EXPEDITED PUBLICATIONS

Effect of Intravenous FX06 as an Adjunct to Primary Percutaneous Coronary Intervention for Acute ST-Segment Elevation Myocardial Infarction

Results of the F.I.R.E. (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) Trial

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Objectives   The purpose of this study was to investigate whether FX06 would limit infarct size when given as an adjunct to percutaneous coronary intervention.

Background   FX06, a naturally occurring peptide derived from human fibrin, has been shown to reduce myocardial infarct size in animal models by mitigating reperfusion injury.

Methods   In all, 234 patients presenting with acute ST-segment elevation myocardial infarction were randomized in 26 centers. FX06 or matching placebo was given as intravenous bolus at reperfusion. Infarct size was assessed 5 days after myocardial infarction by late gadolinium enhanced cardiac magnetic resonance imaging. Secondary outcomes included size of necrotic core zone and microvascular obstruction at 5 days, infarct size at 4 months, left ventricular function, troponin I levels, and safety.

Results   There were no baseline differences between groups. On day 5, there was no significant difference in total late gadolinium enhanced zone in the FX06 group compared with placebo (reduction by 21%; p = 0.207). The necrotic core zone, however, was significantly reduced by 58% (median 1.77 g [interquartile range 0.0, 9.09 g] vs. 4.20 g [interquartile range 0.3, 9.93 g]; p < 0.025). There were no significant differences in troponin I levels (at 48 h, −17% in the FX06 group). After 4 months, there were no longer significant differences in scar size. There were numerically fewer serious cardiac events in the FX06-treated group, and no differences in adverse events.

Conclusions   In this proof-of-concept trial, FX06 reduced the necrotic core zone as one measure of infarct size on magnetic resonance imaging, while total late enhancement was not significantly different between groups. The drug appears safe and well tolerated. (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury [F.I.R.E.]; NCT00326976)   (J Am Coll Cardiol 2009;53:720–9) © 2009 by the American College of Cardiology Foundation

Immediate reopening of acutely occluded coronary arteries by primary percutaneous coronary intervention (PCI) is the treatment of choice to salvage ischemic myocardium in the setting of ST-segment elevation myocardial infarction (STEMI) (1). However, the sudden reinitiation of blood flow can lead to further endothelial and myocardial damage (reperfusion injury) (2). Although reperfusion injury has been recognized for >30 years, its underlying mechanism is not fully known. Membrane damage induced by oxygen radicals, intracellular calcium overload, opening of mitochondrial permeability transition pores, and tissue injury related to infiltration of white blood cells may play crucial roles (3,4).

Most pharmacological strategies to mitigate reperfusion injury have not been successful in clinical trials. These include beta-blockers, glucose-insulin-potassium infusion, sodium-hydrogen exchange inhibitors, adenosine, calcium-channel blockers, potassium–adenosine triphosphate channel openers, antibodies against leukocyte adhesion molecules, and oxygen free radical scavengers (5). Recent trials, with a monoclonal antibody directed against complement C5 (6) and a protein kinase C inhibitor (7), were disappointing. Adenosine reduced anterior infarct size when used at high doses (8); however, a review of 5 trials (including the AMISTAD [Acute Myocardial Infarction Study of Adenosine] I and II studies) failed to show significant benefit (5). In a report of 2 studies, atrial natriuretic peptide reduced infarct size as estimated by creatine kinase (9). The work in this field has been comprehensively summarized (5). In controlled trials, post-conditioning (10) and cyclosporine (11) reduced infarct size.

FX06 is a naturally occurring peptide derived from human fibrin (sequence $B\beta_{15-42}$) (12). It is a 28 amino acid cleavage product of fibrin, which is released from fibrin E1 fragments by plasmin and is an indicator of fibrinolytic activity (13). In animal models for coronary ischemia/reperfusion, FX06 reduced infarct sizes (14–16). In global reperfusion injury, such as hemorrhagic shock followed by resuscitation, FX06 reduced organ damage (17). FX06 competes with E1 fragments of fibrin for binding to an endothelial specific molecule, VE-cadherin, thereby acting as an anti-inflammatory (14), and it signals through VE-cadherin, thereby reducing plasma leakage into tissues (P. Petzelbauer, personal communication, June 2008).

A phase I study in humans showed that FX06 was safe and well tolerated in doses of 50 to 1,350 mg (15). The plasma half-life ranged from 11 to 17 min. Our aim in the F.I.R.E (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) study was to evaluate the cardioprotective efficacy of FX06 as an adjunct to primary PCI in patients with acute STEMI.

Methods

Patients. Between October 2006 and March 2008, we conducted a randomized, double-blind, placebo-controlled study. The rationale and study design have been published (18). Briefly, this study included patients with a first STEMI from a single culprit lesion and no other serious comorbidities undergoing primary PCI as indicated per standard of care (Fig. 1) (1). Additional inclusion criteria were presentation within 6 h of onset of symptoms, >2 mm ST-segment elevation in at least 3 electrocardiogram (ECG) leads, and a single culprit lesion with TIMI (Thrombolysis In Myocardial Infarction) flow grade 0/1 in the infarct-related artery. Patients with prolonged ischemic symptoms, cardiogenic shock, peripheral vascular disease, and history of kidney (serum creatinine >250 μmol/l) or liver dysfunction were excluded. Written informed consent was obtained

Abbreviations and Acronyms

ANCOVA = analysis of covariance

CMR = cardiac magnetic resonance imaging

ECG = electrocardiogram

IQR = interquartile range

LGE = late gadolinium enhanced

MVO = microvascular obstruction

PCI = percutaneous coronary intervention

STEMI = ST-segment elevation myocardial infarction

Inclusion criteria
First myocardial infarction with PCI indicated per standard of care, within 6 hrs of onset of symptoms, >2 mm ST-segment elevation in at least 3 leads in ECG, single index lesion with TIMI flow 0/1

↓

Informed consent obtained, study drug (active or placebo) in 2 bolus injections 10 min apart at the time of reperfusion during the PCI procedure.

