Inhibition of monoamine oxidase A increases recovery after experimental cardiac arrest

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Abstract

OBJECTIVES: Perioperative myocardial infarction (MI) with ischaemia–reperfusion injury (IRI) is a devastating entity occurring in 1–2% of patients after cardiac surgery. The molecular pathway leading to myocardial cellular destruction after MI may include monoamine oxidases. We experimentally investigated whether moclobemide, a monoamine oxidase inhibitor, enhances myocardial recovery after cardiac arrest and MI.

METHODS: Fifty-six syngeneic Fischer rats underwent heterotopic cardiac transplantation to induce reversible IRI after cardiac arrest. Twenty-eight rats also underwent permanent ligation of the left anterior descending coronary artery to induce MI after cardiac arrest. Twenty-eight rats with or without MI were treated with subcutaneous moclobemide 10 mg/kg/day. Methods used to study myocardial recovery were microdialysis for intramyocardial metabolism, histology and quantitative reverse-transcription polymerase chain reaction for high-mobility group box-1 (HMGB1), haeme oxygenase-1 (HO-1), interleukin-6, hypoxia-inducible factor 1α and macrophages (CD68).

RESULTS: Pyruvate increased in MI treated with moclobemide versus IRI with moclobemide (29.19 ± 7.64 vs 13.86 ± 8.49 µM, P = 0.028), reflecting metabolic activity after cardiac arrest and reperfusion. Myocardial inflammation increased in MI compared with IRI after 1 h (0.80 ± 0.56 vs 0, point score units [PSUs], P = 0.003), but decreased after 5 days in MI treated with moclobemide versus MI alone (0.80 ± 0.83 vs 2.00 ± 0.70, PSU, P = 0.033). Expressions of HMGB1, CD68 and HO-1 decreased in MI treated with moclobemide versus MI alone (1.33 ± 0.20 vs 1.75 ± 0.24, fold changes [FCs], P = 0.028; 5.15 ± 1.10 vs 9.59 ± 2.75, FC, P = 0.050; 10.41 ± 4.17 vs 21.28 ± 10.01, FC, P = 0.047), indicating myocardial recovery and increased cellularity of remote intramyocardial arteries.

CONCLUSIONS: Moclobemide enhances myocardial recovery after cardiac arrest and MI; inhibition of remote myocardial changes may be achieved by targeting treatment against monoamine oxidase.

Keywords: Monoamine oxidase A inhibition • Myocardial infarct • Cardiac arrest

INTRODUCTION

Despite early treatment, perioperative myocardial infarction (MI) with ischaemia–reperfusion injury (IRI) is a devastating entity occurring in 1–2% of patients after cardiac surgery, often leading to myocardial remodelling and subsequent heart failure [1, 2]. Cardiac arrest renders the myocardium susceptible to irreversible ischaemia due to IRI, and subsequent MI globally affects the myocardium. Cardiomyocytes suffer from nutrient and oxygen deprivation, generating reactive oxygen species (ROS) during IRI and MI [3]. Developing MI during IRI may cause remote intramyocardial changes [2]. Remote intramyocardial arteries are vulnerable to early inflammatory and oedematous changes that aggravate myocardial recovery from MI with IRI [4]. Subsequently, remote intramyocardial arteries are susceptible to narrowing of the vessel lumen due to progressive intimal thickening associated with smooth muscle cell proliferation and migration as remote intra-myocardial arteriopathy ensues [4].

IRI is associated with an increase in ROS production originated by several enzyme systems such as oxidases of nicotinamide adenine dinucleotide phosphate (NADPH), xanthine oxidase, electron leakage from the mitochondrial respiratory chain, cyclooxygenases and the uncoupled endothelial nitric oxide synthase.
Among these, monoamine oxidases (MAOs) have recently been under intense investigation, since they may contribute to vascular dysfunction after IRI [5].

