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Anti-Fibrotic Effects of Curcumin and Some of its Analogues in the Heart

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Abstract

Cardiac fibrosis stems from the changes in the expression of fibrotic genes in cardiac fibroblasts (CFs) in response to the tissue damage induced by various cardiovascular diseases (CVDs) leading to their transformation into active myofibroblasts, which produce high amounts of extracellular matrix (ECM) proteins leading, in turn, to excessive deposition of ECM in cardiac tissue. The excessive accumulation of ECM elements causes heart stiffness, tissue scarring, electrical conduction disruption and finally cardiac dysfunction and heart failure. Curcumin (Cur; also known as diferuloylmethane) is a polyphenol compound extracted from rhizomes of *Curcuma longa* with an influence on an extensive spectrum of biological phenomena including cell proliferation, differentiation, inflammation, pathogenesis, chemoprevention, apoptosis, angiogenesis and cardiac pathological changes. Cumulative evidence has suggested a beneficial role for Cur in improving disrupted cardiac function developed by cardiac fibrosis by establishing a balance between degradation and synthesis of ECM components. There are various molecular mechanisms contributing to the development of cardiac fibrosis. We presented a review of Cur effects on cardiac fibrosis and the discovered underlying mechanisms by them Cur interacts to establish its cardio-protective effects.

Keywords: Curcumin; Diferuloylmethane; Theracurmin; C66; J19; Cardiac Fibrosis

Introduction

Cardiac fibrosis is an outcome of a diverse range of conditions including diabetes and cardiovascular diseases (CVDs) resulting in fibrosis of heart and thereby heart failure. In these conditions, fibrosis is primarily aimed to correct the maladaptive developed injury [1,2]. Cardiac fibrosis stems from the expression of fibrotic genes leading to macrophage-mediated trans-differentiation of cardiac fibroblasts (CFs) into active myofibroblasts responsible for secreting proteins involved in contraction such as α-smooth muscle actin (α-SMA) and extracellular matrix (ECM) proteins like collagen and elastin [3-5]. In cardiac tissue, ECM is responsible for supporting the cardiac cells' alignment within the tissue to have efficient coupling with other cells nearby throughout the contraction. Normally, the synthesis and degradation procedures relating to ECM components are highly regulated to keep a balance. In case of conditions such as destruction caused by myocardial infarction (MI), the balance between ECM synthesis and degradation is disrupted to compensate for the damage resulting in excessive ECM accumulation [6,7]. The excessive deposition of ECM in cardiac tissue (cardiac fibrosis) is detrimental by itself causing heart stiffness, electrical conduction disruption, development of tissue scars containing high amounts of collagen, left ventricular hypertrophy and ultimately cardiac dysfunction and heart failure [3].

Although different treatments including angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers have been proposed for the treatment of cardiac fibrosis none of them has been proven effective, especially in heart failure where the incidence of fibrosis is high [8]. Hence, there is an unmet need to find novel strategies against the development and progression of cardiac damage mediated by fibrosis resulting from CVDs.

Curcumin (Cur), also known as diferuloylmethane, is a polyphenolic compound derived from *Curcuma longa* plant rhizome and the main curcuminoid in the Indian spice, turmeric. This phytochemical has been revealed to exhibit different functions in living systems comprising anti-thrombotic, anti-inflammatory, anti-oxidant, anti-proliferative, anti-angiogenic and pro-apoptotic, making it or some of its active substances potential therapeutic agents for selected CVDs [9,10]. The remedial use of this compound goes back to traditional medicine in China and India [11]. Cumulative evidence has suggested curcumin to play a positive role in protection against cardiac fibrosis through modulating different molecular pathways via acting on a variety of cytokines, growth factors, and their relating receptors, transcription factors especially those involved in the regulation of cell proliferation, and enzymes [12,13,11].

Molecular mechanisms underlying cardiac fibrosis development

A fibrotic response is the consequence of a change in expression of genes encoding molecules activating various signaling pathways, including growth factors, cytokines and chemokines that are triggered by internal or external stressors. Some of the most important molecules involved in the process of CF differentiation into myofibroblast and their migration include transforming growth factor β (TGF-β), angiotensin II (AngII), platelet-derived growth factor (PDGF)-D, connective tissue growth factor (CTGF), endothelin-1 and interleukin-18 (IL-18) [14-16]. Figure 1 highlights some of the fibrosis mediators and their role in differentiating fibroblasts into myofibroblasts.

