Published in final edited form as:
Circulation. 2006 March 7; 113(9): 1196–1202. doi:10.1161/CIRCULATIONAHA.105.602094.

Factor XIII Deficiency Causes Cardiac Rupture, Impairs Wound Healing, and Aggravates Cardiac Remodeling in Mice With Myocardial Infarction

Matthias Nahrendorf, MD, **Kai Hu, MD**, **Stefan Frantz, MD**, **Farouc A. Jaffer, MD, PhD**, **Ching-Hsuan Tung, PhD**, **Karl-Heinz Hiller, PhD**, **Sabine Voll**, **Peter Nordbeck, MD**, **David Sosnovik, MD**, **Stefan Gattenlöhner, MD**, **Mikhail Novikov, MD**, **Gerhard Dickneite, PhD**, **Guy L. Reed, MD**, **Peter Jakob, PhD**, **Anthony Rosenzweig, MD**, **Wolfgang R. Bauer, MD, PhD**, **Ralph Weissleder, MD, PhD**, and **Georg Ertl, MD**

Medizinische Klinik und Poliklinik I, Universität Würzburg, Würzburg, Germany (M. Nahrendorf, K.H., S.F., P.N., S.G., W.R.B., G.E.); Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School, Charlestown, Mass (M.N., F.A.J., C.T., D.S., R.W.); Physikalisches Institut (EP5), Universität Würzburg, Würzburg, Germany (K.H., S.V., P.J.); Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Charlestown, Mass (M. Novikov, A.R.); ZLB Behring, Marburg, Germany (G.D.); and Cardiology Division, Department of Medicine, Medical College of Georgia, Augusta (G.L.R.)

Abstract

Background—Identification of key molecular players in myocardial healing could lead to improved therapies, reduction of scar formation, and heart failure after myocardial infarction (MI). We hypothesized that clotting factor XIII (FXIII), a transglutaminase involved in wound healing, may play an important role in MI given prior clinical and mouse model data.

Methods and Results—To determine whether a truly causative relationship existed between FXIII activity and myocardial healing, we prospectively studied myocardial repair in FXIII-deficient mice. All $FXIII^{-/-}$ and $FXIII^{-/+}$ (FXIII activity <5% and 70%) mice died within 5 days after MI from left ventricular rupture. In contradistinction, $FXIII^{-/-}$ mice that received 5 days of intravenous FXIII replacement therapy had normal survival rates; however, cardiac MRI demonstrated worse left ventricular remodeling in these reconstituted $FXIII^{-/-}$ mice. Using a FXIII-sensitive molecular imaging agent, we found significantly greater FXIII activity in wild-type mice and $FXIII^{-/-}$ mice receiving supplemental FXIII than in $FXIII^{-/-}$ mice ($P<0.05$). In $FXIII^{-/-}$ but not in reconstituted $FXIII^{-/-}$ mice, histology revealed diminished neutrophil migration into the MI. Reverse transcriptase–polymerase chain reaction studies suggested that the impaired inflammatory response in $FXIII^{-/-}$ mice was independent of intercellular adhesion molecule and lipopolysaccharide-induced CXC chemokine, both important for cell migration. After MI, expression of matrix metalloproteinase-9 was 650% higher and collagen-1 was 53%

Correspondence to Dr Georg Ertl, Medizinische Universitätsklinik, Universität Würzburg, Josef Schneider-Strasse 2, 97080 Würzburg, Germany. Ertl_G@medizin.uni-wuerzburg.de.

Disclosure

Gerhard Dickneite is a full-time employee of ZLB Behring, Marburg, Germany. The other authors report no conflicts.

lower in FXIII$^{-/-}$ mice, establishing an imbalance in extracellular matrix turnover and providing a possible mechanism for the observed cardiac rupture in the FXIII$^{-/-}$ mice.

Conclusions—These data suggest that FXIII has an important role in murine myocardial healing after infarction.

Keywords

factor XIII; healing; heart failure; myocardial infarction; remodeling

The prevalence of heart failure has increased dramatically over the past decade. Postinfarction remodeling of the left ventricle (LV) is currently one of the most common causes of heart failure, and, despite optimal pharmacological therapy, the prognosis of this condition remains poor.[1] Local expansion of the infarct due to inadequate healing may contribute to LV dilatation, remodeling, and ultimately heart failure.[2] An extreme form of adverse infarct healing is ventricular rupture, often a fatal condition. Strategies designed to directly promote myocardial healing have the potential to limit infarct expansion, aneurysm formation, and the development of heart failure. However, few therapies are available to directly improve local myocardial repair in the postinfarct setting.

Blood coagulation factor XIII (FXIII) is thought to play a role in wound healing and tissue repair. FXIII is a protrans-glutaminase that becomes activated by thrombin in the final stage of the clotting cascade. FXIII is present in plasma, platelets, monocytes, and macrophages, all of which are involved in infarct healing. FXIII deficiency can be caused by a genetic defect or by severe illness and may result in impaired hemostasis.[3] The importance of FXIII for wound healing is suggested by the following: (1) delayed wound healing in FXIII-deficient patients[4] and mice[5]; (2) beneficial effects of FXIII application on wound healing in experimental and clinical studies[3]; (3) antiapoptotic properties of FXIII[6]; (4) effects on cell migration into the wound[7]; and (5) modulation of fibrin and collagen synthesis and deposition.[8]

To determine whether FXIII is essential for acute and chronic infarct scar stability, we investigated a murine model with genetically reduced FXIII levels. Mice deficient in or heterozygous for the gene encoding the enzymatically active subunit A of FXIII have plasma enzyme levels <5% and ≈70%, respectively, of normal human plasma.[9] We used a murine coronary ligation model and high-field cardiac MRI to follow scar formation and the consequent remodeling process of the heart. In addition, we used an ^{111}In-labeled molecular imaging agent capable of detecting in vivo FXIII enzyme activity[10] in healing murine infarcts.

Methods

A total of 116 mice 20 to 30 weeks of age and with equal distribution among both sexes of the following groups were studied: sham-operated wild-type mice (CBA, Harlan-Winkelmann, Germany) (n=8); wild-type mice with myocardial infarction (MI) (n=35); sham FXIII$^{-/-}$ mice (n=10); FXIII$^{-/-}$ mice with MI (n=25); FXIII$^{-/-}$ mice with MI and intravenous substitution of FXIII (n=28); and heterozygous FXIII-knockout mice with MI

(n=10). FXIII-knockout mice lacking the active subunit A of FXIII were kindly provided by ZLB Behring, Marburg, Germany. Breeding of these mice is difficult because of intrauterine bleeding, with a resulting maternal mortality rate of 50%[9]; however, the feasibility of obtaining up to 2.2 pups per homozygous breeding pair has been reported.[11]

Mice underwent left coronary artery ligation or sham surgery as described previously.[12] Sham surgery comprised thoracotomy and passing of a suture under the coronary artery without actual ligation of the artery. This control surgery, which bears a 0% mortality rate, excludes that effects on mortality are induced by these injuries. To minimize bleeding complications in FXIII-deficient mice due to the thoracotomy, FXIII-free fibrin glue (gift from ZLB Behring, Marburg, Germany) was applied to the thoracotomy wound in all animals.

