

Mangafodipir as a cardioprotective adjunct to reperfusion therapy: a feasibility study in patients with ST-segment elevation myocardial infarction

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Aims

The aim of the present study was to examine the feasibility of applying the catalytic antioxidant mangafodipir [MnDPDP, manganese (Mn) dipyridoxyl diphosphate] as a cardioprotective adjunct to primary percutaneous coronary intervention (pPCI) in patients with ST-segment elevation (STE) myocardial infarction (STEMI). Both MnDPDP and a metabolite (Mn dipyridoxyl ethyldiamine) possess properties as mitochondrial superoxide dismutase mimetics and iron chelators, and combat oxidative stress in various tissues and conditions.

Methods and results

The study tested MnDPDP ($n = 10$) vs. saline placebo ($n = 10$), given as a brief intravenous (i.v.) infusion prior to balloon inflation during pPCI in patients with STEMI. Mangafodipir was well tolerated and did not affect heart rate or blood pressure. Despite longer ischaemic time (205 vs. 144 min, $P = 0.019$) in the MnDPDP group, plasma biomarker releases were identical for the two groups. With placebo vs. MnDPDP, mean STE resolutions were 69.8 vs. 81.9% ($P = 0.224$) at 6 h and 73.1 vs. 84.3% ($P = 0.077$) at 48 h. Cardiac magnetic resonance revealed mean infarct sizes of 32.5 vs. 26.2% ($P = 0.406$) and mean left ventricular (LV) ejection fractions of 41.8 vs. 47.7% ($P = 0.617$) with placebo vs. MnDPDP. More LV thrombi were detected in placebo hearts (5 of 8) than MnDPDP-treated hearts (1 of 10; $P = 0.011$).

Conclusions

Mangafodipir is a safe drug for use as an adjunct to reperfusion therapy. A tendency to benefit of MnDPDP needs confirmation in a larger population. The study revealed important information for the design of a Phase II trial.

Keywords

Cardiac reperfusion injury • Primary PCI • STEMI • Oxidative stress • Mitochondrial superoxide dismutase • Small molecular catalytic antioxidants

Introduction

Reperfusion therapy by thrombolysis or primary percutaneous coronary intervention (pPCI) has reduced infarct size (IS) and improved clinical outcome^{1,2} in acute myocardial infarction (AMI). Still there is a need for improvement,³ particularly in ST-segment elevation (STE) AMI (STEMI). Importantly, the benefit of pPCI may be increased by avoiding reperfusion injuries^{4–6} when blood and oxygen return to the ischaemic myocardium. Of recent concern is a lethal injury, which nonetheless may be ameliorated experimentally by activating endogenous protection in the form of conditioning.^{7,8}

Oxidative stress with uncontrolled release of reactive oxygen species (ROS) and impaired calcium homeostasis followed by inflammation are main causes behind reperfusion injury.^{4–6} Unfortunately, targeting ROS and calcium by drugs have largely failed.³ However, agents acting via nitric oxide (NO) may be more promising by activating survival pathways⁹ when applied within the time limits for viability of the ischaemic myocardium.¹⁰ Also, measures taken after the onset of STEMI, or just prior to pPCI or during the first few minutes thereafter, may reduce the final IS. Thus, stepwise reperfusion¹¹ and inducing postconditioning by remote ischaemia¹² or by drugs¹³ are cardioprotective.^{7,14}

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Essential in cell death and protection are mitochondrial permeability transition pores (MPTPs).^{7,8} Accordingly, inhibition of MPTP opening by cyclosporine A in STEMI treated with pPCI reduced myocardial IS.¹³ If effective on a broader scale, pharmacological activation of postconditioning¹⁴ may become an adjunct principle in the treatment of STEMI and in the prevention of secondary heart failure.