Over-expression of AngII activates the Ang II type 1 (AT1) receptor giving rise to cardiac fibrosis by inducing CF proliferation and migration, elevated level of myocardial apoptosis and excessive accumulation of collagen type I (Col I), collagen type III (Col III) and fibronectin (FN) ECM elements [17-20]. In the presence of Ang II, plasminogen activator inhibitor (PAI)-1 is over-expressed in cardiac fibroblasts and the myocardium resulting in an increment in ECM synthesis by fibroblasts, decrement in ECM degradation and metalloproteinase inhibition [21,22].

Transforming growth factor β1 (TGF-β1) is another molecule involved in triggering fibrosis and hypertrophy [23][24]. This fibrotic activity of TGF-β1 seems to be modulated by acetyltransferase (HAT) activity of transcriptional co-regulator of p300 via the mediation of Smad, leading to enhanced collagen production and fibrotic response initiation [25].

Several *in vivo* studies demonstrated that the expression of connective tissue growth factor (CTGF) expression is increased in arteries and left ventricle of patients with atherosclerosis and hypertension suggesting both the vascular and cardiac fibrosis are amplified as a consequence of CF and induction of vascular smooth muscle cell proliferation [26,27].

Peroxisome proliferator-activated receptor-γ (PPAR-γ or NR1C3) is another molecule involved in the development of cardiac fibrosis [28]. Once PPAR-γ turns into its activated form as a result of binding to its relevant ligand, it is able to form a heterodimer with retinoid X receptor (RXR) and bind the DNA through PPAR-responsive regulatory elements to regulate the expression of a variety of genes involved in a wide range of biological activities [29]. More specifically, PPAR-γ regulates fibrotic and hypertrophic processes in cardiovascular apparatus in response to stress signals [30].

The family of serine/threonine protein kinases C (PKC) encompasses different isozymes. Their activity is associated with pathogenic cardiac issues, including cardiac fibrosis [31]. The activation of a PKC is triggered in response to an extracellular signal activating phospholipase C (PLC) leading to the formation of diacylglycerol (DAG) and inositoltriphosphate (IP3) elevating the intracellular ca^{2+} content. The PKC is then activated in response to high ca^{2+} level in the cytosol by binding to DAG located in membrane inducing several downstream signaling pathways such as mitogen-activated protein kinase (MAPK) pathway contributing in a range of different intracellular effects involving modulating cell growth and proliferation [32,33]. Various *in vitro* and *in vivo* investigations suggest that in certain conditions such as high amounts of glucose (hyperglycemia) or free fatty acid (FFA) in the bloodstream, the production of DAG increase with associated pathological changes in the cardiac muscles [31,34]. The regulatory effects of PKCs on matrix metalloproteinase (MMP) quantity and function have also been proven to be evident as they enhance the activity of MMP-2 and MMP-9 via MAPK and MMP-9 through JNK signal transduction pathways [34].

In case of exposure to a stress signal like hypertension-induced pressure overload, it has been shown that fibrogenic gene expression is stimulated by the activity of sequence-specific DNA binding transcription factors comprising SMAD2/3, serum response factor (SRF), myocardin-related transcription factors (MRTFs) and nuclear factor of activated T cells (NFAT) [35].

Mechanisms of curcumin effect on cardiac fibrosis

Curcumin (Cur), is a natural polyphenol found in turmeric and is derived from *Curcuma longa*. The potential pharmacological and therapeutic effects of Cur have been studied extensively in a wide range of conditions [36-38]. It has pleiotropic effects in a variety of cells including cardiac cells. Given its hydrophobic nature, presence of a β-diketone moiety and active methylene group Cur is metabolized in the liver via aldo-keto reductase and has poor bioavailability [39,40]. To circumvent these various modified forms of native curcumin [41-44] as well as its synthetic analogs [45] and derivatives [46] have been used in various studies. The Cur derivatives and analogues which have potential anti-fibrotic effects on heart are (2E,6E)-2,6-bis(2-(trifluoromethyl)benzylidene) cyclohexanone, also known as C66 [47], J17 [(2E,5E)-2-(3-Hydroxy-4-methoxybenzylidene)-5- (2 nitrobenzylidene) cyclopentanone][48] and tetrahydrocurcumin (THC) [49]. In the following section, we have reviewed various effects of Cur and its metabolites on cardiac fibrosis and potential molecular mechanisms.

Inhibition of MMPs expression

It has been shown that Cur restored the reduced expression of MMP-2 and MMP-9 after HF induction in New Zealand rabbits. Also, the high level of collagen accumulation was reduced in Cur-subjected animals suggesting an anti-fibrotic activity for this compound [50].