The group of $FXIII^{-/-}$ mice with MI and substitution of FXIII (in the following called reconstituted $FXIII^{-/-}$ mice) received 200 U Fibrogammin per kilogram body weight (ZLB Behring) via tail vein for 5 days, starting 1 day before MI. As previously determined, this dosage results in a FXIII plasma level of >175±19% activity in $FXIII^{-/-}$ mice 1 hour after application. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, rev 1996).

Magnetic Resonance Imaging

For assessment of scar thickness and LV remodeling, cine MRI was performed on days 1, 7, and 42 after coronary ligation on a 7-T Biospec (Bruker, Germany) device with the use of an ECG-triggered fast low-angle shot sequence with the following imaging parameters: echo time, 1.5 ms; repetition time, 4.3 ms; field of view, 30 mm^2; acquisition matrix, 128×128; and slice thickness, 1.0 mm.[13] At midventricular level, an additional slice with a higher resolution (acquisition matrix, 256×256; field of view, 30 mm^2) was acquired for exact determination of scar thickness to assess infarct expansion. Data analysis was performed as described before.[14]

Molecular Imaging of In Vivo FXIII Activity

To assess the in vivo enzyme activity of FXIII within the healing infarct, an ^{111}In-labeled affinity peptide was used. This agent is based on the N-terminus of α_2-antiplasmin, which is covalently cross-linked to fibrin by FXIII.[10] Because FXIII is also known to cross-link collagen fibers,[15] the previous optical imaging agent was developed into a scintigraphic imaging agent to image FXIII activity during infarct healing. FXIII recognizes the probe as a substrate and cross-links it to collagen, leading to local entrapment of the radio-active labeled peptide in the healing infarct. The chemical synthesis has been described in detail.[16] Briefly, the FXIII peptide substrate NQEQVSPLTLLK, based on the N-terminus of α_2-antiplasmin, was synthesized with a chelator, DOTA, on the side chain of C-terminal lysine residue. Before injection, the peptide (10 nmol) was labeled with 1 mCi of $^{111}InCl_3$ in glycine/HCl buffer (50 mmol/L, pH 3.5) at 80°C for 2 hours. After reaction, the probe was diluted in PBS buffer. The ^{111}In-labeled probe (1 mCi/animal) was injected into the tail vein of each of 5 wild-type mice 3 days after coronary ligation. In 3 mice with MI, free ^{111}In (1

mCi/animal) was injected as a control experiment. Twenty-four hours later, the mice were euthanized and perfused with saline. The hearts were excised and cut into myocardial rings of 1-mm thickness, which were stained with 2-3-5-triphenyl tetrazolium chloride (TTC) to demarcate the infarct. Thereafter, the rings were exposed on a PhosphorImager for 18 hours. The target-to-background ratio (TBR) was determined in 3 to 4 myocardial rings per mouse as follows: TBR=(infarct–noise)/(remote myocardium–noise). These regions were identified with the use of digital images of the TTC-stained myocardial rings. The noise was measured in a region of interest next to the tissue. In additional control experiments, the active probe was injected into 5 $FXIII^{-/-}$ and 4 reconstituted $FXIII^{-/-}$ mice. These experiments were performed on day 2 or 3 after MI, before the onset of spontaneous death due to scar rupture.

Histology

To assess neutrophil invasion, hematoxylin-eosin and periodic acid–Schiff staining was performed. For the determination of leukocyte count in the border zone, an additional 5 $FXIII^{-/-}$, 5 reconstituted $FXIII^{-/-}$, and 5 wild-type mice were euthanized 2 days after coronary ligation, well before spontaneous death of the knockout mice. Within a 2-mm distance of the infarct border, neutrophil granulocytes were counted in a midventricular slice with the use of ×400 magnification. Twenty high-power fields were counted in each of 3 slices per heart.

To evaluate the presence of FXIII within the healing infarct, we performed immunohistochemical analysis on fresh-frozen, 6-μm-thick sections using a rabbit anti-mouse anti-FXIII antibody as a primary (omitted on control slides). The secondary was a biotinylated goat anti-rabbit (Vector Laboratories, Burlingame, Calif). Furthermore, avidin-coupled horseradish-peroxidase (Vector) and a substrate chromogen (DakoCytomation) were used.

Biochemical and Molecular Measurements

To determine the expression of intercellular adhesion molecule-1 (ICAM-1) and lipopolysaccharide-induced CXC chemokine (LIX), myocardial RNA isolation of 6 wild-type and 7 $FXIII^{-/-}$ mice, which were euthanized 2 days after MI, was performed as previously described.[12] Again, this time point was chosen to avoid spontaneous death of knockout mice, which started 3 days after MI. In addition, we determined the expression of collagen type 1-α_2 to examine the influence of FXIII on collagen synthesis. After synthesis of cDNA with random hexamers (Superscript, Invitrogen), a real-time polymerase chain reaction (PCR) was performed (iCycler, BioRad) with commercially available TaqMan probes for 18S and murine ICAM, LIX, and collagen type 1-α_2 (Applied Biosystems). PCR parameters were used as recommended for the TaqMan universal PCR master mix kit (Applied Biosystems). RNA samples were normalized to 18S rRNA.

Matrix metalloproteinase-9 (MMP-9) was measured in duplicate by a commercial ELISA (R&D Systems) according to the manufacture's protocol after mechanical lysis of myocardial tissue with a buffer containing 50 mmol/L Tris-HCl, 75 mmol/L NaCl, 1 mmol/L phenylmethylsulfonyl fluoride, pH 7.5. Collagen and MMP-9 expressions were also assessed in 9 reconstituted $FXIII^{-/-}$ mice.

Statistical Analysis

Results are expressed as mean±SEM. Statistical comparisons among various groups were evaluated by ANOVA, followed by the Duncan test to isolate significance of differences. Survival was analyzed by a Kaplan-Meier plot; a log-rank test was performed to test for differences between groups. $P<0.05$ was considered to indicate statistical significance.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

100% Mortality in FXIII-Deficient Mice After Experimental MI Due to Ventricular Rupture

All FXIII$^{-/-}$ and FXIII$^{-/+}$ mice that were subjected to coronary ligation died within 5 days after surgery because of rupture of the LV (Figure 1A), whereas no deaths were observed in the FXIII$^{-/-}$ sham-operated mice. Daily intravenous replenishment of FXIII for 5 days restored survival to the level seen in the wild-type controls (numbers of animals, Table; Kaplan-Meier-survival curve, Figure 2).

Histology

The typical finding on autopsy in FXIII$^{-/-}$ was a hemothorax, and myocardial rupture of the LV free wall was confirmed on histology (Figure 1A).

Immunohistochemistry with an anti-mouse FXIII polyclonal antibody showed distinct staining for the presence of the enzyme in the healing infarct of wild-type mice (Figure 1B). The noninfarcted remote zones, the control sections with omitted primary antibody (not shown), and the MI in sections from FXIII$^{-/-}$ mice did not stain positive for FXIII (Figure 1C). Positive staining for the presence of FXIII was observed in reconstituted FXIII$^{-/-}$ mice (Figure 1D).

