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Research

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Dimethyl Sulfoxide Reduces Microvascular Obstruction and Intramyocardial Hemorrhage in a Porcine Ischemia-Reperfusion Model

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ABSTRACT

Background: Microvascular obstruction (MVO) and Intramyocardial hemorrhage (IMH) are associated with myocardial reperfusion injury and recognized as predictors of adverse left ventricular remodeling in acute myocardial infarction. The pathophysiology of reperfusion injury is characterized by release of reactive oxygen species and inflammation. We investigated whether post-ischemic reperfusion with Dimethyl sulfoxide (DMSO), an organic solvent with therapeutic anti-inflammatory and antioxidant capabilities, could diminish or even abrogate the development of MVO and IMH in a porcine myocardial ischemia/reperfusion model.

Methods and Results: Myocardial ischemia was induced in 20 pigs by balloon occlusion of the Left anterior descending artery (LAD) for 65 minutes. The pigs were allocated to one-hour reperfusion with DMSO or placebo. Eight days post-injury, IMH, MVO, left ventricular function, and myocardial salvage were assessed by Cardiovascular Magnetic Resonance (CMR) imaging; and IMH and myocardial salvage were also assessed by gross pathology. All pigs in the placebo group (100%) but only 10% of the pigs in the DMSO group had IMH. CMR imaging showed presence of MVO in all placebo-treated pigs (100%) and 88% of the DMSO-treated pig and the MVO size was 45% (p=0.03) smaller in DSMO treated pigs. No difference in myocardial salvage between the placebo and the DMSO group was found by CMR, and pathological investigation and global left ventricular function examination showed no difference between the two study groups.

Conclusion: Reperfusion with a DMSO-containing solvent applied during ischemic reperfusion protected against IMH and MVO in a porcine myocardial ischemic/reperfusion model.

KEY WORDS: Myocardial infarction; Reperfusion injury; Hemorrhage; Microcirculation.

ABBREVIATIONS: MVO: Microvascular obstruction; IMH: Intramyocardial hemorrhage; AMI: Acute Myocardial Infarction; CMR: Cardiovascular Magnetic Resonance; LV: Left Ventricular; DMSO: Dimethyl sulfoxide.

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INTRODUCTION

Timely reperfusion is the predominant way to salvage ischaemic myocardium in Acute Myocardial Infarction (AMI).[1] However, reperfusion itself can paradoxically cause additional injury; a phenomenon termed 'reperfusion injury'.[2] Reperfusion injury may cause myocardial Microvascular obstruction (MVO), which prevents sufficient myocardial tissue perfusion despite complete coronary artery revascularisation.[3-5] Reperfusion injury may also cause Intramyocardial hemorrhage (IMH) by extravasation of erythrocytes through a severely damaged microvasculature.[3,6] Both conditions predict poor outcome, and are independent markers of adverse Left Ventricular (LV) remodelling.[7-9] The pathogenesis of ischemic reperfusion injury is complex and remains only partially understood. Still, oxidative stress, inflammation and intracellular Ca^{2+} overload are considered to be essential components of ischemia-reperfusion injury.[10-12] A treatment that prevents the pathophysiologic responses of ischaemia-reperfusion may also be able to prevent MVO and IMH and improve the clinical outcome in patients with successful revascularization after AMI.

Dimethyl sulfoxide (DMSO) is an amphipathic molecule widely used as a solvent in biological studies and as a vehicle for drug administration. DMSO has also demonstrated therapeutic potential by anti-oxidative and anti-inflammatory properties[13-15] and a capability to reduce intracellular Ca^{2+} overload following ischemia.[16-18]

We hypothesised that reperfusion with DMSO following myocardial ischemia may diminish ischemia-reperfusion injury and reduce or even prevent the development of MVO and IMH. We tested this hypothesis in a porcine myocardial ischaemia- reperfusion model[19] using gross pathology and Cardiovascular Magnetic Resonance (CMR) imaging for validation.

METHODS

Animal Model

We studied twenty Danish female landrace pigs (40 kg) treated in accordance with the Danish law on animal experiments.