TGF-β suppression

Cur reversed changes resulted from TGF-β treatment by suppressing the augmented expression of PAI-1 protein in human liver-derived HepG2 cells [51]. In this study, Cur was able to exert almost the same effects as simvastatin, a lipid-lowering medication [51]. In neonatal SD rat CFs reported that Cur administration blocks the pro-fibrotic activity of TGF-β1 through reduction of α-SMA and Col I at both mRNA and protein levels and suppression of Smad2 and p38 MAPK activation levels [52].

A recent investigation on TGF-β1 stimulated human CFs pre-treated with Cur [12] showed a dramatic reduction of α-smooth muscle actin (α-SMA), collagen type Iα (COLA)-1 and COLA3 expression. Cur was also able to suppress CF proliferation following TGF-β1 treatment and stimulate G2/M phase cell cycle arrest. Cur exposure inhibited Smad2/3, p38 MAPK and ERK phosphorylation and consequently down-regulated the expression of cell cycle protein. Conversely, CFs incubated only with Cur did not exhibit any of these changes and the outcomes were consistent with the anti-proliferative and anti-collagen accumulation activities of Cur activities through TGF-β1 pathway [12].

Cur dramatically reduced the excessive accumulation of collagen, the expression of TGF-β and CTGF pro-fibrotic genes as well as the protein levels of collagen I and matrix metalloproteinase-9 (MMP-9) in Cur-treated C57BL/6 mice fed on a high-fat diet (HFD) [53]. This suggests that Cur neutralizes the adverse effects of HFD on the cardiac tissue [53]. Furthermore, curcumin preexposure of H9c2 embryonic rat heart-derived cells followed by treatment with palmitate (PA) resulted in a reversal of hypertrophy induced by PA [53]. The PA-stimulated high expression level of TGF-β was shown to be down-regulated in cells pre-treated with Cur [53].

Altering MAPK phosphorylation

Soetikno, and colleagues [54] assessed the cardio-protective effect of curcumin in high glucose (HG)-related cardiomyopathy in SD rats by streptozotocin (STZ) injection. As a result of the induction of diabetes, the PKC-α and -β2 isozymes were translocated to the membrane as an indication of their activation, which was inhibited following Cur treatment. The increased amount of phosphorylated p38MAPK and ERK1/2 in diabetic animals-related tissue samples from left ventricle was reduced by following treatment with Cur [54]. The expression of TGF-β, osteopontin and p300 transcriptional coactivator as an indicative of anti-fibrotic Cur activity was decreased which was shown by reduced ECM deposition and shrinkage of fibrotic areas [54].

In another study diabetes was induced in SD rats by feeding them on a high energy diet and a low-dose streptozotocin (STZ) injection [55]. Heart specimens from Cur-treated diabetic rats exhibited lower collagen type I and III accumulation compared to Cur-untreated animals. Additionally, TGF-β1, TβR II and phosphorylated Smad 2/3 were detected only at remarkably low levels. However, the expression of Smad 7 was enhanced in those rats [55]. When human CFs were subjected to Cur, accompanied by high glucose (HG) or treatment with TGF-β1. The over-activity of AMPK/p38 MAPK stimulated by HG or TGF-β1 was found to be suppressed and the collagen synthesis was attenuated in those cells as a result of Cur treatment [55].

Suppression of Smads phosphorylation

Bugyei-Twum et al. pre-treated H9c2 rat cardiomyoblast cells using 25 μM Cur followed by HG administration. The Cur pre-treatment led to inhibition of the HG-stimulated p300 activity. When neonatal rat fibroblasts were treated with Cur there was a significant reduction in collagen synthesis confirming its counter-HG/ TGF-β behavior [56]. Cur also reduced the level of acetylated Smad2 in TGF-β-stimulated H9c2 cardiomyoblasts. The up-regulated Smad7 mRNA, induced by treatments with HG or TGF-β, was suppressed by Cur both *in vitro* and *in vivo*. The Cur-treated diabetic Ren-2 rats showed a reduced amount of hypertrophy in heart, ECM synthesis and restoration of diastolic function [56].

The cardiac fibroblasts isolated from Sprague-Dawley (SD) rats were treated with Cur along with TGF-β1 or Ang II [57]. It was found that Cur enhanced the activity of matrix metalloproteinase (MMP)-2 and diminished the levels of the phosphorylated extracellular signal-regulated kinase (ERK) 1/2 in the presence of Ang II. Analyzing CFs co-treated with Cur and TGF-β1 showed reduced expression levels of phosphorylated Smad2/3 and Akt [57]. This study demonstrated that Cur administration attenuates CF proliferation and migration and keeps their collagen production at baseline level regardless of the presence of TGF-β1 or Ang II [57].