MRI Shows Enhanced Post-MI Remodeling in Reconstituted FXIII$^{-/-}$ Mice

FXIII$^{-/-}$ sham mice displayed a normal cardiac phenotype. LV mass and volumes, ejection fraction, and cardiac output were similar to those in wild-type mice (data not shown). No differences in thickness of the infarct area were observed between wild-type and reconstituted FXIII$^{-/-}$ mice 1 day after MI. However, 7 and 42 days after MI, the scar was significantly thinner in reconstituted FXIII$^{-/-}$ mice (Figure 3A to 3E). LV end-diastolic volume from day 1 to 42 increased more in reconstituted FXIII$^{-/-}$ MI than in wild-type MI (Figure 3F, Table). Comparison of infarct size revealed no significant differences between wild-type and reconstituted FXIII$^{-/-}$ mice (day 42, Table).

Molecular Imaging Detects Elevated FXIII Activity in MI of Wild-Type Mice

With the use of a radioactive labeled molecular imaging agent capable of detecting in vivo FXIII activity, a higher enzyme activity was found within the infarct of wild-type mice compared with the remote myocardium (Figure 4A, 4D). In contradistinction, wild-type mice injected with untargeted, free ^{111}In showed no autoradiographic enhancement of the infarct (Figure 4D). Furthermore, FXIII$^{-/-}$ mice were injected with the active probe, but no

specific binding was detected in the infarct of FXIII$^{-/-}$ (Figure 4B, 4D). Conversely, when resubstituted with the enzyme, the infarct areas of the FXIII$^{-/-}$ mice showed strong enhancement, proving that reconstituted FXIII is active in the healing MI of these mice (Figure 4C, 4D).

Reduced Inflammatory Response in FXIII-Deficient Infarcts

The number of granulocytes in the vicinity of the infarct border was reduced in FXIII$^{-/-}$ mice, which was partly reversed by FXIII resubstitution in FXIII$^{-/-}$ mice (Figure 5A).

Myocardial RNA of LIX showed an upregulation in homozygous FXIII$^{-/-}$ mice 2 days after MI compared with wild-type mice (Figure 5B), whereas mRNA levels of ICAM were not significantly changed (Figure 5C).

Imbalance of Extracellular Matrix Turnover

Myocardial expression of mRNA for collagen type 1-α_2 was lower in the infarct area of FXIII$^{-/-}$ mice euthanized 2 days after coronary ligation and was not improved by resubstitution of FXIII (Figure 6A).

In wild-type mice, myocardial tissue levels of MMP-9 were higher 2 days after coronary ligation than after sham operation (wild-type, 0.08±0.02; wild-type MI, 0.30±0.08; $P<0.05$). MMP-9 was 7-fold higher in FXIII$^{-/-}$ MI than in wild-type MI, but resubstitution normalized these levels (Figure 6B).

Discussion

The major novel finding of this study is that mice lacking FXIII suffer from impaired wound healing and fatal rupture of the LV after MI. This phenomenon was observed in 100% of homozygous and, interestingly, in 100% of the heterozygous FXIII-knockout mice, despite a FXIII plasma level of 70%.[9] Replenishment of FXIII during the 5-day acute and subacute period of infarct healing restored survival rates of FXIII-deficient mice to that of wild-type mice, further confirming the specific importance of FXIII in the prevention of cardiac rupture via improved myocardial wound healing. A novel molecular imaging approach to image wound healing demonstrated increased FXIII activity within the infarct (Figure 4). In this experiment, a radiolabeled agent was cross-linked by FXIII to extracellular matrix proteins and therefore trapped at the site of high FXIII activity. This signal enhancement was lost in FXIII$^{-/-}$ mice but was recovered in reconstituted FXIII$^{-/-}$ mice, indicating that the resubstituted enzyme is active in the healing infarct. The role of FXIII was further evidenced in immunohistochemical studies, demonstrating strong FXIII presence in wild-type murine infarcts but not in FXIII-deficient infarcts (Figure 1B and 1C). Efficacy of resubstitution therapy was further proven by positive immunohistochemical staining in reconstituted FXIII$^{-/-}$ mice (Figure 1D).

Cardiac rupture and consequent death of the FXIII-deficient mice occurred between days 3 and 5 after MI, which is the typical time window for this complication.[17] Infiltration of neutrophils represents an important step in the local inflammatory response triggered by a wide variety of chemoattractants, including inflammatory cytokines.[18] The finding of

reduced neutrophil migration, which was substantially improved by resubstitution of FXIII, to the infarct wound (Figures 1A and 5A) suggests an impaired inflammatory response. Interestingly, this was associated with an increased expression of LIX and unchanged expression of ICAM, both important for cell migration.[19] Myocardial expression of LIX is increased in a rat model of ischemia, and its inhibition reduces neutrophil infiltration.[20] Expression of ICAM, an adhesion molecule, is also upregulated in ischemia and associated with induction of neutrophil infiltration.[21] Thus, impaired neutrophil invasion in FXIII$^{-/-}$ mice occurred despite an increased expression of the potent neutrophil-chemotactic cytokine LIX. These observations suggest an ICAM- and LIX-independent impairment of neutrophil recruitment and perhaps a compensatory upregulation of LIX in FXIII deficiency.

Necrotic debris is cleared by phagocytosis, while fibroblasts migrate to the site of injury.[22] MMPs degrade the preexisting collagen network.[23] Similar to reported values,[17,24] we also found a 4-fold increase in MMP-9 levels in wild-type mice 2 days after MI. In FXIII$^{-/-}$ mice, these levels were further increased by ≈650%. To the contrary, resubstitution of FXIII normalized these increased levels to wild-type values. A causal relationship between the MMP-9 levels and rupture of the infarct has been established by the work of Heymans et al,[25] who showed that targeted gene deletion of MMP-9 protects against cardiac rupture in mice. Thus, prevention of upregulation of MMP-9 by FXIII appears to contribute to protection against cardiac rupture.

Wound healing requires deposition of fibrin and subsequently other extracellular matrix proteins such as collagen, which contribute essentially to a mechanically stable scar.[23] In FXIII-deficient mice, collagen synthesis was found to be downregulated. These data correspond to previous reports on control of fibroblast proliferation by FXIII[26] and its ability to regulate collagen synthesis in vitro.[8] In addition, FXIII provides cross-linking of fibrin and collagen fibers.[15] The reduced mechanical strength and rupture of the infarct region in FXIII deficiency are most likely caused by an imbalance of enhanced degradation of the preexisting extracellular matrix by MMP-9 and the insufficient creation of a durable collagen network consisting of lower collagen synthesis and absent cross-linking of collagen fibers.

Resubstitution therapy did not augment collagen synthesis, which may have contributed to thinner infarct scars found by MRI in reconstituted FXIII$^{-/-}$ mice (Figure 3A to 3E). The exact mechanism of how FXIII interferes with collagen synthesis remains unclear; however, a potential explanation for the lack of rescue of collagen synthesis by resubstitution may be related to intracellular FXIII. Intracellular FXIII resides in platelets and inflammatory cells such as monocytes,[3] and intravenous replenishment may not necessarily increase intracellular FXIII levels. In addition, substitution was stopped 5 days after MI, but development of a sufficient collagen scaffold takes longer.[23] In the end, MI expansion and thinning led to higher wall stress and consecutively to more dilatation of the ventricle (Figure 3F).