Mangafodipir, manganese (Mn) dipyrrodoxyl diphosphate (MnDPDP, PledPharma AB, Stockholm, Sweden), is a new candidate for trial as a cardioprotective adjunct to reperfusion therapy. Mangafodipir, developed as a water soluble i.v. contrast agent for MRI of liver and pancreas (TeslascanTM, GE Healthcare), has been in diagnostic use for more than a decade¹⁵ until withdrawal due to a low market earning. Mangafodipir is a prodrug for lipid soluble Mn dipyrrodoxyl ethyldiamine (MnPLED).^{16,17} Both agents act as enzyme mimetics and metal-binding agents *in vitro* and represent small molecular catalytic antioxidants as a new type of drugs.^{16,18–20} Preclinical and early clinical studies indicate that MnDPDP or MnPLED protects various tissues, including the myocardium, during severe oxidative stress.^{16,19–23} The present small study was undertaken to obtain information about the feasibility of MnDPDP as a safe and potentially effective adjunct to pPCI in patients with STEMI.

Methods

Twenty patients, admitted to the County Hospital of Jönköping, Sweden, were recruited to MANAMI 1-09²⁴ between December 2009 and June 2013. They all suffered their first attack of AMI and were apparently free from angina pectoris. Inclusion criteria were: STE in two neighbouring leads covering the left ventricular (LV) anterior, inferior or posterior wall; thrombolysis in myocardial infarction group (TIMI) grade 0–1 coronary blood flow (CBF) at angiography; and ischaemic (symptom-to-balloon) time <6 h. The primary endpoint was peak release at 6 h of high-sensitive cardiac troponin T (TnT) and creatine kinase isoenzyme muscle-brain (CK-MB). Secondary endpoints included accumulated biomarker release, resolution of STE, and IS and LV ejection fraction (LVEF) assessed by cardiac magnetic resonance (CMR). Baseline characteristics of patients are provided in Table 1.

The study was performed according to the guidelines of the regional ethical review board in Linköping and the Helsinki declaration. Patients received a study number after admission and were preliminary enrolled after the diagnosis of STEMI was made. They were then informed orally about potential participation in the study. Oral and written consent was obtained after angiographic detection of an occluded coronary artery or artery branch. Patients were then allocated into a treatment group ($n = 10$) or a placebo group ($n = 10$) following a randomized enrolment list kept in numbered envelopes in the PCI laboratory. Allocation was blinded for both the patient and the interventionist. However, the interventionist, but not the (well-hidden) patient, might become unblinded after an i.v. infusion of test substance (yellow MnDPDP vs. colourless saline). Investigators of endpoints were only informed about the study number of each patient and were thus blinded to group allocation.

Immediately prior to balloon inflation, the treatment group received a hand-held i.v. infusion of MnDPDP 2.0 $\mu\text{mol/kg}$ b.w. (0.2 mL/kg b.w.) over 2–5 min, whereas the placebo group received saline (0.2 mL/kg b.w.). The pPCI procedure was regarded as satisfactory when a TIMI grade 2–3 was achieved. Further treatment of both groups was given according to the current ESC guidelines for management of STEMI.²⁵

Blood samples were drawn for analysis of TnT and CK-MB prior to pPCI and intermittently over 48 h thereafter. Troponin T and CK-MB were analysed by conventional techniques and results obtained according to hospital routine. Maximal STE above the isoelectric line was

Table 1 Baseline characteristics

Characteristics	Placebo	MnDPDP
Number	10	10
Age (years) ^a	60.1 \pm 10.8	64.1 \pm 8.7
Male/female	10/0	8/2
Body mass index (%) ^a	25.9 \pm 2.5	27.7 \pm 3.3
Previous CHD	1	0
Smoking	2	4
Hypertension	1	3
Haemoglobin (g/L) ^a	155 \pm 11	152 \pm 14
Creatinine ($\mu\text{mol/L}$) ^a	87 \pm 22	82 \pm 17
Total cholesterol (mmol/L) ^a	4.9 \pm 0.7	5.6 \pm 0.7
LDL-cholesterol (mmol/L) ^a	2.9 \pm 0.7	3.4 \pm 0.6
Occluded vessel	LAD 6/RCA 4	LAD 6/RCA 3/LCX 1
TIMI grade 1 before pPCI	3	0
TIMI grade 2 after pPCI	1	0
Ischaemic time (min) ^a	144 \pm 72	206 \pm 82

Blood chemistry at admission.

