Basic Res Cardiol 102:63–72 (2007)
DOI 10.1007/s00395-006-0610-7

ORIGINAL CONTRIBUTION

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Regional cardiac dysfunction is associated with specific alterations in inflammatory cytokines and matrix metalloproteinases after acute myocardial infarction in sheep

Received: 7 February 2006
Returned for 1. revision: 28 February 2006
1. Revision received: 1 June 2006
Accepted: 19 June 2006
Published online: 4 July 2006

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Abstract Cardiac remodeling following myocardial infarction (MI) is a maladaptive process, fundamental to the progression of ischemic heart failure. The extent of remodeling is influenced by mechanical stress, inflammatory response and activation of matrix metalloproteinases (MMPs). This study examined regional association between these parameters in response to acute MI.

Coronary ligation was performed in ten sheep. Sonomicrometer transducers measured segmental length in the infarcted, border and non-infarcted region. Regional tissue samples obtained 3 h post MI from six sheep were analysed using RT-PCR, gelatin zymography and Western blot. Six sham-operated sheep served as controls.

Region-specific dilation and reduced contraction was associated with corresponding alterations in matrix molecules. IL-6 and MMP-9 mRNA were increased in the infarcted and border regions compared to controls. MMP-2 and TIMP-1 mRNA increased in non-infarcted myocardium and both correlated positively with segmental shortening. IL-6 mRNA levels, in contrast, were negatively associated with segmental shortening.

In summary, inflammatory cytokines and MMPs are altered early after MI in a region-specific manner, and these changes correspond to acute regional myocardial dysfunction. Therapies for LV remodeling from the time of reperfusion may benefit from further understanding this portfolio of acute alterations.

Key words myocardial contraction – extracellular matrix – cytokines – matrix metalloproteinases – myocardial infarction

Introduction

Acute myocardial infarction (MI) results in structural and molecular alterations to both cardiac myocytes and the extracellular matrix (ECM). These alterations, referred to as cardiac remodeling, originate as an adaptive process as the heart attempts to compensate for acute loss in contractile function. Over time, however, cardiac remodeling becomes maladaptive, self-sustaining, and is a fundamental occurrence in the progression to heart failure.

The ECM serves as a vital supporting scaffold providing structural integrity to adjoining myocytes and translating myocyte shortening into LV pump performance. The extent of ECM remodeling following MI depends on multiple factors, including the magnitude of initial damage (infarct size), the extent of the inflammatory response (infarct healing) and the ensuing hemodynamic and mechanical stresses.

Inflammation plays an important role in healing after tissue injury. Clinical and experimental studies have shown that the post MI inflammatory response is associated with induction of cytokines such as tumour

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necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1B) and interleukin-6 (IL-6) [6, 17]. The acute remodeling process and initiation of wound repair is mediated by inflammatory cytokines, and involves resorption of necrotic tissue, hypertrophy of surviving myocytes, proliferation of myofibroblasts and angiogenesis. Acute, appropriate cytokine activation is required for effective wound healing; however excessive and sustained elevation of cytokines will result in chronic ECM remodeling, ventricular dilation, and progression to cardiac failure.

The endogenous family of enzymes responsible for ECM degradation are the matrix metalloproteinases (MMPs), and alterations in this enzyme system are known to occur rapidly and extensively following MI [5]. Inflammatory cytokines are known to induce activation of MMPs [28], as is mechanical deformation of cardiac fibroblasts in culture [30]. MMP activity is controlled by the tissue inhibitors of metalloproteinases (TIMPs). Experimental studies have provided a cause-and-effect relationship between MMP induction and the post-MI remodeling process.

MI results in irreversibly damaged cardiac tissue, surrounded by a border zone that may either recover or also suffer irreversible damage. While it has been demonstrated that inflammatory cytokines and MMPs are involved in both the wound healing response and chronic ventricular remodeling, their regional distribution has not been fully established. Ischemic stress represents a potent trigger for cytokine production and MMP activation, but direct myocardial mechanical stretch – maximal in the infarct and peri-infarct zone – is also a potent regulator. Since these parameters will vary throughout the heart following MI, it is likely that cytokine and MMP production will follow a similar pattern of distribution.

The aim of this study was to examine alterations in inflammatory cytokines and MMPs in the infarcted, border and non-infarcted myocardium during the acute period following MI, and to examine these changes with respect to regional cardiac geometry and contractile function.

Methods

Sixteen Merino-cross ewes (body weight 44 ± 6 kg) were used in this study approved by the institutional Animal Care and Ethics Committee. All animals received care in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication 85–23, revised 1996).

Surgical preparation

Anesthesia was induced with sodium thiopentone (20 mg/kg) followed by intubation and mechanical ventilation (Bird model 8, Bird Australia, Chatswood, NSW, Australia) with 2 L/min of oxygen, 2 L/min of nitrous oxide and 1.4–1.8 % isoflurane inspired. Rectal temperature was monitored and maintained throughout the experiment and warmed Hartmann's solution with additional 30 mM KCl and 20 mM $MgSO_4$ was given intravenously as maintenance fluid.

The heart was exposed through a left anterolateral thoracotomy at the fifth rib level via pericardiotomy. A transit-time ultrasonic flow probe (model 20A, Transonic Systems, Ithaca, NY) was positioned around the ascending aorta for cardiac output measurement. Four piezo-electric sonomicrometer crystals (Sonometrics Corporation, London, ON, Canada) were inserted into the mid-myocardium of the LV as shown in Fig. 1. The coronary artery to be ligated was identified and crystals placed 10–15 mm apart in the anticipated infarcted, peri-infarcted (border) and non-infarcted regions. Segmental length between the crystals was measured using sonomicrometry 'time of flight' principle. Segmental shortening was calculated as (end-diastolic length – end-systolic length)/end-diastolic length and expressed as a percentage.

