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Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2015 February 23.

Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2013 September ; 33(9): 2180–2186. doi:10.1161/ATVBAHA.113.301633.

Compartmentalized protective and detrimental effects of endogenous MIF mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion

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Abstract

Objectives—Here we aimed to clarify the role of CXCR2 in the macrophage migration inhibitory factor (MIF)-mediated effects after myocardial ischemia and reperfusion (I/R). As a pleiotropic chemokine-like cytokine, MIF has been identified to activate multiple receptors including CD74 and CXCR2. In models of myocardial infarction (MI), MIF exerts both pro-inflammatory effects and protective effects in cardiomyocytes. Likewise, CXCR2 displays opposing effects in resident versus circulating cells.

Approach and results—Using bone marrow (BM) transplantation, we generated chimeric mice with CXCR2$^{-/-}$ BM-derived inflammatory cells and wild-type resident cells (wt/CXCR2$^{-/-}$), with CXCR2$^{-/-}$ cardiomyocytes and wild-type BM-derived cells (CXCR2$^{-/-}$/wt) and wild-type controls reconstituted with wild-type BM (wt/wt). All groups were treated with anti-MIF or isotype control antibody before they underwent myocardial I/R. Blocking MIF increased infarction size and impaired cardiac function in wt/wt and wt/CXCR2$^{-/-}$ mice but ameliorated

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Disclosures
None

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functional parameters in CXCR2$^{-/-}$/wt mice, as analyzed by echocardiography and Langendorff perfusion. Neutrophil infiltration and angiogenesis were unaltered by MIF blockade or CXCR2 deficiency. Monocyte infiltration was blunted in wt/CXCR2$^{-/-}$ mice and reduced by MIF blockade in wt/wt and CXCR2$^{-/-}$/wt mice. Moreover, MIF blockade attenuated collagen content in all groups in a CXCR2-independent manner.

Conclusions—The compartmentalized and opposing effects of MIF after myocardial I/R are largely mediated by CXCR2. Whereas MIF confers protective effects improving myocardial healing and function through CXCR2 in resident cells, complementing paracrine effects through CD74/AMPK, it exerts detrimental effects on CXCR2-bearing inflammatory cells, increasing monocyte infiltration and impairing heart function. These dichotomous findings should be considered, when developing novel therapeutic strategies to treat MI.

Keywords

Macrophage migration inhibitory factor; CXCR2; neoangiogenesis; myocardial ischemia/reperfusion; myocardial infarction

Introduction

Macrophage migration inhibitory factor (MIF) was one of the first cytokines to be identified almost 40 years ago[1]. Today, MIF is known as a structurally unique, pleiotropic inflammatory cytokine with chemokine-like properties that functions as a potent mediator of several inflammatory conditions[2–4]. However, the role of MIF in cardiovascular diseases, especially in myocardial infarction (MI), has not yet been definitively clarified.

MIF is up-regulated in endothelial cells and smooth muscle cells of atherosclerotic arteries, where it contributes to macrophage accumulation and plaque formation[5, 6]. It appears that MIF acts sequentially by first triggering monocyte arrest through the CXCR axis and then promoting monocyte transmigration through the intermediate production of CCL2[7, 8]. Being predominantly expressed in vulnerable plaques and inducing collagen-degrading matrix metalloproteinases[9, 10], MIF has been implicated in plaque destabilization.

In the context of MI, the levels of MIF were found to be significantly elevated in patients with reversible myocardial ischemia[11]. A model of acute MI revealed that both MIF mRNA and MIF protein are constitutively expressed at low levels in myocytes of normal and sham-operated rats but rapidly up-regulated by surviving cardiomyocytes in the infarcted versus the non-infarcted regions, thereby increasing macrophage infiltration one day after acute MI[12]. Moreover, it seems that cardiomyocytes can secrete MIF through a protein kinase C-dependent pathway in response to reactive oxygen species and hypoxia in the myocardium[13, 14]. Of note, MIF released by cardiomyocytes in a model of myocardial injury induced by 15 mins of ischemia followed by reperfusion (I/R) injury exerts cardioprotective effects via the CD74/AMPK/JNK axes[15, 16], and the protective effect can be enhanced by S-nitrosylation of MIF[17]. CD74 is the cell surface form of the major histocompatibility complex class II-associated invariant chain (Ii) and was identified to bind MIF by high-affinity interaction and to mediate MIF-induced ERK1/ERK2 phosphorylation and cell proliferation[18, 19]. However, some of the cell types targeted by MIF (e.g. neutrophils or the

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cell lines HEK293 and HeLa) do not express CD74 at the surface[7], raising the need to further clarify the molecular action and functional receptors of MIF in inflammation. Despite the beneficial role of MIF in cardiomyocytes, it was indeed shown that MIF deficiency protected the heart from prolonged, severe I/R injury by suppressing inflammatory responses[20]. Thus, the exact role of MIF in healing after MI and the receptors involved therein are thus far from being completely understood.

Recent data have provided evidence that MIF is a non-cognate ligand of the CXC chemokine receptors CXCR2 and CXCR4, thus extending the range of binding partners for MIF[7]. MIF harbors ELR- and N-loop-like motives typically required for CXCR2 activation[21, 22] and has chemoattractant activity towards monocytes and neutrophils through CXCR2, both crucial mechanisms in inflammatory pathologies like atherosclerosis[7]. Although MIF can bind to CXCR2 or CD74 individually, binding to a CXCR2/CD74 complex appears to further enhance G-protein-coupled receptor (GPCR) activation and atherogenic functions[7]. In addition, MIF can also bind the CXCL12 receptor CXCR4, thereby promoting T-cell and endothelial progenitor cell recruitment[7, 23], enhancing angiogenesis[24, 25] and inducing phosphorylation of Akt and mitogen-activated protein kinase (MAPK) p42/44[26].

