Transglutaminase activity in acute infarcts predicts healing outcome and left ventricular remodelling: implications for FXIII therapy and antithrombin use in myocardial infarction

Matthias Nahrendorf1*, Elena Aikawa1, Jose-Luiz Figueiredo1, Lars Stangenberg1, Susanne W. van den Borne2, W. Matthijs Blankesteijn2, David E. Sosnovik1,3, Farouc A. Jaffer3, Ching-Hsuan Tung1, and Ralph Weissleder1

1Center for Molecular Imaging Research, Massachusetts General Hospital and Harvard Medical School, Building 149, 13th Street, Room 5406, Charlestown, MA 02129, USA; 2Department of Pharmacology and Toxicology, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands; 3Cardiology Division, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA

Received 7 September 2007; revised 25 October 2007; accepted 5 November 2007

See page 427 for the editorial comment on this article (doi:10.1093/eurheartj/ehm610)

Aims
The transglutaminase factor XIII (FXIII) emerges as a key enzyme in healing after myocardial infarction (MI). Here we assess the impact of transglutaminase-modulating therapies on healing and evolution of heart failure using a novel, non-invasive molecular imaging technique.

Methods and results
Immunoblotting revealed lower FXIII levels in the myocardium of nine patients with infarct rupture when compared to MI patients without rupture (P < 0.0045). In a murine model of MI, we assessed healing while modulating local FXIII activity. Infarct tissue activity was monitored with molecular in vivo single photon emission computed tomography-computed tomography (SPECT-CT) imaging, and activity was found to be increased by 80% in FXIII-treated mice (400 IU FXIII/kg iv.), and decreased by 65% in dalteparin (DP)-treated mice (600 IU/kg DP sc., P < 0.05). DP-treated mice exhibited increased mortality due to infarct rupture (64% by day 7, P < 0.018). Serial Magnetic Resonance Imaging (MRI) showed that left ventricular dilation post-MI was attenuated by FXIII treatment when compared to saline control-treated mice with MI (P = 0.04). Quantitative histological and reverse transcription–polymerase chain reaction analyses revealed that FXIII treatment induced a faster resolution of the neutrophil response, enhanced macrophage recruitment, increased collagen content and augmented angiogenesis in the healing infarct (P < 0.05 vs. control-treated mice with MI).

Conclusion
FXIII tissue levels are decreased in patients with insufficient healing. Therapeutic strategies that modulate FXIII activity impact murine myocardial healing. Molecular imaging of FXIII activity predicts prognosis in mice with experimental MI.

Keywords
Imaging • Inflammation • Wound healing • Myocardial infarction • Factor XIII

Introduction
Adverse left ventricular (LV) remodelling and the subsequent evolution of heart failure remain common after myocardial infarction (MI) and result in a poor prognosis.1 While many factors contribute to remodelling,2–4 poor infarct healing and infarct expansion alter LV geometry, increase wall tension and lead to progressive remodelling.4,5 The transglutaminase factor XIII (FXIII) may play an important role in infarct healing, since it is involved in extracellular matrix turnover and regulation of the inflammatory response after ischaemic injury.6 This plasma protein circulates in its inactive precursor form and is primarily activated by thrombin.7
Genetically altered mice with 50% reduced FXIII level show impaired healing, enhanced ventricular dilation, and infarct rupture. Clinically, FXIII gene variant associated with higher plasma activity reduced cardiovascular mortality and the incidence of heart failure at 1 year follow-up after MI. The aim of this study was to evaluate the clinical significance of reduced FXIII levels in acute MI. This involved the measurement of FXIII levels in myocardial tissue specimens from nine patients with fatal infarct ruptures, an examination of the effects of prolonged heparin therapy on myocardial FXIII levels and infarct healing, and assessing the therapeutically pertinent potential of intravenous administration of FXIII pro-enzyme.

During the first week after MI, an acute phase reduction in FXIII plasma levels has been described. In addition, patients who are admitted with acute MI are often treated with heparin to inhibit clotting. Since all heparin formulations are thrombin inhibitors, they also inhibit FXIII activation and there have been reports that heparin may interfere with the inflammatory response after injury and delay collagen synthesis during wound healing.

