well as activation of matrix metalloproteinases-9 (MMP-9), which plays an important role in migration and proliferation of vascular smooth cells and into the intima [23]. On the other hand, CT was found to reduce calcium flux and calcium induced contraction in rat coronary artery [25] and inhibit thromboxane A2 analogue U46619-induced constriction of the porcine coronary artery [76].

TABLE 1: Pharmacological actions of tanshinones *in vitro*.

Compounds	Cells	Concentrations	Effects	Mechanisms	Reference
Cryptotanshinone	HUVECs	1–9 μM	Antiatherosclerosis action against TNF- α	ET-1, NF- κB $\downarrow$ NO/eNOS $\uparrow$	[26]
		1–5 μM	Anti-inflammatory actions against H_2O_2 , TNF- α	NF- κB activity $\downarrow$ VCAM-1, ICAM-1 $\downarrow$ MMP-9 $\downarrow$	[24]
	HASMCs	2.5–30 $\mu\text{g/mL}$	Antiatherosclerosis action against TNF- α	NF- κB , AP-1 $\downarrow$ p-ERK1/2, p-p38, p-JNK $\downarrow$	[23]
	Rat coronary blood vessels	0.5–50 μM	Vasodilator action	Calcium influx $\downarrow$ Mitochondrial $\cdot\text{O}_2^-$ $\downarrow$	[25]
	H9c2 cells	3 μM	Antioxidant action against hypoxia	Mitochondrial SOD $\uparrow$ NO $\downarrow$, $[\text{Ca}^{2+}]_i$ $\downarrow$	[35]
Dihydrotanshinone		0.3–3 μM	Antiapoptotic effects against hypoxia	Bcl-2/Bax ratio $\uparrow$ Cytochrome C release $\downarrow$ Caspase 3 $\downarrow$	[36]
	Rat coronary artery	IC50 10.4 μM	Vasorelaxation action	Calcium influx $\downarrow$ Platelet aggregation $\downarrow$	[28]
	Rabbit platelet	IC50 8.7 μM	Antiplatelet action against collagen	$[\text{Ca}^{2+}]_i$ $\downarrow$ Arachidonic acid release $\downarrow$ Thromboxane B_2 $\downarrow$	[29]
Tanshinone	VSMCs	0.4–50 $\mu\text{g/mL}$	Antiproliferation action	p-ERK1/2, cyclin D1 $\downarrow$ p21 ^{waf1/cip1} $\uparrow$	[33]
HUVECs		1–50 μM	Anti-inflammatory actions against TNF- α	GATA-6 $\downarrow$ IRF-1 $\downarrow$ VCAM-1 $\downarrow$	[37]
		7.5–60 $\mu\text{g/mL}$	Cytoprotection against H_2O_2	SOD, NO $\uparrow$ CD40 $\downarrow$	[38]
		1–10 μM		p53, caspase-3 $\downarrow$ ATF-3 $\uparrow$	[39]
		100 μM	Vasodilator action	NO $\uparrow$	[17]
		0.6–60 μM		NO, AMPK/PI3K/Akt $\uparrow$	[40]
	Rat coronary arterioles	10–100 μM	Vasodilator action	NO, BK _{Ca} $\uparrow$	[16]
	HASMCs	0.05–5 μM	Antioxidative actions against TNF- α , AngII, H_2O_2	Cytochrome P450 $\uparrow$ GSH, NADPH $\uparrow$	[41]
				Nrf-2 $\uparrow$	
	VSMCs	0.1–1 $\mu\text{g/mL}$	Inhibition of proliferation	p-ERK1/2 $\downarrow$ c-fos $\downarrow$	[42]

TABLE 1: Continued.