Notably, CXCR2 has been found to exert opposing effects on myocardial viability during I/R with the recruitment of damaging inflammatory cells prevailing over direct myocardial protection promoted in resident cells[27], suggesting that CXCR2 may prominently contribute to mediating the effects of MIF in myocardial I/R injury. To shed light on the precise mechanisms enacted by MIF during the myocardial healing response after MI, we aimed to dissect and elucidate the compartment-specific role of CXCR2 in mediating the effects of endogenous MIF in a mouse model of I/R.

Materials and Methods

See Supplementary File.

Results

The effect of anti-MIF-antibody on heart parameters one week after I/R

To examine the role of the MIF-Cxcr2 pathway in healing following I/R, we obtained chimeric mice with wild-type resident cardiomyocytes and Cxcr2$^{-/-}$ BM-derived inflammatory cells, mice with Cxcr2$^{-/-}$ cardiomyocytes and wild-type inflammatory cells and wild-type controls (Table 1). Each group received either anti-MIF or isotype control antibody. The high mortality of Cxcr2$^{-/-}$ mice after irradiation (50%) reduced the number of mice in these groups (4 per group).

One week after I/R, both wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice treated with anti-MIF antibody showed a significant increase in the size of the infarcted area as compared to isotype control, whereas the Cxcr2$^{-/-}$/wt BM mice displayed no effect with anti-MIF (Figure 1). In contrast to a previous report[27], Cxcr2 deficiency in BM cells itself only slightly but not significantly reduced the size of the infarcted area after one week. In

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addition, heart function was evaluated by echocardiography before and after myocardial I/R. Before MI, no significant differences were observed among the groups. One week after I/R, anti-MIF antibody treatment significantly decreased the ejection fraction in wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice, as compared to the untreated mice (Table 2, Figure 2A). By contrast, treatment with anti-MIF antibody led to a significant increase in ejection fraction in the Cxcr2$^{-/-}$/wt BM chimera mice as compared to isotype control (Table 2, Figure 2A). Analysis of cardiac output showed good compensation in all groups (Table 2). Heart rate and weight did not differ among BM chimeras (Table 2).

The analysis of cardiac function by Langendorff perfusion one week after myocardial I/R revealed corresponding results. Whereas treatment with anti-MIF antibody decreased left ventricular developed pressure (LVDP) in wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice as compared to isotype control, it significantly improved and restored the impaired LVDP in Cxcr2$^{-/-}$/wt BM mice (Figure 2B). The maximal (dP/dt_{max}) and minimal (dP/dt_{min}) pressure change in left ventricle measurements confirmed these results. Treatment with anti-MIF antibody impaired contraction (dP/dt_{max}, Figure 2C) and relaxation (dP/dt_{min}, Figure 2D) in wt/wt BM mice and wt/CXCR2$^{-/-}$ BM mice but preserved myocardial function in Cxcr2$^{-/-}$/wt BM chimeras.

These data demonstrate that in the presence of wild-type cardiomyocytes, blocking MIF aggravates myocardial healing and function, thus highlighting that the protective effect of MIF is mediated by a Cxcr2-dependent pathway in resident cardiomyocytes. In contrast, neutralizing MIF in the presence of Cxcr2-deficient cardiomyocytes only blocked the detrimental effects of MIF mediated by Cxcr2 on BM-derived cells.

Histological and immunohistochemical analysis of infarcted area

We next analyzed the recruitment of inflammatory cells, neo-angiogenesis and collagen synthesis in the infarcted area one day after myocardial I/R. One day after MI, neutrophil infiltration did not significantly differ between the groups (Figure 3A, Table 3), suggesting an involvement of receptor axes other than MIF/Cxcr2$^{-/-}$. In contrast, monocyte infiltration into the infarcted area was reduced by treatment with anti-MIF antibody in wt/wt BM and Cxcr2$^{-/-}$/wt BM mice compared with isotype control (Figure 3B, Table 3). In wt/Cxcr2$^{-/-}$ BM mice, monocyte infiltration was substantially impaired and not further attenuated by treatment with anti-MIF antibody (Figure 3B, Table 3), suggesting that the recruitment of monocytes towards the area of the infarction is strictly dependent on Cxcr2 in our model. Using CD14/CCR2 double staining, we could now show that most of the infiltrated monocytes are CCR2-positive inflammatory monocytes. This indicates that MIF promotes the recruitment of inflammatory CCR2+ monocytes into the heart tissue (Figure 3C, Table 3), which dominate the early phase of healing after infarction and exhibit phagocytic, proteolytic, and inflammatory features[28] Of note, it has already been shown that MIF can recruit inflammatory monocytes in a CCL2-dependent manner[29], indicating a functional interaction between CXC and CC receptors in the myocardial recruitment of inflammatory monocytes.

Analysis of neo-angiogenesis in the infarcted area revealed no significant differences in the level of CD31-positive capillaries between the groups one week after MI (Figure 4A, Table

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3), indicating that it was independent of Cxcr2 and MIF. Similarly, Langendorff measurements failed to reveal changes in coronary flow in any of the groups (Figure 4B).

