Emerging evidence for the role of cardiotrophin-1 in cardiac repair in the infarcted heart

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Abstract

Ischemic heart disease is the most common cause of mortality worldwide. Cardiac fibroblasts and myofibroblasts, i.e., the hypersecretory, muscular, and contractile fibroblastic phenotype variant, play an important role in myocardial healing and are responsible for accumulation of collagen in the infarct scar as well as in viable myocardium. Thus, cardiac fibroblasts and myofibroblasts directly contribute to cardiac stiffness, altered performance, and ultimately to the onset of systolic and diastolic heart failure. Cardiotrophin-1 (CT-1) is a member of the IL-6 superfamily and is elevated in the serum of patients with ischemic heart disease and valvular heart disease; it is also known to induce cardiomyocyte hypertrophy in vitro.

The recent, burgeoning awareness of the functions of IL-6 superfamily of cytokines within cardiovascular diseases predicates this summary of CT-1’s effect in cardiac wound healing, and particularly after the induction of myocardial infarction. Further, we summarize recent results of cardiac CT-1 expression post-myocardial infarction (post-MI) as well as the effect of CT-1 on cultured primary adult rat cardiac fibroblasts with respect to proliferation and collagen secretion. It would appear that CT-1 plays an important and heretofore largely unrecognized role in infarct scar formation and angiogenesis in the rat model of chronic MI. Further work is required to determine factors that induce CT-1 expression, its interplay with other mediators of cardiac infarct wound healing in the setting of acute cardiac ischemia and chronic post-MI heart failure, and ultimately whether it confers a beneficial effect or contributes to maladaptive cardiac fibrosis.

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1. Introduction

Myocardial infarction (MI) is a major contributor to mortality and morbidity in all developed nations [1]. If the initial infarct is relatively large, the prognosis of post-MI patients is grim due to the relatively rapid development of congestive heart failure. Myocardial healing after infarction is characterized by complex time-dependent alterations of ventricular architecture of the remnant (non-infarcted) myocardium as well as the infarct zone itself [2–4]. A component of this process is interstitial (or extracellular matrix) remodeling of the remnant heart and wound healing of the infarct scar and is under the coordinated control of multiple trophic factors and chemokines [3]. In the post-MI heart, turnover of the extracellular matrix is accelerated [4–6], and is modulated largely by myofibroblasts, which are phenotypic derivatives of interstitial fibroblasts [7].

The primary function of adult cardiac myofibroblasts is to synthesize fibrillar collagens to maintain the integrity of the cardiac matrix. While fibrosis contributes to the development of heart failure, the mechanisms by which these cells come to populate the infarct scar (i.e., systemic cells homing to a specific signal and phenotypic switching at the site of cardiac damage) are not well understood. In the early period after infarction, the infarct scar becomes repopulated with cells [8] via migration and proliferation of interstitial fibroblasts from the adjacent non-injured...
myocardium. Little information about fibroblast migration and proliferation is available in the literature, and the mechanisms governing fibroblast and myofibroblast movement to the infarct site are largely unknown.

Cardiotrophin-1 (CT-1) is present in low concentration (~30 fmol/ml) in plasma samples from patients without ischemic heart disease [9], and the immunoreactive protein is expressed at low levels in normal heart [10]. At higher levels, it is known to induce hypertrophy of cardiac myocytes via induction of sarcomeric proteins in series [11]. While the hypertrophic effect of CT-1 on cardiac myocytes is fairly well characterized [11–15], its expression in the post-MI heart and its influence on nonmyocyte behavior has only very recently been defined [10,16]. Other members of the IL-6 superfamily are overexpressed in a highly transient manner, i.e., within days after infarction [17]. We have recently carried out a series of investigations based on the premise that CT-1 influences cardiac myofibroblast function, migration and proliferation in a manner which is essentially opposed to effects of profibrotic hormones such as those associated with angiotensin II (angiotensin) and TGF-β1. While CT-1 exerts a relatively weak influence over the synthesis and secretion of collagen by myofibroblasts, it strongly induces migration and proliferation in these cells. Thus CT-1 may stimulate the repopulation of the infarct scar associated with the early phase of post-MI wound healing and may antagonize the effects of angiotensin and TGF-β1 signaling. In demonstrating elevated CT-1 in the remnant heart in the chronic state [10], we suggest that CT-1 contributes to the ongoing wound healing and the pathogenesis of post-MI heart failure.