Compounds	Cells	Concentrations	Effects	Mechanisms	Reference
Tanshinone IIA	Neonatal cardiomyocytes	0.1–10 μM	Cytoprotective effects against H_2O_2	DNA fragmentation $\downarrow$	[19]
		0.1–3 μM	Antiapoptotic/antioxidant effects against doxorubicin	ROS $\downarrow$ Bcl-xl $\uparrow$, caspase 3 $\downarrow$ p-Akt $\uparrow$	[21]
		0.5–2 μM	Antioxidant action against H_2O_2	H_2O_2 , O_2^- $\downarrow$	[43]
		100 μM		Bcl-2/Bax ratio $\uparrow$	[44]
		2–8 μM	Anti-inflammatory effects against TNF- α	$[\text{Ca}^{2+}]_i$ $\downarrow$ MMP $\uparrow$ MCP-1 $\downarrow$	[18]
		10 μM	Antiapoptosis action against hypoxia	TGF- β 1 secretion $\downarrow$ miR-133 $\uparrow$ p-ERK1/2 $\uparrow$	[45]
		10 μM	Cytoprotective effects against hypoxia	p-p38MAPK $\downarrow$ SRF, MEF2 $\downarrow$ Cx43 $\uparrow$	[46]
		10–100 μM	Antihypertrophic action against isoproterenol, AngII	$[\text{Ca}^{2+}]_i$ $\downarrow$ Calcineurin, NFATc3 $\downarrow$ ANP, BNP, β -MHC $\downarrow$	[47]
		10 ⁻⁸ g/L	Antiapoptotic action against AngII	Cell size $\downarrow$ Protein synthesis rate $\downarrow$ c-fos, c-jun $\downarrow$	[48]
		0.1–10 μM		p-Akt, Bcl-xl $\uparrow$	[49]
		10–100 μM	Antifibrosis action against TGF- β	Cytochrome c, caspase 3 $\downarrow$ p-Smad3, CTGF, COL1 $\downarrow$ Smad7 $\uparrow$	[50]
	Neonatal cardiac fibroblasts	0.1–10 μM	Antiproliferation against AngII	ET-1 expression $\downarrow$ p-ERK1/2 $\downarrow$ NO, p-eNOS $\uparrow$ Collagen type 1 $\downarrow$ MMP-1 $\downarrow$	[51]
		3–30 μM	Antifibrosis action against AngII	ROS $\downarrow$ NADPH activity $\downarrow$ P47 $\downarrow$	[52]
		0.1–10 μM	Antifibrosis action against H_2O_2	Collagen synthesis $\downarrow$ NADPH oxidase $\downarrow$ O_2^- $\downarrow$	[53]
	Human cardiac fibroblasts	0.1–10 μM	Inhibition of AngII-induced extracellular matrix remodeling	TGF- β 1 $\downarrow$, pSmad 2 $\downarrow$, p-P38 $\downarrow$, MMP9 $\downarrow$, p-ERK $\downarrow$, p-IkB $\downarrow$, p65 $\downarrow$, interfering Smad-mediated recruitment of CBP1 via the activation of CREB	[54]

TABLE 1: Continued.

Compounds	Cells	Concentrations	Effects	Mechanisms	Reference
	H9c2 cells	0.3–3 μ M	Antiapoptotic effects against hypoxia	Bcl-2/Bax ratio $\uparrow$	[36]
				Cytochrome C release $\downarrow$	
		3 μ M	Antioxidant action against hypoxia	Caspase 3 $\downarrow$	[35]
				HIF-1 α $\downarrow$	
Tanshinone VI	Neonatal cardiomyocytes/cardiac fibroblast	0.1–10 μ M	Antihypertrophic action against AngII, IGF-1, and ET-1	Mitochondrial $\bullet$ O $_2^-$ $\downarrow$	[55]
				Mitochondrial SOD $\uparrow$	
				NO, [Ca $^{2+}$] $_i$ $\downarrow$	
				ATP level $\uparrow$	
		1–10 μ M	Protection against injury induced by oxygen-glucose deprivation/recovery	Apoptosis $\downarrow$, TNF- α $\downarrow$, caspase-3 $\downarrow$,	[56]
				Bax/Bcl-2 $\downarrow$	
				NF- κ B $\downarrow$, p-JNK $\downarrow$	
				PI3k	
		0.1–10 μ M	Antihypertrophic action against AngII, IGF-1, and ET-1	p-ERK1/2 $\downarrow$	[56]
				Natriuretic peptide $\downarrow$	