Notably, collagen content was reduced in all three groups by treatment with anti-MIF antibody but not altered by Cxcr2 deficiency in BM or resident cells, demonstrating that collagen deposition one week after myocardial I/R was regulated by MIF, but not by Cxcr2 (Figure 5A, Table 3). The number of myofibroblasts did not differ between the groups (Figure 5B, Table 3), implying that MIF rather affects their function in collagen synthesis. Hence, protective effects of MIF are in part attributable to increased collagen deposition, whereas its deleterious effects are due to a stimulation of myocardial monocyte infiltration.

Discussion

Our data establish a double-edged role of endogenous MIF in the myocardial healing response after I/R injury. To analyze the MIF-Cxcr2 pathway, chimeric mice were obtained by reconstitution of wild-type mice with wild-type or Cxcr2$^{-/-}$ BM, and of Cxcr2$^{-/-}$ mice with wild-type BM. We demonstrate that MIF exerts opposing effects on resident cardiac cells versus BM-derived inflammatory cells mediated through Cxcr2. Whereas blocking endogenous MIF acting through Cxcr2 on cardiomyocytes impaired heart function and increased infarction size, blocking endogenous MIF acting through Cxcr2 on circulating inflammatory cells protected heart function and decreased infarction size Notably, the latter effect was not related to an infiltration with neutrophils, which did not require Cxcr2 or MIF, but due to the control of monocyte infiltration in the infarcted area mediated by MIF and Cxcr2. Since we did not observe significant differences between the infarction size in isotype-treated wt/wt BM and Cxcr2$^{-/-}$/wt BM, it is conceivable that Cxcr2$^{-/-}$ mice harbor compensatory mechanisms, which may substitute for CXCR2 functions, e.g. the CXCR4/CXCL12 axis, CCL2, TNFα or HIF[30]. The oxidoreductive function of MIF has been elegantly shown to provide an anti-oxidative and cardioprotective capacity in myocardial infarction[31] and could therefore be another compensatory MIF-based protective mechanism in myocardial ischemia/reperfusion injury.

It has previously been shown that MIF released by cardiomyocytes during I/R injury exerts cardioprotective effects by activating AMPK (JNK) through CD74[15]. We observed a reduction in the functional parameters of the heart (ejection fraction, LVED, contraction and relaxation) and increased infarction size after anti-MIF antibody treatment in wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice. MIF blockade improved ejection fraction, LVDP, contraction and relaxation without influencing infarction size in Cxcr2$^{-/-}$/wt BM mice, which do not express Cxcr2 on cardiomyocytes and therefore show impaired heart function following I/R injury, as compared to controls. It thus appears that protective effects of endogenous MIF secreted by cardiomyocytes can prevent severe functional loss in heart tissue in wt/CXCR2$^{-/-}$ BM mice with Cxcr2-deficient circulating cells. Considering what is known about the CD74/AMPK-mediated MIF protection[15], these results are surprising. Hence, we can assume that CD74 and CXCR2 are both required for the myocardial protection in ischemia injury. Although MIF is able to exert his biological effects by binding to CD74 and CXCR2 individually, it was shown that MIF binding to the CXCR2/CD74 complex enhances G-protein-coupled receptor activation and downstream cardiovascular effects[7]. Accordingly,

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predictably the deletion of one or both of the receptors would attenuate the protective effect in cardiomyocytes exposed to ischemia. Therefore, we suppose that the deletion of both receptors similar to that of Cxcr2 alone would decrease the protective MIF effect. However, as Cxcr2/Cd74-DKO mice are not available, we can currently not verify this notion and thus cannot exclude the compensation of this effect by other MIF-independent protective mechanisms in cardiomyocytes. Nevertheless, the CXCR2/CD74 complex should play a crucial role in mediating the protective effects of MIF in cardiomyocytes after MI.

Notably, deficiency in Cxcr2 in cardiomyocytes and in BM-derived inflammatory cells caused only moderate alterations in the size of the infarcted area one week after I/R, which contrasts previous findings in bone marrow chimeras after one day, as reported by Tarzami et al[27]. Since one day after MI the infarction size is largely defined by cardiomyocyte death, this discrepancy may be explained by adaptive remodeling of the infarction area following the inflammatory reaction over the course of one week after I/R injury.

In general, MIF blockade reduced the number of inflammatory cells, namely monocytes, infiltrating the site of injury. Mononuclear cells infiltrating the site of inflammation release cytokines and chemokines, which further enhances the recruitment and activation of these pro-inflammatory cells[8, 32]. It has been shown that MIF can induce monocyte arrest through CXCR2[7], and accordingly myocardial monocyte infiltration was also dependent on CXCR2 in our study. Studies in animal models characterized by impaired monocyte infiltration have consistently shown preserved heart function following experimental induction of MI[33–35]. Since we performed our analysis one day after I/R, most of the recruited monocytes should be Gr-1^{high} (also known as Gr-1^{+}CCR2^{+}CX3CR1^{lo}) monocytes, which dominate the early phase and exhibit phagocytic, proteolytic, and inflammatory features[28]. Reducing inflammatory monocytes following MI seems to beneficially promote cardiac remodeling[30]. Since CXCR2 is knocked out in inflammatory cells in the wt/Cxcr2$^{-/-}$ BM mice, and since the number of infiltrating monocytes was not further reduced by MIF blockade in these mice, we can assume that MIF is able to control the recruitment of inflammatory monocytes. Indeed, MIF has been shown to recruit monocyte/macrophage in a CCL2-dependent manner, as CCL2 deficiency or anti-CCL2 antibody treatment significantly inhibited MIF-induced monocyte adhesion and transmigration in mice[29]. This reveals the importance of CXCR in the myocardial recruitment of inflammatory monocytes.