The carotid artery and jugular vein were isolated through a transverse incision in the left neck. Sheep were instrumented for arterial blood pressure, central venous pressure and LV pressure as previously described [16].

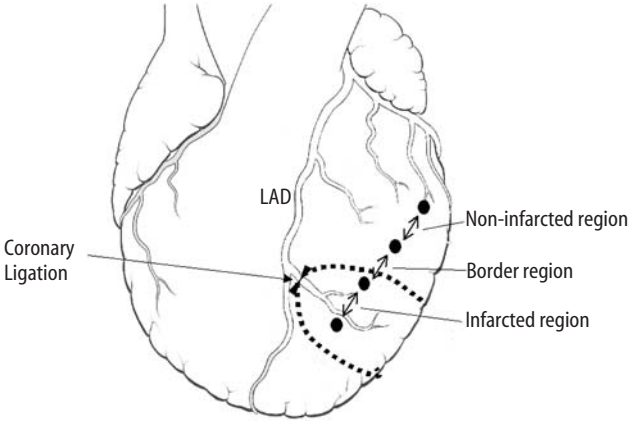

Fig. 1 Graphical illustration of sonomicrometer crystal placement on the LV. The four crystals were placed along the same plane as closely as possible. The dashed line perimeters the infarcted region and the arrow points to the suture used for ligation of the major diagonal branch of the left anterior descending coronary artery

Myocardial infarction

Myocardial infarction was induced by ligation of the major diagonal branch of the left anterior descending coronary artery (LAD). While two diagonals of the LAD were commonly observed, one was usually more dominant (major). Perfusion of 24 ex-vivo merino-cross hearts with methylene blue revealed the major diagonal branch supplied 19 ± 6 % of the left ventricle, while the major and minor combined supplied 25 ± 4 %. These results are consistent with those described previously [24]. All sheep received 50 mg lidocaine as prophylaxis; additional anti-arrhythmic and inotropic therapy was administered as required (see results for details).

Study protocol

Following surgical preparation, sheep were allowed to stabilise for 10–15 minutes. Baseline blood samples were drawn for creatine kinase analysis and baseline hemodynamic and sonomicrometer parameters recorded. Within 5 minutes of baseline measurements, coronary ligation (or sham ligation, with the suture passed under the artery but not tightened) was performed and data recorded every minute for 5 minutes, every 5 minutes until 1 h and every 15 minutes until 3 h. At 3 hours post ligation a second blood sample was taken for creatine kinase analysis and the sheep were euthanised by exsanguination as the heart was removed.

Infarct size measurement

In four sheep, infarct size was measured using differential staining of non-infarcted myocardium with triphenyl tetrazolium chloride. Following removal of the atria and right ventricle, the left ventricle was sectioned at 5 mm intervals along the long axis. Slices were incubated at 37 °C with 1 % triphenyl tetrazolium chloride (Sigma-Aldrich, Saint Louis, MO) for 15 minutes, followed by fixing in 10 % buffered formalin for 15 minutes. Slices were photographed digitally and the slices separated into stained (non-infarcted) and unstained (infarcted) myocardium and weighed. Infarct size as a percentage of the LV was calculated as unstained/(unstained + stained) × 100.

Tissue collection

In twelve sheep (6 ligated and 6 sham), tissue samples were collected from the infarcted, border and non-infarcted regions (samples taken between appropriate sonomicrometer crystals from mid-myocardium) and cryopreserved in isopentane suspended in liquid nitrogen. Samples were transported in liquid nitrogen to a –80 °C freezer and stored for future molecular analysis.

RT-PCR

Steady-state mRNA levels were measured by real-time RT-PCR. Total RNA was isolated using Trizol reagent (Gibco BRL, Gaithersburg, MD) and the absence of contaminating genomic DNA ensured through DNase treatment (DNase I Amp Grade, Invitrogen, Carlsbad, CA). Single-stranded cDNAs were synthesised from total RNA using Random Primers (Invitrogen, Carlsbad, CA) and Superscript II RNase H-reverse transcriptase (Invitrogen, Carlsbad, CA).

Real time PCR amplification was performed in duplicate on a Rotor-Gene 3000A (Corbett Research, Mortlake, NSW, Australia) using Syber Green fluorescence and ImmoMix reaction mixture (Bioline (Aust) Pty Ltd, Alexandria, NSW, Australia). Oligonucleotide primers for RT-PCR were designed using primary cDNA sequences obtained from Genbank™ and are listed in Table 1. Cycling conditions were as follows: 95 °C for 10 min followed by 40 cycles of 95 °C for 20 s, 60 °C for 20 s, 72 °C for 20 s. Specificity of the PCR reactions were confirmed with heat dissociation profiles and running of the products on an agarose gel.