AngII, angiotensin II; ANP, arterial natriuretic peptide; ATF-3, activating transcription factor-3; BK $_{Ca}$, high conductance Ca $^{2+}$ activated K $^{+}$ channels; β -MHC, β -myosin heavy chain; BNP, brain natriuretic peptide; CD40, cluster of differentiation 40; Cx43, connexin 43; [Ca $^{2+}$] $_i$, intracellular calcium; ET-1, endothelin-1; ERK, extracellular signal-regulated kinase; G6PDH, glucose 6-phosphate dehydrogenase; GSH, glutathione; GPx, glutathione peroxidase; ICAM-1, intracellular adhesion molecule-1; IGF-1, insulin-like growth factor-1; I/R, ischemia reperfusion; IRF-1, interferon regulatory factor-1; MEF2, myocyte enhancer factor 2; MMP, mitochondrial membrane potential; MMP-9, matrix metalloproteinases-9; NFATc3, nuclear factor of activated T-cells cytoplasmic 3; NF- κ B, nuclear factor- κ B; Nrf2, nuclear factor-E2-related factor; NO, nitric oxide; HUVECs, human umbilical vein endothelial cells; HASMCs, human aortic smooth muscle cells; p38MAPK, p38 mitogen-activated protein kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; SRE, serum reaction factor; VCAM-1, vascular cell adhesion molecule-1; VSMCs, vascular smooth muscle cells.

TABLE 2: Pharmacological actions of tanshinones *in vivo*.

Compounds	Animals/tissues	Dosages	Effects	Mechanisms	Reference
Cryptotanshinone	Rat heart	125, 250 μ g/kg	Ameliorating I/R injury	Neutrophil infiltration $\downarrow$ NF- κ B, TNF- α , IL-1 β $\downarrow$ MPO $\downarrow$	[24]
Tanshinone	Mouse carotid artery	0.3 and 0.6 g/kg	Suppressing intimal hyperplasia	Intimal thickening $\downarrow$ PCNA $\downarrow$	[34]
	Rat heart	120 mg/kg	Improving acute myocardial infarction	Infarct size $\downarrow$ Systolic function $\uparrow$ [Ca ²⁺] _i $\downarrow$ Cell adhesion $\downarrow$	[57]
Tanshinone IIA	Rat heart	10 mg/kg	Protection of I/R injury	Bcl-2, SOD $\uparrow$ Caspase 3 $\downarrow$, MDA $\downarrow$	[19]
		8 mg/kg	Reducing myocardial infarct size and I/R injury	HO-1 $\uparrow$, TNF- α $\downarrow$, MDA $\downarrow$, SOD $\uparrow$, GSH $\uparrow$, p-NF- κ B $\downarrow$	[58]
		8 mg/kg	Protection of isoproterenol-induced myocardial infarction	HO-1 $\uparrow$, Nrf2 $\uparrow$ MDA $\downarrow$, SOD $\uparrow$, GSH $\uparrow$ p-ERK1/2/ERK $\uparrow$	[59]
		5, 10, 20 mg/kg	Reducing myocardial infarct size and I/R injury	p-AKT $\uparrow$, p-eNOS $\uparrow$, p-NF- κ B $\downarrow$	[20]
		5 mg/kg	Protection of STZ-induced diabetic cardiomyopathy	p-AKT $\uparrow$, GSK-3 β $\uparrow$, MPTP $\downarrow$, TNF- α $\downarrow$, IL-6 $\downarrow$, MPO $\downarrow$	[60]
		10, 20, 40 mg/kg		SOD $\uparrow$, GSH-Px $\uparrow$, MDA $\downarrow$, TNF- α $\downarrow$, IL-6 $\downarrow$, iNOS $\downarrow$, HMGB1 $\downarrow$	[61]
		60 mg/kg	Reducing myocardial infarct size and biochemical markers	TNF- α , CD68 $\downarrow$ MCP-1, TGF- β 1 $\downarrow$	[18]
		20 mg/kg		LVSP $\uparrow$, LVEDP $\downarrow$ p-p38MAPK $\downarrow$ SRE, MEF2 $\downarrow$, Cx43 $\uparrow$	[46]
		20 mg/kg	Reducing infarct size and apoptotic cells	Caspase 3 $\downarrow$, p-Akt $\downarrow$, FOXO3A $\downarrow$, Bim $\downarrow$	[62]
		10, 20 mg/kg		[Ca ²⁺] _i , ATIR $\downarrow$	[63]
		10, 20 mg/kg		PKC $\downarrow$, eNOS $\uparrow$	[64]
		5, 10, 20 mg/kg		Heart muscle index $\downarrow$ p-Akt, p-Gsk3 β $\downarrow$	[65]
		10, 20 mg/kg		LVML, MFD $\downarrow$ Smad 3, TGF- β 1 $\downarrow$ Smad7 $\uparrow$	[66]
		35, 70 mg/kg	Antihypertrophic action	LV fibrosis $\downarrow$ Diastolic dysfunction $\downarrow$ MMP-9, TIMP-1, TIMP-2 $\downarrow$	[67]
		35, 70 mg/kg		CVF, PVCA $\downarrow$ NAD(P)H oxidase, $\cdot$ O ₂ ⁻ $\downarrow$	[68]
	Rat aorta	5, 10, 15 mg/kg		MDA $\downarrow$ SOD $\uparrow$ Bcl-2/Bax ratio $\uparrow$ Caspase 3 $\downarrow$ LV fibrosis $\downarrow$ Smad 3 $\downarrow$, TGF- β 1 $\downarrow$	[69]
		35, 70 mg/kg	Attenuation of atherosclerotic calcification	oxLDL production $\downarrow$ $\cdot$ O ₂ ⁻ $\downarrow$, Cu/Zn SOD $\uparrow$ MDA $\downarrow$	[70]