Inhibition of toll-like receptor 2 expression

To gain insight into the cardioprotective effects of Cur after ischemia/reperfusion (I/R) injuries, SD rats were first orally administered with 300 mg/kg/day Cur for seven days before undergoing I/R injury [58]. The expression of toll-like receptor 2 (TLR2), known to contribute in myocardial infarction, was prominently amplified in the infarct zone of IR rat models; however, the expression of TLR4 showed a constant pattern [58]. The up-regulation of TLR2 was reversed in Cur-treated animals. There was a reduction of macrophage infiltration (CD68) and high mobility group box 1 in Cur-treated IR rat models, whereas, their levels increased in the absence of Cur post the IR injury [58]. Furthermore, comparing changes in neonatal rat-derived myocardial cells stimulated by tumor necrosis factor (TNF)-α, peptidoglycan (PGN) or hypoxia/reoxygenation (H/R) in the presence and absence of 10 μM Cur, the inhibitory effect of Cur on over-expressed TLR2 and monocyte chemoattractant protein (MCP)-1 became apparent [58].

Increasing Akt phosphorylation

Experimental diabetes was induced in Wistar rats via a high-fat diet (HFD) and intraperitoneal (I.P.) injection of STZ [59] followed by administration of Cur. The ratio of fibrosis area to the entire myocardial area in diabetic rats was attenuated by curcumin [59]. Cur declined diabetic cardiomyopathy by promoting protein kinase B (Akt) and GSK-3β phosphorylation [59].

PPAR-γ activation

In spontaneously hypertensive rats (SHRs) Cur treatment reduced the Ang II levels in the blood, the ratios of heart weight/body weight and left ventricle weight/body weight, systolic blood pressure and the expression levels of CTGF, PAI-1, Col III, and FN [13]. There was also upregulation of A PPAR-γ after Cur treatment. Left ventricle samples showed a reduction of collagen accumulation after daily oral treatment of Cur. In groups concomitantly treated with Cur and GW9f662 (PPAR-γ antagonist), the anti-fibrotic activity of curcumin was overturned (Figure 2) [13]. Pretreatment of cardiac fibroblasts with Cur suppressed the Ang II-promoted expression of CTGF, PAI-1, collagen III (Col III/COLA-3), FN, TGF-β1 and also inhibited phosphorylation of Smad2/3. It also increased the PPAR-γ expression and binding capability in a dose-dependent manner. GW9662 pre-treatment, on the other hand, exerted negative effects on Cur-induced anti-fibrotic activities [13] suggesting that Cur can suppress the cardiac fibrosis in SHRs via modulating PPAR-γ and TGF-β1/Smad2/3 signaling interaction [13].

When SD rats after left coronary artery ligation were exposed to Cur [60] there was notable shrinkage of the fibrosis area due to myocardial infarction (MI) after oral treatment of Cur in a dose-dependent manner [60].

Affecting angiotensin receptors expression

Ang II-perfused Sprague Dawley (SD) rats were used to investigate the anti-fibrotic effect of dietary Cur post-Ang II infusion [61]. Cur was found reduce the fibrosis in the intra-cardiac vessels and myocardium by dramatic suppression of AT1 receptor expression after four weeks and, inversely, up-regulation of AT2 receptor expression enhancing through time suggesting dual effects of Cur on AT1 and AT2 receptors [61]. The elevated numbers of macrophages and alpha-SMA-expressing myofibroblasts accumulated in specimens from Ang II-injected rats was significantly decreased following administration of dietary Cur over 28 days [61]. Cur treatment also down-regulated the expression of TGF-β1 and phosphorylated-Smad2/3, suppressed the synthesis of collagen I and reduced the collagen-rich areas [61]. Finally, the reduced ACE2 levels after Ang II injection was abrogated by Cur intake [61].

Reducing inflammation

An *in vivo* study after intraperitoneal (I.P.) injection of Cur on hind limb ischemia mouse model revealed amelioration of cardiac fibrosis damages occurred by ischemia [62]. This study demonstrated that Cur-induced cardio-protective outcomes are mediated by inhibition of NF-kB activation and macrophage infiltration and down-regulation of inflammatory markers (TNF-α, IL-1 and IL-6) [62,38].