↓

In-hospital monitoring
CMR with LGE 5 days after PCI, adverse events, rhythm monitoring, laboratory.

↓

Follow-up
Telephone contacts at months 1, 2 and 3
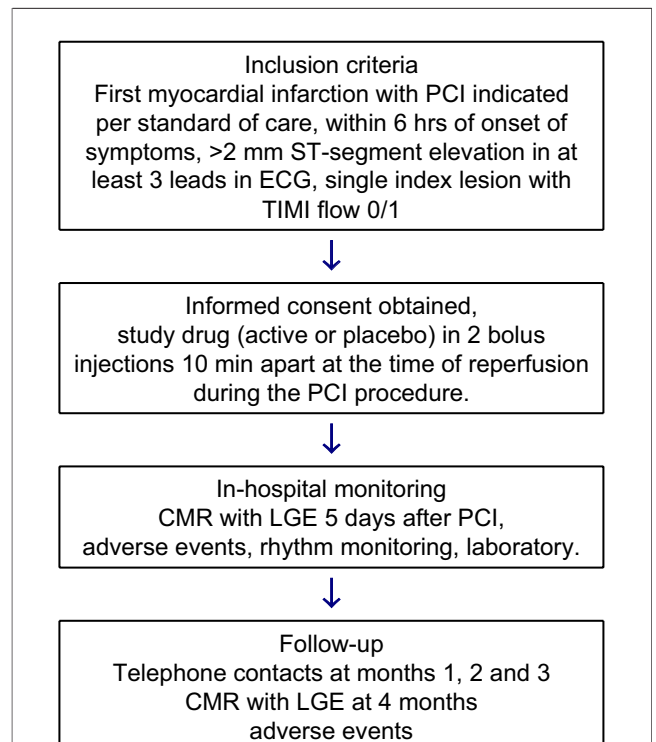CMR with LGE at 4 months
adverse events

Figure 1   **Study Design**

CMR = cardiac magnetic resonance imaging; ECG = electrocardiogram; LGE = late gadolinium enhanced; PCI = percutaneous coronary intervention; TIMI = Thrombolysis In Myocardial Infarction.

Table 1	Patient Characteristics: Intent-to-Treat Population (n = 227)	
	FX06 (n = 110)	**Placebo (n = 117)**
Age, yrs, mean (SD)	59.8 (11)	59.6 (11)
Men	80 (73%)	95 (81%)
Body mass index, kg/m², mean (SD)	27.6 (4.2)	27.4 (3.8)
Hypercholesterolemia	15 (14%)	19 (16%)
Diabetes mellitus	13 (12%)	12 (10%)
Hypertension	44 (40%)	45 (39%)
Regular smoker	48 (44%)	58 (50%)
Family history of coronary artery disease	20 (18%)	20 (17%)
Infarct location		
Anterior	50 (46%)	55 (47%)
Nonanterior	59 (54%)	61 (52%)
Time to PCI, h, median (IQR)	3.1 (2.2–4.5)	3.1 (2.1–4.4)
<3 h	53 (48%)	55 (47%)
At least 3 h	55 (50%)	60 (51%)
TIMI flow before PCI (grade)		
0	90 (82%)	103 (88%)
1	19 (17%)	14 (12%)
2 or 3	1 (1%)	0 (0%)
TIMI flow after PCI (grade)		
0 or 1	0 (0%)	3 (3%)
2	6 (6%)	8 (7%)
3	103 (94%)	105 (90%)
Presence of collaterals		
Yes	26 (24%)	20 (17%)
No	82 (75%)	95 (81%)
Systolic BP at screening, mm Hg, mean (SD)	136 (25)	139 (24)
Diastolic BP at screening, mm Hg, mean (SD)	83 (14)	81 (17)
Concomitant medication		
Acetylsalicylic acid	103 (94%)	111 (95%)
Clopidogrel	110 (100%)	117 (100%)
Beta-blockers	110 (100%)	116 (99%)
Agents acting on RAS	100 (91%)	111 (95%)
Intravenous GP IIb/IIIa inhibitors	59 (54%)	64 (55%)

BP = blood pressure; GP = glycoprotein; IQR = interquartile range; PCI = percutaneous coronary intervention; RAS = renin-angiotensin system; TIMI = Thrombolysis In Myocardial Infarction.

from all patients. All patients were followed up clinically for 4 months.

Procedures. Eligible patients were randomly assigned to 1 of the 2 treatment groups using a computer-generated randomization scheme. FX06 (400 mg) was administered in 2 intravenous bolus injections of 200 mg each during PCI, the first immediately before the guidewire passed the occlusion and the second 10 min (±5 min) later (Fig. 1). Concomitant therapies were allowed except for thrombolytics and adenosine (Table 1). Infarct size was measured at 5 days (range 5 to 7 days) and 4 months after PCI using late gadolinium enhanced (LGE) cardiac magnetic resonance imaging (CMR).