TABLE 2: Continued.

Compounds	Animals/tissues	Dosages	Effects	Mechanisms	Reference
	Mouse aorta	10, 30, 90 mg/kg	Antiatherosclerotic action	Cholesterol level ↓ CD36, SRA, PPAR γ ↓	[22]
	Mouse heart	20 mg/kg	Amelioration of I/R injury	eNOS/NO ↑ AMPK/PI3K/Akt ↑	[40]
	Rabbit aorta	6.25, 15.00, 37.50 mg/kg	Antiatherosclerotic action	CD40, MMP-2 ↓ SOD ↑	[71]

AT1R, angiotensin II type I receptor; $[Ca^{2+}]_i$, intracellular calcium; CD36, cluster of differentiation 36; CD68, cluster of differentiation 68; CVF, collagen volume fraction; Cx43, connexin 43; Dox, doxorubicin; IL-1 β , interleukin-1 beta; IL-6, interleukin-6; I/R, ischemia reperfusion; LV: left ventricular; LVMI, left ventricular mass index; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; MDA, malondialdehyde; MEF2, myocyte enhancer factor 2; MFD, myocardial fiber diameter; MI, myocardial infarction; MMP-2, matrix metalloproteinases-2; MPO, myeloperoxidase; NF- κ B, nuclear factor-kappa B; PPAR γ , peroxisome proliferator activated gamma; PCNA, proliferating cell nuclear antigen; PVCA, perivascular collagen area; ROS, reactive oxygen species; SOD, superoxide dismutase; SRA, scavenger receptor A; SRF, serum reaction factor; TGF- β 1, transforming growth factor-beta 1.

Another tanshinone, DHT, was shown with a vasodilator activity by reducing $CaCl_2$ elicited contraction in rat coronary artery [28]. The calcium regulation effect of DHT was also demonstrated in its inhibition of collagen-induced rabbit platelet aggregation, which involves intracellular calcium mobilization, arachidonic acid liberation, and thromboxane B2 generation mechanisms [29].

3. Effects of Tanshinones on Myocardial Infarction

Myocardial infarction (MI) is caused by disruption of blood supply to the heart and often accompanied by ventricular remodeling and cardiac hypertrophy, which can lead to cardiac cell death [77]. Cardiomyocytes apoptosis is an important mechanism in MI [78], and it is closely associated with cellular inflammatory reaction, intracellular calcium homeostasis, and NO and oxidative stress [79].

There is evidence that tanshinones reduced cardiac infarct size and improved cardiac function in MI via inhibiting intracellular calcium, cell adhesion molecules, apoptotic protein, and inflammation protein expressions. An *in vivo* study demonstrated that acute myocardial infarction induced by occlusion of rat coronary vessel was decreased by post-treatment of a tanshinone extract [57]. This improvement was partially mediated by modulation of expression of genes which regulate intracellular calcium, cell adhesion molecules, and alternative complementary pathways (i.e., neuronal pentraxin 1, intercellular adhesion molecule 1, and C3 convertase) [46, 57]. The anti-inflammatory and antiapoptotic properties of tanshinones may play an important role in their anti-MI actions [18, 46]. TIIA was shown to inhibit TNF- α induced monocyte chemoattractant protein-1 (MCP-1) and transforming growth factor- β 1 (TGF- β 1) expression in cardiac fibroblasts *in vitro* [18]. It reduced TNF- α , NF- κ B, MCP-1, and TGF- β 1 expressions [18] and improved cardiac dysfunction with inhibition of expressions of p-38, serum response factor (SRF: a transcription factor that has a role in modulating muscle proliferation and cell migration and apoptosis), and myocyte enhancer factor 2 (MEF2: a transcription factor that regulates tissue remodeling in cardiac muscle) [46].