Standard curves for each reaction were generated from a dilution series of cDNA known to contain large amounts of the gene of interest. Relative quantification of each target gene was determined by comparison of

Table 1 Primer oligonucleotide sequences for RT-PCR

GenBank Accession No.	Target mRNA		Primer sequence
X62501	IL-6	S	CCTTCAGTCCACTCGCTGTC
		AS	TCAAGCAAATCGCCTGATTG
X55966	TNF-α	S	AACTCTCCCTTCCTGCCAAT
		AS	GGACACCTTGACCTCCTGAA
X56972	IL-1B	S	CGAACATGTCTTCCGTGATG
		AS	GAAGCTCATGCAGAACACCA
AF26715	MMP-2	S	CTGATGGCGCCCATTTATAC
		AS	GATGAACCGGTCCTTGAAGA
X78324	MMP-9	S	GCACCACCACAACATCACCTACTG
		AS	ATCACAACGCCTTTGCCCAGAGAC
AF267160	MT1-MMP	S	CCATCATGGCACCCTTTTAC
		AS	CAAACATCTCCCCTCGAAGC
S67450	TIMP-1	S	CCACCCCACCCACAGACG
		AS	CGCAGGGACTGCCAGGTG
AY894419	28S	S	GCGAAAGACTAATCGAACCA
		AS	AACTGCGTGGGTTGCGAGAG

All primers were selected from ovine specific sequences where available. The genetic sequence for ovine MMP-9 was not available at the time of this study, thus primers selected from the bovine sequence were used

the amount of target gene to 28S rRNA (housekeeping gene) expression [34].

Due to low expression levels and large amounts of primer-dimer formation, TNF-α and MMP-9 were amplified with RT-PCR and visualised with ethidium bromide on a 1.8 % agarose gel. Fluorescence intensity was semi-quantitated using image analysis software. PCR amplification was performed in duplicate on a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA) using Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA). Cycling conditions were as follows: 94 °C for 2 min followed by 40 (TNF-α) or 50 (MMP-9) cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s.

Gelatin zymography

MMP-2 and MMP-9 protein expression and activation were detected by gelatin zymography under non-reducing conditions as described previously [14]. Protein extracts were loaded (20 μg protein per lane) onto a 10 % SDS gel containing 0.5 mg/ml gelatin. Gelatinolytic enzymes appeared as clear bands against the blue background of the stained gel. Relative levels of MMP-2 and MMP-9 were semi-quantitated using image analysis software.

Western blot

MT1-MMP and TIMP-1 protein expression was determined using Western blot. Protein extracts (20 μg protein per lane) underwent SDS-PAGE electrophoresis and were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). Membranes were blocked with 5 % skim milk and incubated in 0.33 μg/ml antisera to MT1-MMP or TIMP-1 (Chemicon AB815 and AB8116 respectively) for 1.5 h. Following incubation with secondary antibody, immunoreactive signals were detected by chemiluminescence with ECL Plus Detection Kit (Amersham Biosciences, Piscataway, NJ).

Data analysis

All results are expressed as mean ± standard error. Where hemodynamic and sonomicrometry results are expressed as one value for the post infarction period, this value is an average calculated through the summed area under the curve divided by the time period recorded.

Statistical analyses were performed using SigmaStat software (SPSS, Chicago, IL). Post infarction values were compared to post sham-ligation (control) values using Student's t-tests. Regional differences (infarcted vs border vs non-infarcted region) were compared using repeated measures ANOVA followed by Bonferroni pairwise comparisons. Any non-normally distributed data was analysed with ANOVA on ranks and post hoc Dunn's comparisons.

Results

Coronary ligation was associated with immediate ECG changes and a well-defined ischemic area within the open chest. Creatine kinase at 3 hours tended to be elevated compared to sham controls (1.4 ± 0.2 fold, P = 0.097) and infarct size was determined to be 17 ± 4 % LV.

Despite 50 mg lidocaine given as prophylaxis, 45 ± 12 mg additional lidocaine and 105 ± 50 mg amiodarone were administered to infarcted sheep for arrhythmias in the 3 h study period. In addition, four of the ten infarcted sheep required cardioversion for ventricular fibrillation. Epinephrine was required in two sheep (total 0.4 mg) and aramine in three (total 2 mg). Hemodynamic and sonomicrometry recordings were not made for at least 20 minutes following treatment, when drug effects had dissipated.

To correct for any drug effect on tissue molecular parameters, control sheep also received additional 45 mg lidocaine and 105 mg amiodorone. Two control sheep received epinephrine (total 0.4 mg) and one received aramine (1 mg). (Two ligated sheep receiving aramine were used to assess infarct size and not included in the molecular analysis.)

Hemodynamic parameters throughout the study period are summarised in Table 2. The only significant differences observed between ligated and control sheep were LV systolic and end-diastolic pressure, with both being elevated in the MI group (P = 0.033 and P = 0.049 respectively).

Significant alterations in segmental length and segmental shortening followed MI (Figs. 2–4). The infarcted

Table 2 Hemodynamic parameters for the study period

	Control (N = 6)	Myocardial infarction (N = 10)
Mean arterial blood pressure (mmHg)	63 ± 2	70 ± 3
Cardiac output (L/min)	4.8 ± 0.4	4.6 ± 0.4
Heart rate (bpm)	90 ± 3	90 ± 5
Stroke volume (ml)	54 ± 4	53 ± 6
Central venous pressure (mmHg)	3.9 ± 0.6	3.6 ± 1.2
LV systolic pressure (mmHg)	77 ± 2	84 ± 2 *
LV end-diastolic pressure (mmHg)	6.8 ± 1.0	9.9 ± 0.9 *
Max LV dP/dt (mmHg/s)	1376 ± 100	1288 ± 73

Values represent the average for the 3 h period following coronary ligation (or sham ligation). * P < 0.05 compared to control