The pigs were pre-sedated with an intramuscular injection of Stressnil (4 mg/kg) and midazolam (1 ml/kg). Anaesthesia was induced with intravenous propofol (5 mg/kg) allowing endotracheal intubation, and it was maintained with sevofluran (2.5%) in oxygen and continuous-rate infusion of fentanyl (3 mg/kg/hr). The pigs were mechanically ventilated with a tidal volume of 425 ml (respiratory rate 12/min).

An 8 F introducer sheath was inserted into the right common femoral artery under ultrasound guidance. This was followed by an intravenous bolus injection of heparin (100 IU/kg). We induced coronary occlusion by placing a 2.5-mm angioplasty balloon in the LAD distal to the second diagonal branch artery and inflating it to 10 atm. The balloon occluded the LAD for 65 minutes and was deflated and removed. A coronary angiogram was performed after the balloon had been inflated to confirm the occlusion and repeated after the balloon had been deflated to confirm the reperfusion. Within one minute after balloon deflation, a guidance catheter was placed just outside the left coronary ostium, and a 5% DMSO solution or a placebo solution consisting of 0.9% NaCl was injected through the catheter at a flow rate of 16 ml/min for 60 min. The DMSO solution was prepared by dissolving 50 ml DMSO in 1.0 L NaCl (0.9%).

During the procedure, the heart rate, Electrocardiogram (ECG), blood pressure, temperature and oxygen saturation were constantly monitored. Ampicillin (2 mg) was administered intravenously before and after the procedure, and acetylsalicylic acid (100 mg/d) was given orally after the procedure and continued until euthanasia. To prevent ventricular fibrillation, 150 mg of amiodarone was administered intravenously before the induction of myocardial infarction. If ventricular fibrillation was encountered, non-synchronised direct current defibrillation (200 J) was performed. The paddles were pressed against the anterior chest wall above the sternum on the right side and below the sternum on the left side.

At the end of the experiment, the pigs were awakened and returned to their stables where they stayed for five to eight days (average 7.2 days) before CMR imaging, harvesting and gross pathology were performed.

CMR Imaging

Fifteen of the pigs underwent CMR imaging before euthanasia (3 pigs were not scanned due to limited access to the MR-scanner and 2 pigs died doing the balloon procedure). The sedation protocol was as described above, except that continuous propofol infusion (120 mg/hr) was used instead of sevofluran. CMR was performed on a 1.5 T MR system (Intera, Philips Medical Systems, Best, The Netherlands) with a five-element cardiac synergy coil. All pigs were imaged in the supine position. First, a survey scan was performed to localise the heart and the diaphragm. Then, the LV function was assessed using a retrospective, Electrocardiogram (ECG)-triggered Balanced-Steady-State-Free-Precession (B-SSFP) breath-hold cine sequence in the cardiac short-axis, vertical long axis and horizontal long axis plane. In the cardiac short-axis, LV volume was completely encompassed by contiguous 8 mm slices with a spatial resolution of 1.22 mm x 1.22 mm with a Field of View (FOV) of 288 mm x 288 mm. The following imaging parameters were used: repetition time (TR) 3.0 ms; echo time (TE) 1.5 ms; flip angle 60º; 30 heart phases.

T2 weighted Short Tau Inversion Recovery (T2-STIR) fast spin echo sequence was obtained in the previously mentioned short-axis orientation to assess AAR. The sequence was navigator-gated, free-breathing and cardiac-triggered. The fol-

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lowing imaging parameters were used: TR 2400 ms, TE 100 ms, echo train length 20, fat inversion time 180 ms, flip angle 90º, spatial resolution 0.54 mm × 0.54 mm in-plane, number of averages 2, slice thickness 8 mm, FOV 320 mm × 320 mm and 14 slices.

T1W IR (GRE), imaging was obtained in the same short-axis slices for the purpose of identifying IMH as previously described by our group.[19] The sequence was navigator-gated, free-breathing and cardiac-triggered. The following imaging parameters were used: TR 3.5 ms, TE 1.13 ms, flip angle 30º, spatial resolution 1 mm × 1 mm in-plane, slice thickness 8 mm (over contiguous slices), FOV 320 mm × 320 mm and 14 slices. Before the acquisition of the T1W IR sequence a TI scout (Look Locker sequence) was performed for the purpose of obtaining the most appropriated TI to null the signal intensity from blood. Typically, the TI was found to be optimal at approximately 500 ms.