Restoring sirtuin protein 1 inhibition

Xiao et al. [8], studied the Cur-induced changes in C57BL/6J wild-type male mice about one month after MI induction. Four weeks post-MI induction, the experiments revealed that there was significant shrinkage of interstitial areas affected by fibrosis in Cur-received animals. The expression levels of collagen I, collagen III and TGF-β1 were found to be down-regulated. The Cur treatment led to the restoration of post-MI inhibition of sirtuin protein 1 (SIRT1), a histone deacetylase. Cur suppressed the proliferation and migration of Ang II-exposed CFs, decreased the deposition of collagen and down-regulated the expression of matrix metalloproteinase (MMP)-9 and -2 [8]. Furthermore, the siRNA-SIRT1-mediated down-regulation of SIRT1 in Ang II-incubated CFs suggested the involvement of SIRT1 in anti-fibrotic property of Cur [8].

Inhibiting expression of autophagy markers

Another recent study investigated the contribution of autophagy in anti-fibrotic and anti-hypertrophic activities of Cur in ISO-induced rat models of cardiac hypertrophy and fibrosis [63]. The heart weight/body weight ratio in Cur-treated hypertrophic rat models decreased by 13.1% and reversed the ISO-induced expression changes in hypertrophic markers including atrial natriuretic peptide (ANP), α-myosin heavy chain (α-MHC or MYH6), β-MHC (MYH7) and MYH7B [63]. The extent of interstitial fibrosis area formed following ISO exposure was limited by Cur intervention. The expression of genes encoding fibrotic markers of procollagen I and procollagen III, which were increased by ISO, decreased to roughly more than 50% as a result of treatment with Cur. Although ISO suppressed mTOR expression, treatment with Cur restored mTOR expression. The expression of autophagy markers, including LC3 and Belin-1, was up-regulated the presence of ISO, while Cur treatment completely abolished this effect [63].

Cur pharmacokinetics and safety

Cur is known to have poor bioavailability limiting its application as a therapeutic agent. Its relatively low absorption, rapid metabolism and clearance from the body contribute to its poor bioavailability [64]. The high lipophilic property of Cur contributes to its low solubility in aqueous environments [65]. Cur is poorly absorbed when orally administered (almost undetectable in plasma, liver and brain after 30 min), while it is detectable (at low levels) in animals with parenteral administration [66]. Cur is both chemically and metabolically unstable [67]. Once administered in neutral to alkaline environments, Cur rapidly (within 30 min) degrades to form mainly bicyclopentadione and autoxidation products, and to a less extent ferulic acid, feruloyl methane and vanillin [68]. In acidic environments, on the other hand, the degradation rate is significantly lower [64].

After oral ingestion, only a low proportion of Cur is absorbed through the intestinal tract which undergoes rapid metabolism in plasma and liver and the rest are excreted in feces [64]. While being metabolized, the absorbed Cur go through two different phases including reduction and conjugation. In the reduction phase, the double bonds are reduced *via* NADPH-dependent curcumin/dihydrocurcumin reductase. In the next phase, the previously reduced metabolites of Cur and Cur itself undergo β-glucuronidase/sulfatase enzymes- mediated conjugation with glucuronic acid or sulfuric acid-producing glucuronides and sulfates in the liver. A proportion of these water-soluble products are then excreted into the duodenum *via* bile, and the rest is released into the blood and excreted through the urine [64,65]. Only the free form of Cur is active while the conjugated forms of Cur are inactive and are rapidly eliminated from the body [65,68]. Cur is well tolerated and causes no harm even when administered at very high doses [67].

Curcumin analogues and cardiac fibrosis

In view of the poor bioavailability of Cur after oral administration [69], several studies have been conducted on the evaluation of anti-fibrotic properties of Cur derivatives to address this limitation. Pang and colleagues [47] used (2E,6E)-2,6-bis(2-(trifluoromethyl)benzylidene) cyclohexanone, also known as C66, a synthetic curcumin derivative, in type 1 diabetic mice to validate its cardioprotective potential. The elevated heart weight/body weight ratio was decreased after a two-month treatment suggesting a beneficial role of C66 in preventing pathological changes in cardiac tissue and potentially reversing diabetic cardiomyopathy [47]. The C66 compound was also used in another study performed on streptozotocin-injected C57BL/6 mice to evaluate its protective effects against diabetic cardiomyopathy [70]. The three-month administration of C66 at a concentration to diabetic mice reduced cardiac fibrosis and cardiac function decrement compared to C66-untreated diabetic mice. The cardioprotective function of C66 was suggested to be due to down-regulation of c-Jun NH2-terminal kinase (JNK) activation [70].

To shed more light on molecular mechanisms behind the cardio-protective action of C66 when diabetic JNK2−/− and wild-type (WT) mouse models were fed with C66 [71] there was a reduction in diabetes-induced cardiac fibrosis due to its inhibitory effect on the JNK2 activity. In contrast to non-treated WT diabetic mice, there was a reduction in expression of TGF-β1, CTGF, and PAI-1 pro-fibrotic factor in C66-treated WT diabetic mice resulting in reduced collagen deposition in the interstitial areas. On the other hand, there were no fibrotic changes in cardiac tissues from JNK2−/− mouse models [71].

