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Beneficial Effects On Cardiac Performance and Cardioprotective Properties of Milrinone after Cold Ischemia

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Abstract

Background: Cold Ischemia-Reperfusion Injury (CIRI) is regarded as the major cause of early graft dysfunction after cardiac transplantations and is associated with rejection episodes. Consequently, it is one of the main therapeutic targets in order to improve survival after heart transplantation. The aim of this study was to evaluate hemodynamic effects of milrinone and its influence on the markers of myocardial damage when used in a piglet working heart model with a cold ischemia-reperfusion setting.

Methods: Hearts of 18 piglets were examined in a homologous blood-perfused, working heart model to get baseline measurements. After hypothermic cardioplegic arrest and storage on ice for 60 minutes, the hearts received either milrinone or served as controls. All hearts were examined for 45 minutes in the working heart model. Hemodynamic parameter changes, h-FABP levels and myocardial oxygen consumption were assessed.

Results: Significant difference between the groups was observed in cardiac output (MIL +14% vs. CON -33%; $p<0.05$), coronary sinus blood flow (MIL +84% vs. CON +17%; $p<0.05$) and relaxation (MIL +5% vs. CON -22%; $p<0.01$). In addition, significantly higher h-FABP (heart fatty acid binding proteine) levels after cold ischemia were measured in CON group (CON: 18.75 ng/ml; MIL 6.29 ng/ml; $p<0.01$).

Conclusions: Milrinone has a positive effect on cardiac function after cardioplegic cardiac arrest with following cold-ischemia period in an isolated piglet heart model. Its use in a heart transplantation setting induces an improved hemodynamic performance and a better preservation from reperfusion injury.

Keywords: Milrinone; Heart transplantation; Ischemia

Introduction

The shortage of donor organs presents a global problem. Hence, there is a necessity for the ideal protection of donor organs to optimize the transplantation results. The protection begins with organ preservation techniques after organ explantation. Cold Ischemia Reperfusion Injury (CIRI) after heart transplantation is an important cause of graft dysfunction and myocardial damage [1]. It is the primary trigger to the recipient's immune system activation with consequent inflammatory response involving cytokines, endothelial cell factors and leukocytes, which promote the development of graft coronary artery disease [2]. Furthermore several studies observed myocardial apoptosis as an early event in the setting of CIRI [3]. After reperfusion systolic and diastolic function of the graft is detoriated. Additionally, calcium overload, cellular swelling and interstitial edema due to the cold ischemic storage worsen the cardiac performance [4]. The dysfunction may result in a postoperative low cardiac output syndrome, with insufficient perfusion of kidneys, liver and other organs causing organ failure [5].

Phosphodiesterase III inhibitors, with combined inotropic, lusitropic and vasodilatory effects, are agents whose efficacy in the management of patients with low cardiac output syndrome after cardiac surgery is clinically proven in both adult patients and also in children [6,7]. Effects of phosphodiesterase III inhibitors are based on the inhibition of the Cyclic Adenosine Monophosphate (cAMP) - specific phosphodiesterase III isoenzyme in the myocardium and vascular smooth muscle cells. Thus, unlike catecholamines, phosphodiesterase III inhibitors increase the intracellular c-AMP concentration by non-ß1-adrenergic pathways. By increasing intracellular c-AMP concentration in myocytes, milrinone increases both the permeability of slow calcium channels in the sarcolemma, which consequently leads to an increase in intracellular ionized calcium, and a more intensive calcium storage and release from the sarcoplasmatic reticulum [8]. By those pathways milrinone increases inotropy and improves diastolic relaxation. Additionally Sanada et al. demonstrated in an animal model direct cardioprotective properties of milrinone with limitation of the infarct size [9].

The aim of our study was to evaluate the effects of milrinone in blood-perfused piglet hearts after cardioplegic arrest with following cold ischemia in an isolated heart model. That model with both a constant left atrial preload and left ventricular afterload, allowed us to observe the milrinone-induced hemodynamic effects on left ventricular myocardium.

h-FABP (heart fatty acid binding proteine), the main cytosolic fatty acid transporter in the myocardium, was first described as myocardial necrosis marker in 1998. Because of its high concentrations in the myocardium, early release into the circulation and high sensitivity it is the ideal marker to detect early cardiac damage. Its utility in the field of

cardiac surgery was proved in several studies as the concentration peak one hour after aortic declamping could early indicate intraoperative ishemic injury [10,11].

H-FABP was measured in our setting in order to evaluate cardioprotective potential of milrinone in the reduction of CIRI when applied during reperfusion.