The CMR examination involved an ECG-triggered acquisition of a stack of short-axis slices covering the entire left ventricle from the base to the apex using a state-of-the-art, steady-state, free-precession pulse sequence. The imaging parameters were as follows: slice thickness of 8 mm, no gap between slices, temporal resolution <50 ms, matrix 224 to 256 × 224 to 256 using magnetic resonance scanners from all major vendors operating at 1.5 Tesla. In addition, long-axis 2-, 3-, and 4-chamber views were acquired with the same parameter settings. Then, a contrast medium dose of 0.25 mmol/kg was used (19,20). After 20 min, ECG-triggered segmented inversion-recovery gradient echo images were acquired in the same slice orientations. The dose of contrast medium was chosen to expand the window for viability imaging, facilitate data acquisition, and account for the multiple centers (with variable experience in CMR).

To acquire LGE images, inversion time was adjusted in each patient to null the signal of normal myocardium (19,21). The LGE imaging was obtained in mid to late diastole to minimize motion by adjusting the trigger delay (~450 ms). Other imaging parameters were as follows: slice thickness of 8 mm without gap, matrix 192 to 256 × 192 to 256, and flip angle 20°.

Before participation, centers were asked to submit a series of consecutive CMR images for quality control purposes. All images were analyzed at the central site by a single, blinded, experienced CMR reader followed by blinded review by a level III CMR expert (P.B. or J.B.). In the case of discordance between the primary and expert reviewer, consensus was reached. Intraobserver variability was assessed in a subset of 40 randomly chosen studies and the intraclass correlation was 0.85 for 26 studies from day 5. That is higher than the critical value of 0.7 that was fixed before this examination (22).

Using short-axis cine loops of the left ventricle from base to apex, endocardial and epicardial contours were traced manually in each slice at end systole and end diastole to measure areas. Left ventricular end-systolic and -diastolic volumes, ejection fraction, and myocardial mass were calculated in a standard fashion (23).

All short-axis slices were assessed for areas of signal enhancement. Areas with a very bright signal, namely, white areas, were designated as the necrotic core zone, where the majority of myocytes are damaged beyond repair (24,25). This necrotic bright area often contains a dark core where there is no penetration of contrast medium, designated as the microvascular obstruction (MVO) zone (26,27). The MVO zones were included in the measurement of the necrotic core zone. In addition, an infarct border zone was detected with signal intensity higher than in normal myocardium with "nulled" signal, but lower than in the necrotic core zone. This infarct border zone characterizes an injury zone with a variable portion of irreversibly damaged myocytes (25,28). All 3 zones were separately measured, and total volumes were obtained by summing compartments of all slices covering the entire left ventricle. All volumes and masses were also expressed as a percentage of left ventricular myocardial mass.
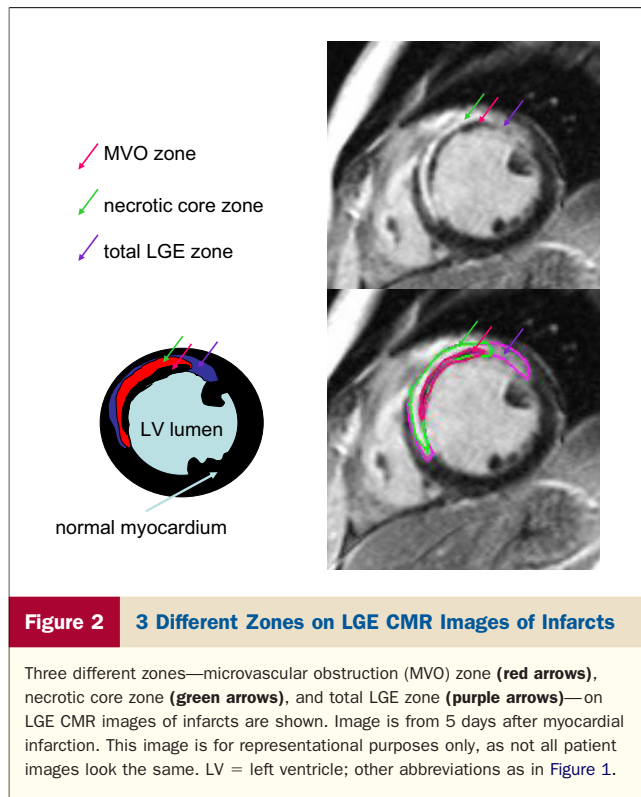
Figure 2   **3 Different Zones on LGE CMR Images of Infarcts**

Three different zones—microvascular obstruction (MVO) zone **(red arrows)**, necrotic core zone **(green arrows)**, and total LGE zone **(purple arrows)**—on LGE CMR images of infarcts are shown. Image is from 5 days after myocardial infarction. This image is for representational purposes only, as not all patient images look the same. LV = left ventricle; other abbreviations as in Figure 1.

The pre-defined primary end point was the difference between FX06- and placebo-treated patients in infarct size, defined as the total LGE mass (necrotic core, MVO, and infarct border zones) after 5 days (Fig. 2). Secondary end points were prevalence of MVO zone at 5 days, as well as left ventricular function, troponin I levels, and safety at 5 days and 4 months.

Statistical analysis. Sample size calculations for the primary end point were based on an expected infarct size of 18% of the left ventricle in the placebo group. With an expected reduction of 25% and a common SD of 11%, 95 patients in each group were required to detect a 4.5% treatment difference with a 2.5% 1-sided significance and 80% power.