Following this, gadolinium enhanced first-pass myocardial perfusion and Late Gadolinium Enhancement (LGE) was performed for the purpose of identifying areas of MVO and myocardial infarction. An intravenous bolus dose of 0.2 mmol/kg Gd-DTPA (Gadobutrol, Gadovist, Bayer Schering Pharma, Berlin) was administered manually. First-pass perfusion imaging was performed using a fast gradient echo sequence with the following parameters: (TR) 2.5 ms, echo time (TE) 1.3 ms, flip angle 18º, spatial resolution 2.8 mm × 3.0 mm × 10 mm, FOV 256 mm × 256 mm, 3 slices acquired in the LV short-axis using a 5 mm inters lice gap.

Fifteen minutes after gadolinium injection, the 'Look Locker' sequence was repeated to obtain the most appropriate TI to null the signal intensity of normal myocardium. The TI was in the range of 300-350 ms. Subsequently, LGE was acquired using a 3D phase sensitive inversion recovery-prepared T1-weighted gradient echo sequence with the following parameters: TR 5.78 ms, TE 2.78 ms, echo train length, flip angle 25º, spatial resolution 1.5 mm × 1.5 mm × 8 mm, FOV 350 mm times 350 mm, 14 slices was acquired in the LV short-axis and no interslice gap.

Following CMR, the pigs were kept under anaesthesia and moved to the operating room for organ harvesting.

Harvesting and Pathology

After a midline sternotomy, a snare was placed around the LAD distal to the second diagonal branch at the same level as the previously performed balloon occlusion. Then, 25 ml 10% Evans blue dye was injected into the left auricle to delineate the AAR. Subsequently, the animal was euthanized, and the heart was excised. The heart was then cut into consecutive 8 mm-thick slices in short-axis planes. Each slice was photographed with a digital camera (Nikon, Tokyo, Japan) for the purpose of registering myocardial infarction, IMH and AAR.

DATA ANALYSIS

CMR Images

One observer (WYK) who was unaware of the intervention during reperfusion analysed all the CMR image using the semi-automatic, freely available software segment version 1.9 R3746 (http://segment.heiberg.se.).[20] First, LV volumes and function were calculated on the end-diastolic and end-systolic phases of the short-axis cine images. Second, myocardial infarct size was determined in LGE images by a semi automated algorithm maccounting for partial volume effects.[21] The infarct size was expressed as a percentage of the LV myocardium (infarction volume/LV myocardium volume x 100%).

We quantified AAR from T2-STIR images by a semi-automated algorithm[22] (22)[22][31] as (AAR/LV myocardium volume x 100%). Finally, the presence of IMH and MVO was assessed using the following semi-automatic approach: A Region of Interest (ROI) was placed in a homogenous region of the myocardium and the relative mean Signal Intensity (SI) was measured. On T1W IR images myocardium with mean signal intensity more than 2 SD above the mean ROI SI was defined as IMH. MVO was visually defined and measured on first-pass perfusion images as myocardium with no contrast perfusion.

Gross Pathology

Two observers (SFP and ESH) analysed all photographed images of the myocardial slices using the Adobe Photoshop software (Adobe Systems Inc., San Jose, CA, USA). The LV myocardial volume was determined by manually tracing the epicardium and endocardium. Secondly, the IMH volume defined as a distinct blood-stained area within the LV myocardium was manually measured and expressed as a percentage of the LV myocardium (IMH volume/LV myocardium volume x 100%). Third, the infarction size, defined visually as paleor white tissue (scar) areas within the AAR together with any IMH areas, was measured and expressed as a percentage of the LV myocardium (infarction volume/LV myocardium volume x 100%). Finally, the myocardial area stained with Evans blue was manually measured, and the AAR was determined by the formula: (LV myocardial volume- Evans blue volume/LV myocardium volume x 100%). Representative images from DSMO and placebo treated animals are shown in Figure 1.