Promoting inflammation, smooth muscle cell proliferation, intimal hyperplasia and apoptosis, oxidative stress induced within the vascular wall may initiate atherogenesis after IRI and MI [6]. Proliferating and migrating vascular smooth muscle cells increase atherogenesis of the intramyocardial arteries. The generated ROS are responsible for numerous signalling responses, inducing activation of tyrosine kinase receptors, the sphingolipid pathway, transcription factors and activation of pro-apoptotic pathways with caspase activation and calcium deregulation, to name but a few [6]. Among these, the mitogenic response to oxidation of biogenic amines by MAO may, at least partly, influence the association of ROS and IRI and trigger alteration of remote intramyocardial arteries [6].

MAOs have been shown to act against the arterial wall during ischaemia by generating ROS [6]. Mitochondrial MAOs are flavoproteins that exist in two isoforms, MAO-A and MAO-B, which are involved in the metabolism of biogenic amines in vascular cells during oxidative stress [6, 7]. ROS generated by smooth muscle cell MAO-A enhance cellular proliferation mediated by serotonin and tyramine [6]. MAO-A activates the sphingolipid pathway, resulting in cardiac cell apoptosis and inflammation [7]. MAO inhibitors may have therapeutic potential in myocardial hypertrophy and cardiac failure [3, 8].

The aim of this study was to investigate the early vascular changes of the myocardium and associated remote intramyocardial inflammation after IRI and MI. We also investigated whether moclobemide (Mo) impacts these changes. We hypothesized that Mo has an impact on remote intramyocardial arteries after IRI and MI in a heterotopic cardiac transplantation model simulating the clinical scenario of MI after cardiac arrest. Mo may inhibit the release of ROS generated by mitochondria of smooth muscle cells because vascular MAO is predominantly expressed in smooth muscle cells [5].

MATERIALS AND METHODS

Rats

One hundred and twelve inbred Fischer 344 rats (F344/NHsd, Harlan Laboratories, Netherlands) weighing 200–250 g served as donors (n = 56) and recipients (n = 56) and underwent a heterotopic cardiac transplantation. In addition, six normal hearts from non-operated rats served as controls. The rats were kept in a vivarium at Tampere University and received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources (National Institutes of Health publication no. 86-23, revised 1996). The study was approved by the Finnish State Provincial Office.

Surgical procedure

Rats were anaesthetized with sevoflurane (Baxter, USA) for inhalation and pentobarbiturate 50 mg/kg (Mebunat Vet, Orion Espoo, Finland) intraperitoneally. The thoracic cavity was surgically opened and the donor heart exposed. The ascending aorta of the cardiac graft was infused with cold cardioplegia fluid (4°C Custodial, Bretschneider HTK solution for cardioplegia and multiorgan protection, Germany; with 100 U Heparin Leo, Vianex S.A., Greece). The graft was preserved in the cold solution while the recipient rat was prepared. Heterotopic cardiac transplantation was performed intra-abdominally by joining the graft aorta to the abdominal aorta and the graft pulmonary artery to the inferior vena cava of the recipient, as previously described [9]. From the recipient aorta, the transplanted heart received oxygenated blood that was introduced into the coronary arteries of the graft. Via the coronary sinus, this blood circulated into the right atrium and eventually to the right ventricle, from where deoxygenated blood was introduced to the recipient rat throughout the pulmonary artery [9]. The nutritional flow of the myocardium consisted of oxygenated blood, and the transplanted heart was not ischaemic after reperfusion upon transplantation. Since the aortic valve was competent, oxygenated blood was not allowed to fill the left ventricle; therefore, the transplanted heart simulated a non-working resting state of the left side of the graft. This heterotopic transplantation model allowed the study of ischaemia–reperfusion in vivo without interferences from myocardial stress factors. The model thus simulated the clinical concept of acute cardiac arrest resuscitated with initiation of a cardiopulmonary bypass and a left ventricular assist device [10]. The total warm ischaemia time before total graft reperfusion was 30 min after cardiac arrest.