The current study thus tested the following hypotheses: (i) FXIII tissue levels are lower in patients with acute infarct rupture; (ii) supranormal FXIII pro-enzyme plasma levels lead to higher FXIII activity in the MI and improve infarct healing; (iii) treatment with high doses of dalteparin (DP) is associated with decreased FXIII activity in the MI and with impaired myocardial wound healing. We tested hypotheses 2 and 3 in a murine model of MI using a novel, non-invasive molecular imaging approach to directly report FXIII activity in the healing infarct in vivo.

Methods

Myocardial transglutaminase factor XIII levels in patients

FXIII levels were studied by western blotting in myocardial samples of nine patients who died 3–7 days after MI due to infarct rupture. Inclusion criteria were a history of acute MI, haemothorax, and a clearly visible site of rupture at autopsy. In the control group we used samples from nine patients with a cause of death unrelated to infarct rupture, which was excluded by autopsy. The most common causes of death in this group were heart failure and arrhythmia. Both groups were matched for post-infarction survival time, age, and gender. The frozen samples were homogenized, sonicated and centrifuged. Protein content was measured using the BCA protein assay (Pierce Biotechnology). Ten microgram of total protein was separated on a 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis gel and transferred onto a Hybond C nitrocellulose membrane (Amersham Biosciences). After blocking, membranes were incubated overnight with primary antibodies directed against FXIIIa 1:1500 (Neomarker) and α-tubulin (H-300) 1:500 (Santa Cruz) for loading control. Collection, storage and use of human heart tissue and patient data were undertaken in compliance with the ‘Code for Proper Secondary Use of Human Tissue in the Netherlands’ (http://www.fmwv.nl), and informed consent was given by the families of the patients.

Mouse model and study outline

This study used 110 C57BL6 mice: validation of 111In-DOTA-FXIII for SPECT-CT imaging of FXIII infarct activity, n = 5; FXIII plasma level determination in control-, DP- and FXIII-treated mice, n = 10; ex-vivo assessment of FXIII activity in the infarct of control-, DP- and FXIII-treated mice using 111In-DOTA-FXIII, n = 32; longitudinal SPECT-CT and MRI trial for assessment of FXIII and DP effects, n = 18; survival, histology and polymerase chain reaction (PCR) analyses in respective treatment groups, n = 45. In addition, five FXIII−/− mice were used for in vivo SPECT-CT validation (a gift of Dr Dickneite, ZLB Behring, Marburg, Germany). MI was induced by permanent coronary ligation. First, we validated molecular imaging of FXIII activity in the infarct with SPECT-CT by imaging of FXIII−/− mice, ex-vivo imaging, autoradiography and scintillation counting of excised hearts. Thereafter, we assessed the natural time course of FXIII activity in murine MI. In the in vivo therapy trial as outlined in Supplementary Figure S1 (see Supplementary material online), the following daily treatments were started 2 h after MI and continued for 7 days: (i) DP (Pharmacia, Kalamazoo, MI, USA), 600 IU/kg sc., a dosage comparable to previous studies investigating non-antithrombotic functions of heparin in mice; (ii) factor XIII (ZLB Behring, Marburg, Germany), 400 IU/kg iv.; (iii) saline control, 100 μL sc. Mice were randomly assigned to treatment prior to infarct surgery. The surgeon was blinded with respect to treatment groups. We measured the resulting FXIII plasma levels using a colorimetric assay (Benichrom assay, Behring, Marburg, Germany).

Three hours after injection, FXIII plasma levels were unchanged in the DP group (107 ± 5% of control treatment, P = 0.9), and significantly increased in the FXIII injected mice (170 ± 5%; P < 0.01, n = 4 per group). The impact of this treatment on FXIII activity in the infarct was then studied ex vivo in a separate group of mice on day 3 after MI by scintillation counting. Furthermore, healing was assessed by histopathology and real time reverse transcription–polymerase chain reaction in a group of mice sacrificed on day 7 after MI. Mice were anesthetized for all procedures by inhalation anaesthesia (isoflurane 1–2% v/v + 2 L O2). The institutional subcommittee on research animal care at MGH approved all animal studies.