In our study we did not investigate the impact of supranormal FXIII levels on wound healing (eg, as in FXIII$^{+/+}$ mice treated with FXIII), and genetic deficiency of FXIII is rare. However, in a serial study in 25 patients with acute MI, a mean decrease of initially normal

FXIII plasma values by 25% was reported during the first week after the ischemic event.[27] In light of our findings, this may suggest that a subset of patients who develop an acquired FXIII deficiency after MI may benefit from substitution of FXIII.

We show by these experiments that FXIII-deficient mice suffer from cardiac rupture, infarct expansion, an attenuated inflammatory response, enhanced degradation of the extracellular matrix, impaired collagen synthesis, and aggravated cardiac remodeling. Collectively, these findings suggest that FXIII plays a key role in wound healing after MI. In addition, the importance of wound healing for LV remodeling after MI is highlighted. Future strategies to improve myocardial healing may therefore delay the evolution of heart failure beyond established therapy options.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 355 “Pathophysiologie der Herzinsuffizienz” and in part by R24 CA 92782 (Dr Weissleder), U01 HL 080731 (Dr Weissleder), and R01 HL 078641 (Dr Weissleder). We gratefully acknowledge S. Störck for help with statistics, Aiilyan Houng for preparing anti-FXIII antibody, Elena Aikawa and Vincent Lok for immunohistochemistry, Nan-Hui Ho for synthesizing the FXIII peptide probe, Filip Swirski for assistance with the FXIII molecular imaging studies, and Helga Wagner for biochemical measurements.

References

1. Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Kober L, Maggioni AP, Solomon SD, Swedberg K, Van de Werf F, White H, Leimberger JD, Henis M, Edwards S, Zelenkofske S, Sellers MA, Califf RM. Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. N Engl J Med. 2003; 349:1893–1906. [PubMed: 14610160]
2. Gaudron P, Eilles C, Kugler I, Ertl G. Progressive left ventricular dysfunction and remodeling after myocardial infarction: potential mechanisms and early predictors. Circulation. 1993; 87:755–763. [PubMed: 8443896]
3. Muszbek K, Yee VC, Hevessy Z. Blood coagulation factor XIII: structure and function. Thromb Res. 1999; 94:271–305. [PubMed: 10379818]
4. Vanscheidt W, Hasler K, Wokalek H, Niedner R, Schopf E. Factor XIII-deficiency in the blood of venous leg ulcer patients. Acta Derm Venereol. 1991; 71:55–57. [PubMed: 1676216]
5. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneite G, Modis L, Muszbek L, Inbal A. Impaired wound healing in factor XIII deficient mice. Thromb Haemost. 2005; 94:432–437. [PubMed: 16113836]
6. Dardik R, Solomon A, Loscalzo J, Eskaraev R, Bialik A, Goldberg I, Schiby G, Inbal A. Novel proangiogenic effect of factor XIII associated with suppression of thrombospondin 1 expression. Arterioscler Thromb Vasc Biol. 2003; 23:1472–1477. [PubMed: 12805075]
7. Brown LF, Lanir N, McDonagh J, Tognazzi K, Dvorak AM, Dvorak HF. Fibroblast migration in fibrin gel matrices. Am J Pathol. 1993; 142:273–283. [PubMed: 8424460]
8. Paye M, Nusgens BV, Lapiere CM. Factor XIII of blood coagulation modulates collagen biosynthesis by fibroblasts in vitro. Haemostasis. 1989; 19:274–283. [PubMed: 2777140]
9. Lauer P, Metzner HJ, Zettlmeissl G, Li M, Smith AG, Lathe R, Dickneite G. Targeted inactivation of the mouse locus encoding coagulation factor XIII-A: hemostatic abnormalities in mutant mice and characterization of the coagulation deficit. Thromb Haemost. 2002; 88:967–974. [PubMed: 12529747]
10. Jaffer FA, Tung CH, Wykrzykowska JJ, Ho NH, Houng AK, Reed GL, Weissleder R. Molecular imaging of factor XIIIa activity in thrombosis using a novel, near-infrared fluorescent contrast agent that covalently links to thrombi. Circulation. 2004; 110:170–176. [PubMed: 15210587]

11. Koseki-Kuno S, Yamakawa M, Dickneite G, Ichinose A. Factor XIII A subunit–deficient mice developed severe uterine bleeding events and subsequent spontaneous miscarriages. Blood. 2003; 102:4410–4412. [PubMed: 12933578]
12. Frantz S, Hu K, Widder J, Bayer B, Witzel CC, Schmidt I, Galuppo P, Strotmann J, Ertl G, Bauersachs J. Peroxisome proliferator activated-receptor agonism and left ventricular remodeling in mice with chronic myocardial infarction. Br J Pharmacol. 2004; 141:9–14. [PubMed: 14662734]
13. Ruff J, Wiesmann F, Hiller KH, Voll S, von Kienlin M, Bauer WR, Rommel E, Neubauer S, Haase A. Magnetic resonance microimaging for noninvasive quantification of myocardial function and mass in the mouse. Magn Reson Med. 1998; 40:43–48. [PubMed: 9660551]
14. Nahrendorf M, Wiesmann F, Hiller KH, Han H, Hu K, Waller C, Ruff J, Haase A, Ertl G, Bauer WR. In vivo assessment of cardiac remodeling after myocardial infarction in rats by cine-magnetic resonance imaging. JCMR. 2000; 2:171–180.
15. Akagi A, Tajima S, Ishibashi A, Matsubara Y, Takehana M, Kobayashi S, Yamaguchi N. Type XVI collagen is expressed in factor XIIIa+ monocyte-derived dermal dendrocytes and constitutes a potential substrate for factor XIIIa. J Invest Dermatol. 2002; 118:267–274. [PubMed: 11841543]
16. Tung CH, Ho NH, Zeng Q, Tang Y, Jaffer FA, Reed GL, Weissleder R. Novel factor XIII probes for blood coagulation imaging. Chem Bio Chem. 2003; 4:897–899.
17. Tao ZY, Cavasin MA, Yang F, Liu YH, Yang XP. Temporal changes in matrix metalloproteinase expression and inflammatory response associated with cardiac rupture after myocardial infarction in mice. Life Sci. 2004; 74:1561–1572. [PubMed: 14729404]
18. Nian L, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res. 2004; 94:1543–1553. [PubMed: 15217919]
19. Niessen HW, Krijnen PA, Visser CA, Meijer CJ, Hack CE. Intercellular adhesion molecule-1 in the heart. Ann N Y Acad Sci. 2002; 973:573–585. [PubMed: 12485931]
20. Chandrasekar B, Smith JB, Freeman GL. Ischemia-reperfusion of rat myocardium activates nuclear factor-κB and induces neutrophil infiltration via lipopolysaccharide-induced CXC chemokine. Circulation. 2001; 103:2296–2302. [PubMed: 11342480]
21. Altavilla D, Deodato B, Campo GM, Arlotta M, Miano M, Squadrito G, Saitta A, Cucinotta D, Ceccarelli S, Ferlito M, Tringali M, Minutoli L, Caputi AP, Squadrito F. IRFI 042, a novel dual vitamin E-like anti-oxidant, inhibits activation of nuclear factor-kappaB and reduces the inflammatory response in myocardial ischemia-reperfusion injury. Cardiovasc Res. 2000; 47:515–528. [PubMed: 10963724]
22. Schaffer CJ, Nanney LB. Cell biology of wound healing. Int Rev Cytol. 1996; 169:151–181. [PubMed: 8843654]
23. Cleutjens JPM, Blankesteijn WM, Daemen MJAP, Smits JFM. The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. Cardiovasc Res. 1999; 44:232–241. [PubMed: 10690298]
24. Chen J, Tung CH, Allport JR, Chen S, Weissleder R, Huang PL. Near-infrared fluorescent imaging of matrix metalloproteinase activity after myocardial infarction. Circulation. 2005; 111:1800–1805. [PubMed: 15809374]
25. Heymans S, Luttun A, Nuyens D, Theilmeier G, Creemers E, Moons L, Dyspersin GD, Cleutjens JP, Shipley M, Angellilo A, Levi M, Nube O, Baker A, Keshet E, Lupu F, Herbert JM, Smits JF, Shapiro SD, Baes M, Borgers M, Collen D, Daemen MJ, Carmeliet P. Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat Med. 1999; 5:1135–1142. [PubMed: 10502816]
26. Bruhn HD, Pohl J. Growth regulation of fibroblasts by thrombin, factor XIII and fibronectin. Klin Wochenschr. 1981; 59:145–146. [PubMed: 6259408]
27. Alkjaersig N, Fletcher AP, Lewis M, Ittyerah R. Reduction of coagulation factor XIII concentration in patients with myocardial infarction, cerebral infarction, and other thromboembolic disorders. Thromb Haemost. 1977; 38:863–873. [PubMed: 579691]