Study protocol

The rats were randomized into four groups. Fourteen grafts underwent heart transplantation only, to serve as controls with IRI. In 28 grafts, the left anterior descending coronary artery (LAD) was also ligated permanently at its proximal part with a single 7-0 suture to cause MI. The ligation knot for LAD obstruction was placed at the bifurcation of the first diagonal branch due to coronary vessel distribution, since the apex is most vulnerable to ischaemic changes leading to MI. The ligation knot was placed before onset of reperfusion while the heart remained still. A confined blue ischaemic area after reperfusion of the left anterior ventricular wall corresponding to the area nourished by the LAD was ensured to confirm the desired anatomical area size of the completely ischaemic myocardium. We approximated the confined ischaemic area to 10 × 10 mm. Twenty-eight rats were treated with Mo, an MAO-A inhibitor administered at 10 mg/kg/day. The first dose (5 mg/kg) was given with cold cardioplegia and the second (5 mg/kg) was given i.p. just prior to reperfusion. At postoperative days 2–5, Mo was given subcutaneously. Fourteen of the grafts with IRI and 14 of the grafts with IRI and MI were treated with Mo. A detailed protocol of the experimental setting is provided in Fig. 1. After the procedure, buprenorfin 0.1 mg/100 g (Vetergesic, Orion Espoo, Finland) and carprofen 0.5 mg/100 g (Norocarp, Norbrook Laboratories Ltd, Newry, Northern Ireland) were given subcutaneously for pain relief.

Microdialysis

Assessment of metabolic activity was performed immediately after reperfusion to demonstrate the effect of IRI [9]. To explore the early myocardial outcome with or without Mo, we performed microdialysis to evaluate metabolic parameters associated with energy consumption, such as lactate and pyruvate after reperfusion. Together with energy consumption, functional recovery after IRI may also be predicted by evaluating the release of interstitial glutamate and glycerol associated with ongoing ischaemia [9]. Glutamate, pyruvate, lactate and glycerol were investigated.
Immediately after surgery, a single microdialysis probe (CMA 70; 20,000 Da cut-offs, 0.6 mm diameter; CMA Microdialysis AB) was implanted in the left ventricular wall of the graft tangentially to the LAD, as previously described [9]. The microdialysis catheter was carefully implanted away from the distal ischaemic area, in the basal remote myocardial part of the heart. The microdialysis probe was connected to a CMA 107 micro-infusion pump at a flow rate of 0.2 μl/min. After onset of reperfusion and the 15-min tissue stabilization period, samples were collected without interruption for 10 min and for three cycles in 30 min, and immediately stored at −20°C. Analysis was performed by a CMA 600 Microdialysis Analyzer.

Tissue samples

The recipient rats were sacrificed at two time points: 1 h after reperfusion (n = 32) and 5 days after reperfusion (n = 24). Six normal hearts from non-operated rats were also taken. The basal part of the grafts were separated and stored in RNA later (Applied Biosystems, Foster City, CA, USA) for analysis by quantitative reverse-transcription (RT) polymerase chain reaction (PCR). The apex part of the graft was fixed in formalin and embedded in paraffin. Three hearts (one with IRI + Mo, one with IRI + MI and one with IRI + MI + Mo) were excluded due to technical failure.

Histology

For histology, 5-µm sections were cut and stained with haematoxylin and eosin. The following variables were evaluated from all of the samples: presence of myocardial oedema, haemorrhage and ischaemia. As the vacuolization of nuclei in the media layer of intramyocardial arteries reflects oedema, a representative cross-sectional intramyocardial artery was chosen randomly from the left anterior, septum and posterior ventricular walls representing remote myocardium. Normal, vacuolated and sharp-edged media are shown in Figure 1.