Surprisingly, infiltration of the infarcted myocardial tissue with neutrophils was unaffected by MIF blockade or Cxcr2 deficiency. Neutrophils have been found to express CXCR2 but not CD74[3]. The moderate chemotactic activity towards MIF in neutrophils has been related to a lack of CD74 in these cells[7]. Since the function of MIF depends on the CXCR2/CD74 complex[7], the absence of CD74 might explain why neutrophil infilitration was unaltered in our myocardial I/R model. Furthermore, this is consistent with findings showing that early neutrophil infiltration following I/R injury was greatly diminished by Ccr1 deficiency and is hence rather dependent on the presence of the CCL5 receptor CCR1[36].

Unexpectedly, our evaluation of neoangiogenesis in the infarcted tissue one week after myocardial I/R injury failed to revealed changes in coronary flow or CD31-positive capillaries in any of the experimental groups. This is surprising, given that MIF and CXCR2

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have been implicated in angiogenic responses[5] under hypoxic conditions by initiating tube formation and differentiation towards endothelial cell phenotypes or stimulating endothelial proliferation and capillary-like structure formation, respectively[23, 24, 37]. Our data support the notion that the MIF-CXCR2 pathway plays a minimal role in angiogenesis after MI, and rather indicate the involvement of other factors such as vascular endothelial growth factor or the CXCR4 ligand stromal-cell derived factor-1, acting through receptors other than CXCR2[25].

Another unexpected effect of MIF blockade was the CXCR2-independent reduction of collagen content in the infarcted area. Until recently, it was believed that reduced collagen content in the infarcted area might facilitate cardiac function. Our results rather indicate that increased collagen content in the infracted area can lead to a more stable myocardial scar and improved heart function[30, 38]. Therefore, we assume that different collagen subtypes can be synthesized in response to MIF and, together with other factors, influence myocardial function. Accordingly, we have shown that MIF can favor the differentiation of smooth muscle-actin (SMA)-positive cells *in vivo*[24]. Moreover, MIF in conjunction with CD74 can antagonize myocardial hypertrophy and fibrosis[39] and experimental liver fibrosis[40]. Thus, MIF may contribute to fibroblast differentiation towards SMA-positive myofibroblasts, which is a crucial event in myocardial healing and scar formation[30]. Because the number of myofibroblasts did not differ in our groups, MIF seems to exert a direct effect on myofibroblast function and collagen synthesis. However, the mechanism underlying MIF-dependent fibrosis and scar formation following MI remain to be clarified in detail.

In conclusion, this study provides novel insights into the mechanisms of MIF and its functional receptor CXCR2 in myocardial regeneration and scar formation. Without affecting neutrophil infiltration or angiogenesis, MIF protects cardiac tissue via CXCR2. This benefit of MIF is counteracted by the CXCR2-dependent recruitment of monocytes associated with an impaired heart function. Our findings should be taken into account, when developing tailored therapeutic strategies for improved remodeling and preservation of heart function after MI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We want to thank Susanna Kintig, Christiane Herold, Manfred Dewor, and Birgitt Lennartz for excellent technical assistance.

Source of funding

This study was supported by the Deutsche Forschungsgemeinschaft (Forschergruppe 809, TP01 and TP04; and IRTG1508, project P13 to J.B., C.W., and I.K.), and the Interdisciplinary Centre for Clinical Research IZKF Aachen (junior research group to E.L., project K5 to J.B. and junior career funding to I.K.) within the faculty of Medicine at RWTH Aachen University.

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Abbreviations

MIF	Macrophage migration inhibitory factor
I/R	ischemia and reperfusion
BM	bone marrow
wt	wild-type
MI	myocardial infarction
GPCR	G-protein-coupled receptor
MAPK	mitogen-activated protein kinase
SMA	smooth muscle-actin

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Significance

Myocardial infarction remains the most common cause of death in the western countries, despite the extensive research in the last decades. Developing new therapeutic strategies to prevent and treat myocardial damage after an ischemic insult represents a priority for the cardiovascular scientific community. To this end, more detailed and advanced knowledge about the mechanisms of cardiac cell protection and regeneration is urgently required.

Macrophage migration inhibitory factor (MIF) was found to be significantly elevated in patients with myocardial ischemia and also appears to protect the heart, thus possibly serving as a suitable therapeutic concept. However, the underlying mechanisms and its implications for cardioprotection are not completely elucidated.

This study provides novel insights into the mechanisms, by which MIF exerts its effects after myocardial infarction. Whereas MIF protects cardiac tissue via its receptor CXCR2 on resident cells, it exerts detrimental effects on CXCR2-bearing inflammatory cells, associated with an impaired heart function. These findings are of particular importance when developing novel therapeutic strategies to preserve the heart function after myocardial infarction.