CLINICAL PERSPECTIVE

The plasma transglutaminase factor XIII (FXIII) emerges as a major factor in wound healing. In our work, we describe for the first time that the lack of this enzyme invariably leads to the worst-case scenario of healing after myocardial injury: the often fatal left ventricular wall rupture. In FXIII-deficient mice, healing was affected by an imbalance in extracellular matrix turnover and an attenuated inflammatory response to injury. Clinical studies will be necessary to define the role of FXIII in patients, but our results raise the possibility that augmenting FXIII levels might improve myocardial healing and delay the evolution of heart failure after myocardial infarction. In addition, the findings are of importance because they highlight healing as a potential therapeutic target, currently a clinically underexploited time window between acute reperfusion efforts and therapy of cardiac remodeling and chronic heart failure. We cautiously need to keep in mind that FXIII is part of the clotting cascade and stabilizes blood clots, which is an undesirable function before reliable reperfusion after coronary occlusion is established.

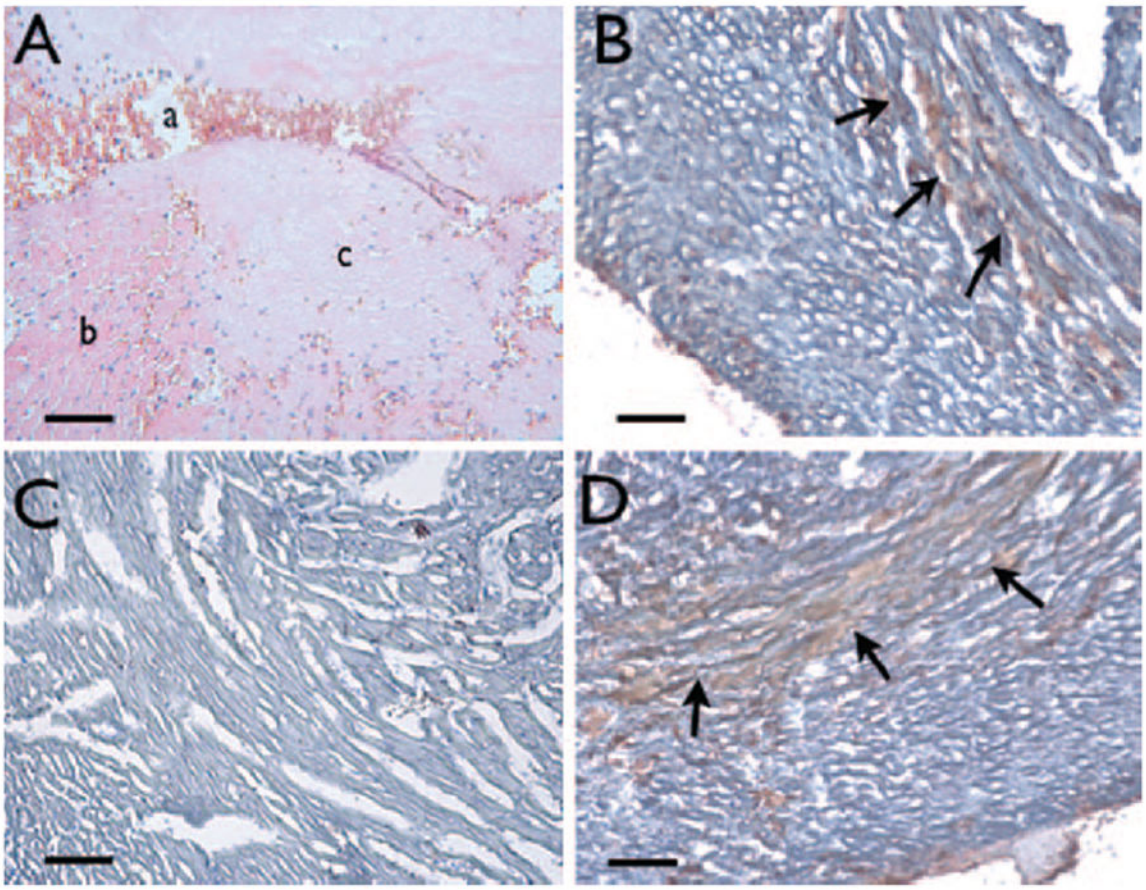

Figure 1.

A, Hematoxylin-eosin histology of a FXIII$^{-/-}$ mouse with a ruptured LV free wall 4 days after induction of MI. a, Ruptured channel filled with erythrocytes connects the LV cavity (right side of image) and the epicardial surface (toward left side of image). b, Remote zone, surviving myocardium. c, Necrotic area within infarct; note the absence of inflammatory cells. Bar=50 μm. B, Immunohistochemical staining with the use of anti-FXIII antibody in a wild-type mouse 2 days after MI. The infarct region shows enhanced staining for the FXIII presence. Bar=50 μm. C, Immunohistochemical staining with the use of anti-FXIII antibody in a FXIII$^{-/-}$ mouse (control, 2 days after MI). There is no staining for FXIII. Bar=50 μm. D, Immunohistochemical staining with the use of anti-FXIII antibody in a reconstituted FXIII$^{-/-}$ mouse 2 days after MI. There is positive staining for FXIII presence in the border zone of the infarct. Bar=50 μm.