Figure 1: Schematic description of the experimental setting of the study. IRI: hearts undergoing transplantation with ischaemia–reperfusion injury only; IRI + Mo: transplanted cardiac grafts with moclobemide; IRI + MI: transplanted grafts with left anterior descending coronary artery (LAD) ligation; IRI + MI + Mo: transplanted cardiac grafts with LAD occlusion and moclobemide. The sequence of the experimental protocol was as follows: infusion of cold (4°C) cardioplegia (2 ml) with or without moclobemide into the aortic root of the surgically prepared donor rat heart (1) inducing immediate cardiac arrest (2) and onset of a 15-min cold ischaemic time, during which the heart was surgically dissected and procured (3) and immersed into cold (4°C) physiological saline fluid (4) and the anaesthetized recipient rat was surgically prepared to reveal the abdominal cavity with its aorta and vena cava (5). These vessels were clamped (6), and heterotopic heart transplantation was initiated (7), which defined the onset of a 30-min warm ischaemic time. Depending on the study group, the LAD was first ligated (IRI + MI and IRI + MI + Mo), after which moclobemide was given intraperitoneally (IRI + Mo and IRI + MI + Mo) before the application of the microdialysis catheter in all hearts. Warm ischaemic time was terminated by releasing of the recipient abdominal aorta and vena cava clamps to initiate the onset of reperfusion. Immediately after the onset of reperfusion, a waste microdialysis sample was discarded during a 15-min stabilization period, as required for the technique utilizing microdialysis. The stabilization period ensures that the microtrauma of the inserted microdialysis catheter had a minimal effect to interfere with the collected parameters. Thereafter, the three phases of the subsequent 10-min microdialysis samples were collected. Finally, after a 60-min reperfusion time, the heart was transected, the basal part was snap frozen in liquid nitrogen for RNA analysis, and the middle part of the heart was procured for histology.
cell nuclei were manually counted separately. Periadventitial inflammation was graded according to an arbitrary scale from 0 to 2 and expressed as point score units (PSUs): 0 = no inflammation, 1 = presence of occasional inflammatory cells and 2 = groups of inflammatory cells. Intimal thickness was graded using an arbitrary scale from 0 to 2 and expressed as PSU: 0 = normal endothelial layer, 1 = occasional thickness and 2 = concentric intimal thickness. Evaluation of histology was performed by two investigators blinded to the study protocol.

Quantitative reverse-transcription polymerase chain reaction analysis

We investigated as markers of inflammation the expressions of high-mobility group box-1 (HMGB1) protein, macrophages (cluster of differentiation [CD68]) and interleukin (IL)-6 [11–13]. Hypoxia-inducible factor 1α (HIF1α) and haeme oxygenase-1 (HO-1) were chosen to mirror the molecular cascade activation related to recovery from IRI, reflecting induced ischaemia and protection from oxidative stress, respectively [13, 14]. These parameters were chosen to confirm the effect of IRI on the remote myocardium by seeking for delicate changes in gene expressions influencing the remote vascular endothelium after MI. The frozen tissue of the base of the heart was randomly chosen from each group and was homogenized and RNA extraction was carried out with the GenElute Mammalian Total RNA Miniprep kit (Sigma Aldrich, St Louis, MO, USA) with proteinase K treatment. Total RNA was then reverse-transcribed to cDNA using TaqMan Reverse Transcription reagents and random hexamers (Applied Biosystems). The cDNA obtained from the RT reaction (amount corresponding to 1 ng of total RNA) was subjected to quantitative PCR using QuantiTect Primer Assays (Qiagen, Valencia, CA, USA) for HMGB1, HIF1α, IL-6, CD68, HO-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA) and the ABI PRISM 7000 Sequence detection system (Applied Biosystems). The cDNA obtained from the RT reaction (amount corresponding to 1 ng of total RNA) was subjected to quantitative PCR using QuantiTect Primer Assays (Qiagen, Valencia, CA, USA) for HMGB1, HIF1α, IL-6, CD68, HO-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The values of six control samples were used as a calibrator, and the expression levels of HMGB1, HIF1α, IL-6, CD68 and HO-1 were normalized against GAPDH.

