Eprosartan improves cardiac function in swine working heart model of ischemia-reperfusion injury

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Eprosartan is an angiotensin II receptor antagonist used as an antihypertensive. We sought to evaluate the regional effect of Eprosartan on postinfarct ventricular remodeling and myocardial function in an isolated swine working heart model of ischemia-reperfusion injury.

Material/Methods: 22 swine hearts were perfused with the Langendorff perfusion apparatus under standard experimental conditions. Myocardial ischemia was induced by a 10-min left anterior descending artery ligation. Hearts were reperfused with either saline (control group, n=11), or Eprosartan (treatment group, n=11). Left ventricular pressure (LVP) and regional heart parameters such as intramyocardial pressure (IMP), wall thickening rate (WTh), and pressure-length-loops (PLL) were measured at baseline and after 30 min of reperfusion.

Results: Measured parameters were statistically similar between the 2 groups at baseline. The administration of Eprosartan led to a significantly better recovery of IMP and WTh: 44.4±2.5 mmHg vs. 51.2±3.3 mmHg, p<0.001 and 3.8±0.4 µm vs. 4.4±0.3 µm, p=0.001, respectively. PLL were also significantly higher in the treatment group following reperfusion (21694±3259 units vs. 31267±3429 units, p<0.01). There was no difference in the LVP response to Eprosartan versus controls (63.6±3.0 mmHg vs. 62.5±3.1 mmHg, p=0.400).

Conclusions: Pre-treatment with Eprosartan is associated with a significant improvement in regional cardiac function under ischemic conditions. Pharmacological treatment with eprosartan may exert a direct cardioprotective effect on ischemic myocardium.

MeSH Keywords: Eprosartan • Langendorff perfusion • Reperfusion Injury

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Background

Ischemia-reperfusion injury (IRI) is a well-known phenomenon in thrombolysis, percutaneous coronary intervention, coronary artery bypass grafting, and cardiac transplantation [1]. IRI is clinically manifested as myocyte damage by myocardial stunning, microvascular injury, and, rarely, lethal myocyte necrosis [1]. Various mechanisms are involved in this complex process: production of reactive oxygen species, alterations in calcium homeostasis, endothelial cell dysfunction, neutrophil activation, and complement cascade [1].

Over the past decades, numerous pharmacological agents have been studied as potential treatment options for IRI. Several studies have demonstrated beneficial effects of angiotensin receptor blockers (ARB) on endothelial function, oxidative stress, antioxidant properties, and ventricular remodeling [2]. It was shown that angiotensin II-mediated AT1 receptor stimulation enhances oxidative stress by stimulating NADPH oxidase, with consequent generation of reactive oxygen species [3,4], which, in turn, enhances inactivation of endothelial nitric oxide and accelerates the progression of IRI [5]. Furthermore, AT1 receptors may trigger intracellular reactions contributing to myocyte and vascular hypertrophy, fibrosis, and apoptosis [6]. It is also known that diastolic stress, which induces programmed cell death *in vivo* and *in vitro*, is accompanied by an increase in angiotensin II and may be inhibited by AT1 antagonism [7,8].

In light of these considerations, the aim of the present study was to evaluate the hypothesis that treatment with Eprosartan, an AT1 receptor antagonist, has protective effects on postinfarct ventricular remodeling and improves myocardial function in an isolated swine working heart model of IRI.

Material and Methods

Animals

Twenty-two male Landrace pigs with a mean weight of 27.6±0.8 kg were used in this study. All animals received humane care in accordance with Animal Welfare Regulations published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996). The local Ethics Committee for Animal Experimentation approved the experiments.

Cardiectomy

Preoperative and anesthetic procedures

An intravenous catheter was placed in a superficial ear vein of the animal for intravenous infusion. The right cervical vein was cannulated with an 8 Fr catheter, and Ringer lactate solution

Table 1. Composition of modified Krebs-Henseleit solution.