Methods

The procedures and animal care conform to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985) and the German "Law on the Protection of Animals".

Operative procedure

The hearts were explanted from German land-race piglets weighing 10.5 ± 0.9 kg. Preoperatively, all animals received an intramuscular (i.m.) preanesthetic medication including atropine and azaperone. Under i.m. sedation with diazepam and ketamine, the animals were transported to the operating room. After placement of the central venous line and pre-oxygenation, standardized general anesthesia was induced with fentanyl, propofol and vecuronium. The animals were intubated endotracheally and ventilated. Anesthesia was continued with dose maintenance infusions of flunitrazepam and propofol. Continuous analgesia was ensured with fentanyl. Before cardioplegia and exsanguination, the animals received the triple induction dose of flunitrazepam, fentanyl and propofol.

The thorax was opened through a midline sternotomy, and the ascending aorta was cannulated with a 16 G cardioplegia cannula. The animals then received 2500 I.U. heparin and a narcotic bolus. The inferior caval vein was opened for exsanguination and one pulmonal vein was opened as well to relieve pressure from the left ventricle. When the right ventricle was visibly empty the ascending aorta was cross-clamped. The crystalloid cardioplegic infusion (St. Thomas solution) was started and the pericardium was filled with cold saline (4°C). After 5 minutes of cardioplegic infusion the heart was explanted.

Isolated heart perfusion and study protocol

After cardioplegia and explantation, the hearts were weighed and the great vessels were connected with the perfusion model (Isolated Heart Model Size 9 type 842/1, Hugo Sachs Elektronik, Harvard Apparatus GmbH, March-Hugstetten, and Germany). Pacemaker electrodes were sutured to the left ventricular epicardium and connected to the device (Medtronic® 53755, Medtronic Inc. Minneapolis, U.S.A. The hearts were perfused with diluted homologous whole pig blood from the slaughterhouse. Blood from one donor pig had been heparinized (50 I.U./ml) and diluted to a hematocrit of 25% with potassium free Krebs Henseleit solution (pharmacy of the University Hospital Tuebingen) in which gentamicin sulfate, insulin, hydrocortisone and glucose had been added.

The blood was transported from the heated reservoir to the membrane oxygenator (Hollow Fiber Membrane Oxygenator®, Maquet Medizintechnik, Hirrlingen, Germany) using a nonpulsatile roller pump and directed to the preload chamber. In the working heart mode the oxygenated blood from the preload chamber filled directly the left atrium. In contrast, during the Langendorff reperfusion, a retrograde roller pump transported the oxygenated blood from the preload to the afterload chamber.

The pO_2 and the pCO_2 were adjusted using the Sho-rate device (Sho-rate®, Brooks Instruments, Veendal, Holland) of the oxygenator. If necessary, pH values were normalized by changes of pCO_2.

All hearts were first perfused in the Langendorff mode with a perfusion pressure of 40 mmHg, measured by the Isotech-pressure-transducer® (HSE, March-Hugstetten, and Germany). Initially, in the working heart phase, a MIKRO-TIP® pressure-transducer catheter (Millar instruments, Inc., U.S.A.) was placed via the ascending aorta in the left ventricle. Heart rate (HR), left ventricular systolic (LVSP) and end-diastolic (LVEDP) pressure, contractility (+dp/dt max) and relaxation (-dp/dt max) were recorded via the MIKRO-TIP® pressure-transducer catheter, whereas Cardiac Output (CO) and Coronary Sinus Blood Flow (CF) were measured by flowprobes based on ultrasonic technique.

Both measured and derived hemodynamic parameters were registered on-line with the Haemodyn® Word software for Microsoft® Windows® 95/98/NT (Hugo Sachs Elektronik, March-Hugstetten, and Germany). Left ventricular-stroke work was calculated and normalized for heart weight as Stroke Work Index (SWI). Pulmonary vein pressure (preload), measured by the pressure transducer, and was adjusted from 6-8 mmHg and the aortic blood pressure (afterload) to 60 mmHg (Isotech-pressure-transducer®, HSE, March-Hugstetten, and Germany).

After registration of baseline measurements in the first working heart mode St. Thomas solution was administered and afterwards the hearts were stored on ice in cold St. Thomas solution (4°C). Meanwhile blood temperature was adjusted to 30°C.

After 1 h of cold ischemia on ice, the hearts were subjected to the Langendorff mode with a pressure-controlled perfusion (40 mmHg). The blood was rewarmed during this reperfusion period from 30°C to 38°C. Twenty minutes later, the second working heart stage was started and the hemodynamic data were collected.