Molecular imaging of in vivo transglutaminase factor XIII activity

To assess the in vivo enzyme activity of FXIII within the infarct, we employed an 111Indium labelled affinity peptide (111In-DOTA-FXIII). FXIII recognizes the probe as a substrate and cross-links it to extracellular matrix proteins, leading to local entrapment of 111In-DOTA-FXIII in the healing infarct. After validation experiments in five FXIII−/− mice with MI, the imaging agent (100 μCi/animal iv.) was employed to determine the time course of endogenous FXIII activity in the MI. We measured radioactivity of excised hearts in sham operated animals and at day 2, 3 and 4 after coronary ligation with a gamma counter, with subsequent exposure on the phosphor imager. These myocardial rings were also stained with triphenyltetrazolium chloride (TTC), to assess the exposure of the signal to the infarct.

Non-invasive therapy trial

We performed late enhancement and cine MRI volumetry on day 2 and day 21 after MI, and SPECT-CT imaging of FXIII activity on day 3 after MI to assess the impact of FXIII modulating treatment as outlined in Supplementary Figure S1. Ten minutes after iv. injection of 0.4 mmol/kg Gd-DPTA, a 7 Tesla Pharmascan (Bruker, Billerica, MA, USA) and a gradient echo FLASH-sequence were employed to capture the time course of endogenous FXIII activity in the MI. We measured radioactivity of excised hearts in sham operated animals and at day 2, 3 and 4 after coronary ligation with a gamma counter, with subsequent exposure on the phosphor imager. These myocardial rings were also stained with triphenyltetrazolium chloride (TTC), to assess the exposure of the signal to the infarct.
USA) 3 days post-MI. Isotropic resolution was 72 μm for CT and 2 mm for SPECT. Imaging was performed 1 h after injection of 1mCi 111In-DOTA-FXIII. Directly prior CT imaging, 250 μL iodinated CT contrast agent was injected intravenously. The peak FXIII activity was determined as a target to background ratio by drawing regions of interest in the infarct and skeletal muscle in fused SPECT-CT images. Analysis of MRI and SPECT-CT data was performed in a blinded fashion.

Real time polymerase chain reaction
In an additional group of mice, we determined the impact of DP and FXIII treatment on mRNA levels of TSP-1, TSP-2, vascular endothelial growth factor (VEGF), MMP-2, MMP-9, and collagen 1-α2 on day 7 after MI using TriZol (Invitrogen) to isolate RNA and Oligo(dT) primers to transcribe mRNA into cDNA (StrataScript, Stratagene). Quantitative PCR was performed on an SDS 7000 system (ABI) in triplicates with β-Actin as an internal calibrator. Primer was generated with PrimerExpress.

Quantitative histopathological analysis
On day 7, mice of all treatment groups were sacrificed and hearts excised for histological assessment (see Supplementary material online, Figure S1). In addition, neutrophil content was also analysed on day 2 after MI. Serial 6 μm thick sections were produced for immunoreactive staining of neutrophils, macrophages, myofibroblasts, and endothelial cells forming capillaries with appropriate secondary antibodies. The collagen content of the scar was visualized with picrosirius red stain, which was assessed under polarized light.21 We quantified neutrophils, macrophages, and myofibroblasts using five high power fields (x400) per section and per animal with IP Lab Software (Scanlytics, Fairfax, VA, USA).

Statistics
Results are expressed as mean ± SD. The data sets were tested for normality using the Kolmogorov–Smirnov test with the Dallal–Wilkinson–Lilliefors correction, and for equality of variances using the F-test. If normality and equality of variances could not be rejected at 0.05 significance level, the group means were compared using parametric tests. Unpaired data were compared using the unpaired two-sided t-test, and for paired data using the paired two-sided t-test. If either normality or equality of variances were rejected, the non-parametric Mann–Whitney test was used. For comparison of multiple groups, variance was analysed by ANOVA, followed by Bonferroni’s multiple comparison test. Survival was analysed by a Kaplan–Meier plot with a log-rank test for differences between groups. P < 0.05 indicates statistical significance. We used Graphpad Prism 4.0c for Macintosh (GraphPad Software, Inc., San Diego, CA, USA) for statistical analysis.

Results
Transglutaminase factor XIII levels are diminished in patients after infarct rupture
FXIII protein levels were significantly diminished in the myocardium of nine patients who died due to acute infarct rupture when compared to patients who also died acutely after MI, but unrelated to infarct rupture (Figure 1, P = 0.0045).