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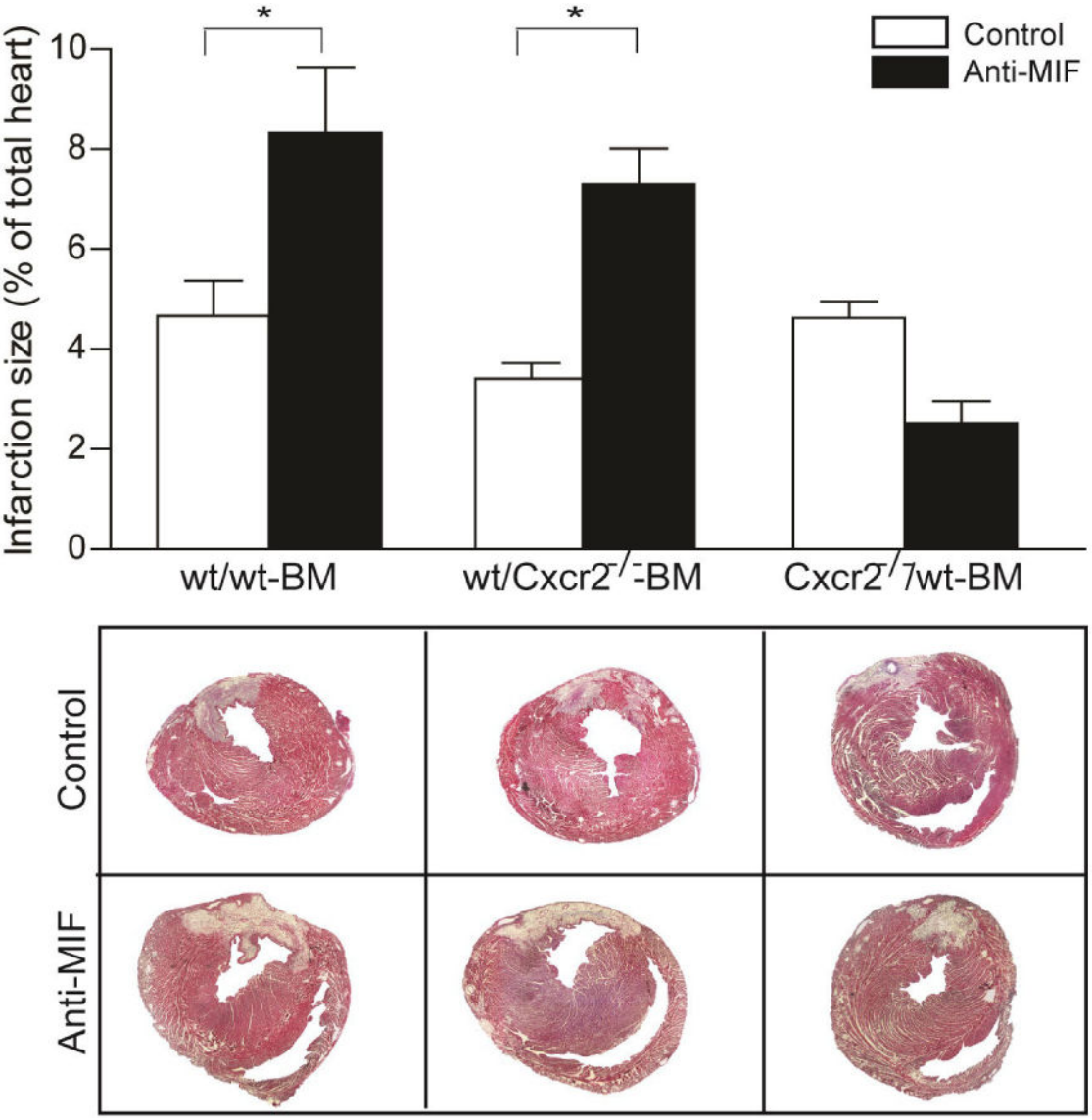

Figure 1. The effect of anti-MIF antibody on infarction size after I/R

Infarction size was analyzed in wt/wt BM, wt/Cxcr2$^{-/-}$ BM, Cxcr2$^{-/-}$/wt BM treated with anti-MIF antibody (dark bars) or isotype control (open bars) one week after I/R. Both wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice treated with anti-MIF antibody showed a significant increase in the size of the infarcted area as compared to isotype control, whereas the Cxcr2$^{-/-}$/wt BM mice displayed no effect after anti-MIF treatment (*$p<0.05$, n=4–6).

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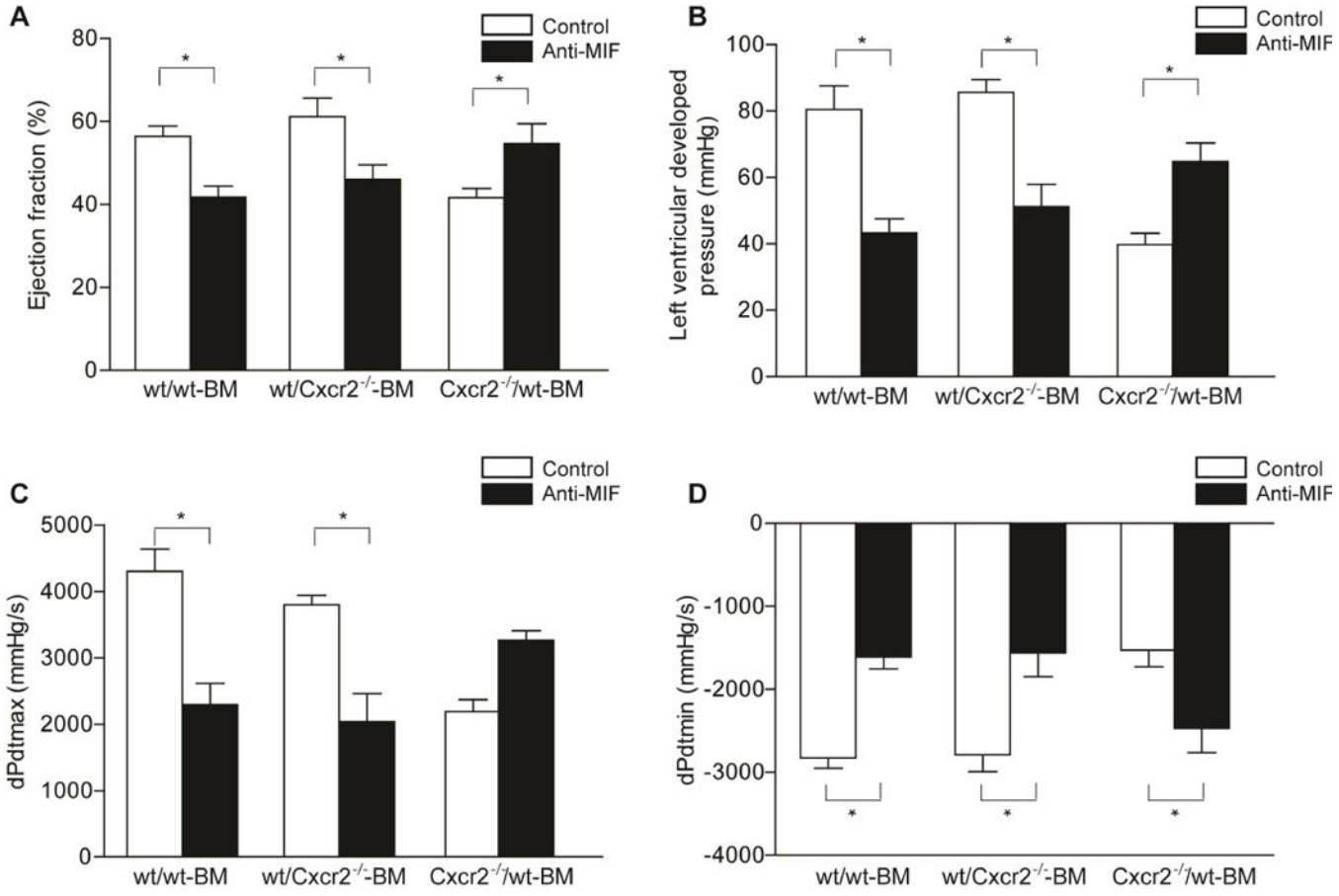