The piglet hearts were randomly assigned to the control group CON without milrinone application and to the group MIL receiving milrinone. Milrinone was administered as a bolus dose of 0.6 mg into the blood reservoir containing 3 liters of diluted homologous blood taking into account the protein binding (50-80%) of the drug and the findings of Zausig et al. in their study analyzing milrinone dosage and effects in an isolated guinea pig heart model, as well as our experience from previous studies [12].

h-FABP determination

After the second working heart period blood samples were taken from the perfusion reservoir. After centrifugation and separation from the clot, the serum samples were frozen in liquid nitrogen and stored at -20°C. H-FABP plasma levels were determined with the aid of a sandwich-type ELISA kit (HK 403 kit, Hycult® Biotech, Uden, and The Netherlands).

Statistical analysis

Milrinone and control groups were compared on each parameter using repeated-measures mixed-model analysis of variance (ANOVA) in order to account for multiple measurements within each animal over the time course. Therefore, means and standard errors were used to summarize the results of each of the seven ANOVAs (CO, CF, LVSP, LVEDP, +dt/dp max, -dt/dp max, SWI and HR). ANOVA was also used to evaluate statistical significance of the results of myocardial oxygen consumption between the study groups. H-FABP levels were

analysed using student´s t-test. Analysis of the data was performed using the SPSS software package (version 15.0, SPSS Inc., Chicago, IL, USA). Change from baseline values are presented in terms of the mean and standard error. Two-tailed values of p<0.05 were considered statistically significant.

Results

A total of 25 piglet hearts were assigned randomly either to the group MIL, which was treated with milrinone or to the group CON without milrinone application. Four hearts from the CON and two hearts from the MIL group showed a cardiac failure with an irreversibly akinetic left ventricle after the ischemia period with a cardiac output of less than 0.1 l/min. To prevent statistical bias those experiments were terminated and excluded from the further analysis. Beyond, one experiment in the CON group failed because of a technical problem with the online data collection. Consequently the results of two experimental groups of non-identical size (CON: n=7; MIL: n=11) were statistically analysed.

Averaged percent changes in hemodynamic parameters of each heart during the working heart period were compared relative to the baseline values (before drug application) (Table 1) and the statistical significance was evaluated (+means increase, -means decrease compared to baseline parameters).

The two groups were comparable for LVSP (MIL: -6% ± 2; CON: -10% ± 5; Figure 1) and contractility (+dP/dt) (MIL: -13% ± 3; CON -9% ± 9; Figure 1). LVSP and +dP/dt decreased in both groups compared to the measurements before the cold ischemia but the difference between CON and MIL was not significant (LVSP: p=0.63; +dP/dt: p=0.65).

Significant differences between the groups were observed in CO, -dP/dt and CF. The CO increased in the milrinone-treated group by 15% ± 12 whereas a decrease in the control group (CON: - 33% ± 10; Figure 1) could be measured. The statistical analysis showed significant difference between the study groups (p value < 0.05).

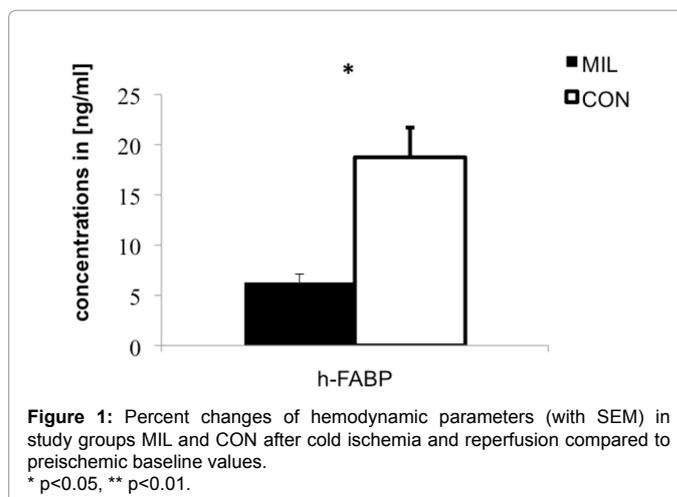The study groups also differed significantly (p < 0.01) with regard to –dP/dt. There was a 6% ± 6 increase in the MIL and a 22% ± 9 decrease in the CON group compared to the measurements before the cold ischemia (Table 1 and Figure 1). Coronary flow increased in both groups but only the increase in the MIL group reached statistical significance (MIL: +83 ± 11; CON: +43 ± 21; p<0.05, Figure 1).