2. The cardiac myofibroblast

While fibroblasts are abundant in the healthy heart [18], cardiac wound healing and fibrosis as well as interstitial kidney fibrosis, pulmonary fibrosis and liver sclerosis are mediated primarily by myofibroblasts [19–24]. During wound healing, circulating myofibroblast progenitors and normal interstitial fibroblasts transform into myofibroblasts that are hypervascular, less migratory and possess contractile properties [7,21,25]. This phenotype is marked by expression of α-smooth muscle actin, vimentin, AT1 receptors, TGF-β receptors, LIFR/gp-130, ACE, and fibrillar collagens [22,26–32]. Additional features of myofibroblasts include well developed rough endoplasmic reticulum, myofilaments (stress fibres) with focal densities, collagen secretion granules [33] and gap junctions [21]. A marker of myofibroblasts that may be more specific than α-smooth muscle actin expression is expression of the embryonic isoform of smooth muscle myosin, Smemb [34]. This protein has been found to be expressed in infarct scar [34], and in hibernating myocardium [35]. Unlike dermal myofibroblasts which disappear during the transition from granulation tissue to scar [36], cardiac myofibroblasts can be found in the infarct scar many years after injury [37]. The main stimuli for fibroblast–myofibroblast transformation is PDGF, SCF and most importantly, TGF-β [7,21], whereas LIF may antagonize this phenotypic change [38]. In cultured cells, this phenotype is induced by TGF-β1 and in vitro culture seeding at low density [39–41]. We have demonstrated the predominance of myofibroblasts in the infarct scar [20] in post-MI rats and in adult cells when plated at low initial density (Fig. 1). Cultured adult myofibroblasts are phenotypically stable and hypervascular [39].

Myofibroblast motility and proliferation contribute to net matrix deposition in the pathogenesis of cardiac fibrosis [8]. Myofibroblasts contract and produce isometric tension within granulation tissue in vivo and in cultures [7]. Tension is exerted at the level of focal adhesions (FAs), which connect cells to matrix [41]. TGF-β1 stimulation leads to FA maturation facilitating tension transmission from the cell to the matrix [41]. Myofibroblast contraction likely mediates infarct scar thinning that occurs in the late stages of post-MI wound healing [42]. Stimulation of focal adhesion kinase (FAK) facilitates FA turnover, which leads to enhanced cellular motility [43]; increased FAK activity also promotes cell cycle progression [44]. Mature and supermature FAs favour enhanced cellular anchoring, which tends to retard myofibroblast motility and inhibit cell proliferation [41]. It is possible that this “anchoring” phenomenon is an adaptation for efficient collagen synthesis and scar contraction.

3. Cytokine expression in post-MI heart

Upon cessation of blood flow, there is rapid degranulation of preformed mast cells in the heart. These cells release histamine and TNF-α, which is considered to be important in initiating the inflammatory cascade [45]. This results in an increase in IL-6 expression in mononuclear cells as well [25] with subsequent activation of adhesion molecules, leukocyte trapping and neutrophil-induced injury [45–47]. In addition to its effects on adhesion, IL-6 may also act as a nitric oxide-dependent cardiac depressant, and therefore may be associated with stunned myocardium [25,45]. Furthermore, IL-6 plays a role in tissue repair, since knock-out mice demonstrate significantly delayed cutaneous wound healing [48]. There are several cytokines that are expressed during the various stages of post-MI wound healing, including members of the IL-6 family, IL-1β [17], TGF-β [49,50], EGF and FGF [51]. In the subacute and chronic phase, these are elevated, at least in part, by activation of the renin–angiotensin–aldosterone system (RAAS) [52–54]. Angiotensin has been shown to increase TGF-β expression in post-MI heart, and its antagonism results in decreased cardiac fibrosis and improved ventricular function [32,49,55]. Cardiotrophin-1 has also been
shown to be elevated in the myocardium after myocardial infarction [56], but the relationship of its expression to the RAAS is not clear.