Figure 2. The effect of anti-MIF-antibody on functional parameters after I/R

Ejection fraction was analyzed by echocardiography (A, *p<0.05, n=4–6). One week after I/R, anti-MIF antibody treatment (dark bars) significantly decreased the ejection fraction in wt/wt BM and wt/Cxcr2$^{-/-}$ BM mice, as compared to the isotype treated mice (open bars), while treatment with anti-MIF antibody led to a significant increase in ejection fraction in the Cxcr2$^{-/-}$/wt BM chimera mice as compared to isotype control. Similar results are observed after analysis of cardiac function by Langendorff perfusion, as shown by left-ventricular developed pressure (LVDP) (B, *p<0.05, n=4–6), maximal − dP/dt_{max} (C, *p<0.05, n=4–6) and minimal − dP/dt_{min} (D, *p<0.05, n=4–6) pressure changes in the left ventricle.

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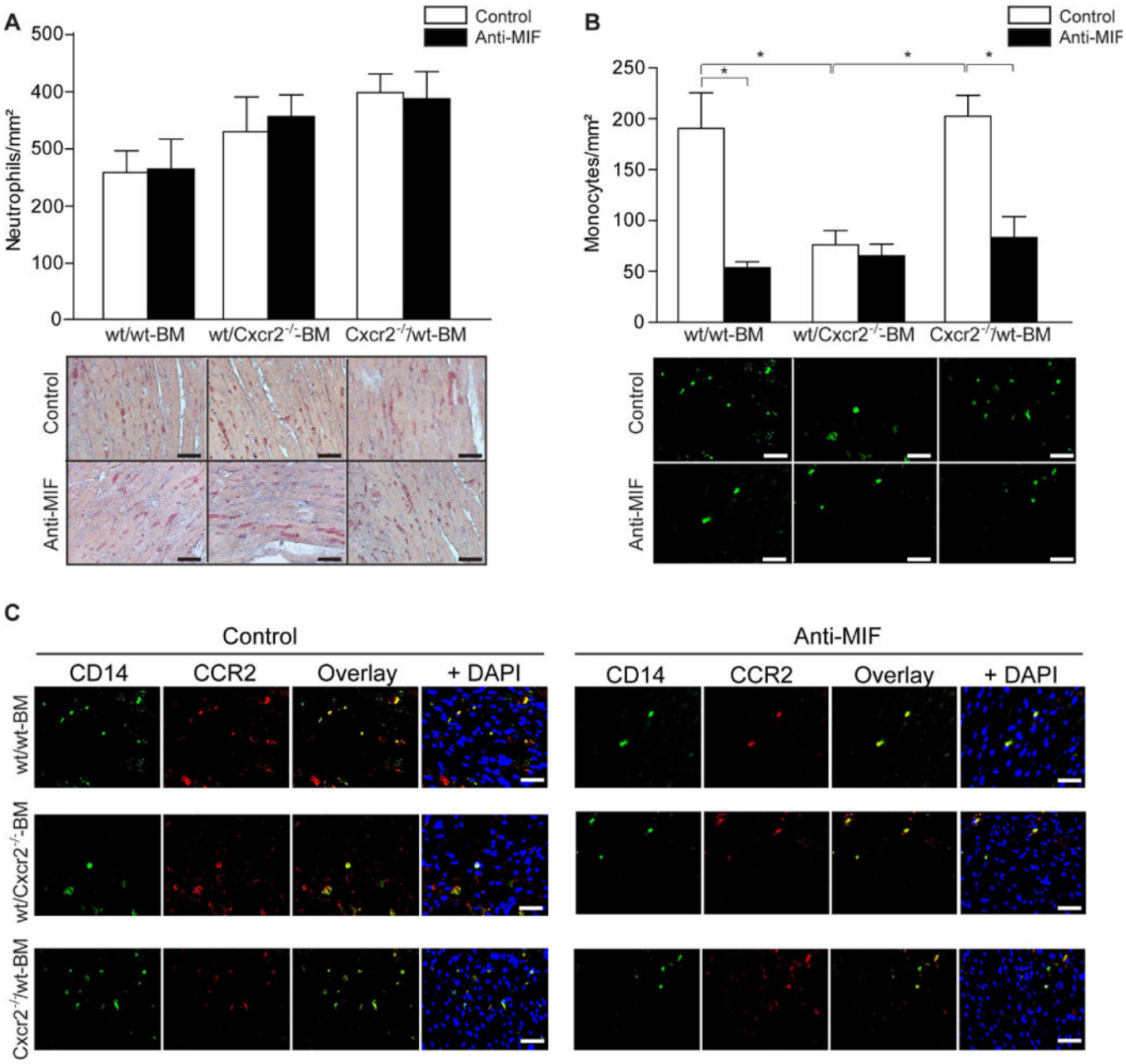