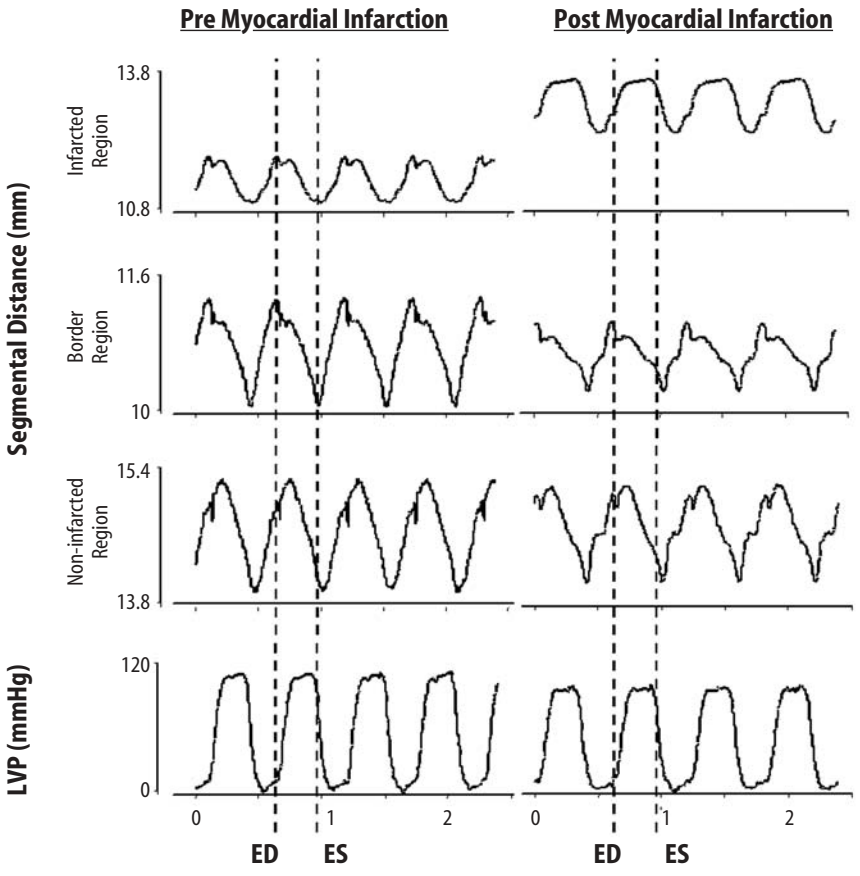

Fig. 2 Representative trace of sonomicrometer recordings pre and post (3 h) MI. Dashed vertical lines represent end-diastole (ED) and end-systole (ES). End-diastolic segmental length is increased in the infarct due to acute dilation of the region, and a paradoxical lengthening of the segment occurs during systole. Shortening of the border segment remains in phase with the non-infarcted region post infarction, however is markedly reduced

region demonstrated increased end diastolic segmental length (1.08 ± 0.02 fold control, $P = 0.017$) which was also significantly lengthened compared to border and non-infarcted regions ($P < 0.001$ and $P = 0.025$ respectively) (Fig. 4A). Segmental shortening was reduced in the infarcted (-0.36 ± 0.25 fold control, $P = 0.001$) and border regions (0.21 ± 0.11 fold control, $P = 0.076$), and these were also significantly reduced compared to non-infarcted region ($P < 0.05$ and $P < 0.05$) (Fig. 4B).

Expression of mRNA for inflammatory cytokines IL-6, TNF-α and IL-1B is shown in Fig. 5. Compared to control sheep, IL-6 mRNA levels were significantly elevated in the infarcted (54 ± 22 fold, $P = 0.004$) and border region (50 ± 19 fold, $P = 0.004$). There were no significant differences in mRNA levels of TNF-α or IL-1B, although levels tended to be elevated in infarcted and border regions.

The results of mRNA expression for MMP-2, MMP-9, MT1-MMP and TIMP-1 are displayed in Fig. 6. MMP-2 and TIMP-1 mRNA exhibited similar patterns of expression post MI, with increases moving away from the infarct zone, being highest in the non-infarcted region (MMP-2 $P = 0.020$, TIMP-1 $P = 0.003$ compared to control, MMP-2 $P = 0.019$ non-infarcted compared to infarcted). MT1-MMP mRNA was unchanged compared to control sheep and was not significantly different between regions. MMP-9, in contrast to other MMP subtypes, was significantly increased in the infarcted (9.9 ± 3.2 fold, $P = 0.020$) and border region (14.6 ± 4.8 fold, $P = 0.010$).

Gelatin zymography was used to determine the levels of pro and active MMP-2 and MMP-9 protein (Fig. 7). MMP-9 was detected in the pro form only, and despite variable levels between animals there was a significant elevation in the border region (3.4 ± 0.8 fold, $P = 0.017$) compared to controls (Fig. 7B). MMP-2 was produced in abundance, mainly in the pro MMP-2 form with only minor bands representing the active form. Unlike the decrease in MMP-2 mRNA in the infarcted compared to non-infarcted myocardium (Fig. 6), there was no significant change in pro MMP-2 protein levels (Fig. 7C). Interestingly, active MMP-2 was increased in both the infarcted ($P = 0.009$) and border ($P = 0.002$) regions.

Western blots were performed to investigate effects of MI on MT1-MMP and TIMP-1 protein levels. No change in protein levels could be observed at this timepoint, neither between control and infarcted sheep, nor between infarcted, border and non-infarcted regions (data not shown).