CHD, coronary heart disease; LAD, anterior branch of left coronary artery; LCX, left circumflex artery; LDL, low-density lipoprotein; pPCI, primary percutaneous coronary intervention; RCA, right coronary artery.

^aMean \pm SD.

measured 60 ms after the J point using a computer diagnostic program (Marquett SL-12, GE Healthcare). ST-segment elevation prior to reperfusion and at 6 and 48 h thereafter was expressed in μV .

Cardiac magnetic resonance protocol and analysis

Cardiac magnetic resonance with measurements of IS²⁶ and LVEF²⁷ was scheduled to be performed at routine clinical follow-up (6–10 weeks) after the coronary attack (late CMR). After eight patients had been included, the time point for CMR was changed to within 1 week (7 ± 1 days) post-STEMI (early CMR). The reason for change of the time point was to gain information about the myocardium at risk (MAR), utilizing a then recently adopted T_2 -weighted imaging sequence.²⁸ All 10 patients in the MnDPDP group underwent CMR (six early and four late), but only 8 patients in the placebo group (four early and four late) as two patients refrained from scanning.

Cardiac magnetic resonance was performed on a 1.5-T scanner (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany) with use of a six-element phased-array body matrix coil. Ten to 12 short-axis slices were applied to cover the left ventricle from base to apex, with identical positions for all relevant examinations. Left ventricular chamber volumes, LVEF, and LV muscle volume and mass were assessed in short- and long-axis images by use of an ECG-triggered balanced steady-state pre-cession cine CMR sequence. Late gadolinium-enhanced (LGE) images for measurement of IS were obtained 10 min after i.v. injection of 0.2 mmol/kg b.w. gadopentate dimeglumine (Magnevist, Bayer Schering Pharma, Berlin, Germany). An ECG-triggered phase-sensitive inversion recovery and a T_2 -weighted imaging sequence were used to measure the MAR. All CMR sequences were analysed using a computer freeware 'Segment' v 1.9 R2939 (<http://segment.heiberg.se>).²⁹ One observer analysed all CMR images blinded from clinical data. T_2 -weighted measurements of the MAR were also undertaken by a second investigator blinded from the other investigator's results.

Myocardial volumes (mL) were converted to mass (g) by using the factor 1.05. Infarct size and MAR were expressed in percentage of the total LV myocardium. The myocardial salvage index (MSI) was calculated as follows: $MAR - IS/MAR$. The presence of microvascular obstruction (MVO) and thrombi in the LV cavity was noted in a yes or no manner without quantification of volumes.

Analysis of data

All data and results obtained were analysed by use of standard non-parametric and parametric programs using the StatPrism software version 6.03 (Graph Pad Software, Inc., La Jolla, CA, USA). Statistical significance was noted when Mann–Whitney tests reported two-tailed $P < 0.05$. Results are presented as the median \pm interquartile range in figures and tables, and as the mean values in the text.

Results

Background characteristics

Patient background was similar in both groups, except for two main factors (Table 1). First, duration of ischaemia (symptom-to-balloon time) was significantly ($P = 0.037$) longer in the MnDPDP group (mean 206 min) than the placebo group (mean 144 min). Secondly, some small residual CBF before pPCI was present in placebo hearts (TIMI grade 1 in three patients), but not in MnDPDP hearts. Also, the placebo group consisted of 10 males, whereas the MnDPDP group included eight males and two females. At admission, two patients in the placebo group were treated with statins for cardiovascular disease and a third patient was treated with corticosteroids for Crohn's disease of the colon. In the MnDPDP group, two patients were on treatment with angiotensin-converting enzyme inhibitors either combined with a calcium antagonist or with a statin and a β -adrenergic antagonist.