In vivo imaging of transglutaminase factor XIII activity by SPECT-CT is feasible and specific
SPECT-CT fusion imaging detected areas of high FXIII activity within the healing infarct (Figure 2). To validate the source of the signal, we performed ex vivo SPECT-CT on an explanted mouse heart that had been embedded in agar, confirming the cardiac source of activity (Figure 2A and B). The infarct or the thoracotomy wound did not enhance in FXIII−/− mice, demonstrating the specificity of 111In-DOTA-FXIII (Figure 2D). Scintillation counts yielded a 356% higher % injected dose per gram tissue in explanted hearts from wild-type than in FXIII−/− mice (P = 0.0014).

Transglutaminase factor XIII activity peaks on day 3 after myocardial infarction
We next investigated the natural time course of FXIII activity by injecting 111In-DOTA-FXIII on days 0, 2, 3 and 4 after MI using three mice per time point. FXIII activity peaked on day 3 after coronary ligation (Figure 3). Autoradiography corroborated these findings and located the imaging agent to the healing MI.

Dalteparin impairs and exogenous transglutaminase factor XIII boosts uptake of 111In-DOTA-FXIII
Treatment with DP significantly reduced FXIII activity in the healing MI (Figure 4A). Conversely, intravenous FXIII pro-enzyme supplementation increased 111In-DOTA-FXIII uptake. These results were corroborated by target to background ratios calculated from autoradiography (control: 2.92 ± 0.88, DP: 1.75 ± 0.89, FXIII: 4.18 ± 1.85, P < 0.0001).

Dalteparin reduces survival
Daily subcutaneous injections with DP increased mortality significantly (Figure 4B). Autopsy established macroscopic rupture of the infarct in nine DP-treated mice (Figure 4B) and one control-treated mouse and did not show any thoracotomy related bleeding.
as source of death. DP-treated sham-operated mice survived the thoracotomy without increased mortality.

**In vivo imaging of infarct healing and left ventricular remodelling**

LV remodelling was imaged by serial MRI in control and FXIII-treated mice only, since DP-treated mice died before the second MRI could be performed (Figure 5A–I). On day 2 we determined infarct size as enhanced % of the LV by late enhancement MRI (control 27 ± 5%, DP 26 ± 5%, FXIII 26 ± 5%, P = 0.95). SPECT-CT showed lower FXIII activity with DP treatment, and higher activity in FXIII-treated mice (Figure 5K). Serial MRI on day 2 and 21 post-MI revealed attenuated LV dilatation in FXIII treated mice (Figure 5L). LV mass increased due to post-MI hypertrophy, and this change was only significant in the control group (Table 1). Representative MR and SPECT-CT images are shown in Figure 5, and cine MRI movies are provided as Supplementary material online.

**Altered recruitment of inflammatory cells**

Immunoreactive staining revealed that treatment with DP significantly delayed neutrophil recruitment. In a subgroup of mice that was sacrificed on day 2 after MI, fewer neutrophils were observed (positive % area of high power field, DP 0.1 ± 0.03%, FXIII 1.3 ± 0.2%, P = 0.0004); however, on day 7 the staining was most intense in the DP group, thus establishing a delayed time course of neutrophil influx (Figure 6A–C, G). Macrophages as assessed by MAC-3 staining were less numerous in mice that had received DP treatment and showed an enhanced presence in FXIII-treated mice at day 7 after MI (Figure 6D–F, H).

**Angiogenesis**

Seven days after induction of MI, DP-injected mice exhibited a significantly lower capillary density in the infarct, whereas treatment with FXIII increased formation of new microvessels (Figure 7A–D). TSP-1, which has been described as downregulated in FXIII mediated angiogenic activity in a transplant model, showed similar expression levels in the treatment groups (control: 0.25 ± 0.08, DP: 0.21 ± 0.12, FXIII: 0.22 ± 0.03, P = 0.80). TSP-2 was also unchanged by either treatment (control: 1.49 ± 0.71, DP: 2.53 ± 2.52, FXIII: 2.35 ± 0.35, P = 0.72). VEGF was upregulated four-fold in FXIII-treated mice, and was unchanged in DP-treated mice (Figure 7E).

![Figure 2](image-url) SPECT-CT imaging of transglutaminase factor XIII (FXIII) activity. (A) CT of an explanted heart with myocardial infarction (MI). Arrows indicate the infarct; (B) fusion of CT with SPECT reveals focal signal in the infarct; (C) SPECT-CT shows high (FXIII) activity in the MI. Some SPECT signal is observed in the thorax wall, also a site of active wound healing; (D) SPECT-CT of a FXIII−/− mouse shows no signal in the infarct.