Statistical analysis

The data are presented as mean ± SD. Statistical analyses were performed with the commercial statistical software (SPSS 22.0, Chicago, IL, USA). Kruskall–Wallis tests were used to compare independent groups. Non-normally distributed data between two groups were analysed with the Mann–Whitney U test. A value of \( P < 0.05 \) was considered significant. For clarity, statistically significant comparisons are shown in the figures.

RESULTS

Microdialysis

Throughout observation, there were no significant changes in glutamate, glycerol, pyruvate or lactate in IRI when compared with IRI + MI (Fig. 2). In contrast, pyruvate increased after 15 min of reperfusion in IRI + MI + Mo compared with IRI + Mo (29.19 ± 7.64 vs 13.86 ± 8.49 μM, \( P = 0.028 \)) and glycerol increased in IRI + Mo compared with IRI (38.70 ± 10.03 vs 21.70 ± 15.57 μM, \( P = 0.047 \)). Concomitantly, after 15 min of reperfusion, lactate was higher in IRI + Mo than in IRI (2.40 ± 0.28 vs 1.02 ± 1.28 mM, \( P = 0.047 \)) and in IRI + MI + Mo than in IRI + MI (2.10 ± 0.39 vs 0.37 ± 0.29 μM, \( P = 0.004 \)). Lactate remained increased until 35 min after reperfusion in IRI + Mo compared with IRI (2.25 ± 0.86 vs 1.10 ± 0.98 μM, \( P = 0.040 \)). Glutamate was increased after 35 min of reperfusion in IRI + Mo compared with IRI (17.46 ± 11.86 vs 4.53 ± 4.80 μM, \( P = 0.028 \)), whereas glutamate was lower in IRI + MI compared with IRI + Mo (4.76 ± 0.50 vs 17.46 ± 11.86 μM, \( P = 0.046 \)).

Histology

There were no haemorrhagic differences between the cardiac grafts 1 h (Fig. 3) and 5 days (Fig. 4) after reperfusion. The media layer of the remote intramyocardial arteries was preserved in all groups. One hour after reperfusion, periadventitial inflammation of the intramyocardial arteries of the left anterior ventricular wall and ventricular septum was observed in IRI + MI but not in IRI (0.80 ± 0.56 vs 0, PSU, \( P = 0.003 \)). Periadventitial inflammation was also seen in the remote left posterior ventricular wall area in IRI + MI in contrast to IRI (0.80 ± 0.56 vs 0.13 ± 0.36, PSU, \( P = 0.045 \)). The periadventitial inflammation of the infarction area was lower in IRI + MI + Mo than in IRI + MI (0 vs 0.80 ± 0.56, PSU, \( P = 0.009 \)) and in the remote area (0 vs 0.80 ± 0.56, PSU, \( P = 0.009 \)). Five days after reperfusion, the left anterior ventricular wall and septum revealed a well-developed granulation area corresponding to the evolving MI in IRI + MI + Mo and IRI + MI + Mo (Fig. 5). Concomitantly, periadventitial inflammation of remote intramyocardial arteries was decreased in IRI + Mo compared with IRI (0.40 ± 0.54 vs 1.50 ± 0.57, PSU, \( P = 0.036 \)), and in IRI + MI + Mo compared with IRI + MI (0.80 ± 0.83 vs 2.00 ± 0.70, PSU, \( P = 0.049 \)). The periadventitial inflammation of the infarction area was lower in IRI + MI + Mo than in IRI + MI (0 vs 0.80 ± 0.56, PSU, \( P = 0.009 \)) and in the remote area (0 vs 0.80 ± 0.56, PSU, \( P = 0.009 \)).