4. Effects of Tanshinones on Ischemia Reperfusion Injuries

Myocardial ischemia reperfusion (I/R) injury is caused by insufficient oxygen supply to the cardiac tissue which can occur in various conditions, such as thrombosis, coronary atherosclerotic plaques, and diabetes complications [80]. Sudden oxygen supply during reperfusion after prolonged ischemia can lead to impairment of myocardial cells via *reactive oxygen species* (ROS) dependent mechanisms [81]. The myocardial I/R injury can be affected by a number of cellular factors/regulators including calcium, pH, ROS, and in particular mitochondrial function. For example, mitochondrial membrane transition pore (MPTP) opening has been a research focus and potential therapeutic target for developing new treatments for I/R injury in recent years [82]. I/R injuries are likely to be the main cause of MI and subsequently heart failure due to imbalance in redox signaling [83]. Therefore, regulating redox signaling during reperfusion may be an important turning point in treating MI and heart failure [84].

Tanshinones have been well demonstrated with protective actions on reducing ischemia I/R injuries involving inhibition of free radical formation and mitochondrial apoptosis, as well as regulating eNOS/NO and AMPK/PI3K/Akt pathways. In a rat model of I/R injury, TIIA (20 mg/kg) was shown to reduce infarction size, possibly by upregulating eNOS/NO pathway and AMPK/PI3K/Akt pathway [40]. TIIA was also shown to enhance glutathione peroxidase and SOD activities and decrease production of free radicals (H_2O_2 and O_2^-) [19, 44, 85]. Since glutathione peroxidase, rather than SOD, is the major antioxidant in the cardiomyocytes [86], TIIA may be a promising agent to treat I/R injuries and MI due to its better permeability to cell membranes.

TIIA has been shown to inhibit hydrogen peroxide induced cell apoptosis in neonatal cardiomyocytes *in vitro*, with a 40% reduction of cell death as evidenced by decreased Hoechst 33342 staining and DNA fragmentation [19]. Similar findings were reported for its inhibition of H_2O_2 induced cardiomyocytes cell apoptosis and intracellular calcium increase [44]. However, the exact role of TIIA in I/R injuries still needs to be elucidated due to its complex interactions of H_2O_2 with

other signaling pathways. Additionally, antiapoptotic actions of TIIA have been shown in neonatal cardiomyocytes under hypoxia condition, with reducing p38 mitogen-activated protein kinase (p38MAPK) phosphorylation and miR-1 level [46], and enhanced ERK1/2 phosphorylation [45]. Our study has also demonstrated that TIIA (3 μ M) reduced hypoxia induced late apoptosis in H9C2 cells, the effect linked to decreasing hypoxia inducible factor-1 α (HIF-1 α) translocation, balancing in Bcl-2/Bax ratio, reducing cytochrome c release and caspase 3 activity [36]. A recent study also found that the protection by TIIA on hypoxic ischemia-induced injury could be enhanced by the JAK2/STAT3 inhibitors [87]. Additionally, the antiapoptosis effects of TIIA were associated with regulation of intracellular calcium, ATP, mitochondrial superoxide, and SOD levels but not intracellular H₂O₂ [35]. Studies *in vivo* found that TIIA inhibited I/R induced apoptosis (about 50% reduction of TUNEL staining), reduced caspase 3 activity, increased SOD level and Bcl-2/Bax ratio [19], and restored I/R induced diminished eNOS/NO production and coronary dysfunction [40]. Thus, it is likely that the protection of TIIA on myocardial I/R injuries involves multiple mechanisms including inhibition of intrinsic apoptosis pathway and anti-inflammatory and antioxidant activities. Interestingly, recent studies on cerebral I/R injury also indicate that TIIA may act by inhibiting intrinsic death pathway, inflammatory response, and ROS [7, 88].