Figure 3. The analysis of anti-MIF-antibody effect on inflammatory reaction after I/R

The infarcted area of chimeric wt/wt BM, wt/Cxcr2$^{-/-}$ BM and Cxcr2$^{-/-}$/wt BM mice treated with anti-MIF (dark bars) or isotype control (open bars) antibody were analyzed one day after myocardial I/R. Neutrophil infiltration (specific esterase – red) did not significantly differ between the groups (A, n=4–6, Scale bars 50 μm), while monocyte infiltration (CD14 – green) was reduced by treatment with anti-MIF antibody in wt/wt BM and Cxcr2$^{-/-}$/wt BM mice as compared to isotype control. In wt/Cxcr2$^{-/-}$ BM mice the monocyte recruitment was significantly impaired and anti-MIF antibody treatment did not further reduce the monocyte infiltration (B, *$p<0.05$, n=4–6, Scale bars 50 μm). The most infiltrated

monocytes are CCR2 positive inflammatory monocytes, as shown by CD14 (green)/CCR2 (red) double staining (C, Scale bars 50 μm).

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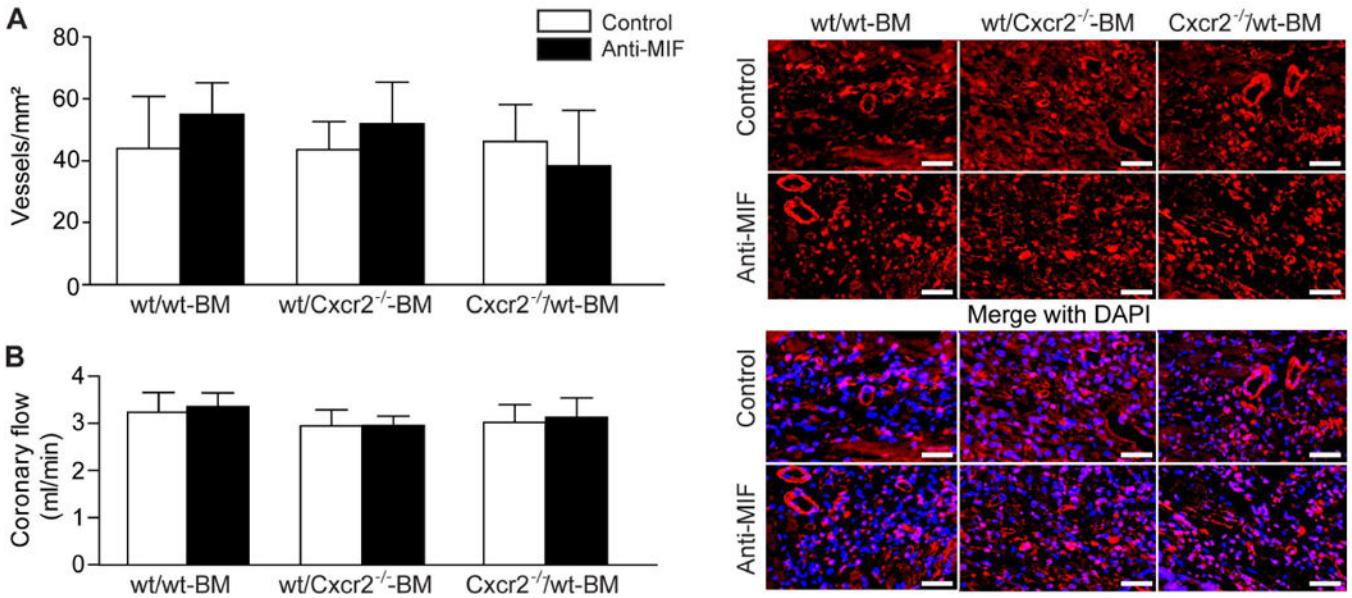

Figure 4. The analysis of angiogenesis in the infarcted area after anti-MIF-antibody after I/R

Analysis of neo-angiogenesis in the infarcted area (A) revealed no significant differences in the level of CD31-positive capillaries (red rings, right panel) between the groups one week after MI (n=4–6, Scale bars 50 μm). Similarly, Langendorff measurements failed to reveal changes in coronary flow in any of the groups (B, n=4–6).