![Figure 3](image-url) Time course of transglutaminase factor XIII (FXIII) activity. Endogenous FXIII activity in infarct healing peaks on day 3 after myocardial infarction (MI) as assessed by scintillation counting and the target to background ratio of the phosphor imager. Comparison to TTC staining shows that the imaging agent targets the infarct.
Dalteparin and transglutaminase factor XIII treatment modulate collagen synthesis

The presence of myofibroblasts, the source of collagen 1-α2, increased in the infarct of FXIII-treated mice, and diminished by DP treatment (Figure 8A–C, G). In FXIII-treated mice, the mRNA level of collagen 1-α2 significantly increased, and decreased in the DP group (Figure 8H). Collagen staining of the infarcts demonstrated increased amounts of collagen fibres in FXIII-, but less collagen in DP-treated animals (Figure 8D–F, I). Levels of MMP-2 (control: 4.01 ± 0.53, DP: 6.00 ± 2.95, FXIII: 6.74 ± 2.92, P = 0.49) and MMP-9 (control: 0.32 ± 0.27, DP: 0.41 ± 0.47, FXIII: 0.27 ± 0.19, P = 0.87) were not affected by treatment.

Discussion

In patients with acute infarct rupture, FXIII tissue levels were significantly diminished. These data provide clinical evidence for the role of FXIII in cardiac healing. In a murine model of MI, DP treatment is associated with lower FXIII activity and impaired wound healing. Therefore, heparin therapy in acute infarction might have differential effects in the infarct related artery (beneficial) and the injured myocardium (detrimental). Conversely, supranormal FXIII zymogen plasma levels lead to higher local FXIII activity in the MI and improve infarct healing. We furthermore demonstrate that molecular imaging of FXIII activity allows direct assessment of wound healing in vivo and predicts prognosis after MI.

We assessed the time course of endogenous FXIII activity in the healing murine infarct using an isotope labelled peptide. FXIII specifically recognizes this peptide as a substrate and cross-links it to extracellular matrix proteins, leading to local entrapment of the radioactive labelled peptide in the healing infarct. The specificity of this imaging agent has been compared to scrambled control peptides and investigated in FXIII−/− mice (Figure 2). The peak activity occurred on day 3 after MI (Figure 3), coinciding with the beginning of the proliferative phase of infarct healing. This indicates that FXIII participates in the organization of the new extracellular matrix forming the infarct scar, rather than serving in its ‘traditional’ clotting-factor role by cross-linking fibrin monomers. If the latter was true, activity would peak during haemostasis, which occurs acutely after MI. One limitation of this study is the surgical induction of MI as opposed to thrombotic occlusion in the clinical setting, which is frequently followed by reperfusion. Clinical or large animal studies will have to define how the present data predicts the situation in patients with MI, also because surgical ligation of the coronary artery neutralizes both antithrombotic benefits of heparin and potential prothrombotic effects of increased FXIII levels.

Serial MR imaging revealed attenuated LV remodelling in FXIII treated mice as indicated by reduced LV dilation and less myocardial hypertrophy. Also, we found a trend towards preserved EF, and less infarct thinning and expansion (Table 1). It is therefore conceivable that improved healing in FXIII-treated mice introduced a geometrical advantage with smaller initial LV volumes and lower wall stress. Since wall stress is a major determinant of LV remodelling, this may have attenuated the evolution of heart failure in FXIII-treated mice. In a previous study, we found accelerated post-MI remodelling still occurred in FXIII−/− mice resubstituted with FXIII (200 U/kg bodyweight), suggesting that the resubstitution was only partial. In the present study, we treat wild-type mice, with normal basal levels of FXIII, with FXIII injections at 400 U/kg in an attempt to create supranormal levels. In addition, wild-type mice do not lack intracellular FXIII as FXIII−/− mice. These differences may explain why attenuated LV remodelling was observed in the present study and not in the previous one.
Table 1