Component	Amount
NaCl	128 mmol/l
KCl	3.6 mmol/l
NaHCO$_3$	25 mmol/l
MgSO$_4$	0.6 mmol/l
CaCl$_2$	1.25 mmol/l
KH$_2$PO$_4$	1.3 mmol/l
Glucose	11.2 mmol/l
Insulin	10 IE/l
BDM	30 mmol/l

was infused to maintain euvolemia. The right cervical artery was cannulated with a 14 G tube. After intramuscular injection of 4 mg Azaperone (Stresnil®, Janssen, High Wycombe, UK) and 0.01 mg/kg Fentanyl, 3–4 mg/kg Hypnomidate was administered intravenously, followed by intubation and ventilation with 40% FiO$_2$. Muscle relaxation was achieved with Pancuronium (0.3 mg/kg/h.

Surgical procedure and cardioplegia

Following thoracotomy, 1 L of blood was collected and anti-coagulated with citrate (50 ml, 3.2%). The blood was then filtered (Biotest TNSB-3 Transfusion Device, 200 µm), packed in commercially available blood bags, and stored at cold temperature for subsequent use in the lab. The heart was then procured and arrested with combined antegrade and retrograde cardioplegia using cold (4°C) Custodiol® solution (HTK solution, Dr. Franz Köhler Chemie GmbH, Alsbach-Hähnlein, Germany). A specially designed polyvinyl chloride cone was placed in the aortic root to achieve rapid delivery of antegrade cardioplegia and promptly reduce cardiac oxygen consumption. The heart was subsequently transported to the laboratory at 4°C in cardioplegic solution comprising 2,3-butanedione monoxime (BDM, Sigma, Deissenhofen, Germany) 30 mM; CaCl2 2.5 mM; glucose 11.2 mM; Heparin (Liquemin®N 25.000, Hofmann-La Roche, Grenzach-Wyhlen, Germany) 2000 I.U./l; insulin (Insuman® Rapid, Hoechst Marion Roussel, Frankfurt am Main, Germany) 10 I.U./l; KCl 2.3 mM; KH2PO4 1.3 mM; MgSO4 0.6 mM; NaCl 128 mM, and NaHCO3 25 mM) (Table 1). Butadione Monoxime (BDM) was specially employed for its superior myocardial protection, which permits storage/transport for several hours in an ice-cold solution (4°C) [9–11]. Solution without BDM was used for subsequent dialysis in the laboratory.

The cadaver of the animal was then discarded, and all further experiments were performed on the isolated heart. Anesthesia, surgery, and general care of the animals strictly conformed to

the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health, and the local Animal Care Committee approved the protocol.

Instrumentation of isolated hearts

Cardiac pacer leads, ECG electrodes, and sensors for pO2 measurement and microdialysis were placed. Sonocrystals, used to measure wall thickness (WTh) (Ultrasonic Triton®) and intramyocardial pressure (IMP) (microtip-manometer, SPC-320), were placed midway between the apex and base of the LV in the LAD bed. Pressure-length-loops (PLL) were obtained from these parameters, with the area of the loop demonstrating regional cardiac work. A tip-manometer (Millar, SPC-751, Houston, Texas) surrounded by a latex balloon was positioned inside the left ventricle (LV) to measure left ventricular pressure (LVP).

Hearts were prepared for perfusion via the coronary arteries as follows: the right coronary artery and the left anterior descending branch (LAD) as well as the circumflex coronary artery (CX) of the left coronary artery were cannulated using 3 flexible silicon catheters with a 3-cm Teflon tip and an inner diameter of 3 mm. A T-connector (3/4", Cole-Parmer) was inserted into the aorta for secure placement of the organ in the perfusion system.

Perfusion circuit

The perfusion system (Mediport Biotechnik GmbH, Berlin, Germany) consisted of a blood circuit and a dialysis unit (Figure 1). Two computer-controlled pumps (Watson Marlow 505U, Cornwall, UK) placed before and after the dialysis circuit prevented volume exchanges between blood and dialysis circulation.