Linear regression analysis of segmental shortening

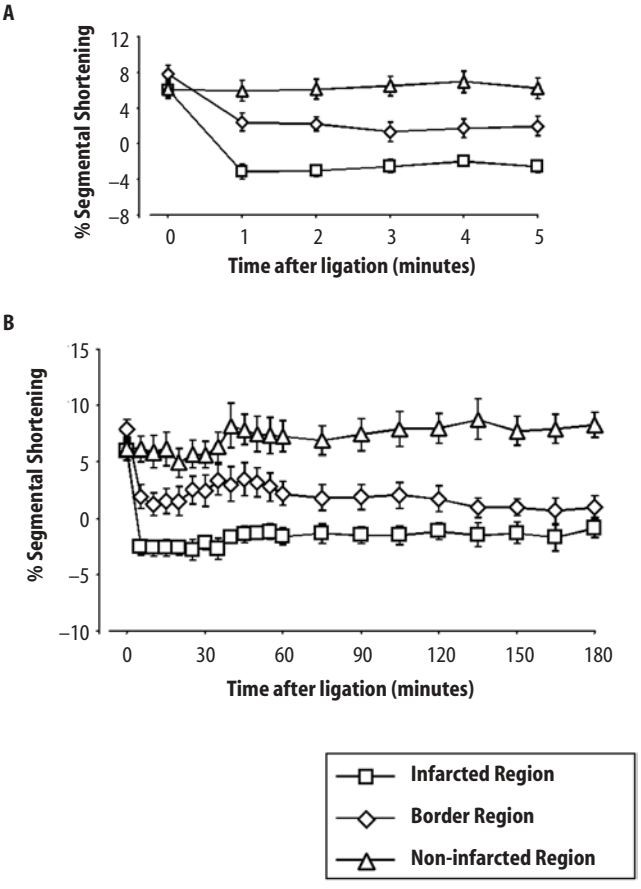

Fig. 3 Time-course of alterations in regional segmental shortening. An immediate reduction in segmental shortening was observed in the infarcted and border regions (**A**). While the reduction in shortening appeared to improve in the infarcted region over the 3h-study period, the border region appeared to deteriorate in function (**B**). N = 7–10 for all time points

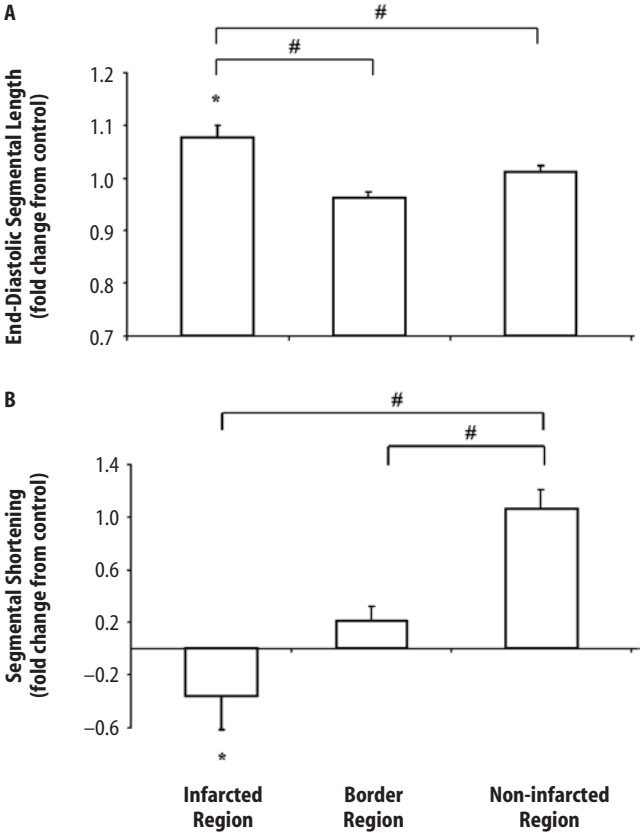

Fig. 4 Regional end-diastolic segmental length (**A**) and segmental shortening (**B**) following MI. Post myocardial infarction values represent the average for the 3 h period following coronary ligation, and have been expressed as fold change from control. * $P < 0.05$ compared to control, # $P < 0.05$ compared to indicated region. N = 10 for MI group, N = 6 controls

and molecular parameters (Fig. 8) revealed significant correlations with IL-6 (negative), MMP-2 and TIMP-1 mRNA ($P = 0.034$, $P < 0.001$ and $P = 0.017$ respectively). End-diastolic segmental length correlated with TIMP-1 only ($P = 0.266$, $P = 0.080$ and $P = 0.027$) while end-systolic correlated with MMP-2 and TIMP-1 ($P = 0.073$, $P = 0.002$ and $P = 0.002$) (segmental length data not shown).

Discussion

Myocardial infarction results in acute loss of contractile function closely followed by cellular changes to both cardiac myocytes and extracellular matrix. The extent of mechanical dysfunction, inflammatory response and ECM remodeling will all contribute to patient prognosis. Recent research highlights the involvement of inflammatory cytokines and MMPs in both the acute post MI period and in progression to chronic heart failure. The role of variations in regional distribution of inflammatory cytokines and MMPs and their inhibitors, however, remains uncertain.