STATISTICS

The significance of group differences was evaluated with t-tests. Data are presented as mean +/- 95% Confidence Intervals (CIs). A value of $p<0.05$ was considered statistically significant. Performing a q-q plot tested normal distribution of the data. The association between IMH and MVO and treatment with DMSO or placebo was tested with a 2-tailed Fischer exact test.

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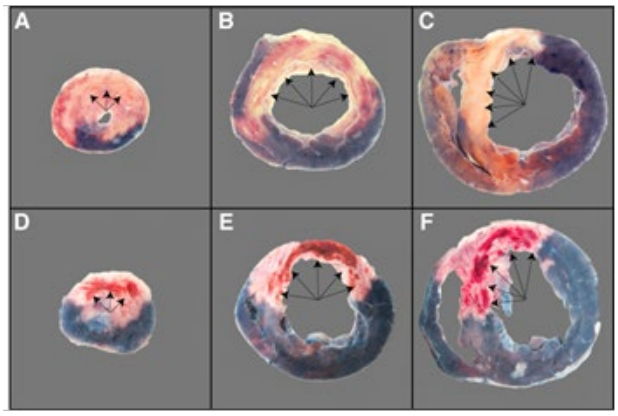

Figure 1: Top row: Photographs of a heart treated with DMSO obtained seven days following ischemic-reperfusion in the apical- (A), mid- (B), and basal-ventricular (C) short-axis view. In the antero-septal myocardium infarction without IMH (arrows) is present. Bottom row: Photographs of a heart treated with placebo eight days following ischemic-reperfusion in the apical- (D), mid- (E), and basal-ventricular (F) short-axis view. In the antero-septal myocardium severe IMH (arrows) is present.

Statistical analysis was performed with SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Two pigs died during coronary occlusion and were excluded from the study leaving eighteen pigs for further investigation. CMR imaging was only performed in 15 of the eighteen pigs due to limited access to the MR-scanner. All CMR examinations and pathological procedures were successfully performed. The results are summarized in Table 1. Hemodynamic variables did not differ between groups during the intervention. Global LV function and volumes were similar in the two study groups. Neither CMR nor gross pathology demonstrated significant differences in AAR, infarct size or myocardial salvage index between groups even though the CMR showed a non-significantly increased myocardial salvage index in the DSMO treated group. Both CMR and gross pathology revealed a significant difference in the occurrence of IMH. CMR showed that 0 out of 8(0%) had IMH in the DMSO treated group compared to 7 out of 7(100%) pigs in the placebo group ($p<0.01$). The gross pathology showed that 1 out of 10(10%) animals had IMH in the DMSO group compared to 8 out of 8(100%) in the placebo group ($p<0.01$). The only case in the DSMO group that demonstrated IMH by gross pathology was not investigated by CMR. By CMR, MVO was demonstrated in 7 out of 8(88%) animals in the DSMO and in 7 out of 7(100%) in the placebo group ($p=1.00$).The MVO size was 45% ($p=0.03$) smaller in DSMO treated group compared to the placebo group. Figure 2 show representative gross pathological pictures and CMR images of hearts subjected to DSMO and placebo.

DISCUSSION

The results of this study show that DMSO can prevent the formation of IMH and diminishing MVO in porcine myocardium exposed to prolonged ischemia-reperfusion injury. Several studies have recently shown that DMSO attenuates ischemic reperfusion injury in a variety of organs.[13,23-26] The present study expands these findings by demonstrating that DMSO can protect the myocardial microvasculature against ischemia-reperfusion injury by preventing IMH and diminishing MVO development even in the absence of infarct size reduction.

The protective effect of DMSO against MVO and IMH may be attributed to its anti- inflammatory effect.[13,27] However, the pathophysiology of reperfusion injury is complex, and the protective effect of DMSO may also be attributed to other factors such as its anti-oxidant properties and its capability to inhibit intracellular calcium overload.[17] The porcine model used in the present study did not, however, allow identification of the underlying mechanisms involved in MVO and IMH prevention and additional studies will therefore be needed in order to dissect the molecular pathways.