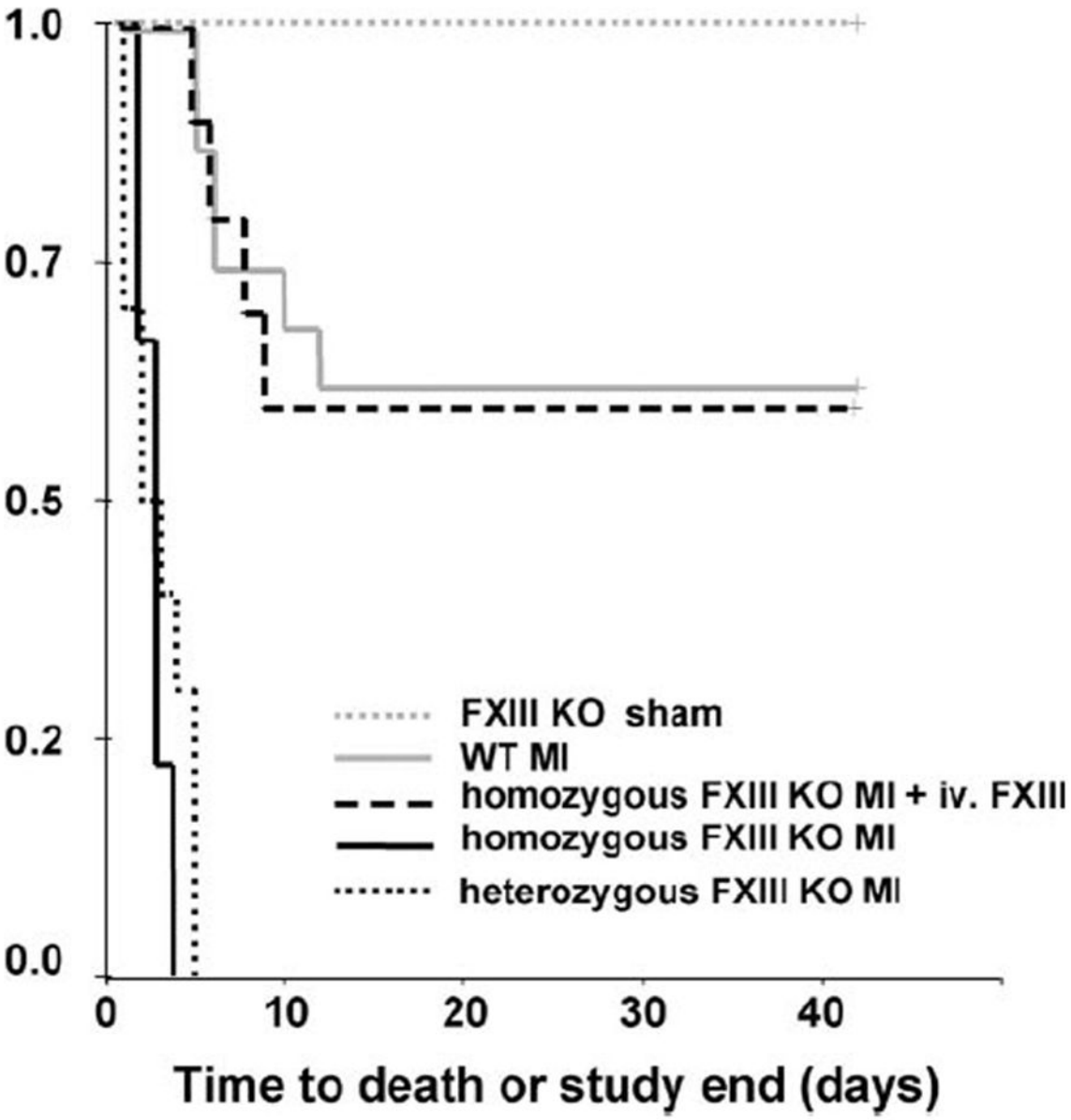

Figure 2.

Kaplan-Meier-survival curve. Homozygous and heterozygous FXIII-knockout (KO) mice do not survive the first week after MI because of a ruptured LV. This effect is abolished by intravenous substitution of FXIII. Sham $FXIII^{-/-}$, n=10; MI $FXIII^{-/+}$, n=10; number of animals for all other groups are stated in the Table. $P<0.05$ for wild-type MI vs $FXIII^{-/-}$ MI and $FXIII^{-/+}$ MI; $P<0.05$ for reconstituted $FXIII^{-/-}$ MI vs $FXIII^{-/-}$ MI and $FXIII^{-/+}$ MI.

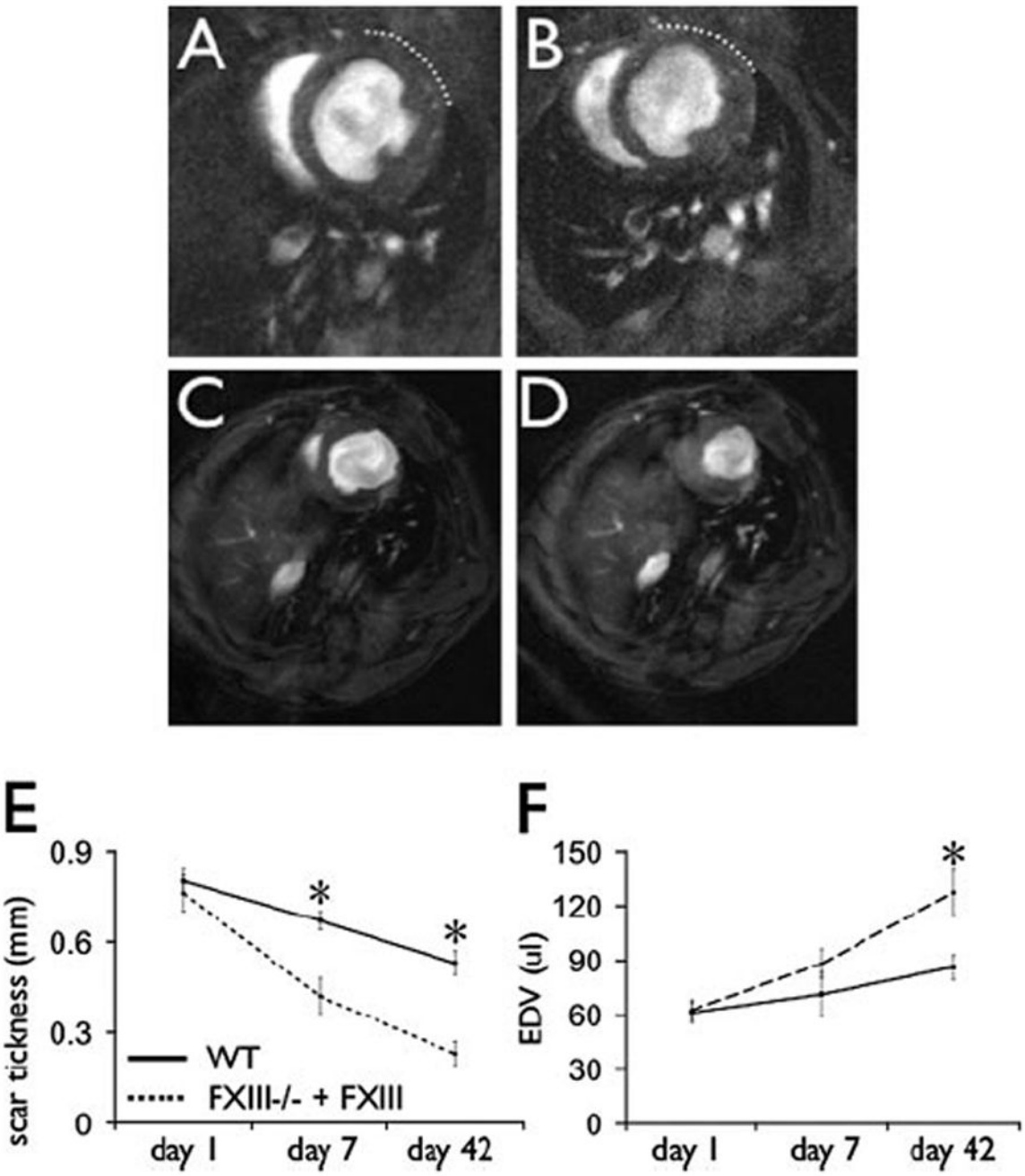

Figure 3.