Clinical observations

The 2–5 min i.v. infusion of MnDPDP prior to balloon inflation was well tolerated in all patients without any changes recorded in

blood pressure, heart rate or ECG. Hypotension or arrhythmias were not encountered during administration of MnDPDP as well as saline placebo. During the stay in hospital after pPCI, no differences in haemoglobin, creatinine, liver enzymes, blood pressure, occurrence of arrhythmias or heart failure were observed. Especially, the incidence of heart failure routinely assessed by echocardiography was similar in both groups as expressed by the scale no/mild/moderate, placebo 2/3/5 and MnDPDP 2/4/4. There were no differences in medical treatment at peri-PCI or at discharge.

Biomarker release

Peak release to plasma of cardiac TnT (cTnT) and CK-MB revealed no differences between groups, whether prior to reperfusion or thereafter (Figure 1). Mean cTnT values were: at 6 h placebo 6063 ng/L and MnDPDP 6473 ng/L; and at 48 h placebo 3532 ng/L and MnDPDP 2906 ng/L. Mean CK-MB values were: at 6 h placebo 258 μ g/L and MnDPDP 288 μ g/L; and at 48 h placebo 14 μ g/L and MnDPDP 13 μ g/L. The mean accumulated (0–48 h) releases were also much similar: for cTnT, placebo 211 526 ng/L and MnDPDP 207 094 ng/L; and for CK-MB, placebo 4850 μ g/L and MnDPDP 4730 μ g/L.

ST-segment elevation and resolution

ST-segment elevation was closely similar in both groups prior to pPCI (Figure 2). Following reperfusion, a tendency (n.s.) to a more rapid (6 h) and more complete (48 h) STE resolution might be observed with MnDPDP. Mean STE resolutions were: at 6 h, placebo 69.8% and MnDPDP 81.9% ($P = 0.138$); and at 48 h, placebo 73.1% and MnDPDP 84.3% ($P = 0.077$).

Cardiac magnetic resonance examinations

The mean IS was lower (n.s.) in the MnDPDP group, 26.2%, than in the placebo group, 32.5%, and the mean LVEF was higher (n.s.) with MnDPDP, 47.7%, than with placebo, 41.8% (Figure 3). Left ventricular volume assessments revealed higher (n.s.) mean values of LV end-diastolic volume and LV end-systolic volume in placebo hearts than

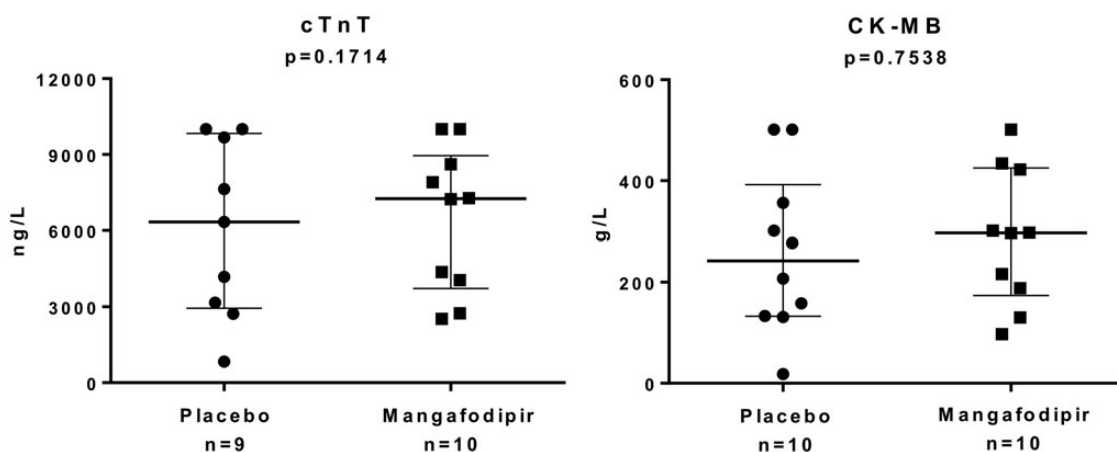


Figure 1 Release of cardiac troponin T (cTnT; ng/L) and creatine kinase isoenzyme muscle-brain (CK-MB; μ g/L) at 6 h of reperfusion. Values are presented as the median and interquartile range.