4. Cardiotrophin-1

Cardiotrophin-1 (CT-1), a member of the IL-6 family of cytokines, was isolated in 1995 based on its ability to induce hypertrophy of neonatal cardiac myocytes in vitro [57]. CT-1 induces hypertrophy more potently than other members of the IL-6 family of cytokines or other known mediators of cardiomyocyte hypertrophy [57]. The 1.4 kb band was observed in adult mouse mRNA from heart, skeletal muscle, liver, lung and kidney [57] and in adult rat heart, lung, liver, kidney, skeletal muscle, stomach and urinary bladder [58]. In humans, a 1.7 kb mRNA encoding CT-1 was found in heart, skeletal muscle, prostate and ovary [59].

Expression of CT-1 has been observed in normal and disease states [9]. Increased CT-1 mRNA was observed in the ventricles of genetically hypertensive rats [58,60] and in the ventricles of rats subjected to pressure overload [61]. Elevated CT-1 and gp130 mRNA and protein were observed in the ventricles from rats with myocardial infarction, and increased expression persisted into the chronic phase of wound healing [56]. Elevated CT-1 was observed in the ventricles from dogs with pacing induced heart failure, and the degree of CT-1 mRNA expression correlated with left
ventricular mass index [62]. Mechanical stretch of cardiomyocytes has been shown to increase CT-1 mRNA expression [63], as has norepinephrine stimulation [64], isoproterenol stimulation [65] and hypoxic stress [66]. Angiotensin II was shown to induce CT-1 expression in cardiac fibroblasts [67], and cardiac non-myocytes (mainly fibroblasts) were observed to have 3.5 times higher CT-1 mRNA expression than cardiac myocytes [68]. CT-1 expression has been observed in rats with Chagasic cardiomyopathy (Trypanosoma cruzi infection) [69] and in mice with acute Coxsackievirus B3 myocarditis [70].

In humans, elevated serum levels of CT-1 have been observed in patients with unstable angina [71], acute myocardial infarction [72] and heart failure [73]. The level of CT-1 expression was correlated with the degree of left ventricular systolic dysfunction. Elevated CT-1 was also observed in patients with aortic stenosis [74] and mitral regurgitation; the degree of regurgitation correlated with the level of CT-1 in serum [75]. There has been a report of a mutation/polymorphism in the promoter region and coding region of the human CT-1 gene in some patients with idiopathic dilated cardiomyopathy [76]. The significance of this is unknown, although elevated CT-1 was observed in the serum of patients with dilated cardiomyopathy and its expression was positively correlated with left ventricular mass index [77]. Elevated mRNA and protein levels of CT-1 were observed in the hearts from patients with end stage cardiomyopathy undergoing heart transplantation. This increased expression was accompanied by a decrease in gp130, suggesting that receptor downregulation plays a role in balancing enhanced CT-1 expression [78].

When administered intravenously, CT-1 induces a drop in mean arterial pressure and reflex increase in heart rate without affecting cardiac output, an effect that is mediated by iNOS [79]. This effect was also seen in spontaneously hypertensive rats [80]. Male Wistar rats became resistant to repeated injections of CT-1, by induction of endogenous suppressors of IL-6 family cytokine signaling, namely Jak-binding protein (JAB)/Suppressor of Cytokine Signaling-1/STAT induced STAT Inhibitor-1 and CIS3/SOCS-3/SSI-3 [81]. Chronic administration of CT-1 to mice by intraperitoneal injection led to increased heart to body weight ratio, as well as increased liver, spleen and kidney weight, but induced a reduction of thymus weight [82].