MR short-axis images. A, Wild-type (WT) mouse 1 week after induction of MI. The dotted white line marks the epicardial border at the area of the MI. B, Reconstituted $FXIII^{-/-}$ mouse 1 week after MI. The infarct is thinner than in wild-type mice. C and D, Reconstituted $FXIII^{-/-}$ mouse, 42 days after MI, diastolic and systolic short-axis. A thin infarct scar is visible at the high-resolution MRI (field of view, 30 mm^2; matrix, 256×256). E and F, Scar thickness and end-diastolic volume at days 1, 7, and 42 after induction of MI. In reconstituted $FXIII^{-/-}$ mice, induction of MI leads to thinner scars and larger LVs. *P<0.05 vs wild-type MI. EDV indicates end-diastolic LV volume.

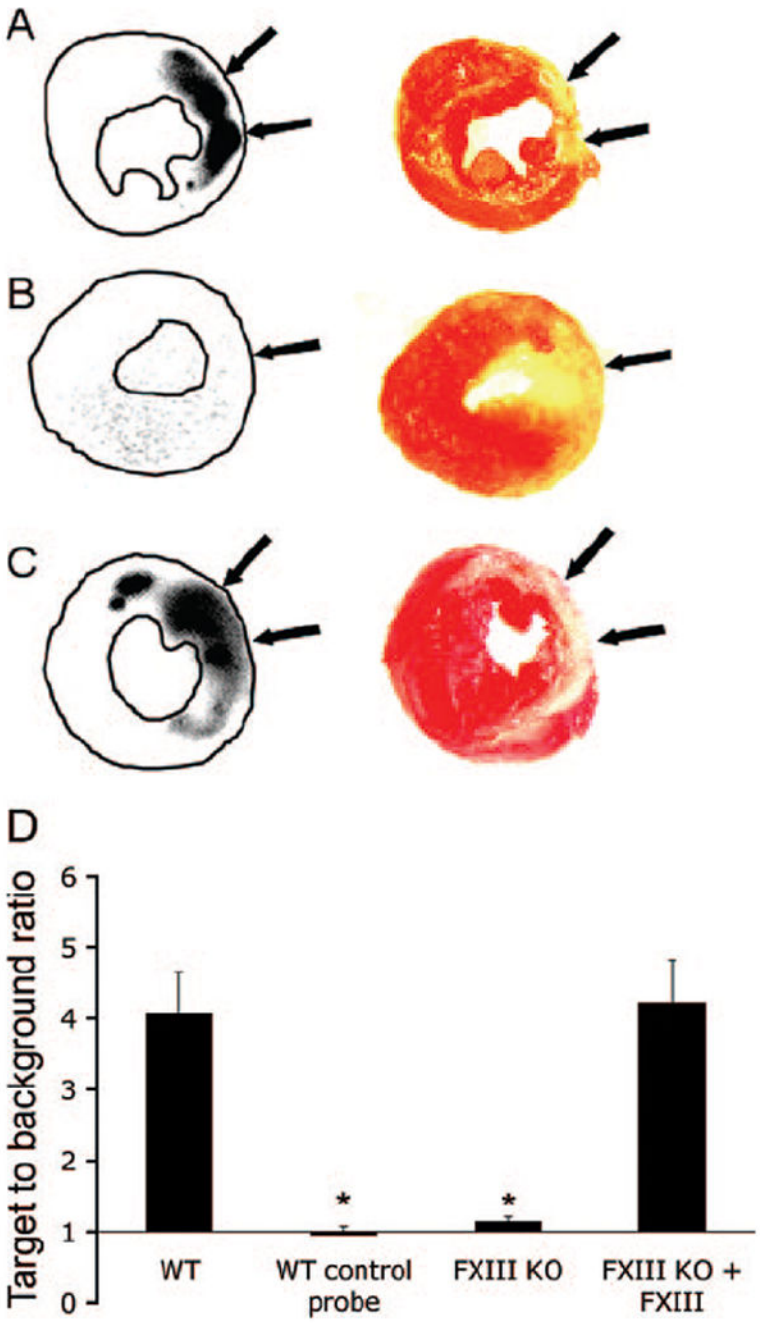

Figure 4.

A to C, Molecular imaging of FXIII activity. Representative autoradiography (left) of myocardial short-axis ring showing remote myocardium and MI (arrows). The contrast agent, which reflects in vivo FXIII activity, distributes to the healing infarct in wild-type mice (A) and FXIII$^{-/-}$ mice that were resubstituted with the enzyme (C). There is no probe accumulation in the infarct of FXIII$^{-/-}$ mice (B), proving the specificity of the FXIII-targeted agent. The pale area of the infarct in the TTC stain (middle) colocalizes with an area of higher FXIII activity in the autoradiography compared with the remote myocardium in wild-type mice (A) and reconstituted FXIII$^{-/-}$ mice (C) but not in FXIII$^{-/-}$ mice (B). D,

TBR (infarct–noise)/(remote myocardium–noise) measured in 3 to 4 myocardial rings of wild-type mice injected with FXIII-targeted ^{111}In, wild-type mice after injection of untargeted ^{111}In (control probe), FXIII$^{-/-}$ injected with FXIII-targeted ^{111}In, and reconstituted FXIII$^{-/-}$ injected with FXIII-targeted ^{111}In. In the wild-type mice injected with the active probe, there is a significantly higher TBR, consistent with raised FXIII activity within the healing infarct. The absent signal in the control experiments (wild-type control probe and FXIII$^{-/-}$ active probe) proves the specificity of the agent. Resubstitution of the enzyme in knockouts (KO) reestablishes a high TBR, which demonstrates the efficacy of the intravenous replenishment. *P<0.05 vs wild-type active probe and reconstituted FXIII$^{-/-}$.