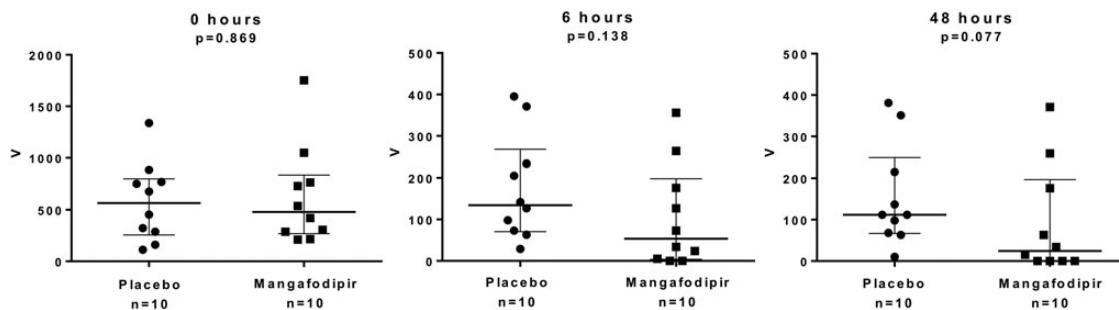


Figure 2 ST-segment elevation (μV) prior to and following reperfusion. Values are presented as the median and interquartile range. Observe that the Y-axis represents 0–2000 μV at 0 h and 0–500 μV at 6 and 48 h.

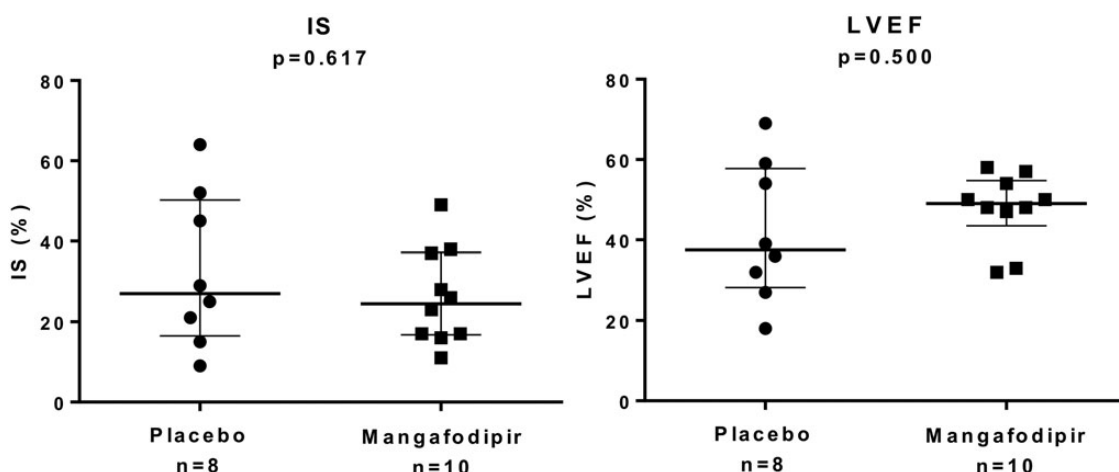


Figure 3 Infarct size (IS) and left ventricular ejection fraction (LVEF). Infarct size was measured by LGE–CMR and expressed as percentage of LV muscle mass. Left ventricular ejection fraction in % was calculated from measurements of LV end-diastolic (LVEDV) and end-systolic (LVESV) volumes by cine CMR. Values presented are the median and interquartile range.

in the MnDPDP-treated hearts (Table 2). Microvascular obstruction was present in 3 of the 8 placebo hearts and in 3 of the 10 MnDPDP hearts. Thrombi in the LV chamber were significantly more frequent in placebo hearts (5 of 8) than in MnDPDP hearts (1 of 10). Collected data from both groups ($n = 18$) showed a highly significant correlation ($P = 0.0009$) between IS and LVEF.