Similarly, CT was shown to reduce hypoxia induced H9c2 cell apoptosis by balancing Bcl-2/Bax ratio, reducing hyperpolarisation in mitochondria membrane potential and caspase-3 activity [36]. Additionally, CT prevented the hypoxia induced changes of intracellular calcium, mitochondrial SOD, superoxide level, and NO [35]. A study in I/R model *in vivo* demonstrated that CT reduced myocardial infarction and inhibited proinflammatory cytokines including TNF- α , IL-1 β (interleukin-1 beta), and IL-6 (interleukin-6) production, NF- κ B translocation, and neutrophil infiltration [24].

TVI has also been demonstrated with protective action on hypoxia/reoxygenation injury and improvement of cardiac function [89]. TVI treatment improved myocardial contractility in reoxygenated rat heart, accompanied by reduced release of ATP metabolites and decrease in cellular calcium contents, but further study is required to elucidate its actions on ROS mechanisms [89].

5. Effects of Tanshinones on Cardiac Hypertrophy

Cardiac hypertrophy is defined as an enlargement of cardiac tissue or myocardium, resulting in a decrease in size of ventricle chambers. The pathological cardiac hypertrophy is associated with increased interstitial fibrosis, cell death, and cardiac dysfunction and influenced by many factors/conditions such as hypertension, ischemic injury, and genetic polymorphism [90, 91]. The main cellular and molecular regulators of cardiac hypertrophy include calcineurin/nuclear factor of activated T-cells (NFAT), G protein-coupled receptors (GPCR), MAPKs and apoptosis pathways, and cytokines activated by various stress stimuli including AngII, endothelin-1, and

insulin-like growth factor-1 (IGF-1) [91, 92]. Pathological cardiac hypertrophy has been shown to be more susceptible to proapoptotic changes, including modulations of Fas, Bcl-2 family proteins and caspases [93], and apoptosis occurring during hypertrophy may act as a cardiac adaptive response [94, 95]. Additionally, hypertrophic stimuli (i.e., angiotensin II (AngII) and endothelin-1 (ET-1) and catecholamines) have also been shown to induce hypertrophy by stimulating ROS production (via NADPH oxidase, xanthine oxidase, and nitric oxide synthase) [96]. It has been suggested that NADPH oxidase plays an importance role regarding redox-signaling involvement in pathological hypertrophy [92].

The anticardiac hypertrophy actions of tanshinones have been demonstrated *in vitro* and *in vivo* (see Tables 1 and 2). TIIA inhibited the size of enlarged cell induced by isoproterenol [47] and AngII [48, 49] and inhibited mRNA expressions of c-fos, c-jun (immediate-early genes of hypertrophic markers) induced by AngII [48]. The antiapoptotic actions of TIIA were shown via enhancing Bcl-xl expression and Akt phosphorylation, decreasing apoptotic markers (cytochrome c protein, cleaved caspase 3 levels) and reducing ROS production [49]. Furthermore, TIIA inhibited isoproterenol-induced increase in intracellular calcium level and reduced calcineurin/nuclear factors derived from activated T-cells cytoplasmic 3 (NFATc3) proteins expression [47]. TIIA has also been shown with antifibrosis effects by reducing TGF- β [50]/hydrogen peroxide [53] induced collagen synthesis in cardiac fibroblasts. The antifibrosis of TIIA may involve regulating Smad proteins expression (increasing Smad-7 and decreasing p-Smad 3) [50] and NAD(P)H oxidase activity (reducing Nox2, Nox4, and p47phox protein levels) [53]. The antihypertrophy actions of tanshinones have been confirmed *in vivo* in various animal models including those by abdominal aorta constriction [63, 64, 66], thoracic aorta partial constriction [65], and two-kidney-two-clip hypertensive rats [67, 68]. The main finding from *in vivo* studies is that TIIA reduced left ventricular mass index but did not alter blood pressure. The actions of TIIA may involve its interactions with various signaling pathways including protein kinase C (PKC), calcium, phosphoinositide 3-kinase (PI3K)/Akt (reducing p-Akt and p-Gsk3 β), TGF- β /Smad (decreasing Smad 3, TGF- β 1 and increasing Smad 7), and oxidant signaling. TIIA has been shown to inhibit intracellular calcium [63], PKC [64] and its downstream c-fos/c-jun [48], and matrix metalloproteinases 9 (MMP9)/tissue inhibitor of metalloproteinases type 1 (TIMP1) [67]. However, it is still not clear how TIIA interacts with calcineurin/NFAT proteins (downstream of calcium signaling) and GPCR (upstream of calcium signaling). Furthermore, it has been suggested that TIIA acts by regulating NADPH oxidase activity [92] and its subunits (decreasing Nox2, Nox4, and p47phox proteins levels) [68] and inhibiting eNOS-mediated endothelial dysfunctions [64].