The harvested autologous blood was treated with a leukocyte filter (Jostra Medizintechnik, Hirrlingen, Germany) to avoid myocardial damage by activated leukocytes. The blood circuit contained 1 L of this leukocyte-depleted blood, heparinized (10.000 I.U.) and diluted 1:1 with modified Krebs-Henseleit buffer solution to allow for near-normal hematocrit without the need for donor or support animals. The "working" model was designed to simulate actual working conditions of an *in vivo* heart, with modulation of preload and afterload. The preload line (polyethylene glycol, 20 mm diameter) was securely inserted in the left atrium. A variable-length (60–100 cm) afterload line (polytetrafluoroethylene [PTFE], 24 mm diameter) was connected to the aorta using a PTFE cone 25–36 mm diameter. Preload could be manipulated by changing the height of the preload reservoir relative to the level of the mitral valve, and afterload could be modified by adjusting the length of the afterload line (Figure 1, afterload). Preload and afterload were maintained at 10–20 mmHg and 100–120 mmHg, respectively.
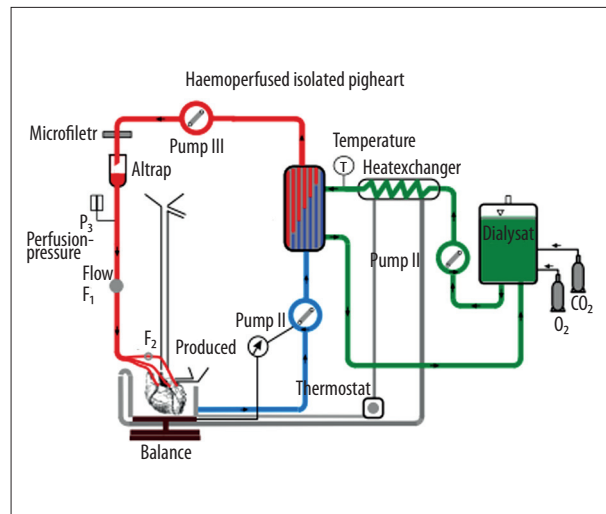

Figure 1. Perfusion circuit with the hemoperfused isolated swine heart. Schematic representation of the perfusion circuit.

Through an overflow tube in the afterload reservoir, the perfusate was collected into another reservoir and pumped by a roller-pump into the venous reservoir, where it then passed through a filter. A centrifugal pump routed the perfusate into the oxygenator, which contained 95% oxygen and 5% carbon dioxide. The oxygenator was connected to a laboratory thermostat (Haake Technik GmbH, Vreden, Germany) and a heat exchanger (D720 Helios C, Dideko, Mirandola, Italy) to maintain perfusate temperature at 37°C. This normothermic, oxygenated perfusate was then pumped by a second roller-pump back into the preload reservoir, where its temperature was monitored. From the preload reservoir, the left atrium was supplied with the oxygenated perfusate.

The dialysis unit (Fresenius Polysulfone® UF 6.4, Haemoflow F7, Bad Homburg, Germany) was used to assess oxygenation of the circulating blood, for filtration of metabolites, and as a source of glucose and insulin to ensure euglycemia. An air trap (dialysis set: Gambro® medical line, arterial line: A-5. 126-B8, Hechingen, Germany) was interposed for an air-free hemoperfusion. Five liters of dialysate was admixed with oxygen bubbles to maintain pO_2 and pCO_2 at 420–450 mmHg and 35–45 mmHg, respectively, before the dialysate entered the heart. An automated blood gas analyser (ABL™505 Blood Gas and Electrolyte System and OSM™3 Hemoximeter™, Radiometer Copenhagen, Denmark) was used for analysis of blood gases and electrolytes. To facilitate rapid metabolic substance exchange, a Heidolph pump (pump I) was used, which had a maximum pump volume of 19 L/min – 10 times that of the blood pumps (pump II and III). The pH of the solution was measured with a digital pH meter (GPH 014, Greisinger electronic, Regenstauf, Germany) and corrected with bicarbonate or CO_2 as needed to maintain mean pH values of 7.4±0.08.