The primary end point was analyzed using an analysis of covariance (ANCOVA), with treatment, location of infarct (anterior/nonanterior), TIMI flow grade 0/1 before PCI (assessed in a blinded fashion by the investigator), presence of collaterals (assessed in a blinded fashion by the investigator using the Rentrop scale), time between onset of symptoms and first treatment injection (>3 or <3 h), and interactions between treatment and the other factors as control variables. Missing values for control variables were replaced by pre-established rules that represent a worst-case scenario: missing ECG at baseline was replaced with ECG shortly after PCI to determine location of infarct, missing TIMI flow grade before PCI was set as TIMI flow grade 1, missing data on presence of collaterals was set as collaterals present, and missing data on time between occurrence of symptoms and first injection of therapy was set as >3 h.

The final ANCOVA model was derived using a stepwise procedure. The complete model was analyzed, and all factors with an impact on the primary variable (p < 0.2) and the treatment were pre-selected. Using the pre-selected factors, a stepwise backward selection was performed until all remaining factors other than treatment showed a p value <0.1. This final model was used to calculate the treatment effects and test for the superiority of FX06 using adjusted means. In addition, the 1-sided 97.5% confidence interval was calculated.

The values of the zones of damaged left ventricular mass and the other left ventricular function measurements were compared between treatments using a 1-sided Wilcoxon rank sum test at the 2.5% level (i.e., significant at p < 0.025) after 5 days and 4 months. Mean, SD, median, and interquartile range (IQR) were derived but only the latter 2 are reported, as we used a nonparametric testing approach. For biomarker levels, mean and SD are given. For safety outcomes, frequencies were calculated and compared between treatments by the Fisher exact test at the 2-sided 5% level. All efficacy results are reported for the intent-to-treat population, and adverse events are reported for the safety population. All p values for secondary analyses are only interpreted in an exploratory manner.

Results

As shown in Figure 3, 252 patients were screened, and 234 were randomized at 26 European centers. Thirteen patients were excluded who had no single index lesion and complete occlusion (TIMI flow grade 0/1) of 1 target vessel, 2 patients in whom PCI was not possible or indicated, 1 patient whose myocardial infarction was not the first, 1 patient who needed coronary artery bypass graft surgery, and 1 patient who had an implanted device.

There were no significant differences between the groups in patient characteristics (Table 1). Median time from symptom onset to treatment was 3.1 h in both groups. Approximately 45% of patients had anterior infarcts. Essentially, all patients received aspirin, clopidogrel, and beta-blockers, and nearly one-half received glycoprotein IIb/IIIa inhibitors.

Overall, 94 patients treated with FX06 and 104 patients treated with placebo completed the trial, 78 patients with FX06 and 89 patients with placebo, respectively, had repeat CMR studies (Fig. 3). For analysis by the ANCOVA model, 93 patients treated with FX06 and 103 patients treated with placebo were included (1 patient in each group was lost due to protocol violations). The location of infarct (p < 0.001) and the presence of collaterals (p = 0.029) influenced infarct size. The adjusted means for the total LGE zone (in % of left ventricle) were not different between groups (19.71% for FX06 vs. 19.79% for placebo, 97.5% confidence interval: −4.08% to ∞; p = 0.4849). However, based on data distribution in total LGE zone, outliers may have had an influence on results. A box plot analysis
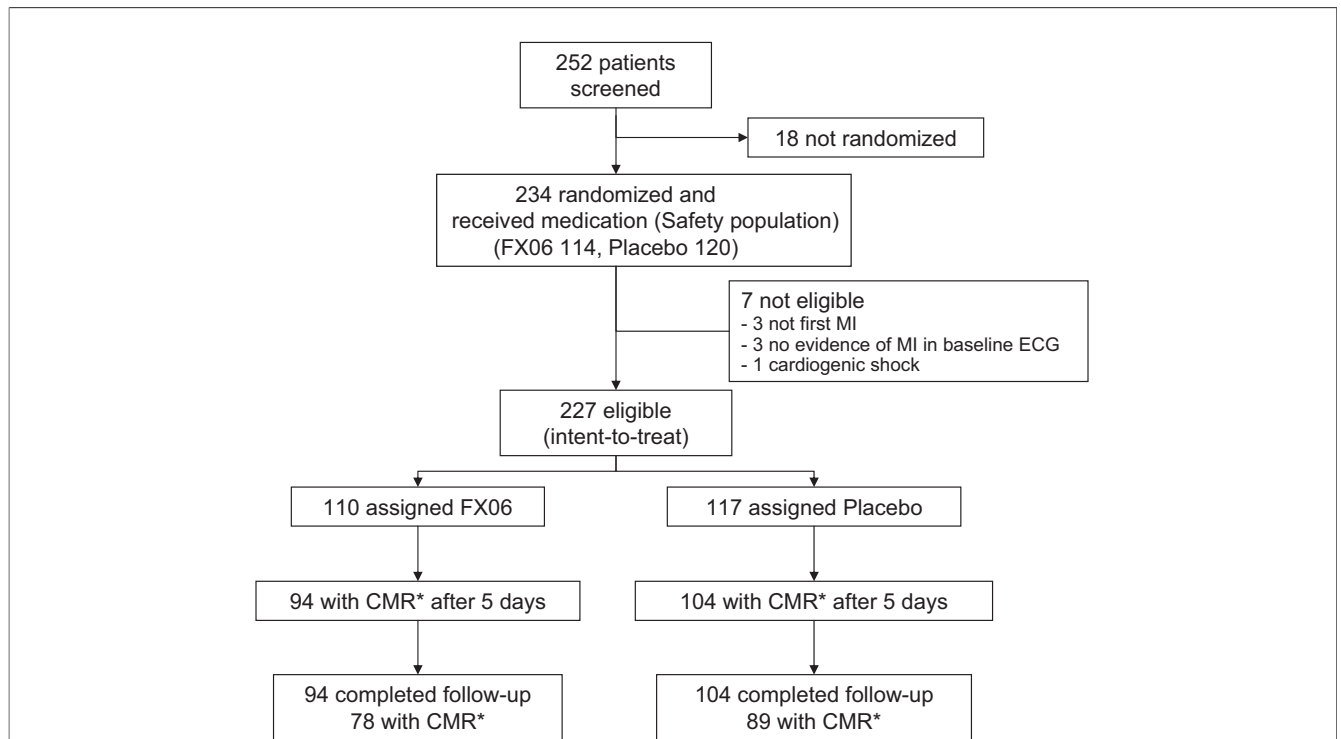
Figure 3   Trial Profile