In the present study, MI in sheep resulted in immediate regional myocardial dysfunction, most prominent in the infarct region, but also evident in the peri-infarct, or border region. Three hours after coronary occlusion, region-specific alterations in inflammatory cytokines and MMPs were demonstrated, some of which correlated to functional measures. This study shows that regional cardiac dysfunction following acute myocardial infarction is associated with region-specific alterations in inflammatory cytokines and matrix metalloproteinases.

The acute inflammatory response after MI is now regarded as an essential component of infarct healing. In this study, stimulation of inflammatory cytokines IL-6 and IL-1B are in keeping with this response, which is evident post MI throughout the injured heart, but in a graded fashion according to the degree of damage. Increases in IL-6 and IL-1B mRNA have previously been demonstrated in rats over 24 hours following coronary ligation [6], and IL-6 serum levels are elevated in pa-

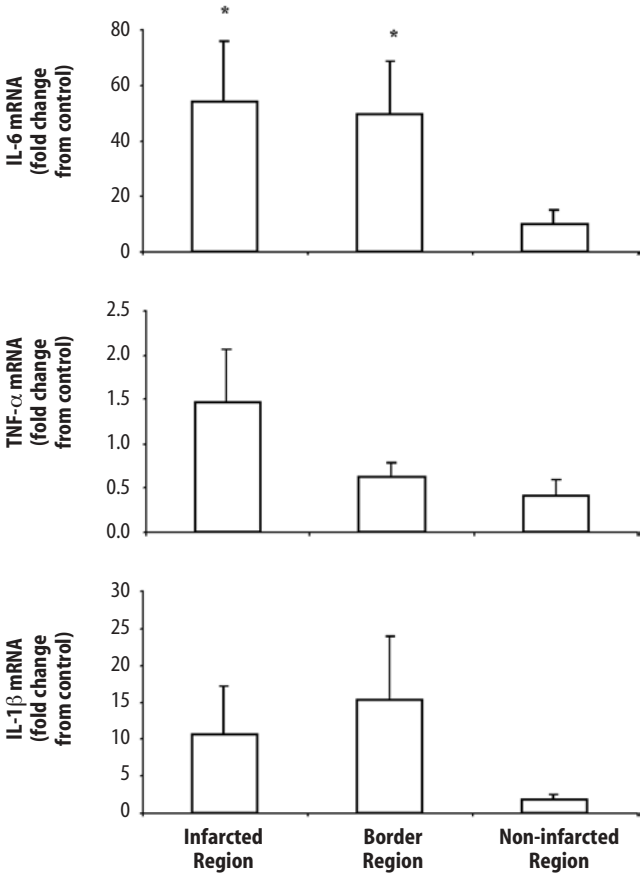

Fig. 5 Regional mRNA levels of inflammatory cytokines IL-6, TNF-α and IL-1B. Results are expressed as fold change from control, and have been normalised to 28S rRNA level. * $P < 0.05$ compared to control, N = 5–6 in each group

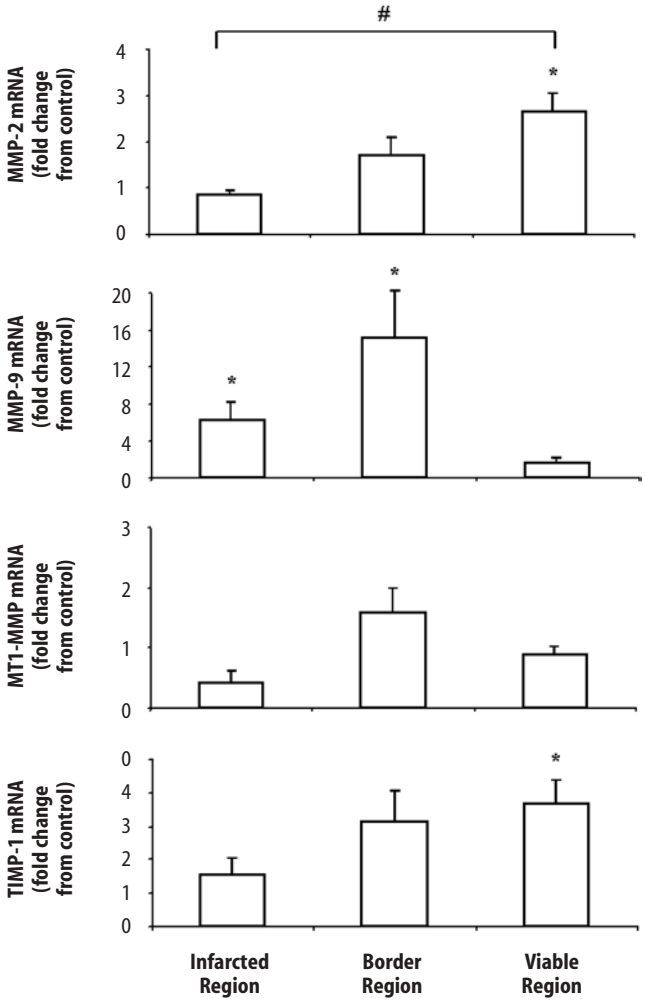

Fig. 6 Regional mRNA levels of matrix metalloproteinases MMP-2, MMP-9, MT1-MMP and tissue inhibitor of metalloproteinases TIMP-1. Results are expressed as fold change from control, and have been normalised to 28S rRNA level. * $P < 0.05$ compared to control, # $P < 0.05$ compared to indicated region. N = 5–6 in each group

tients after acute MI. In contrast to a previous report that IL-6 induction is dependent on reperfusion of ischemic myocardium [19], the present study provides evidence that ischemia alone will induce this response.