Early CMR measured the MAR to provide a calculation of the MSI while also measuring IS (Figure 4). Despite the small number, a tendency (n.s.) to a higher mean MAR value might be seen in the placebo group (54.5%, $n = 4$) than in the MnDPDP group (42.2%, $n = 6$). Mean MSI values were almost similar in both groups (MnDPDP 32.2% and placebo 28.8%).

Discussion

The main finding was that MnDPDP can be safely administered i.v. as an adjunct to pPCI in patients during AMI. This was expected from prior clinical reports,^{15,21,22} but the study became the first

documentation of MnDPDP given safely as a brief i.v. infusion to critically ill cardiac patients. Reported adverse reactions with MnDPDP as a contrast agent releasing paramagnetic Mn ions for MRI are mild and related to NO-induced vasodilation like flushing and headache.^{15,20} Also, the drug may induce hypotension followed by an adrenergic activation (by Mn ions) with transient rise in blood pressure and heart rate.²⁰ Neither of these possibilities nor allergic reactions were observed. However, the administered dose was lower (2 $\mu\text{mol}/\text{kg}$) than commonly applied for MRI (5–10 $\mu\text{mol}/\text{kg}$).

The primary endpoint, reduction of biomarker release to plasma, was not met, whereas other findings, in light of a skewed population (see below), appeared more positive and justify testing in a larger clinical trial. Thus, ECG and CMR examinations may indicate a tendency to potential improvement by MnDPDP. Importantly, valuable information was obtained for planning of a Phase II trial.

Baseline characteristics differed between groups with a longer symptom-to-balloon time with MnDPDP and a tendency to residual CBF prior to pPCI with placebo. However, ischaemic time based on

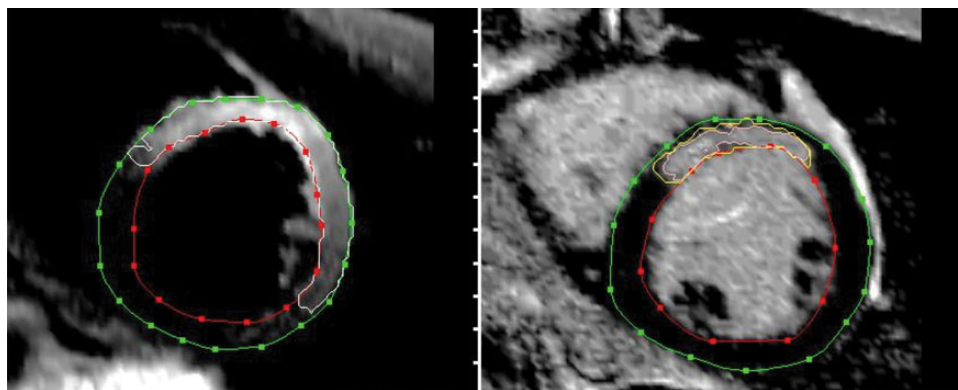


Figure 4 Cardiac magnetic resonance: T_2 -weighted image of the myocardium at risk (MAR) to the left and LGE- and T_1 -weighted image of infarct size (IS) to the right. Short-axis slice imaged at the midventricular level. The T_2 -zone extends laterally and transmurally outside the LGE zone. Measurements revealed these values: MAR 60%; IS 25%; and MSI 58%. A left ventricular thrombus at the apex was observed below the image plane. The patient is from the placebo group.