*Number of patients for whom infarct sizes as measured by cardiac magnetic resonance imaging (CMR) are available; the number of patients for whom other results are available may differ. ECG = electrocardiogram; MI = myocardial infarction.

revealed 4 outliers with an infarct size >60%: 1 in the FX06 group and 3 in the placebo group. Therefore, in the following data analyses, a nonparametric approach was used.

The results of the CMR measures for both 5 days and 4 months are shown in Table 2. As there was a slight imbalance

of total left ventricular mass between the treatment groups after 5 days, both the absolute weight of the zone and the normalization with respect to the mass of the left ventricle are given. After 5 days, the median value of the total LGE zone was not significantly different between groups (reduced by 21% for

Table 2   Results for Measures of Cardiac Magnetic Resonance Imaging After 5 Days

	FX06 (n = 94)	Placebo (n = 104)	Treatment Effect (1-Sided p Value)
After 5 days			
Total LV mass, g	145.0 (121.5–167.4)	154.3 (129.2–174.1)	0.185*
Total LGE zone, g	21.7 (8.3–47.1)	27.3 (11.7–44.9)	−21% (0.207)
Total LGE zone, % of LV	16.1 (6.1–29.1)	17.3 (9.3–27.1)	−6.5% (0.311)
Necrotic core zone, g	1.77 (0–9.09)	4.20 (0.3–9.93)	−58% (0.019)
Necrotic core zone, % of LV	1.37 (0–6.12)	2.80 (0.18–6.22)	−51% (0.023)
LV ejection fraction, %	47.2 (40.3–55.4)	46.8 (41.2–53.0)	+0.8% (0.455)
Cardiac output, ml/min	4,406 (3,662–5,252)	4,474 (3,910–5,282)	−1.5% (0.749)
After 4 months	(n = 78)	(n = 89)	
Total LV mass, g	134.7 (116.7–158.4)	135.4 (110.1–156.5)	0.907*
Total LGE zone, g	15.4 (5.7–38.3)	19.3 (7.5–31.4)	−20% (0.363)
Total LGE zone, % of LV	11.4 (5–24)	14 (6.9–23.9)	−19% (0.226)
Necrotic core zone, g	2.31 (0–9.06)	2.67 (0.33–7.00)	−14% (0.328)
Necrotic core zone, % of LV	1.67 (0–6.06)	2.01 (0.21–4.82)	−17% (0.289)
LV ejection fraction, %	49.6 (42.4–56.4)	48.6 (42.4–55.5)	+2.1% (0.306)
Cardiac output, ml/min	4,563 (3,717–5,325)	4,503 (3,692–5,221)	+1.3% (0.314)

Data shown as median (interquartile range). *2-sided p value.
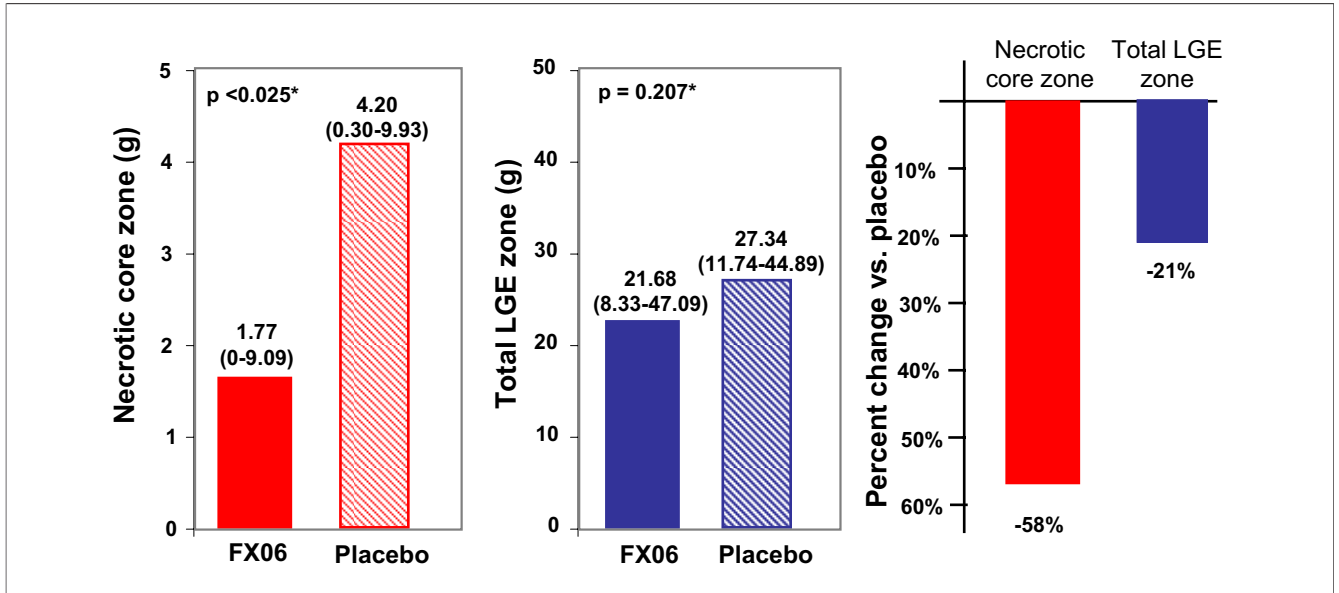LGE = late gadolinium enhanced; LV = left ventricle.