The absence of a concomitant increase in the inflammatory cytokine TNF-α mRNA is somewhat unexpected, as TNF-α has been implicated in both acute MI and chronic ischemic heart failure [31]. However TNF-α has also been found unchanged or only minimally altered in both clinical [25] and animal studies [7]. Discrepancies between species in post MI TNF-α expression have been described [7]; however the use of large animal models, such as sheep, should minimise deviation from human pathology [16, 24].

During the acute post MI period, MMP-9 mRNA expression is elevated [6] and its protein is released from endogenous myocardial cells and neutrophils. MMP-9 is known to have important pro-inflammatory actions, partly due to its ability to activate pro-TNF-α and pro-IL-1B [11, 27]. In addition to its association with the inflammatory response, MMP-9 retains its function in matrix degradation as mice deficient in MMP-9 are partially protected against myocardial rupture [15] and LV dilation within 8 days post infarction [9].

Interestingly, while MMP-9 mRNA and protein levels were both elevated after MI in this study, alterations in the other gelatinase, MMP-2, were less consistent. MMP-2 mRNA expression was unchanged in the infarcted region and significantly elevated in the non-infarcted region. At the protein level, pro MMP-2 was unaltered; however active MMP-2 was significantly increased, in this instance in the infarcted and border regions. It has been suggested that post-translational activation of stored extracellular latent MMPs plays a more important role in the regulation of collagenase activity following MI than the synthesis of MMP mRNA [3]. However there is also evidence that while MMP-9 is important in early post MI remodeling, MMP-2 is more involved in later

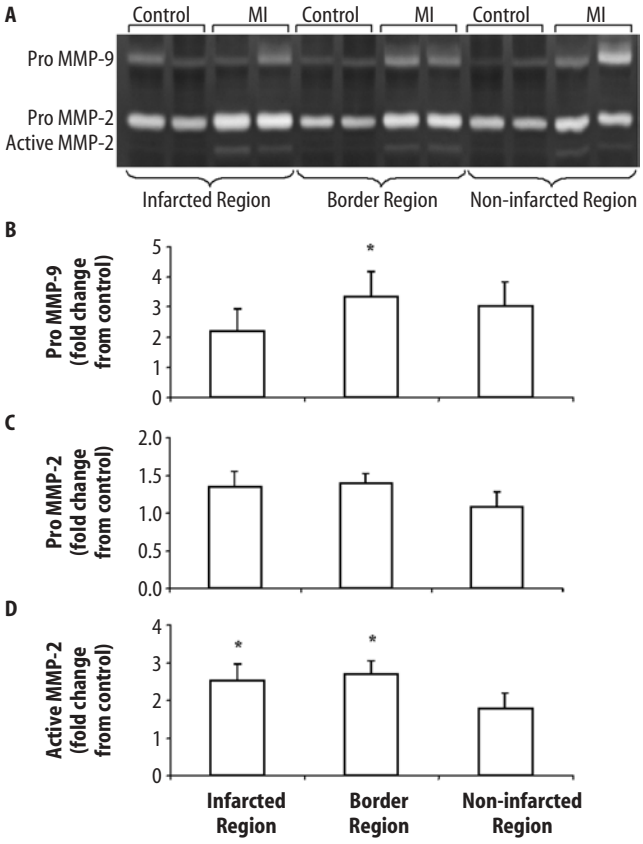

Fig. 7 Gelatin zymography of regional myocardial samples from control (sham ligated) and myocardial infarction (MI) sheep. A representative zymogram at the top of the figure (**A**) displays regional samples from four sheep. Semi-quantitation of zymography using image analysis is displayed in the graphs below (**B–D**). * $P < 0.05$ compared to control, $N = 6$ in each group