After 5 days, there were no differences in the gene expression levels of HIF1α and IL-6, CD68 and HO-1 (Table 1). After 5 days, there were no differences in the gene expression levels of HIF1α and IL-6. Expressions of HMGB1, CD68 and HO-1 decreased in IRI + MI + Mo compared with IRI + MI (1.33 ± 0.20 vs 1.75 ± 0.24, fold changes [FCs], \( P = 0.028 \), 5.15 ± 1.10 vs 9.59 ± 2.75, FC, \( P = 0.050 \), 10.41 ± 4.17 vs 21.28 ± 10.01, FC, \( P = 0.047 \)).

DISCUSSION

In this study, Mo decreased adventitial inflammation and evolution of intimal thickness of the remote intramyocardial arteries in hearts exposed to IRI and MI.
IRI may lead to myocardial remodelling due to release of ROS [1-3]. An important consequence of intramyocardial remodelling includes the intramyocardial arterial wall insult associated with endothelial swelling and smooth muscle cell proliferation. The acute developing MI has long-term effects on the remote intramyocardial arteries after IRI, and arteriosclerosis may ensue. The generation of ROS and inflammation is associated with the sphingolipid pathway that mediates the association of MAO with IRI [7]. The mitochondrial MAO-A metabolizes serotonin, which is released by activated platelets during IRI [3]. Vascular smooth muscle cell proliferation is dependent on serotonin metabolism [16] and MAO [17]. The development of cardiac hypertrophy is associated with the presence of MAO and associated hydrogen peroxide production by oxidative stress [8]. The target of MAO inhibition is the mitochondria that react upon IRI; selective MAO inhibition blocks the release of hydrogen peroxide and concomitant production of detrimental ROS [18].

It is beyond the scope of this study to define the exact mechanisms associated with MAO-A inhibition with Mo. The gene expressions chosen for investigation reflected the overall outcome after IRI and MI. Previously, activation of the mitochondrial electron transport chain, NADPH oxidase and xanthine oxidase have been speculated to increase during ROS generation after IRI. However, IRI may occur in organs known to be deficient for xanthine oxidase and in a mouse model without a functional NADPH oxidase [18]. The sphingolipid pathway seems plausible in our model; it may be possible to intervene with cell apoptosis, proliferation and migration by inhibiting MAO activity. MAO has a critical role in the initiation of renal cell injury after IRI; MAO regulates through ROS the lipid peroxidation of cell membranes, protein and enzyme oxidation and some irreversible DNA changes leading to cell death [18]. In a recent experimental study by Sturza et al. [5], it was shown that MAOs are responsible for vascular endothelial damage; MAO-induced peroxidation is decreased by MAO inhibition and...
abolishes endothelial nitric oxide release, again suggesting the involvement of MAO activity during atherogenesis.

We may speculate that the prevention of apoptosis does not necessarily lead to decreased migration or proliferation of smooth muscle cells during early atherogenesis in our model. The prevention of atherogenesis in our model may include different mechanisms compared with volume overload-induced congestive heart failure. Abassi et al. [19] found that a non-MAO inhibitor, TVP 1022, attenuated experimental cardiac remodelling, presumably by inhibiting cytochrome c release from the mitochondria and caspase 3 activation. In another study [20], IRI was induced by temporal occlusion of the LAD and early reperfusion to study reduced infarct size; again, cardioprotection was assumed by decreased cytochrome c release from the mitochondria and caspase 3 activation. However, the subcellular location of MAO at the outer mitochondrial membrane may explain the importance of MAOs in the induction of cell apoptosis as well. The mitochondria have a key role in apoptosis by the generation of ROS and the release of cytochrome c and calcium [18].

It was therefore essential to establish a model simulating the concept of cardiac arrest and MI that could be followed up for 5 days while initiation of remote intramyocardial vascular changes occurred. As observed with early microdialysis, the establishment of our experimental model was successful for the 5-day follow-up; there were no statistical differences in glutamate, glycerol, pyruvate or lactate releases in hearts with IRI or IRI + MI, suggesting that functionally the heart was not endangered acutely despite the presence of the developing MI. Early histology confirmed the well-preserved myocardium and occasional inflammation of the hearts with IRI and MI. Ligation of the LAD produced an irreversible developing infarction area distal to the ligation that was responsible for developing remote myocardial changes.