The nature of cardiomyocyte hypertrophy produced by CT-1 differs from that of other hypertrophic stimuli. Whereas hypertrophy typically results in assembly of sarcomeres in parallel, CT-1 induced hypertrophy resulted in sarcomere assembly in series with subsequent increase in cardiomyocyte cell length [11,57]. This hypertrophic response was transduced by a receptor complex composed of the leukemia inhibitory factor receptor subunit β (LIFRβ) and gp130. Upon CT-1 stimulation, both LIFRβ and gp130 become tyrosine phosphorylated by Janus kinases (Jaks), since neither gp130 nor LIFRβ contain any inherent kinase activity [11,83,84]. Because Janus kinases are activators of multiple signaling pathways, there is activation of STATs, the mitogen activated protein kinase pathway, the phosphoinositol-3-kinase pathway and the Src pathway [85,86]. Activation of these pathways results in hypertrophy and protection from ischemia/reperfusion injury [87].

CT-1 also exhibits cytoprotective effects, i.e., it prevents apoptosis of serum starved neonatal ventricular myocytes in a PI3K-dependent manner [87] and a MAPK dependent manner [88,89]. NF-κB also plays a central role in the cytoprotective effect of CT-1 [90]. CT-1 was also able to protect isolated rat cardiac myocytes from apoptosis when added before simulated ischemia or when added at reoxygenation [91]. The same effect was observed in whole rat heart ex vivo [92]. The protective effect of CT-1 on human right atrium was found to be as effective as ischemic preconditioning when cells were treated for up to 24 h before ischemia [93].

The effects of CT-1 on cardiac fibroblast function are largely unexplored. Tsuruda et al. showed that canine cardiac fibroblasts express CT-1, gp130 and the LIF receptor. CT-1 stimulation of canine cardiac fibroblasts increased proliferation and incorporation of ³H-proline, an effect that could be inhibited by coincubation with a gp130 blocking antibody or the endothelin-1 receptor antagonist BQ123 [30]. Further, we have recently sought to determine the time course of CT-1 expression in post-MI rat heart, and its effect on post-MI wound healing, with particular respect to cardiac myofibroblast proliferation, migration, protein (collagen) synthesis and CT-1 signalling specific to primary cardiac myofibroblasts [10,16].

5. Cardiotrophin-1 expression in post-MI heart

CT-1 is cardioprotective for ischemia–reperfusion injury, even when added at the point of reoxygenation [89,91,92]. Therefore early expression of CT-1 in the ischemic myocardium may represent an adaptive, protective phenomenon that is beneficial in reducing myocyte loss and inducing hypertrophy of remaining myocytes so that overall ventricular function is maintained. However, CT-1 expression in the late phase of wound healing and the onset of heart failure may also contribute to ventricular dilation by inducing hypertrophy of myocytes with increased cell length where sarcomeres are added in series (eccentric hypertrophy), rather than in parallel (concentric cardiac hypertrophy) [11], although this hypothesis remains to be proven. Recent examination of the expression of CT-1 in the post-MI heart revealed elevated expression in the infarct zone from 24 h to 8 weeks as well as elevated CT-1 expression in the viable myocardium in the chronic phase of wound healing (8 weeks—summarized in Fig. 2) [10]. The available evidence supports the suggestion that CT-1 is beneficial during the early period of post-MI wound healing via its effect on proliferation of myofibroblasts [10]. However, in chronic stages post-MI, CT-1 may contribute
Infarct scar-specific CT-1 expression may be beneficial by increasing cellularity, an effect that may be antagonized by angiotensin or TGF-β, both of which have neutral or negative effects on fibroblast proliferation [108, 109, 110]. Unpublished observations]. Indeed, a common theme in explanation of the beneficial effects of popular ACE inhibiting agents (angiotensin-suppressing drugs) is that while angiotensin is suppressed, other systems are derepressed, i.e., ACE inhibition prevents the destruction of bradykinin [111]. Thus the upregulation of a parallel system is invoked and yields a net benefit to the post-MI heart. This mechanism may have the additional beneficial effect of angiotensin antagonism insofar as the CT-1 stimulus is allowed to act unopposed. In addition to opposing the action of these mediators, CT-1 expression may antagonize TNF-α induced myocyte apoptosis [112, 113], thereby preserving overall ventricular performance.