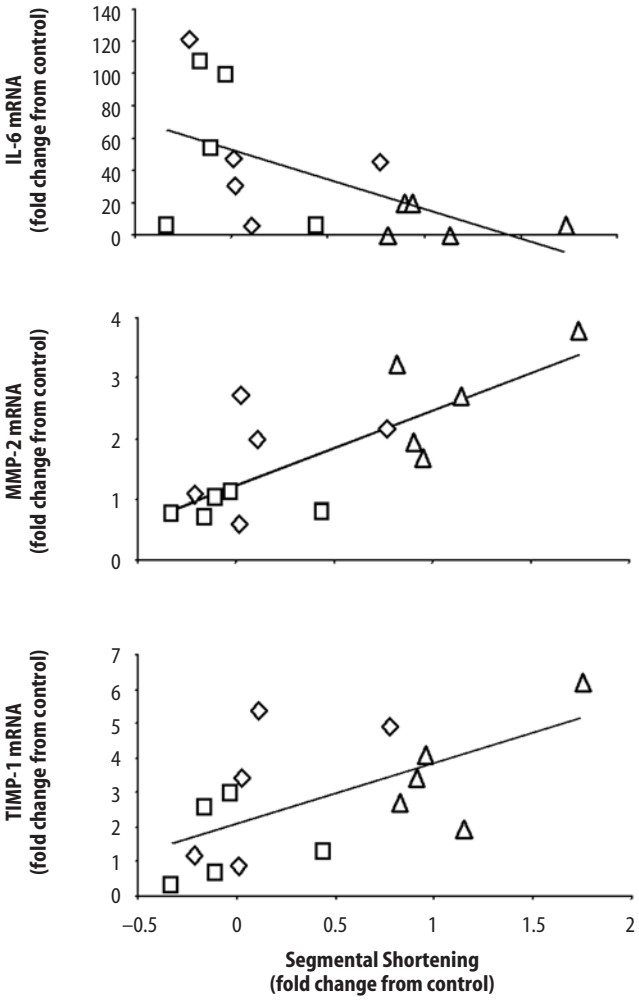

Fig. 8 Linear regression analysis of regional segmental shortening and regional IL-6, MMP-2 and TIMP-1 mRNA level. IL-6 (IL-6 = –36.573 x Segmental Shortening + 52.819, R = 0.548), MMP-2 (MMP-2 = 1.248 x Segmental Shortening + 1.245, R = 0.762) and TIMP-1 (TIMP-1 = 1.776 x Segmental Shortening + 2.071, R = 0.604) correlated significantly with segmental shortening ($P = 0.034$, $P < 0.001$ and $P = 0.017$ respectively). Infarcted, border and non-infarcted regions are represented with squares, diamonds and triangles respectively

phases of wound healing. Deten et al. [5] reported in rodents that MMP-2 mRNA levels in infarcted tissue were similar to, or less than, non-infarcted myocardium for the first 3 days post MI. This pattern then began to shift, and from 12–82 days MMP-2 mRNA was markedly increased in the infarct. This is consistent with a study by Wilson et al. [32], who found pronounced increases in MMP-2 protein levels in infarcted tissue 8 weeks post MI in sheep.

The alterations in MMP-2 mRNA in this study were mirrored by those of TIMP-1 mRNA. The reason for increased transcription of MMP-2 and TIMP-1 in the non-infarcted myocardium is uncertain; however their reported ability to confer myocardial protection and their induction in the non-infarcted tissue may be related.

A study by Matsuska et al. [22] demonstrated that ablation of the MMP-2 gene reduces survival and exacerbates cardiac failure in association with increased myocardial inflammation. This protection may be attributed to the anti-inflammatory actions of MMP-2, as it cleaves monocyte chemoattractant protein-3 to a product that is an antagonist of receptors to this protein [23]. In addition, Lalu et al. [20] demonstrated ischemic-preconditioning of the isolated rat heart prevented MMP-2 release, resulting in increased ventricular MMP-2, suggesting a potential mechanism of cardioprotection and reduction of infarct size [12]. A recent study by Squire et al. [29] found post MI plasma levels of MMP-2 were inversely associated with LV volumes (interestingly MMP-9 demonstrated the opposite). In contrast to these findings, however, Gao et al. [10] found administration of neutralising anti-MMP-2 antibody attenuated cytokine-induced dysfunction of the isolated rat heart, and speculate a role for MMP-2 in degradation of troponin I.

TIMP-1, even more so than MMP-2, is believed to play a protective role following myocardial infarction. Deletion of the TIMP-1 gene exacerbates LV remodeling following infarction [4] and TIMP-1 gene transfer following coronary ligation in rats leads to a preservation of cardiac geometry and function after 6 weeks [18].

The region-specificity of alterations in matrix components observed in the present study may be expected in light of the known upstream regulators. Ischemic stress will be greatest in the infarcted region, and hypoxia itself has been demonstrated to alter MMP expression in cultured cardiac fibroblasts [1]. In addition, ischemia (and infarction) will cause alterations to the regional cell population, as necrosis and apoptosis of cardiomyocytes under oxidative stress will occur more readily than that of cardiac fibroblasts [33].

Associations between functional parameters, myocardial MMPs and LV remodeling have been made in both animal and human studies [8, 26, 29]. The regional difference in mechanical load and stretch, demonstrated in this study using sonomicrometer transducers, may at least partially account for changes in MMP expression. Li et al. [21] have shown that mechanical unloading during LVAD support decreases MMP-1 and MMP-9 expression and increases TIMP-1 and TIMP-3 expression. Guo et al. [13] found increased MMP-2 in cell lysates of mechanically unloaded cardiac fibroblasts. Whether alterations in mechanical load affect expression of these matrix enzymes directly (for example through integrins) or whether autocrine and paracrine signals are required (such as growth factors) remains incompletely investigated.

The aim of the current study was to measure acute changes that occur rapidly (within 3 h) after infarction. Our results found altered gene expressions were often unaccompanied by altered protein levels, which may be explained by the lack of sufficient time to allow for translational changes and secretion. One advantage of measuring functional and molecular alterations at this early time-point, however, is that it approximates the time following infarction when most patients receive reperfusion therapy. For example, in the recent PREMIER clinical trial investigating MMP inhibition to prevent LV remodeling post MI, the average time to reperfusion therapy was 2.9 h and 3.2 h in the treatment and placebo group respectively. The fact that this trial failed to show any benefit with treatment [2] confirms the need for further understanding of matrix remodeling in the acute stages following MI.

In summary, we have shown that immediately after acute infarction in sheep, the damaged regions have elevated mRNA of pro-inflammatory mediators IL-6 and MMP-9 while MMP-2 and TIMP-1 mRNA are decreased relative to non-infarcted areas. This specific portfolio of inflammatory cytokines and MMPs may provide a useful target for therapies for LV remodeling following MI.

Acknowledgments This project was supported by funding from the North Shore Heart Research Foundation (Grant Number 15–04/05). The authors acknowledge the technical assistance of Ray Kearns, Wayne Roach, Catherine Mundy, Chi Ming Lee, James Mau, Meilang Xue, Patrick Thompson and Margaret Smith.

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