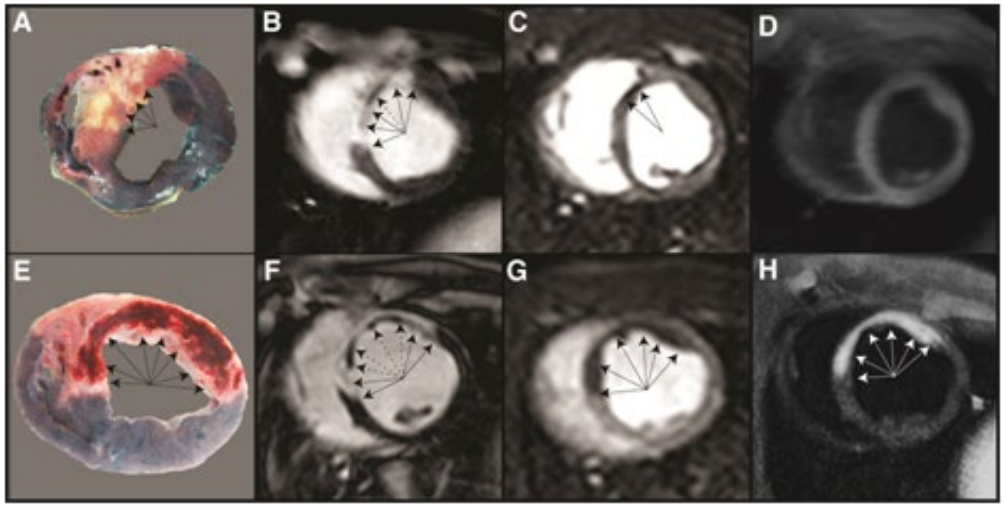

Figure 2: Short axis gross pathology photo of a DMSO (A) and placebo (E) treated heart with corresponding CMR images. Infarction without IMH is present on the DMSO treated heart (A) while IMH is present on the placebo treated heart (E). On the late gadolinium CMR images (B+F) MVO is present as a hypointense core region (dotted arrows) in a hyperintense (arrows) infarction area. On first-pass perfusion MVO is also seen as a hypointense area (Arrows) (C+G). On T1W images IMH is identified in the placebo treated heart (H) as a distinct hyperintense area whereas no hyperintense area is seen on the DMSO treated heart (D).

ISSN 2377-164X Open Journal http://dx.doi.org/10.17140/HROJ-2-114

Table 1	DMSO	Placebo	Difference	P-Value
Observations During Infarction (n=18)	n=10	n=8		
Pulse (heart beats/min)	52	51	1 CI; [-7.1;8.9]	0.82
MAP (mmHg)	64	61	4 CI; [3.7;10.9]	0.31
Temperature (°C)	37.2	36.9	0.3 CI; [-0.3;0.8]	0.27
Oxygen saturation (%)	99.7	99.6	0.1 CI; [-0.1;0.7]	0.73
CMR Functional Parameters (n=15)	n=8	n=7		
LV end-diastolic volume (mL)	89.6	96.4	6.84; [-18.5;32.2]	0.57
LV end-systolic volume (mL)	44.0	52.8	8.79; [-10.2;27.8]	0.34
LV ejection fraction (%)	51.7	46.3	5.38; [-13.9;3.13]	0.20
CMR Infarct Parameters (n=15)	n=8	n=7		
AAR size (% LV)	35.8	36.1	0.29; [-11.1;11.7]	0.96
LGE infarct size (% LV)	15.8	22.7	6.93; [-1.18;15.0]	0.09
Myocardial salvage (% of LV)	20.0	13.4	-6.64; [-13.7;0.44]	0.06
MVO size (% of LV)	3.9	2.1	-1.7; [-3.3;-0.20]	0.03
No. of animals with IMH (count)	0	7		<0.01
No. of animals with MVO (count)	7	7		1.00
Gross Pathological Parameters (n=18)	n=10	n=8		
AAR size (% LV)	37.6	37.1	0.52; [-11.0;10.0]	0.92
Infarct size (% LV)	25.0	26.0	1.00; [-7.00;9.00]	0.79
Myocardial Salvage (% LV)	15.1	11.1	-3.98; [-14.4;6.38]	0.43
No. of animals with IMH(count)	1	8		<0.01

Table 1: Results summary. MAP: Middle Arterial Pressure; LV: Left Ventricle; AAR: Area at risk; LGE: Late Gadolinium Enhancement; IMH: Intramyocardial hemorrhage; MVO: Microvascular obstruction.