Reperfusion

Connection to the perfusion system and reperfusion

Following cannulation, hearts were connected to the perfusion apparatus. Coronary perfusion was controlled by pump flow (constant pressure perfusion) with catheters in the coronary ostia and coronary blood flow (CBF) values measured by a flow sensor (Transonic® Transit-Time Flowmeter, Ithaca, USA). The coronary perfusion pressure (CPP) was initially kept at 50 mmHg and was increased to 70 mmHg after an arbitrary interval of 2 min. In the event of ventricular fibrillation, electrical defibrillation was performed as necessary to achieve sinus rhythm. Following a stabilization period of 10–15 min, CBF was adjusted to reach a CPP of 80–120 mmHg. Control of aerobic flow to the LAD was set by adjusting the calibrated perfusion pump to a flow rate that yielded a perfusion pressure equivalent to mean aortic pressure. Flow to the circumflex and right coronary beds was constant at aerobic levels throughout the experiment.

Measurements on the isolated hearts

LVP, CPP, and CBF were assessed. Arterial blood gas analysis and oximetry were performed every 15 min and included pO_2, pCO_2, pH, hemoglobin, sodium, potassium, calcium, chloride, glucose, and lactate levels.

Experimental protocol

Twenty-two isolated pig hearts were randomly assigned to either of 2 groups. The treatment group (n=11) received Eprosartan (85-mg bolus into the aortic root) before ischemia and after completing the baseline measurements. The control group (n=11) underwent ischemia without Eprosartan treatment. Additional hearts (n=6) were used before the study initiation for dose-dependent tests of Eprosartan and standardization of the perfusion circuit.

Hearts in both groups were subjected to a protocol consisting of a 10-min control flow followed by 10-min ischemia induced by occlusion of the LAD behind the first diagonal branch, followed by a final 30-min phase of reperfusion. LV systolic pressure, IMP, WTh, and PLL were measured to assess cardiac contractile function. At the end of reperfusion, heart weight was measured and compared to pre-experimental values, which were used as the control for this study.

Data collection and analysis

After a hemodynamic steady-state was achieved, all signals were digitalized and collected using an AD-converter (National Instruments: AT MIO 16E) with a sampling rate of 5 ms in a portable personal computer. Data acquisition was performed using Cordat II (Triton°, USA) software, with display of all signals and digital values simultaneously on 2 monitors, and real-time calculation of ECG parameters, pressure, flow, and dimension, including curve areas, as single and mean values.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 21 software (IBM Corp., Ehningen, Germany). All values are reported as mean ± standard deviation (SD). For statistical analysis, the independent Student t test was applied for inter-group comparisons and the paired-samples t test for comparisons of samples at baseline and after induction of ischemia. $P<0.05$ was taken as indicating significance.

Results

Intramyocardial pressure

Intramyocardial pressure (IMP) was measured under ischemic (occlusion) and non-ischemic conditions for direct comparison, with and without administration of Eprosartan. Figure 2 shows changes of intramyocardial pressure (IMP) in response to myocardial ischemia. There was no statistically significant difference in terms of IMP at baseline (p=0.466). Ten minutes of ischemia resulted in a significant reduction of IMP in both groups: from 71.1±3.9 mmHg to 44.4±2.5 mmHg in the control group and from 72.4±4.1 mmHg to 51.2±3.3 mmHg in the treatment group (p<0.001). At this time point, the IMP was significantly higher in the Eprosartan group compared to the control group (p<0.001).

Wall-Thickening rate

The WTh was measured under the same ischemic conditions (Figure 3). While there was no statistically significant difference in WTh between the 2 groups at baseline (p=0.843), WTh was significantly reduced after 10 min of ischemia: 6.7±0.6 μm *vs.* 3.8±0.4 μm in the control group and 6.8±0.5 μm *vs.* 4.4±0.3 μm in the Eprosartan group, p<0.001. WTh was significantly higher after the ischemic period under the pharmacological influence of Eprosartan when compared to 10 min of ischemia in the control group (p=0.01).