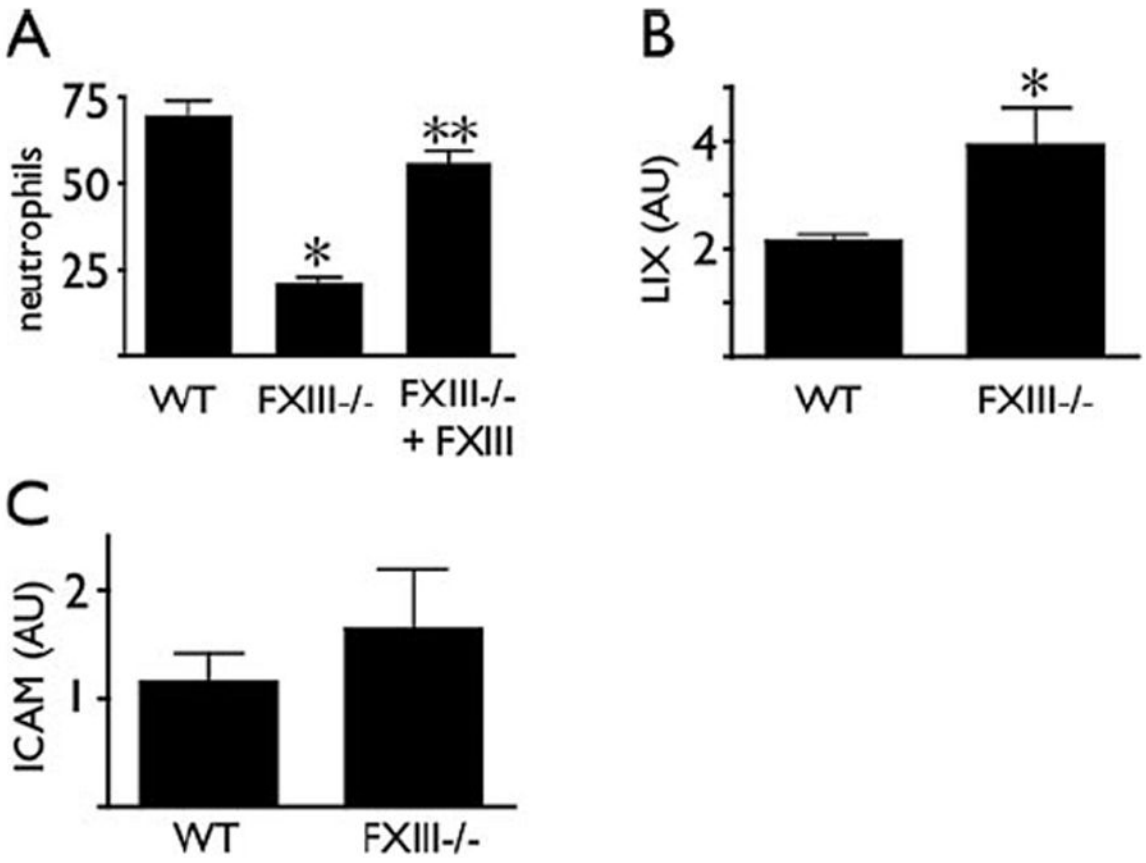

Figure 5.

A, Leukocyte count in border zone of the infarct scar in wild-type (WT) and FXIII$^{-/-}$ mice 2 days after MI. Migration of neutrophils into the ischemic region was significantly diminished in FXIII$^{-/-}$ mice and reestablished to some degree by resubstitution of FXIII. *P<0.01 vs wild-type MI and reconstituted FXIII$^{-/-}$; **P<0.05 vs wild-type MI. B, Two days after MI, the level of LIX mRNA was significantly higher in FXIII$^{-/-}$ mice. Numbers are given in arbitrary units in B and C. *P<0.05 vs wild-type MI. C, No significant difference in ICAM mRNA levels was found.

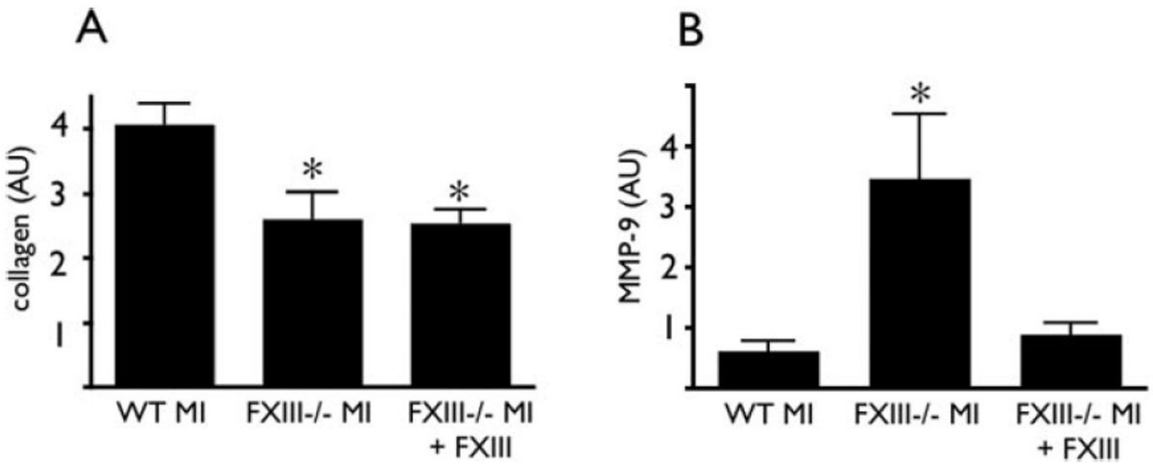

Figure 6.

A, Collagen type 1 mRNA levels were diminished in FXIII$^{-/-}$ mice 2 days after MI. Resubstitution of FXIII did not normalize collagen synthesis. *P<0.01 vs wild-type (WT) MI. B, Myocardial tissue level of MMP-9 was 7-fold higher in FXIII$^{-/-}$ mice 2 days after MI. Resubstitution led to significantly lower levels. *P<0.05 vs wild-type MI and reconstituted FXIII$^{-/-}$ MI.

Table

MRI Data After MI of Wild-Type, Homozygous FXIII$^{-/-}$, and Reconstituted FXIII$^{-/-}$ Mice

	MI Wild-Type	MI FXIII$^{-/-}$	MI Reconstituted FXIII$^{-/-}$
Day 1 after MI	n=16	n=8	n=10
Scar thickness, mm	0.80±0.05	0.80±0.05	0.75±0.05
LV mass, mg	101±4.8	97.8±7.6	108.7±6.0
End-diastolic volume, μL	61.3±5.9	57.9±7.0	62.6±5.7
EF, %	54.7±4.7	43.6±6.1	48.1±5.8
Cardiac output, mL/min	13.8±1	9.3±1.7*	10.2±0.85
End-diastolic wall thickness, mm	0.90±0.05	0.90±0.05	0.10±0.05
Day 7 after MI	n=12	n=0	n=8
Scar thickness, mm	0.70±0.05	…	0.40±0.05*
LV mass, mg	104.5±7.4	…	113.2±4.0
End-diastolic volume, μL	71.3±12.3	…	88.2±8.1
EF, %	54±5	…	48±5
Cardiac output, mL/min	16.2±1.2	…	16.8±1.4
End-diastolic wall thickness, mm	0.95±0.05	…	0.90±0.05
Day 42 after MI	n=10	n=0	n=6
Scar thickness, mm	0.55±0.05	…	0.20±0.05*
LV mass, mg	122.0±7.1	…	123.6±9.5
End-diastolic volume, μL	86.1±6.9	…	127.3±12.4*
EF, %	52±5	…	43±7
Cardiac output, mL/min	17.2±0.7	…	20.4±3.2
End-diastolic wall thickness, mm	1.05±0.05	…	1.00±0.05
MI size	27±4%	…	27±3%

Values are mean±SEM.

*P<0.05 vs wild-type MI.