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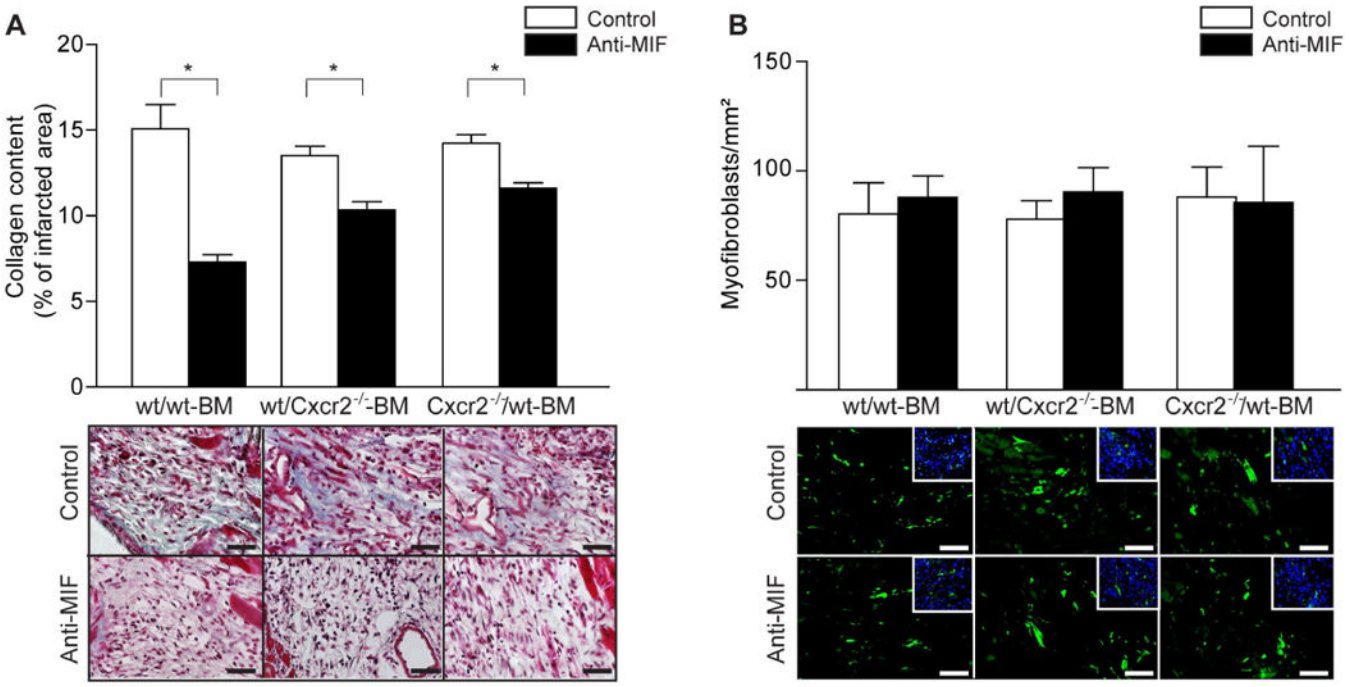

Figure 5. The effect of anti-MIF-antibody treatment on fibrosis after I/R

Notably, collagen content (blue) was reduced in all three groups by treatment with anti-MIF antibody but not altered by Cxcr2 deficiency in BM or resident cells (A, *$p<0.05$, n=4–6, Scale bars 50 μm), while the number of myofibroblasts (green) did not differ between the groups (B, n=4–6, Scale bars 50 μm).

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Table 1

Treatment groups of bone marrow chimeras

Mouse	Bone marrow	Anti-MIF antibody (100 μg/week)	Appellation	Cells resident/recruited	CXCR2
wild-type	wild-type	−	wt/wt BM	cardiomyocytes	+
		+		inflammatory cells	+
wild-type	$CXCR2^{-/-}$	−	wt/$CXCR2^{-/-}$ BM	cardiomyocytes	+
		+		inflammatory cells	−
$CXCR2^{-/-}$	wild-type	−	$CXCR2^{-/-}$/wt BM	cardiomyocytes	−
		+		inflammatory cells	+

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Table 2

Echocardiography and functional parameters

	wt/wt BM		wt/CXCR2 BM		CXCR2/wt BM	
	Control	**Anti-MIF**	**Control**	**Anti-MIF**	**Control**	**Anti-MIF**
Ejection fraction (%)	56.5±2.5	41.8±2.7	61.2±4.5	46.0±3.5	41.6±2.2	54.6±4.8
Cardiac output (ml/min)	10.9±1.5	10.8±0.9	10.5±1.7	9.6±0.7	9.5±0.5	9.7±0.9
Heart rate (bpm)	388±11.8	413±14.5	377±15.0	425±25.7	434±19.0	407±5.0
Heart weight (mg)	89.4±8.8	84.5±9.7	88.4±6.3	83.8±2.6	88.3±20.2	93.7±6.7

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Table 3

Histological and immunohistochemical analysis of infarcted area.

	wt/wt BM		wt/CXCR2 BM		CXCR2/wt BM	
	Control	**Anti-MIF**	**Control**	**Anti-MIF**	**Control**	**Anti-MIF**
Neutrophils/mm^2	250±37	256±50	319±59	345±37	386±31	375±46
Monocytes/mm^2	191±35	54±6	78±10	66±11	202±20	83±20
CCR2$^+$ monocytes (% of CD14$^+$ monocytes)	86±5	72±14	84±7	73±11	86±10	83±9
Vessels/mm^2	44±16	55±10	43±9	52±13	46±12	38±18
Collagen (% infarcted area)	15.1±1.4	7.3±0.4	13.5±0.5	10.3±0.5	14.2±0.5	11.6±0.3
Myofibroblasts/mm^2	80±14	88±10	78±8	91±11	88±13	86±25