Early metabolic changes after IRI have long-term effects on histology [9]. The beneficial effect of Mo on metabolism and remote myocardium is observed when MI is present. The early remote inflammatory changes due to developing MI were decreased with Mo. There is an increase in energy production with Mo, as observed with the increased release of pyruvate. However, this is at the expense of increased lactate release. Eventually though, glutamate decreases in hearts with IRI + MI + Mo. After 5 days of reperfusion, remote inflammation decreased in both IRI + M and IRI + MI + Mo, but the decrease of development of intimal changes was only observed in hearts with IRI + MI + Mo.

The early remote changes as evaluated by the expressions of HMGB1, HIF1α, IL-6, CD68 and HO-1 remained unchanged, thus confirming the stability of the model. The inflammatory cytokine IL-6 and HIF1α indicating ongoing ischaemia remained unchanged among the groups after 5 days of reperfusion, suggesting that the remote inflammatory and ischaemic process subsides with or without the presence of MI. As the expression of HMGB1 decreased in IRI + MI + Mo compared with IRI + MI, it may be suggested that the decreased intimal evolution may also be associated with Mo. Earlier data show that HMGB1 increases inflammation and atherosclerotic-like changes [12, 21]. Decreased HMGB1 expression may inhibit smooth muscle cell migration, which is essential for intimal proliferation during arteriosclerosis [11, 21].

Figure 3: Representative histology of the heart showing a left ventricular intramyocardial artery in cardiac grafts after 1 h of reperfusion in the IRI (A), IRI + Mo (B), IRI + MI (C) and IRI + MI + Mo groups (D). Haematoxylin–eosin staining ×40. Arrows show initial adventitial inflammation of a remote intramyocardial artery associated with ongoing ischaemia. No major histological differences were observed in IRI and IRI + Mo when compared with IRI + MI + Mo.
Figure 4: Representative histology of the heart showing a left ventricular intramyocardial artery in cardiac grafts after 5 days of reperfusion in the IRI (A), IRI + Mo (B), IRI + MI + R (C) and IRI + MI + Mo groups (D). Haematoxylin–eosin staining ×40. White line segments show evolution of intimal bulging reflecting early arteriosclerosis associated with adventitial inflammation (black arrows) during ongoing ischaemia. No major histological differences were observed in IRI + Mo when compared with IRI + MI + Mo.

Figure 5: Representative histology of the heart after 5 days of reperfusion in grafts without LAD ligation (IRI and IRI + Mo; A) and in grafts with LAD ligation (IRI + MI and IRI + MI + Mo; B). Haematoxylin–eosin staining ×10. Note the outer border of myocardial infarction in B (black dotted line).
The decreases in HO-1 and intimal changes observed in IRI + MI + Mo compared with IRI + MI may indicate that no further protection from oxidative stress is needed after 5 days with Mo. The antioxidant HO-1, reflecting early protection from ischaemia [14], has been shown to be beneficial in decreasing atherosclerotic-like changes [22]. There is a reversed association of HO-1 and HMGB1, and the connection of these molecules should be evaluated using at times schedule [22]. It should be remembered that although a decrease of HO-1 was evident in our study in IRI + MI + Mo, all hearts at 5 days had relatively high levels of HO-1 expression in the remote myocardium compared with the values at 1 h after reperfusion. The expressions of HMGB1, IL-6 and HIF1α after 5 days were comparable to the levels at 1 h.