<table>
<thead>
<tr>
<th>Treatment group (imaging time point)</th>
<th>Ejection fraction (%)</th>
<th>Myocardial infarction thickness (μm)</th>
<th>Infarct size (%)</th>
<th>Left ventricular mass (mg)</th>
<th>End-diastolic volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (day 2)</td>
<td>41 ± 11</td>
<td>690 ± 50</td>
<td>27 ± 5</td>
<td>70.4 ± 3.3</td>
<td>77.5 ± 9.8</td>
</tr>
<tr>
<td>Dalteparin (day 2)</td>
<td>40 ± 7</td>
<td>560 ± 20</td>
<td>26 ± 5</td>
<td>81.2 ± 10.2</td>
<td>72.7 ± 4.1</td>
</tr>
<tr>
<td>FXIII (day 2)</td>
<td>41 ± 7</td>
<td>680 ± 40</td>
<td>26 ± 5</td>
<td>88.2 ± 4.4</td>
<td>77.7 ± 6.9</td>
</tr>
<tr>
<td>Control (day 21)</td>
<td>29 ± 10</td>
<td>380 ± 40*</td>
<td>27 ± 5</td>
<td>103.0 ± 2.7*</td>
<td>153.5 ± 36.6*</td>
</tr>
<tr>
<td>FXIII (day 21)</td>
<td>35 ± 5</td>
<td>505 ± 60***</td>
<td>23 ± 5</td>
<td>97.0 ± 3.7</td>
<td>110.1 ± 17.9***</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. day 2. **P = 0.05 vs. control treatment.

Figure 5 In vivo molecular imaging of transglutaminase factor XIII (FXIII) activity predicts survival and evolution of heart failure. (A–I) Longitudinal imaging study (MRI day 2, SPECT-CT day 3, second MRI day 21); on day 2 (A, D, G), late enhancement MRI showed similar infarct size in all groups. FXIII-treatment led to higher SPECT signal (H, K). In dalteparin-treated mice, the SPECT signal was lower (E, K). Serial MRI showed attenuated left ventricular (LV) dilation in FXIII-treated mice (L). Due to reduced survival in dalteparin (DP)-treated mice, the second MRI on day 21 was not acquired (F). *P < 0.05, **P < 0.001
involving FXIII−/− mice. The relative contribution of intracellular FXIII to the wound healing process remains to be elucidated.

Augmented wound healing induced by FXIII treatment was characterized by fast resolution of the neutrophil response and enhanced macrophage recruitment. Conversely, treatment with DP delayed the peak of neutrophil recruitment. Heparin has been previously reported to diminish neutrophil recruitment by inhibition of adhesion molecules.11 DP treatment also decreased macrophage infiltration (Figure 6). These associations of leukocyte recruitment with wound healing are in accordance with previous reports of retarded infarct healing due to impaired monocyte recruitment24-26 and augmented neutrophil recruitment by inhibition of adhesion molecules.27 Sufficient macrophage activity may be necessary to resolve the first line neutrophil response and to facilitate later wound healing stages such as angiogenesis and collagen deposition in the scar, which were also affected by the level of FXIII activity. Further studies are needed to reveal the mechanism of FXIII involvement in leukocyte recruitment, and to better understand how to optimize healing by modulating phagocyte recruitment.

FXIII activity was associated with the density of microvessels in the infarct, consistent with previous reports assessing the involvement of FXIII in angiogenesis in different settings of tissue repair.28 These microvessels supply the newly formed granulation tissue and constitute an important feature of sufficient infarct healing.29 One likely reason for the impact of treatment on capillary density may be the role of macrophages in promoting angiogenesis. Macrophages, which were more numerous in FXIII−/− and less in DP-treated mice, enhance angiogenesis by, for instance, secretion of VEGF.30 In our study, we found a four-fold upregulation of VEGF in FXIII treated mice (Figure 7).

Collagen synthesis to form an organized, mechanically competent infarct scar is crucial for the integrity of the LV. FXIII targeted therapy effects were shown on several levels: (i) it increased the number of myofibroblasts in the healing wound, (ii) it upregulated mRNA levels of collagen 1, and (iii) it increased collagen content as assessed by picrosirius red staining. These findings parallel a report of lower collagen contents in skin wounds of FXIII−/− mice,31 and can be explained by the regulatory function of FXIII on fibroblast proliferation and migration described in vitro.32 Clinical studies have shown a beneficial net effect of the widely used heparin treatment in acute MI,33 which may be due to its antithrombotic action superseding the effect on wound healing. In the mouse model, however, surgical ligation of the coronary artery neutralized any potential antithrombotic benefit. We here report an association of clinical-type DP treatment, lower FXIII