symptoms and signs may be questionable, and a more objective parameter like system-to-balloon time, recorded from first contact to the healthcare system, may be preferable.³⁰ Even then a major confounder in reperfusion–protection studies remains, unnoticed angina inducing preconditioning. Also, TIMI grade 1 prior to pPCI may behave similarly. In a study³¹ on the use of CMR to assess myocardial salvage, there was a tendency to higher MSI with TIMI grade 1 (47%, $n = 118$) than with TIMI grade 0 (40%, $n = 14$). Although speculative, an apparent skewness in disfavour of MnDPDP may explain why plasma TnT and CK-MB failed to show any differences between the groups. In contrast, tendencies to a potential benefit of MnDPDP were at least partly observed in other indices.

STE resolution, seemingly more complete with MnDPDP, is a predictor of patency of reopened culprit arteries,³² indicates that microvascular perfusion is restored, is consistent with a low early mortality in patients with STEMI and has proved to be a predictor of freedom from reinfarction at 30 days and at 1 year.³³ In the present study, STE resolution together with CMR findings may indicate a positive effect on the microcirculation. Especially, the significantly less frequent LV thrombi with MnDPDP may be consistent with improved subendocardial perfusion, possibly mediated by conservation of NO.^{9,18,20}

There was also a tendency (n.s.) to smaller IS and higher LVEF with MnDPDP in pooled data from late and early CMR. Interestingly, the MSI was almost similar, but the MAR was smaller with MnDPDP compared with placebo. Whereas smaller MAR with MnDPDP may be a random consequence of a too limited number of patients, it may also be speculated that it resulted from tissue protection. If so, the present CMR data from a few patients may reflect discrepancies between the MAR–MSI pair and the preferably more robust IS–LVEF pair of parameters, as have recently become a concern in reperfusion–protection trials.^{14,34,35}

In line with the above, how and when CMR is applied in reperfusion studies may be questioned. On the one hand, LGE is the gold standard for measuring IS, and combined with cine CMR, IS and LVEF stand out as the most accurate parameters to assess the outcome of a serious coronary attack.^{26,27} As expected, they also showed a close inter-

relationship in the present study. However, both are time-dependent, IS as the LGE zone shrinks gradually while the LVEF may improve by remodelling. The time-dependence in STEMI patients³⁶ examined at 1, 7, 42, 182 and 365 days indicated a compromise at 42 days when the LGE zone was reduced by $\sim 35\%$ and LVEF increased by $\sim 11\%$.

Early CMR is applied in reperfusion trials mainly to measure the MAR and thereby assess the MSI.²⁹ As seen from Figure 4, the T_2 imprint of the MAR is much larger than the LGE imprint of IS, indicating a considerable MSI. However, as reviewed by Croisille *et al.*³⁴ T_2 -weighted imaging to delineate the MAR is problematic, and Thuny *et al.*³⁵ showed that stepwise reperfusion reduced the MAR when assessed by T_2 -CMR (after pPCI), but not when assessed by X-ray ventriculography (prior to pPCI). Also, a warning has been given by basic physiologists¹⁴ against T_2 -based CMR to measure the MAR in reperfusion–protection studies. Whereas this situation calls for other techniques to measure the MAR, it also questions the need for CMR examination during the acute phase of AMI. Thus, a clinical trial may benefit from a single CMR measurement of IS and LVEF undertaken at an intermediate stage, i.e. after early repair but before remodelling becomes far advanced, like at 3–6 weeks. If desirable, the MAR may be detected before reperfusion by use of ECG³² and X-ray-based techniques.^{11,28,37}