Figure 4   Reduction of Mass of Infarct Size, Necrotic Core, and Total LGE Zones

The mass of affected tissue and median values with 25th and 75th percentile range,
5 days after myocardial infarction, is shown. *Wilcoxon rank sum test. LGE = late gadolinium enhanced.

tissue weight in FX06). MVO was present in 27.6% of FX06 patients versus 37.5% of placebo patients (p = 0.093). A statistically significant difference was seen in both the absolute mass (1.77 g vs. 4.2 g, p < 0.025) (Fig. 4) and the relative size (1.37% vs. 2.8%, p < 0.025) (Fig. 5) of the necrotic core zone with FX06 compared with placebo. The mass and size of this zone was more than halved (−58% and −51%, respectively) (Fig. 4) with FX06 treatment. There

were no major differences between treatments regarding other CMR parameters (Table 2).

After 4 months, CMR revealed no significant differences in total LGE zones (20% reduction in tissue mass or 19% reduction in % of left ventricle in the FX06 group) or necrotic core zones (14% reduction in tissue mass or 17% reduction of % of left ventricle in the FX06 group). Because ~15% of patients did not have a repeat CMR at 4 months,
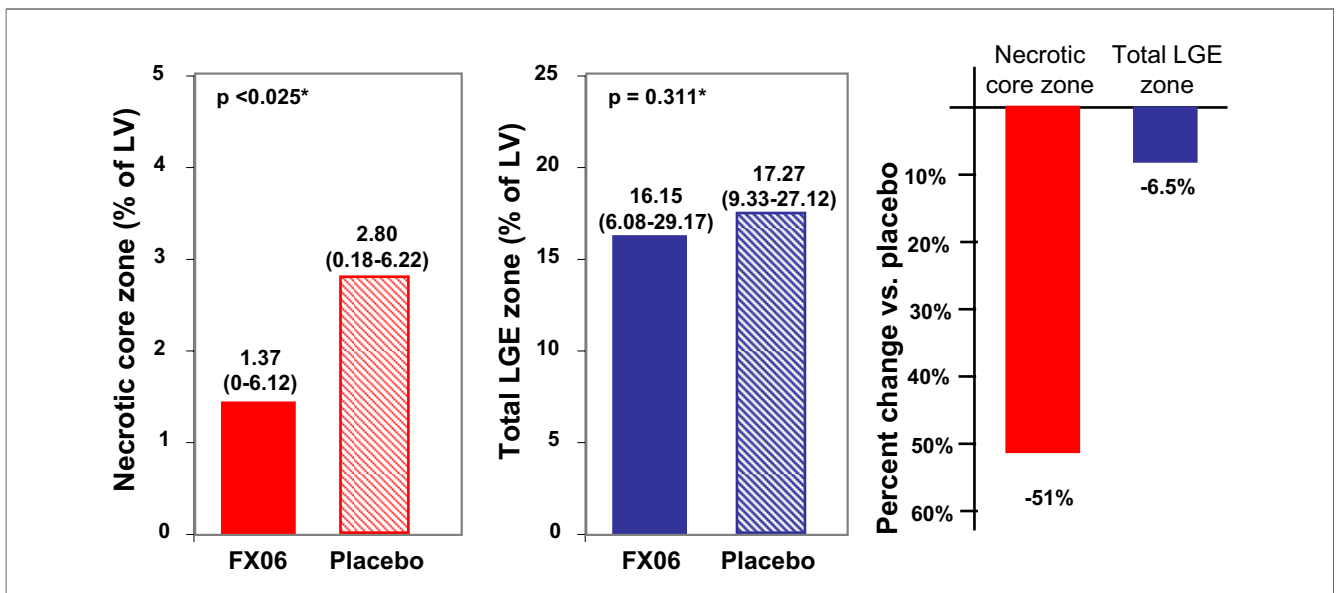

Figure 5   Reduction of Necrotic Core and Total LGE Zone Size as % of LV

Percent of left ventricle (LV) and median values with 25th and 75th percentile ranges
5 days after myocardial infarction is shown. *Wilcoxon rank sum test. LGE = late gadolinium enhanced.

Table 3	Safety Data and Clinical Outcomes		
	FX06 (n = 114)	Placebo (n = 120)	2-Sided p Value
Treatment-emergent adverse events	100 (87.7%)	97 (80.8%)	0.157
Treatment-emergent adverse events leading to premature discontinuation	7 (6.1%)	7 (5.8%)	1.0
Treatment-emergent serious adverse events	37 (32.5%)	31 (25.8%)	0.314
Treatment-emergent serious adverse events leading to premature discontinuation	7 (6.1%)	6 (5.0%)	0.78
Death	3 (2.6%)	5 (4.2%)	0.723
Cardiac death	2 (1.8%)	5 (4.2%)	0.447
Composite of cardiac death and new onset heart failure/pulmonary edema	5 (4.4%)	8 (6.7%)	0.572

Data presented as n (%) of patients.

this result might not be comparable to the early end point owing to disparity between the treatment groups in the characteristics of patients without repeat measurements.