Pressure-length loops

PLL represents intramyocardial pressure and wall-thickening rate as a highly sensitive parameter of regional heart work. There was no statistically significant difference regarding PLL at baseline (p=0.811, Figure 4). Ten minutes of ischemia resulted in a significant reduction of PLL in both groups: 42157±3319 units
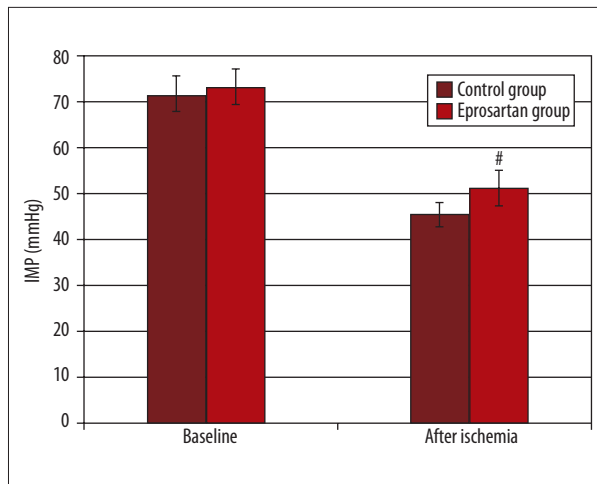
Figure 2. Effect of eprosartan on intramyocardial pressure (IMP) in ischemic swine myocardium. Hearts were reperfused with either saline (control group, n=11), or Eprosartan (treatment group, n=11). All values are reported as mean ± standard deviation (SD). # P<0.05.
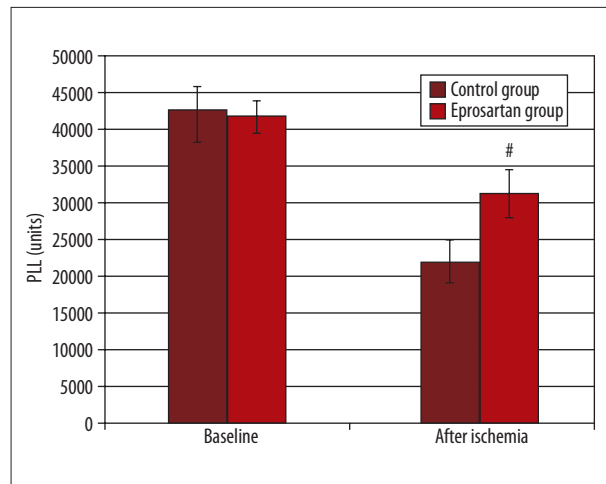

Figure 4. Pressure-Length Loops (PLL) under ischemic conditions before and after application of Eprosartan. Hearts were reperfused with either saline (control group, n=11) or Eprosartan (treatment group, n=11). All values are reported as mean ± standard deviation (SD). # P<0.05.
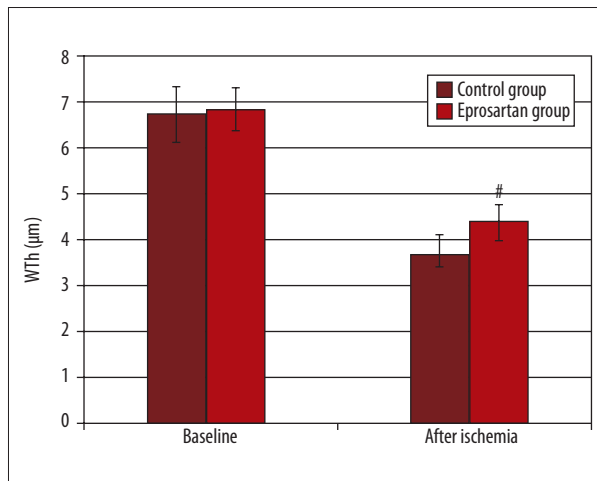

Figure 3. Effect of eprosartan on wall-thickening rate (WTh) in ischemic swine myocardium. Hearts were reperfused with either saline (control group, n=11), or Eprosartan (treatment group, n=11). All values are reported as mean ± standard deviation (SD). # P<0.05.
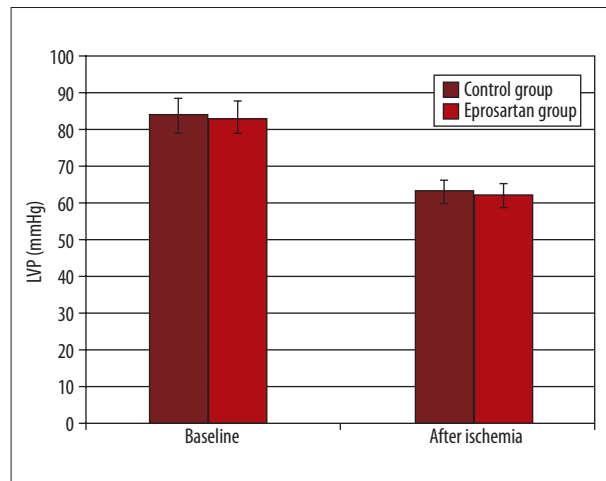