Since chronic kidney diseases (CKD) are accompanied by CVD-related complications like cardiac fibrosis, a compound called theracurmin with the similar formulation as Cur was fed to CKD SD rat models by gavage [72]. After treatment with theracurmin, both cardiac structure and function improved and cardiac fibrosis and hypertrophy were reduced in rats with CKD. The assessment of expression of pro-fibrotic and pro-hypertrophic genes in heart tissues isolated from the treated rats showed the suppressive effects of theracurmin on TGF-β1, β-MHC, and type I collagen. Besides, theracurmin lowered the phosphorylation level of Smad2 [72].

A Cur analog called J17 [(2E,5E)-2-(3-Hydroxy-4-methoxybenzylidene)-5- (2 nitrobenzylidene) cyclopentanone] [48] was tested as a possible Cur alternative to reverse cardiomyopathy and fibrosis established by diabetes. There was a reduction of fibrosis via AKT signal transduction silencing in H9C2 rat myoblast cells cultured in the presence of DMSO-dissolved Cur and J17. These were added thirty minutes before high glucose-mediated fibrosis stimulation. There was a stronger dose-dependent inhibitory activity with J17 compared to the Cur-induced inhibitory effects [48]. Male C57BL/6 mice were subjected to either J17 solubilized in 0.5% sodium carboxyl methyl cellulose (CMC-Na) at a concentration of 10 mg/kg or Cur at a concentration of 50 mg/kg administered by gavage eight days after the induction of diabetes mellitus [48]. The heart tissue sections from which received Cur- and J17 showed a significant attenuation of collagen deposition and cardiac fibrosis. The over-expression of collagen type I and TGF-β were attenuated in diabetic mice treated with J17 to physiological level [48]. Similarly, the level of TNF-α and ICAM-1 transcripts in heart specimens were reduced to their relevant normal physiological levels following the administration of J17 [48]. This suggests a protective effect of J17 against fibrosis and other cardiac pathological changes after initiation of a fibrosis response due to diabetic hyperglycemia [48].

When a metabolite of Cur, tetrahydrocurcumin (THC), was orally administered to STZ-induced diabetic mice [49], there was improvement of cardiac function. THC treatment also attenuated fibrosis within myocardium in THC-received diabetic mice by up-regulation of SIRT1 signaling pathway expression. THC treatment was also accompanied by suppression of acetylation and stimulation of deacetylation of SOD2, a SIRT1 downstream molecule reinforcing the anti-oxidative capacity. The administration of THC inhibited TGFβ1/Smad3 signaling pathway activated by reactive oxygen species (ROS). The collagen deposition was significantly reduced after THC exposure in the cardiac tissue of diabetic mice [49].

Conclusion

Mounting evidence, both *in vitro* and *in vivo*, support the anti-fibrotic functions of Cur and its analogues in the presence of various pro-fibrotic factors (Table 1). Cur reverses the effect of pro-fibrotic factors through altering the expression and activation of numerous intracellular molecules. These evidences suggest that curcumin and its metabolites could potentially act as an effective adjuvant to inhibit the progression of myocardial damage resulting from various conditions that may lead to heart failure. It can be implied that Cur is a safe herbal medication that merely targets the cells responsible for the disease, while leaving normal ones unaffected. Various measures including the use of altered formulations of Cur, concomitant administration of Cur with agents reducing its metabolism and designing oral delivery systems using structures such as liposomes and nanoparticles are necessary to tackle the low bioavailability of Cur.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

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Tables

Table 1: A summary of studies evaluating curcumin's effects on cardiac fibrosis.

Reference	Animal/cell type	Cardiac fibrosis condition induced by	Curcumin concentration(s)/treatment duration	Outcomes
Tang et al. [50]	New Zealand rabbits	Combined aortic regurgitation and aortic stenosis	-100 mg/kg/day -2 months	-Upregulation of MMP-2 - Upregulation of MMP-9 -Reduction of collagen deposition
Sunagawa et al. [73]	Sprague-Dawley (SD) rats	Myocardial infarction (MI)	-50 mg/kg/day (combined with/without enalapril) -6 weeks	-Decreased perivascular fibrosis expansion -Additive effect on fibrosis extent when combined with enalapril -Repressed expression of p300 transcriptional coactivator observed only in animals subjected to enalapril+Cur
Nakayama et al. [51]	Human liver-derived HepG2 cells	TGF-β administration	-10 μmol/l - 30 min	-Blunted PAI-1 protein level
Soetikno et al. [54]	SD rats	-High glucose (HG) -Streptozotocin (STZ) injection (diabetes induction)	-100mg/kg/day -8 weeks	- PKC-α inactivation -PKC-β2 inactivation -Phosphorylation diminution of p38MAPK and ERK1/2 -Downregulation of TGF- β, osteopontin and p300 -Attenuated ECM accumulation
Kim et al. [58]	SD rats	Ischemia/reperfusion (I/R) injury	-300 mg/kg/day -1 week	- Reduced TLR2 expression - Lowered macrophage infiltration (CD68) and high