Pre-specified subgroup analyses were performed using the suspected control variables for selection (i.e., anterior vs. nonanterior infarcts, time to balloon <3 h vs. >3 h). Although infarct location, time to presentation, and presence of collaterals had some influence on the result, there was no deviation from the overall effect in the subgroups. Measurements of biomarkers of myocyte necrosis revealed no significant differences in the placebo group and the FX06 group in mean troponin I values at 24 h (55.4 ng/ml [SD 42.8 ng/ml] and 49.6 ng/ml [SD 39.7 ng/ml], p = 0.195), mean troponin I at 48 h (26.4 ng/ml [SD 22.8 ng/ml] and 21.8 ng/ml [SD 17.1 ng/ml], p = 0.091), and creatine kinase–myocardial band activity at 90 min after PCI (4.34 μkat/l [SD 3.79 μkat/l] and 3.65 μkat/l [SD 3.06 μkat/l]).

Safety data and clinical outcomes at 4 months are shown in Table 3. There were no differences in treatment-related adverse events between groups. Seven adverse events in each group resulted in premature discontinuation. In the FX06 group, these included 6 events assessed as unrelated to the study drug (malignant neoplasm, coronary artery disease with mitral valve incompetence, circulatory collapse, cardiac arrest, cardiac failure, and metastatic neoplasm), as well as 1 event assessed as possibly related to the study drug (cardiogenic shock). The number of cardiovascular-related serious adverse events was numerically lower in the FX06 group than in the placebo group (n = 21 vs. 29) (Table 4).

There was no increased risk of life-threatening arrhythmias (ventricular fibrillation/tachycardia), conduction disorders (third-degree atrioventricular block), stent thrombosis, or hypotension. There were 3 cases of hypotension in the placebo group and 4 (1 orthostatic) in the FX06 group.

All-cause mortality was 2.6% (3 of 114) in the FX06 group and 4.2% (5 of 120) in the placebo group. There were 3 cases of nonfatal heart failure or pulmonary edema in the FX06 group and 5 in the placebo group. All deaths, except 1 in the FX06 group (metastatic prostate cancer not identified at study inclusion), were due to cardiac causes. One death due to bradycardia with pulseless electric activity occurred 13 days after PCI (placebo group). The other 6

cardiac deaths occurred between 1 and 4 days after PCI, primarily due to acute heart failure and cardiogenic shock.

Discussion

The F.I.R.E study, conducted as an exploratory proof-of-concept study among patients with STEMI treated with primary PCI, did not meet the primary end point of LGE zone reduction. However, the necrotic core zone—which might have been a more reasonable definition of "infarct size"—was significantly reduced, suggesting that FX06 tends to reduce at least one, and perhaps the most important, measure of infarct size as predicted from pre-clinical data (14–16). Other measurements and observations were negative in statistical terms; however, they all showed a numerically consistent trend in favor of FX06.

Although a variety of strategies have suggested reductions of reperfusion injury in animal models, most have not produced significant myocardial protection in humans. These failed interventions have been extensively reviewed by Dirksen et al. (5). The DELTA-MI (Direct Inhibition of d-Protein Kinase C Enzyme to Limit Total Infarct Size in Acute Myocardial Infarction) study with protein kinase C inhibitor KAI9803 delivered equivocal results (5,7). Atrial natriuretic peptide (9), post-conditioning (10), and cyclosporine (11) have been associated with reduced infarct size.

Table 4	Cardiovascular-Related Serious Adverse Events	
	Number of Events	
	FX06	Placebo
Cardiac death	2	5
Heart failure (nonfatal)/pulmonary edema	3	5
VT/VF, life-threatening arrhythmia	5	7
Atrioventricular block third-degree	2	2
Acute coronary syndrome (STEMI/non-STEMI)	3	4
Acute stent thrombosis	2	2
Revascularization	2	2
Intraventricular thrombus	1	1
Noncardiac major vascular events	1*	1†
Total	21	29

*Acute peripheral ischemia. †Stroke.
STEMI = ST-segment elevation myocardial infarction; VF = ventricular fibrillation; VT = ventricular tachycardia.

The significant effects on the necrotic core zone with FX06 are likely due to its dual mode of action through VE-cadherin. VE-cadherin regulates traffic of cells and fluids across endothelial barriers from the lumen into tissues (29,30). FX06 competes with fibrin fragments for binding to VE-cadherin, reducing leukocyte migration and tissue inflammation (14). Additionally, FX06 stabilizes endothelial cell junctions by signaling through VE-cadherin, reducing vascular leak and tissue edema (P. Petzelbauer, personal communication, July 2008); both effects may improve capillary flow. Improvement of capillary flow may explain the slightly lower incidence of MVO in FX06 patients. In this context, the presence of MVO has been found to be a strong predictor of unfavorable patient outcome (27).

The periprocedural dosing of FX06 is critical to achieve maximum plasma levels at the time of reperfusion, which is considered a trigger event for injury early after blood flow is re-established (31). The dosing of FX06 was based on pharmacokinetics; with a plasma half-life of 11 to 17 min, 2 injections within 10 min would maintain therapeutic levels for up to 45 min (15). Administration at 60 min after reperfusion did not reduce infarct size in animal models (16).