Histologically, the periadventitial inflammation of the intramyocardial arteries consisted of macrophage-like cells in grafts with IRI and MI. The decreased inflammation and decreased CD68 expression levels in grafts with Mo suggest that macrophages may orchestrate the periadventitial inflammation associated with the increased intimal thickness. Cardiac fibrosis may be attenuated by modulating macrophage phenotype after experimental MI [23]. The irreversible MAO inhibitor pargyline reduces renal fibrosis after IRI when given as a single dose prior to the ischaemic insult [24]. It is tempting to speculate that Mo inhibits macrophage infiltration and plausible intimal thickness after IRI and MI, thus decreasing intramyocardial arteriosclerosis; interestingly Mo seemed to have an impact only when irreversible MI together with IRI was present. Indirect evidence suggests that without permanent MI and ongoing ischaemia, IRI may be reversible without additional MAO inhibition [19].

Limitations of the study include the plausible interaction with catecholamine metabolism due to MAO inhibition [25]. We therefore chose Mo, a selective and reversible MAO inhibitor. The reversibility of MAO inhibition may abolish possible side effects due to catecholamine metabolism. On the other hand, oxygen and saturation values were not included in the experimental protocol. In this acute setting, we did not measure left ventricular ejection fraction.

### Table 1: Remote mRNA expressions (mean fold change ± standard deviation) of the base(s) of hearts

<table>
<thead>
<tr>
<th>Time</th>
<th>mRNA</th>
<th>IRI</th>
<th>IRI + Mo</th>
<th>IRI + MI</th>
<th>IRI + MI + Mo</th>
<th>P-value</th>
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<tr>
<td>1 h</td>
<td>HMGB1</td>
<td>0.73 ± 0.08</td>
<td>0.64 ± 0.07</td>
<td>0.78 ± 0.07</td>
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<td>HIF1α</td>
<td>0.80 ± 0.19</td>
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<td>0.85 ± 0.13</td>
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<td></td>
<td>IL-6</td>
<td>6.61 ± 8.38</td>
<td>6.38 ± 7.91</td>
<td>8.88 ± 5.17</td>
<td>6.51 ± 7.24</td>
<td>0.776</td>
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<tr>
<td></td>
<td>CD68</td>
<td>0.81 ± 0.19</td>
<td>0.84 ± 0.21</td>
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<td>HO-1</td>
<td>0.82 ± 0.14</td>
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<td>5 days</td>
<td>HMGB1</td>
<td>2.23 ± 0.58</td>
<td>2.34 ± 1.33</td>
<td>1.75 ± 0.24</td>
<td>1.33 ± 0.20*</td>
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<td>HIF1α</td>
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<td>IL-6</td>
<td>7.71 ± 8.37</td>
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<td>2.04 ± 0.99</td>
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<td>CD68</td>
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<td>21.28 ± 10.01</td>
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</tbody>
</table>

IRI: isogenic rat heart with ischaemia–reperfusion injury; IRI + Mo: isogenic rat heart with ischaemia–reperfusion injury treated with moclobemide; IRI + MI: isogenic rat heart with ischaemia–reperfusion injury and myocardial infarction of the heart apex; IRI + MI + Mo: isogenic rat heart with ischaemia–reperfusion injury and myocardial infarction of the heart apex treated with moclobemide; HMGB1: high-mobility group box-1; HIF1α: hypoxia-inducible factor 1α; IL-6: interleukin-6; CD68: macrophages; HO-1: hemoxgenase-1.

*P < 0.05 (IRI + MI + Mo versus IRI + MI, Mann–Whitney test), P-value between all groups according to the Kruskall–Wallis test.
fraction to describe cardiac function. IRI includes a dispersed range of histological findings necessitating careful statistical interpretation of the results. The results should be cautiously interpreted, as this study is experimental and may be categorized as translational; the findings and conclusions should not be simply translated to humans in the real clinical setting.

In conclusion, MAO may be associated with MI after IRI. We suggest further studies of MAO inhibition after MI during IRI. Despite these promising experimental results, clinical studies will be necessary to evaluate the therapeutic properties of MAO inhibitors after IRI, especially in organs such as the heart containing a large amount of MAOs [16].

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**REFERENCES**