Figure 5. Left ventricular pressure (LVP) under ischemic conditions before and after administration of Eprosartan. Hearts were reperfused with either saline (control group, n=11) or Eprosartan (treatment group, n=11). All values are reported as mean ± standard deviation (SD). There was no significant difference between the groups at baseline and 10 min after induction of ischemia.

vs. 21694±3259 units in the control group and 41853±2495 units *vs.* 31267±3429 units in the treatment group (p<0.001). At this time point, PLL was significantly higher in the Eprosartan group compared to the control group (p<0.001).

Left ventricular pressure

The systolic LVP was measured as a parameter for global left heart function. LVP was significantly reduced after ischemia in both groups (84.1±5.1 mmHg *vs.* 63.6±3.0 mmHg in the control group and 83.0±5.0 mmHg *vs.* 62.5±3.1 mmHg in the

treatment group (p<0.001). However, there was no significant difference between the groups both at baseline (p=0.607) and 10 min after induction of ischemia (p=0.400, Figure 5).

Warm ischemic time from excision to beginning of cardioplegic infusion was less than 1 min in each case. Mean cold ischemic time (from excision to reperfusion) was 136.6±15.5 min. Lactate levels of perfusate were stable throughout the experiments. Mean heart weight was 171.4±5.8 g at baseline and

197.8±5.5 g after perfusion, and was not significantly different between the treatment and control groups.

Discussion

The aim of the present study was to examine *in vitro* the acute effects of Eprosartan, an angiotensin II type I receptor antagonist, during induced ischemia of an isolated, hemoperfused heart in a porcine model using Langendorff perfusion. Both regional and global cardiac function were evaluated and analyzed. This study shows a direct, measurable, and beneficial effect of Eprosartan in reducing IRI and consequent post-ischemic LV dysfunction and remodeling.

AT1-receptor antagonists are a group of pharmaceuticals known to modulate the renin-angiotensin-aldosterone system by blocking the binding of angiotensin II to AT1 receptors. Several studies have addressed the effects of AT1 antagonists on cardiac dysfunction [12]. Whole-animal experiments have shown that Candesartan, an AT1 antagonist, protects the porcine heart from reperfusion damage, improves myocardial functional recovery, and reduces infarct size in the ischemia model [13]. L-158.809, an AT1 antagonist, diminished infarct expansion and left ventricular remodeling in dogs with induced myocardial infarction caused by coronary ligation [14]. Wang et al. showed improved hemodynamic parameters after microembolization of the coronary arteries in rabbit hearts by treatment with AT1 antagonists [15]. Tsutamoto et al. demonstrated – by pharmacologic blockade of the AT1 receptor – an improvement of the global left ventricular function and a decline of the TNFα concentration [16]. Additionally, a selective blockade of the AT1 receptor caused improved cardioprotection in the ischemic/reperfused heart [17].

Eprosartan is a newer AT1-receptor antagonist that has a high affinity for the AT1 receptor on the surface of myocardial cells [18] and has been shown to prevent LV dysfunction and reduce progressive LV remodeling in a canine model [19]. This body of evidence forms the basis of our attempt to investigate the effect of Eprosartan on myocardial protection against ischemic damage and dysfunction [12–20].