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				mobility group box 1
	Neonatal rat-derived myocardial cells	-Tumor necrosis factor (TNF)-α -Peptidoglycan (PGN) -Hypoxia/reoxygenation (H/R)	-10 µM - 18 hours	- Reduced TLR2 expression -Reduced monocyte chemoattractant protein (MCP)-1
Yu et al. [59]	Wistar rats	-High fat diet (HFD) - STZ injection	-100 or 200 mg/kg/day -4 months	-Limiting the fibrosis area - Increased phosphorylated protein kinase B (Akt) and GSK-3β
Meng et al. [13]	Spontaneously hypertensive rats (SHRs)	Hypertension	-100 mg/kg/day -12 weeks	- Blood Ang II reduction - Decreasing CTGF, PAI-1, Col III, and FN expression levels - Downsized heart weight/body weight - Downsized left ventricle weight/body weight - PPAR-γ expression increment and activation -Slight reduction of collagen deposition
	Cardiac fibroblasts (CFs)	Ang II	-5, 10, 20 µmol/L -1 hour	- Downregulation of CTGF, PAI-1, collagen type III, FN, TGF-β1 -Decreased levels of phosphorylated Smad2/3 - Promoted PPAR-γ expression and binding capability -Inhibition of CF proliferation
Sunagawa et al. [60]	SD rats	Left coronary artery ligation	-0.5, 5, and 50 mg/kg/day -24 days	- Concentration-dependent limitation of fibrosis area
Bugyei-Twum et al.[56]	Rat H9c2 myoblasts	- high glucose (HG) - TGF-β1	-25 µM	-Suppression of p300 activity

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				-Reduction of Smad acetylation -Smad7 reduction - Mitigated collagen production
	homozygous TGR (mRen-2) 27 rats	-Tail vein injection of STZ	-2%	- Smad7 down-regulation -Attenuated ECM synthesis -Alleviated cardiac hypertrophy -Restoration of diastolic function
Chung et al. [57]	SD rat- isolated cardiac fibroblasts	- Ang II - TGF-β1	-25 μM -1 day	-Elevated matrix metalloproteinase (MMP)-2 activity -Reduction of ERK1/2 phosphorylation level -Diminished levels of phosphorylated Smad2/3 and Akt - Decrement of CF proliferation and migration -Base line-level production of collagen
Pang et al. [61]	SD rats	- Ang II	-150 mg/kg/day -2, and 4 weeks	- AT1 receptor suppression - AT2 receptor amplification -Lowered numbers of macrophages and myo-fibroblasts expressing α-SMA -TGF-β1 down-regulation - Smad2/3 phosphorylation suppression - Collagen I production diminution - ACE2 expression augmentation
Zeng et al. [53]	C57BL/6 mice	- HFD	- 50 mg/kg/day - 8 weeks	-Diminished collagen deposition

				- Decreased expression level of TGF-β and CTGF -Downregulation of MMP-9 -Diminished collagen I production
	H9c2 embryonic myoblasts	- Palmitate (PA)	-20 µM -1 hour	-Reversal of induced cardiac hypertrophy - TGF-β down-regulation
Liu et al. [62]	C57BL/6J mice	-Ligation of left femoral artery, great saphenous artery, iliac circumflex artery/vein, and muscular branch (left hindlimb ischemia)	- 100 mg/kg -Administered1 hour before the ligating surgery by intraperitoneal (I.P.) injection	- Recovery of cardiac fibrosis injured areas - Restricting NF-kB activation -Inhibiting macrophage infiltration -Decreased expression levels of TNF-α, IL-1, and IL-6
Liu et al. [52]	CFs derived from neonatal SD rats	- TGF-β1	-20 µmol/l - 30 min	-Diminution of α-SMA and Col I expression - Reduced activated forms of Smad2 and p38 MAPK
Xiao et al. [8]	C57BL/6J wild-type mice	- permanent left anterior descending coronary artery ligation (MI induction)	-100 mg/kg/day -5 weeks	-Alleviation of interstitial fibrosis -Decreased levels of collagen I, collagen III, and TGF-β1 -Restored SIRT1 expression
	CFs derived from Wistar rats	- Ang II	- 5, 10, and 15 µM -1 hour	-Repression of CF proliferation and migration -MMP down-regulation -Increment in SIRT1 expression - Decreased levels of collagen I, collagen III, and TGF-β1dose dependently -Decreased expression of