The FX06 treatment was well tolerated in this study, with no unexpected drug toxicity compared with placebo. On the contrary, the composite outcome of cardiac death and new-onset heart failure/pulmonary edema was slightly lower in the FX06 group (4.4%, 5 of 114) than in the placebo group (6.7%, 8 of 120). Although the F.I.R.E. study was not powered to detect a significant difference in clinical outcomes, it is noteworthy that the incidences of cardiac death, all serious adverse cardiac events, and new-onset heart failure were all in favor of the FX06 treatment.

The primary end point showed a trend toward smaller infarct size but did not achieve statistical significance, and that may be due to an inadequate sample size. The sample size was calculated based on an SD of 11% associated with a reduction in infarct size of 25%, whereas the observed SD in the data was 16% and the reduction was 21%. That leads to a post-hoc power of 59%, with an infarct reduction of 25%. With the lower reduction, the statistical power was even lower.

Infarct size measured at hospital discharge predicted short-term (32) and 2-year mortality (33). Until recently, studies correlating infarct size with clinical outcomes used single-photon emission computed tomography, but CMR is now considered the most accurate modality (34,35). A large study evaluating the prognostic value of CMR in 1,493 patients found that age, left ventricular ejection fraction, and extent of scar tissue were independent predictors of all-cause mortality (36). These findings were confirmed in a 2-year trial using CMR in 122 patients (37). As in the F.I.R.E. study, CMR was used in a trial to assess global left ventricular function and infarct size after stem cell transfer after successful primary PCI for STEMI (38).

CMR allows high spatial resolution, and the total LGE zone can be differentiated into necrotic core, MVO, and infarct border zones. The prognostic significance of the infarct border zone is unclear (39), but the necrotic core zone undoubtedly represents nonviable myocardium. This zone was where FX06 exerted its single significant effect after 5 days; however, this effect was not significant after 4 months.

The beneficial effects on the necrotic core zone may represent an important outcome. Although the necrotic core regions were small, they were similar to those in the study by Thiele et al. (40). Evidence suggests that when even a small part of the left ventricle is permanently damaged, it can have serious consequences for remodeling (41). Necrosis and scar size quantified by magnetic resonance imaging are documented to predict improvement of regional function after revascularization and prognosis for major cardiovascular events (19,21,27,42–44). That observation is underlined by MVO, which affects an even smaller part of the myocardium, having also been shown to be an independent predictor of adverse patient outcome (41).

Study limitations. Infarct size was quantified manually, although all evaluations were performed in a blinded fashion, and consequently, a subjective bias was unlikely to occur.

Only a single dose of 400 mg during the PCI procedure was used. Even though we were able to demonstrate a significant reduction in the necrotic core zone, further dose-finding studies are required to determine optimal dosing.

Effects of FX06 were significant on the necrotic core zone, but not on the infarct border zone. In previous trials using CMR, the reported values for infarct size were in accordance with published single-photon emission computed tomography values (45). None of the studies using CMR to correlate infarct size with clinical outcomes or studies that measured infarct size for a treatment effect reported the details of the respective zones of necrosis, MVO, or infarct border. Of note, adverse clinical outcomes correlate with infarct size only in large infarcts >12% of the left ventricle in earlier studies (26,27,32). A correlation between necrotic core size and clinical cardiovascular events has not yet been assessed, which limits the interpretation of our results.

Patients were followed for 4 months primarily for safety reasons, looking for cardiac death and major adverse cardiac events. A second CMR at this visit was done to assess whether FX06 treatment had an effect on scar formation, however, it was unlikely that a significant effect would be demonstrated since we did not control for confounding effects and medication during the follow-up period. This was indeed the case: scar mass was numerically but not significantly lower at the 4-month (visit 3) timepoint compared with placebo. The study design did not allow for any estimation of infarct expansion or shrinkage with respect to necrotic core size at 5 days and scar size at 4 months. More importantly, 15% of patients (FX06 n = 14, placebo n = 16) did not return for repeat CMR at 4

months. This included five patients in the placebo group and 2 patients in the FX06 group who died from a cardiac cause; since they probably had large infarctions, their loss to follow-up leads to a distortion. A further distortion is likely since patients without any symptoms post-PCI were lost to follow-up and larger infarcts were over-represented in the FX06-treated group at 4 months. Analysis of only patients with paired CMR showed no difference in infarct size relative to LV mass in patients treated with FX06 at day 5 and 4 months, whereas there appears to be a shrinkage in the placebo patients. However, analysis of completers only introduces another selection bias. It is possible that the infarcts of patients treated with FX06 were already so small that there was not much apparent shrinkage during remodeling (unlike under placebo), but such an interpretation remains speculative.

Conclusions

Although this study did not show a significant reduction in the total LGE zone, it did show a reduction of necrotic core size after injection of FX06 as an adjunct to standard care in STEMI patients undergoing PCI. There were no significant differences in biomarkers of myocardial necrosis and no significant differences in scar size after 4 months, although trends were positive. There was a lower incidence of serious cardiovascular events in the FX06 group, but with numbers too small to prove beneficial clinical effects. These findings warrant further scrutiny in an adequately powered dose-finding and clinical outcome trial.

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Key Words: acute myocardial infarction ▪ ST-segment elevation ▪ percutaneous coronary intervention ▪ reperfusion injury ▪ FX06 ▪ cardiac magnetic resonance imaging.

▶ **APPENDIX**

For a complete list of investigators, please see the online version of this article.

Effect of Intravenous FX06 as an Adjunct to Primary Percutaneous Coronary Intervention for Acute ST-Segment Elevation Myocardial Infarction: Results of the F.I.R.E. (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) Trial

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