To the best of our knowledge, MANAMI 1-09 is the first clinical study using a small molecular antioxidant with catalytic and metal-binding properties to combat cardiac reperfusion injury. Preclinical studies have revealed that MnDPDP or MnPLED protects against oxidative stress in normal tissues during chemotherapy for cancer and in liver tissue during acetaminophen poisoning.¹⁶ In patients with cancer of colon treated with oxaliplatin, MnDPDP provided neuroprotection and conserved white blood cells.^{16,21,22} Of direct relevance for the present study, MnDPDP reduced enzyme release and improved contractile function during hypoxia–reoxygenation in isolated rat hearts.¹⁹ In anaesthetized pigs²³ with occlusion/deocclusion (30/120 min) of the anterior descending branch of the left coronary artery, MnPLED reduced the ensuing IS by 55%. It also improved cardiac function and abolished arrhythmias. In that study, MnPLED

Table 2 CMR examination

Group	IS (%)	LVEF (%)	LVEDV (mL)	LVESV (mL)	MAR (%)	MSI (%)	MVO present	LV thrombus
Placebo	27.0 (17.1–37.3) n = 8	37.5 (28.3–57.8) n = 8	210 (158–234) n = 8	134 (69–150) n = 8	56.5 (42.5–64.5) n = 4	33.5 (8.3–44.5) n = 4	3 n = 8	5 n = 8
MnDPDP	24.5 (16.8–37.2) n = 10	49.0 (43.5–54.8) n = 10	192 (136–212) n = 10	95 (65–130) n = 10	41.5 (31.0–56.0) n = 6	35.0 (22.5–44.3) n = 6	3 n = 10	1 n = 10
P-value	0.617	0.500	0.395	0.372	0.214	0.971	0.999	0.043

Data presented are the median and interquartile range of values (% or mL) for IS, LVEDF, LVEDV, LVESV, MAR and MSI, and for the incidence (number) of MVO and LV thrombus. CMR, cardiac magnetic resonance; IS, infarct size; LVEF, left ventricular ejection fraction; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; MAR, myocardial salvage index; MSI, myocardial salvage index; MVO, microvascular obstruction; LV, left ventricular.

was applied as an i.v. bolus at the end of ischaemia followed by a continuous infusion.

In explaining protective mechanisms in myocardial ischaemia–reperfusion, MnDPDP and metabolite MnPLED mimic mitochondrial superoxide dismutase (MnSOD), the first-line defence against oxidative stress in mitochondria.^{16,19,23,38–41} Mitochondrial superoxide dismutase converts superoxide to hydrogen peroxide and oxygen and protects beneficial NO from superoxide-induced conversion to damaging peroxynitrite. During ischaemia, however, native MnSOD is inactivated by protein tyrosine nitration.³⁹ Thus, a rationale exists for use of small molecular MnSOD mimetics,⁴⁰ which may access targets inside endothelial cells and cardiomyocytes. Intriguingly, MnDPDP has been shown to prevent MPTP opening in cell models of severe oxidative stress.⁴¹ Being potent iron chelators,¹⁶ MnDPDP and MnPLED also inhibit iron-driven conversion of hydrogen peroxide to highly injurious hydroxyl radicals.^{6,16,23} Thus, they have the advantage to attack reperfusion injury at various levels in the hierarchy of oxidative stress reactions. The lack of a similar broad pharmacodynamic platform may explain why the single target MPTP inhibitor TRO 40303 did not improve the outcome after pPCI in STEMI patients, as recently reported.⁴² This situation may call for other agents or measures or that a combination of drugs are applied. Also the timing, dose and mode of administration need to be considered.

The major limitations of the study were the low number of patients and that, as might be expected from a feasibility study, the collected small material did not allow any firm conclusion to be drawn on the efficacy of the examined drug. Other limitations were an apparent skewedness in the background of patients, and that CMR did not include all patients and was undertaken at either of two time points.

In conclusion, MnDPDP is a safe drug for use as an adjunct to reperfusion therapy. A tendency to benefit of MnDPDP needs confirmation in a larger population. The study revealed important information for the design of a Phase II trial.

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Conflict of interest: R.G.A. is a board member of PledPharma AB. P.J. is a shareholder and advisor on cardiovascular disease in PledPharma AB. The other authors have no conflict of interest.

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