In our study, DMSO did not reduce myocardial infarct size. This observation contradicts previous studies demonstrating that DMSO reduced infarct size in isolated rat hearts.[26] A potential explanation is that we used a very long ischemia time (65 min) for the purpose of inducing MVO and IMH. Furthermore, pigs are known to develop large transmural myocardial infarcts due to the lack of coronary collaterals. Consequently, myocardial injury may have progressed beyond the point of salvage, and therefore all infarcts were transmural and hence did not differ significantly in size between groups when investigated 7 days later. The very low myocardial salvage indices in the order of 11-20% and the large infarct sizes in both groups reflect the effect of the long ischemia time before reperfusion. As such, the infarcts in this study resemble large anterior infarcts in patients.

Although the placebo group had a higher occurrence of MVO and IMH than the DMSO group, we found no significant difference in cardiac function between the groups as determined by ejection fraction. This is in disagreement with previous studies, which found that MVO and IMH were strong predictors of LV remodelling and mortality.[9,28,29] The reason for this discrepancy is likely because cardiac function was assessed by cine MR-imaging an average of seven days after the infarction was induced. This time span is far too short to develop pronounced LV remodelling, and the negative effect of MVO and IMH on LV remodelling has therefore not been fully elucidated in this study.

Another important aspect of this model of ischemia-reperfusion injury is the evaluation by CMR after approximately one week. This time point was chosen since CMR detection of myocardial salvage is considered optimal at one week after AMI.[19,30] The choice of time point is transferable to the clinical setting of CMR evaluation of patients with AMI.

Coronary DMSO perfusion performed *in vivo* has, to our knowledge, not previously been described in the literature, and it was therefore a challenge to decide which DMSO concen-

tration to use. In previous studies, the concentration in the target organ varied with the route of administration. When applied to organs such as urinary bladder and intestine, 1-10% solutions were used. For intravenous use, the solutions were typically 10-40%, and such solutions have been reported to be safe and well-tolerated.[31] Irreversible injury to the heart has, however, been reported in isolated rat hearts exposed to DMSO concentrations exceeding 10%.[32] Consequently, we chose a concentration of 5% and observed no side effects by hemodynamic measurements and occurrence of ventricular arrhythmias.

Various approaches to diminish the deleterious consequences of ischemic reperfusion injury have been tested in the past. Most involved treatment with exogenous substances in the pre-ischemic phase. However, none of these pre-treatments have found their way into the clinic since they need to be initiated before the onset of ischemia, something that obviously cannot be anticipated in a clinical setting. This makes the present study particularly interesting because DMSO was applied in the post-ischaemic phase and therefore has the potential to become a clinically relevant treatment for the prevention of MVO and IMH.

LIMITATIONS

While the data were convincing in demonstrating that DMSO-containing solvent applied during ischemic reperfusion protected against IMH and MVO, the sample size was relatively small. Larger studies would therefore be useful to confirm our findings. MVO and IMH were only assessed by CMR and gross pathology, however further information regarding the mechanism of MVO and IMH could properly be obtained by examining the microvasculature histologically.

CONCLUSION

In conclusion, in an *in vivo* porcine model of myocardial reperfusion injury, we demonstrated that intracoronary DMSO administered after ischemia and onset of reperfusion protects the myocardial microvasculature against reperfusion injury by diminishing IMH. Furthermore, the study demonstrates that CMR performed one week after AMI can accurately detect MVO and IMH. CMR may provide a non-invasive measure of reperfusion injury in patients with AMI beyond infarct size reduction to evaluate the effect of cardioprotective approaches. Additional studies are required to elucidate the underlying protective mechanisms of DMSO and also long term studies are needed in order to evaluate the positive effect of DSMO on cardiac remodelling and function.

CONFLICTS OF INTEREST: None.

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CONSENT

No consent is required to our article publication (It is an animal study and it was conducted in agreement with Danish law).

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