Myocardial ischemia/reperfusion injury

Post-ischemic reperfusion leads to myocardial and coronary endothelial dysfunction. Intracellular acidosis during myocardial ischemia leads to an increase of cytosolic Na^+, which is then exchanged with calcium via ionic pumps, resulting in cellular Ca^{2+} overload [21], with consequent loss of sensitivity of the myofilaments for Ca^{2+} [22,23] and myocardial stunning [38]. The AT1 antagonist Losartan has been shown to prevent this diastolic Ca^{2+} overload [24]. Presumably, Eprosartan similarly stabilizes myocardial sensitivity for Ca^{2+} and leads to diminished myocardial stunning following myocardial ischemia.

Moreover, reperfusion engenders the release of chemokines such as IL 8, platelet activating factor, and C5a, which, in turn, lead to a release of free oxygen radicals, proteases, and neutrophilic lysosomal enzymes. Neutrophilic agglutination can occlude small capillaries, arterioles, and precapillary vessels, causing the so-called "no-reflow" phenomenon [25]. Furthermore, neutrophils have the ability to produce vasoconstrictive substances that downsize the vessel diameter and consequently reduce perfusion [26].

Leucocyte depletion of the harvested autologous blood effectively blocks the aforementioned harmful effects, with thrombocytes and micro-coagulum being removed by thrombocyte filters. Several working groups have already demonstrated the influence of leukocyte depletion on cardiac function, and documented diminished reperfusion damage with preservation of the endothelial function and left ventricular diastolic compliance [27,28].

Isolated swine working heart model

In 1895 Langendorff first described the isolated heart model that has since undergone numerous modifications in the fields of pharmacology, cardiology, and cardiac surgery to quantify and influence cardiac function [29]. This preparation was refined by using whole blood as a perfusate and by either perfusing the coronary arteries directly or filling the atria and chambers in a working heart model in pigs [11,30]. Previous work has been performed in hearts from the abattoir or in surgically explanted hearts [31]. The pathophysiology of cardiac arrhythmias and electrical conduction of endocardial pacing were studied in coronary perfused pig hearts [32,33]. This perfusion mode has also been used to study right and left ventricular performance in neonatal hearts [34] and to compare different techniques of donor heart preservation [30].

Most heart perfusions are performed on small-mammal hearts, which are nevertheless prone to edema and are too small to use in research asking numerous questions [35,36]. The dissociation from neurohormonal influences in the hemoperfusion model utilized herein facilitates an objective evaluation of special pharmacological effects under controlled experimental conditions. A distinct advantage is the model's precision, allowing accurate control of regional and global cardiac perfusion/ischemia and oxygenation, as well as controlled nutrient delivery. Furthermore, in contrast to *in vivo* conditions, isolated heart preparation allows experiments to be continued after fatal events (e.g., infarction-induced cardiac arrest or arrhythmias), which can frequently terminate an *in vivo* experiment.

Of all animal species studied, the porcine heart is the most similar to the human heart as regards anatomy and physiology.

Compared to the common perfusion of smaller (e.g., murine) hearts, the porcine heart also offers the advantage of greater comparability of extrapolation of regional changes demonstrable by imaging and other investigative modalities. The minute collateralization of the porcine heart facilitates examination in ischemia models. Studies with advanced imaging procedures are quite possible, and utilization of catheter materials and probe measurements reliably used in human medicine are almost always feasible in porcine hearts.

The results of the isolated, hemoperfused porcine heart, although not as strictly physiological as those in intact animals, remain largely comparable to those of *in vivo* animal experiments, while offering much greater control and reproducibility. The wide range of examinations that can be conducted on the hemoperfusion model can contribute, in the context of the usage of organs of animals for slaughter, to a reduction of animal testing and cost-saving within the scope of research [37].

Limitations

The findings in this study relate to isolated, hemoperfused porcine hearts without influence of neuronal, humoral, or hemodynamic counter-regulation. These results may differ *in vivo*, with chronic changes that cannot be demonstrated in such experimental models.

Conclusions

The current work demonstrates for the first time a direct, measurable, and beneficial effect of Eprosartan in reducing IRI and consequent post-ischemic LV dysfunction and remodeling in the isolated, hemoperfused swine heart model, which most closely resembles the human heart anatomically and physiologically. Further studies are warranted to facilitate the extrapolation and application of these experimental findings to clinical research scenarios.

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