A cellular scar confers improved survivorship and cardiac function when compared to a hypocellular scar [118]. Thus repopulation of the infarct scar is associated with improved ventricular performance, even though these cells may not contribute to synchronous ventricular contraction [119]. Expression of CT-1 in the infarct zone is likely beneficial given the potent induction of cell proliferation and migration. CT-1 acts to induce proliferation and migration of fibroblasts from adjacent viable myocardium thereby repopulating and maintaining the cellularity of the scar. Additionally, CT-1 may act to maintain a proliferative, migratory phenotype and oppose the actions of angiotensin and TGF-β, which act to induce a contractile, hypertrophic myofibroblast phenotype [7, 41, 109]. LIF, another member of the IL-6 family of cytokines which shares the LIFR/gp130 receptor dimer with CT-1, has been shown to reduce collagen expression and antagonize the switch to a myofibroblastic phenotype [38]. Although the effects of CT-1 on cardiac fibroblast phenotype are unexplored at present, we suggest that it is likely that CT-1 has a similar effect, which again supports the notion that CT-1 has beneficial effects on cardiac fibroblast function in the post-MI heart.

Although the heart is the major source of circulating CT-1 in humans [114], the cellular source of CT-1 in the post-MI heart is not clear. While induction of CT-1 mRNA has been shown in cardiac fibroblasts [67] and myocytes [63, 64, 66] in vitro in response to a variety of stimuli, it is not clear which cell type is primarily responsible for in vivo myocardial CT-1 expression. It is possible that inflammatory cells contribute to CT-1 expression, particularly early on in the course of post-MI wound healing. Irrespective of the cellular source, activation of the gp130 signaling cascade is required for an appropriate myocardial response to injury that allows survival of the animal [115]. It was suggested that cardiac myocyte hypertrophy produced by angiotensin is mediated through CT-1 [116] however recent studies in humans do not support an upstream connection to angiotensin [72]. Conversely, norepinephrine, which is locally and systemically activated after MI [117], is known to
elevate CT-1 expression in cardiac myocytes in vitro and in vivo via a cAMP response element in the 5′ flanking region of the CT-1 gene [64]. This finding suggests the involvement of adrenergic stimulation in post-MI CT-1 expression. Whether CT-1 stimulates the proliferation of other cardiac non-myocytes remains an open question. The infarct scar has been shown to be a vascular structure and myocardial wound healing after infarction invokes a significant upregulation of angiogenesis in the infarct zone [42]. It is suggested that CT-1 induced STAT3 activation as observed in the post-MI heart may act to initiate angiogenesis; for example, STAT3 has been shown to induce VEGF-dependent myotube proliferation.
formation when overexpressed [120]. As STAT3 movement to the nuclei occurs in CT-1 responsive cardiac cells, this may represent another effect of CT-1 signaling in post-MI heart [16]. While translocation of STAT3 may also apply to cells important for cardiac angiogenesis, there is no information regarding the effect of CT-1 on endothelial cell or smooth muscle cell proliferation to date.

Recent examination of CT-1 signaling in cardiac myofibroblasts revealed that CT-1 activates JAK 1 and 2 (but not JAK3 or Tyk2), leading to the phosphorylation of STAT3 and STAT1 which then translocate to the nucleus of myofibroblasts (see Fig. 3) [16]. Others have shown that upon gp130 activation, rapid negative regulation of JAKs occurs via induction of suppressor of cytokine signaling 3 (SOCS3) in heart [121], and thus a balance between positive and negative regulatory loops is attained. While CT-1 exerts cardioprotective effects [11,115], myocardial expression of CT-1 is increased in heart failure models [56,62] both in plasma [9] and cardiac tissues of patients with heart failure [78]. It is becoming clear that CT-1 expression generally precedes the development of pathological hypertrophy [60]. As mentioned previously, this may be a beneficial event, required for an appropriate myocardial response to injury by activating the gp130 signaling cascade and inhibiting myocyte apoptosis [115].