				MMP-9 and MMP-2
Ma et al. [74]	SD rats	- Isoproterenol (ISO) injection	- 150 or 300 mg/kg/day -4 weeks	- Collagen I/III level decrement in myocardial interstitium and perivascular areas - Suppression of α-SMA -Reversal of cardiac weight index (CWI) increment
	3rd to 5th generations of CFs isolated from SD rats	- Ang II	-5, 10, and 20 μmol/L -1 hour	- Collagen I/III reduction - Fibroblast proliferation and differentiation inhibition -Expression decrement relating to TGF-β1, matrix metalloproteinase (MMP)-9 and tissue inhibitor of metalloproteinase (TIMP)-1
Fang et al. [12]	Human CFs	- TGF-β1	-20 μmol/l -1 hour	-Expression reduction of: α-SMA, Col I, Col III - CF proliferation repression -Induction of G2/M phase cell cycle arrest - Deactivation of Smad2/3, p38 MAPK, and ERK
Liu et al. [63]	SD rats	-ISO	-200 mg/kg/day -4 weeks	-Decrement of heart weight/body weight - Restoration of ANP, α-MHC/MYH6, β-MHC/MYH7 and MYH7B expression levels -Shrinkage of interstitial fibrosis area - Decrement in procollagen I/III production

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				- mTOR expression restoration -Suppression of LC3 and Belin-1
Guo et al. [55]	SD rats	- High energy diet - STZ injection	-300 mg/kg/day -16 weeks	-Suppressed Col I/III deposition - Reduced TGF-β1, TβR II and phosphorylated Smad 2/3 -Smad 7 amplification
	Human CFs	- HG or TGF-β1	-25 μmol/L	- Moderation of AMPK/p38 MAPK activity -Repression of collagen production

Figure legends

Figure 1. Epigenetic mechanisms in the formation of pro-fibrotic myofibroblasts. Following an injury or a stress, resident cardiac fibroblasts activate and differentiate into myofibroblasts. Myofibroblasts secrete extracellular matrix components (ECM), such as collagen, laminin, and fibronectin, and form fibrotic tissue. Epigenetics is a key player in this pro-fibrotic response, therefore it can be addressed for therapeutic purposes. Histone deacetylase (HDAC) inhibitors (mocetinostat, trichostatin A and MPT0E014 a pan HDAC inhibitor) have a direct action both on transforming growth factor beta (TGF-β) and cytokines including interleukin 6 (IL-6). Resveratrol activates Sirtuin 3 (SIRT-3) that indirectly blocks TGF-b/Smad3 pathway, thus suppressing fibroblast-to-myofibroblast transformation. Demethylating agents, such as 5-azacytidine can silence Ras association domain family 1 isoform A (RASSF1A), a tumor suppressor gene involved in fibroblast activation, and prevent cardiac fibrosis. Several cardiac microRNAs (miRNAs) have a cardio-protective activity by targeting the expression of TGF-β and pro-fibrotic cytokines (miR-133, miR30, miR15, and miR-378). Anti-miR-208 acts on the TGF-β/Smad3 pathway. Anti-miR-21 and anti-miR-19b can regulate TGF-β1-mediated endothelial-to-mesenchymal transition via PTEN/Akt pathway. In endothelial cell, down-regulation of SET1 by HDAC inhibitors attenuates Ang II-induced cardiac fibrosis. Moreover, bone morphogenetic protein 7 (BMP7), an anti-fibrotic morphogen agent can reactivate Ras protein activator like 1 (RASAL1), thus reducing Ras-GTP activity and endothelial to mesenchymal transition (EndMT). With permission from [1]

Figure 2. Cur decreased collagen deposition in the left ventricles of SHRs. WKY rats were used as controls, and SHRs were treated with saline, Cur (100 mg·kg-1·d-1), or Cur (100 mg·kg-1·d-1)+GW9662 (10 mg·kg-1·d-1) by oral gavage for 12 weeks. Sirius Red staining was used to analyze the levels of collagen deposition in the left ventricles of WKY or SHRs (n=8 rats, each group). Scale bar: 100 μm. WKY: Wistar Kyoto rats; SHRs: spontaneously hypertensive rats. With permission from [13]