**Figure 6** Recruitment of inflammatory cells. (A–C, G) Immunoreactive staining for neutrophil recruitment on day 7 after myocardial infarction (MI) shows a slower resolution of this first line response in dalteparin (DP)-treated mice. *P < 0.0001; (D–F, H) macrophage recruitment in DP-treated mice has not reached control level; whereas it is significantly enhanced in transglutaminase factor XIII (FXIII) treated mice. Magnification 400×, the scale bars depict 50 μm. *P < 0.0001
Figure 7 Capillary density and VEGF expression. (A–D) Microvessel density is reduced by dalteparin (DP). Conversely, transglutaminase factor XIII (FXIII) treatment increased microvessel density. Magnification 400×, the scale bars depict 50 μm. *P < 0.0001; (E) in FXIII-treated mice, the level of VEGF mRNA was increased four-fold, consistent with the increased vessel density observed in Figure 6C.

Figure 8 Collagen synthesis. (A–C, G) Immunoreactive staining for myofibroblasts, the cellular source of collagen, was decreased in dalteparin (DP)-treated and increased in transglutaminase factor XIII (FXIII)-treated mice. Magnification 400×, the scale bars depict 20 nm. *P < 0.0001. In parallel, mRNA levels of collagen 1-α2 were decreased in DP-treated and increased in FXIII-treated mice (H) *P = 0.0026. (D–F, I) Collagen content in polarized light microscopy of picrosirius red stains revealed less collagen deposition in DP-treated and increased collagen content in FXIII-treated mice. Magnification 200×, the scale bars depict 50 μm. *P < 0.0001.
activity in the infarct, and increased infarct rupture. Heparin exerts a wide range of effects modulating the inflammatory, proliferative and fibrotic response, independent of FXIII activation.\textsuperscript{11,13–15,34} Given this broad action of heparin, the detrimental effect of DP may also have been caused or cofounded by mechanisms other than the inhibition of FXIII activation. However, the FXIII activity levels found on day 3 in the infarct of DP-treated mice (65% reduction in scintillation counts) closely resembled the situation in FXIII<sup>−/−</sup> mice, which have a 50% reduction of FXIII plasma levels and were also reported to die from infarct rupture.\textsuperscript{6} Since the observed effects may be species-specific and not directly translatable, further studies in large animal models and humans are needed to confirm these data. While heparin therapy has beneficial anticoagulant properties and promotes the patency of the infarct related artery, it may retard wound healing in the myocardium. Future strategies should thus aim to maintain the beneficial anticoagulatory effects of heparin in the infarct related vessel, but prevent its effects on wound healing.

In summary, FXIII levels are significantly decreased in patients with ruptured infarcts. In the mouse model of coronary ligation, modulation of FXIII activity by therapy impacts myocardial healing. This finding is of clinical importance, since it potentially translates into novel therapeutic strategies to augment infarct healing and retard the genesis of heart failure post-MI. Patients with low FXIII plasma levels, which occur naturally after MI,\textsuperscript{8,9} may potentially benefit from transient and local augmentation of FXIII activity in the infarct.

### Supplementary material

Supplementary material is available at European Heart Journal online.

### Acknowledgements

The authors would like to acknowledge Gerhard Dickneite, PhD, ZLB Behring (Marburg, Germany) for the generous gift of fibrogamin and FXIII knockout mice; Peter Whittaker, PhD, University of Massachusetts; Georg Ertl, MD, Wurzburg University; and Richard T. Lee, MD, PhD, Brigham and Women’s Hospital, Boston, for fruitful discussions; Jan Grimm, MD, PhD, for help with establishing cardiac SPECT-CT imaging; the CMIR Pathology Core (Vincent Lok, BS; Todd Sponholz, MS) for assistance with histology; Nan-Hui Ho, PhD and Hanwen Zhang, PhD, for synthesising the FXIII peptide probe; the CMIR mouse imaging programme (Carlos Rangel, BS; Claire Kaufman, BS; Sheena Hembrador, BS; and Gregory Wojtkiewicz, MS); and the MGH PCR core facility. Support sources: RO1-HL078641 (RW), and UO1-HL080731 (RW).

### Conflict of interest

none declared.

### References

17. Echtenacher B, Weigl K, Lohn N, Mannel DN. Tumor necrosis factor-dependent adhesions as a major protective mechanism