6. Role of cardiotrophin-1 in cardiac fibroblast collagen/protein synthesis

CT-1 induces protein synthesis in cardiac fibroblasts through typical signaling pathways. Proteins that could be induced by CT-1 stimulation include extracellular matrix proteins (collagens, fibronectin, tenascin, etc.), proteins involved in regulation of cell cycle (cyclins, proliferating nuclear antigen, etc.) and proteins required for cell adhesion and migration (primarily integrins) [122–124]. We have observed that the collagen secretion stimulus of CT-1 is modest without cell number normalization and is actually associated with a reduction of collagen secretion if the normalized data are considered (Fig. 4). These findings indicate that CT-1 may not possess a significant profibrotic stimulus insofar as there is not a significant induction of fibrillar collagen synthesis per cell. While this finding appears to be contradictory to other published data [30] the differences may be due to one of the following extenuating experimental parameters: (i) the current dataset is presented in both raw and normalized forms, and it is the former subset that agrees with other published data, which also is not normalized to cell number; (ii) we measured collagen synthesis by procollagen-I-carboxy propeptide (P1CP) expression (secretion) in culture media, as opposed to simple incorporation of $^3$H-proline; (iii) the current data were sourced from samples of culture medium as opposed to cell lysates. Collagen synthesis is a discordant process in cardiac myofibroblasts with respect to peak mRNA and procollagen monomer expression. Thus, changes in collagen type I mRNA abundance may not accurately reflect changes in protein secretion [125], and most importantly, collagen secretion. The latter is mediated through a complex series of steps involving extensive post-secondary modification of procollagen monomers, self-winding of the triple helices, and finally cleavage of both N- and C-terminal polypeptides. In cases whereby myofibroblasts are unstimulated or

![Fig. 4. Effect of CT-1 on mature collagen secretion/synthesis in cultured cardiac myofibroblasts. Mature collagen synthesis was reflected by measurement of secreted, cleaved type I carboxy propeptide fragment, and was determined by radioimmunoassay for procollagen-I carboxy-propeptide (P1CP-Diasorin) in conditioned media from P1 cardiac myofibroblasts stimulated with specified concentrations of CT-1 for 24 h. Conditioned media from three separate experiments was analyzed in triplicate. Panel A shows the P1CP content of media collected from CT-1 stimulated cells. Panel B shows the P1CP concentration in media corrected for the number of cells at the end of the experiment. Quiescent cardiac fibroblasts were extracted from adult (150 g) rat hearts and plated at low initial density [39] in the presence of 10% FCS as described previously [32]. Upon passage to P1, >98% of the cells in culture assumed the relatively synthetic myofibroblastic phenotype, and these cells were used for the current experiment. SMemb and aSMA were present in low abundance in P0 cardiac fibroblasts, while P1 and P2 myofibroblasts were characterized by large induction of these markers (data not shown). Cultured cells exhibited negligible staining for factor VIII and SMM. Results are displayed as the Mean±S.E.M. *P<0.05 vs. non-stimulated control.](image-url)
there is a deficiency of cofactors, a high proportion of collagen monomers are cycled within the cell, degraded shortly after synthesis and therefore never reach the point of secretion [126]. Thus determination of secreted collagen is the preferred method for ascertaining net collagen synthesis in cardiac myofibroblasts, and the advantages conferred by use of the P1CP (or P1NP) method(s) outweigh those that may be realized by addressing collagen type I mRNA expression or incorporation of ^3H-proline alone. On balance, we suggest that the proteins generated in response to CT-1 stimulation are involved in induction of cell functions (such as proliferation or migration) other than synthesis of secreted ECM proteins.

7. Significance of cardiotrophin-1 induced cardiac fibroblast cell migration

The observation that CT-1 induces cardiac fibroblast migration coupled with infarct scar specific expression of CT-1 suggests that it contributes to repopulation of the scar as mentioned previously. Although CT-1 does appear to have a chemotactic effect on porcine coronary artery vascular smooth muscle cells (unpublished observation), there is conflicting evidence for the role of IL-6 family cytokines on angiogenesis. Whereas LIF may act to reduce angiogenesis in vitro [127], oncostatin M induces angiogenesis in vivo and in vitro and is chemoattractant for human microvascular endothelial cells [128]. Clearly the individual effect of CT-1 on angiogenesis needs to be determined.

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