Other tanshinones, such as TVI, have also been shown with antihypertrophy actions in neonatal cardiomyocytes/fibroblasts induced by IGF-1, AngII, and ET-1 [56, 89]. TVI inhibited IGF-1-induced cardiomyocyte hypertrophy and fibrosis of cardiac fibroblasts and collagen synthesis *in vitro* [89]. Further study found that TVI attenuated

FIGURE 2: Molecular targets in cardioprotective actions of tanshinones. This schematic illustration summarizes the mechanisms involved in cardioprotective actions of tanshinones based on studies on TIIA and CT. The targets with amelioration by tanshinones are indicated with the shaded text box. The targets with attenuation by tanshinones are indicated without shaded text box. Main cardioprotective actions of tanshinones were characterized via anti-inflammatory, antioxidant, antiapoptosis, antiproliferation/fibrosis, and vasodilator actions. The anti-inflammatory actions of tanshinones are via inhibition of $\text{TNF-}\alpha$ and $\text{IL-}\beta$ and their downstream signaling pathways including $\text{I}\kappa\text{B}\alpha/\text{NF-}\kappa\text{B}$, MCP-1, STAT3, and Akt, resulting in reduced IRF-1 and GATA-6 proteins expression which in turn decreases VCAM-1. ERK1/2, p38, and JNK are also inhibited. By suppressing ERK1/2 and JNK phosphorylation, CT reduces AP-1 activation. However, it is undetermined whether CT inactivates AP-1 via reduction of c-fos and c-jun. The inhibition of NF- κB 's translocation to nucleus results in decreased MMP-9 expression. Furthermore, TIIA decreases PPAR γ expression and CD36 which inhibits cell formation and subsequently inflammation. TIIA exerts antioxidant effects by enhancing antioxidant enzymes (Cu/Zn SOD, Mn-SOD, and GSH) and decreasing intracellular $\text{H}_2\text{O}_2/\text{O}_2^-$, mitochondrial O_2^- levels, and Nox 2 and Nox 4. The antiapoptosis action of tanshinones is via balancing Bcl-2/Bax protein, reducing cytochrome C release, and inhibiting caspase 3 activation. Bax can be activated by p53 which can be activated by HIF-1 α . TIIA inhibits HIF-1 α translocation to nucleus which in turn could reduce p53-induced Bax activation and ultimately apoptosis. Tanshinones inhibit the apoptosis by reducing mitochondrial superoxide level and enhancing Mn-SOD, increasing NO level, and decreasing intracellular calcium level. Antiproliferation/fibrosis actions of TIIA involve calcineurin-NFAT pathway, TGF- β /Smad pathway, PKC (ERK1/2 and JNK) pathway, and Akt/Gsk3 β signaling. As results of ERK1/2 and JNK inhibition by TIIA, c-fos and c-jun were inhibited by TIIA as well as MMP-2. Additionally, tanshinones present vasodilator action by reducing calcium influx, regulating intracellular calcium and NO levels, which may also involve inhibition of ER stress. AMPK, adenosine monophosphate kinase; AP-1, activator protein-1; AT1R, angiotensin II type 1 receptor; Cyto-C, cytochrome C; Casp 3, caspase 3; CD36, cluster of differentiation 36; Cu/Zn SOD, copper/zinc superoxide dismutase; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; GSH, glutathione; Gsk-3 β , glycogen synthase kinase-3 beta; HIF-1 α , hypoxia inducible factor-1 alpha; ICAM-1, intracellular adhesion molecule-1; IL-1 β , interleukin-1 beta; JNK, c-Jun N-terminal kinases; MCP-1, monocyte chemotactic protein-1; MEKs, MAPK/ERK kinases; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; Mn-SOD, manganese superoxide dismutase; MPTP, mitochondrial permeability transition pore; Mt O_2^- , mitochondrial superoxide; NADPH, nicotinamide adenine dinucleotide phosphate; NFATc3, nuclear factor of activated T-cells, cytoplasmic 3; NO, nitric oxide; Nox 2, NADPH oxidase 2; Nox 4, NADPH oxidase 4; PKC, protein kinase C; STAT3, signal transducer and activator of transcription 3; TNF- α , tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1.

[^3H]-leucine incorporation, ERK1/2 phosphorylation, and atrial natriuretic peptide level but not Akt phosphorylation [56]. However, the actions of TVI on upstream signaling, which regulates ERK1/2 phosphorylation, such as GPCR, ROS, and NADPH oxidase, are still not clear.

In addition, there is evidence for the involvement of microRNAs in the cardioprotection of tanshinones. It was found that TIIA increased the expressions of MiR-133 and ERK1/2 in hypoxic neonatal cardiomyocytes [45]. In rats with acute myocardial infarction, TIIA inhibited postinfarct

cardiac fibrosis and improved impaired left ventricular function, with downregulation of expression of TGF- β 1 and upregulation of expression of miR-29b. The antifibrotic effect of TIIA was blocked by a miR-29b inhibitor and Smad3 siRNA, indicating that miR-29 may play an important role in the action of TIIA in postinfarct cardiac remodeling, possibly mediated by TGF- β -Smad3 signaling pathway [97].

6. Effects of Tanshinone IIA on Adriamycin Induced Cardiomyopathy

Adriamycin (ADR), also called doxorubicin, is an effective anticancer drug. However, its clinical usage is limited by its cardiomyopathy which may lead to heart failure [98]. ADR-induced cardiomyopathy is related to increased oxidative stress [99]. Increasing ROS can reduce mitochondria oxidative phosphorylation, which in turn depletes cellular ATP, resulting in cardiac cell impairment [100]. Other possible mechanisms for ADR-induced cardiomyopathy include activation of mitogen-activated protein kinases (MAPKs) and necrosis and extrinsic pathways [101]. TIIA has been demonstrated to prevent ADR-induced cardiomyocytes death *in vitro* by preventing DNA fragmentation (60% compared to positive control), suppressing ROS production, and increasing Bcl-2/Bax ratio [43]. Hong et al. also showed that TIIA prevented ADR-induced cell apoptosis and ROS production by 33% and 37%, respectively, with enhanced Bcl-x1 protein expression, inhibited cytochrome c release and caspase 3 activity, and restored Akt phosphorylation [21]. Thus, TIIA and other tanshinone analogues may potentially be useful in preventing ADR-induced cardiomyopathy.

7. Effects of Tanshinone IIA on Endotoxin-Induced Cardiac Dysfunction

Tanshinone IIA has recently been shown to attenuate endotoxin-induced cardiac dysfunction in septic mice via inhibiting NADPH oxidase 2-related signaling pathway. It inhibited LPS-induced production of TNF- α , IL-1 β , and ROS and decreased Nox2, p-ERK1/2, and p38 MARK [72].

8. Synthetic Tanshinone Derivatives and Structure Optimization

Natural tanshinones have a unique structure and bioactivity but also some limitations such as low water solubility and poor bioavailability [12]. Approaches have been made to develop synthetic derivatives of tanshinones to optimize their structures for potential clinical applications. For example, sodium tanshinone IIA sulfonate was synthesized as a water soluble derivative of tanshinone IIA, which has been used in China for the treatment of angina pectoris and myocardial infarction [102]. Several tanshinone IIA derivatives with better vasodilatation activity have been synthesized by the reactions of tanshinone IIA with aromatic aldehydes, and the structure-activity relationship indicated that the methoxyl-substituting group in the *meta*-position of aromatic aldehydes presented better activity than that of nitro substitution derivatives [103]. A number of tanshinone derivatives have

been synthesized with modification of tanshinones' four rings with fatty acids, N-containing substituent, and other function groups, resulting in analogues with potential better bioactivity and bioavailability [102].

9. Conclusion

In conclusion, tanshinones have been demonstrated with significant cardiac protective actions, which may be potentially useful as therapeutic agents to treat cardiac diseases. The molecular mechanisms of cardioprotective actions of tanshinones involve anti-inflammatory, antioxidant, and antiapoptosis actions although regulating various signaling pathways, in particular TNF- α signaling, mitochondrial apoptosis, and calcium and NO signaling pathways, as illustrated in Figure 2. Further studies on tanshinones and tanshinone analogues may lead to development of novel drugs for treating cardiovascular diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by grants from Western Sydney University.

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