Higher cardiac output in the study group with milrinone treatment was a result of a better relaxation and was not due to Heart Rate (HR) changes as HR decreased in both groups after baseline measurements and the difference between MIL and CON was not significant (MIL: 98% ± 0.8; CON: 96% ± 0.4; p=0.051).

Stroke work index increased by 12% ± 12 in the MIL group and

Figure 1: Percent changes of hemodynamic parameters (with SEM) in study groups MIL and CON after cold ischemia and reperfusion compared to preischemic baseline values.
* p<0.05, ** p<0.01.

decreased in the CON group by 7% ± 15 without reaching statistical significance (p=0.4).

LVEDP increased in both groups with a trend towards lower LVEDP values in the control group after cold ischemia (CON: + 101% ± 54; MIL: + 41% ± 15; Figure 1; p=0.18).
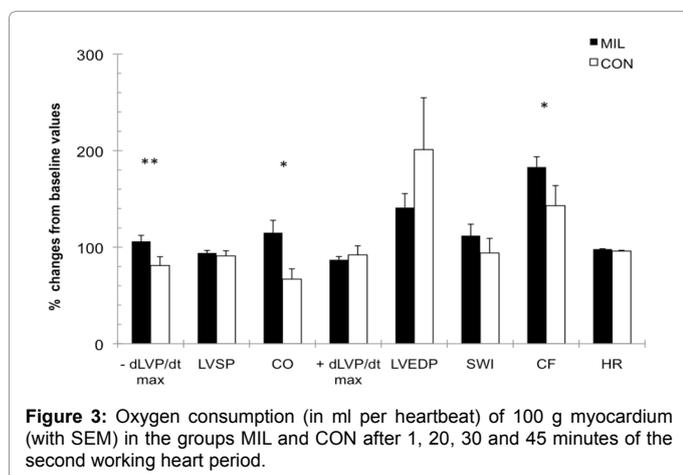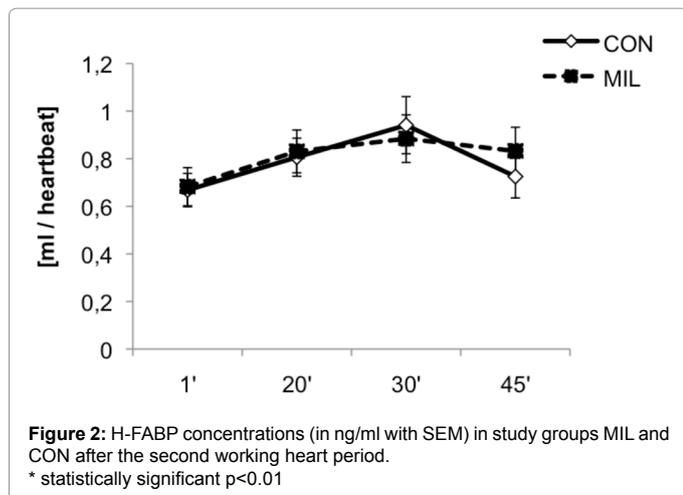
H-FABP concentrations detected after cold ischemia and reperfusion were significantly higher in the CON group (CON: 18.75 ng/ml; MIL 6.29 ng/ml; p<0.01, Figure 2) whereas the oxygen consumption calculated in the working heart period after cold ischemia and reperfusion showed no statistically significant differences between the study groups or between the four intervals of the working heart period (Figure 3).

Discussion

Several studies have assigned cardioprotective properties to milrinone. It reduced the left and right ventricular infarct size in animal studies after selective ligation of coronary arteries [9,13,14]. Cardioprotective properties of milrinone could be affirmed by our study performed in a cardiac transplantation setting. We used heart fatty acid binding protein - an early marker of cardiac necrosis – to detect myocardial injury. Hasegawa et al. proved with their study on pediatric cardiac surgery patients that h-FABP could be detected already after aortic declamping, remarkably earlier than troponine or creatine kinase [10]. They stated that h-FABP is a rapid marker for assessment of myocardial injury and for clinical outcome in pediatric cardiac surgery, so that we regarded the h-FABP as the best parameter for myocardial damage since the hearts in our study have been perfused for 65 minutes after cold ischemic period [10]. In our study milrinone application led to significantly lower h-FABP plasma concentrations indicating a lower level of cold ischemia reperfusion injury.

The evaluation of the underlying molecular mechanism of that effect was not an aim of this study, yet the observations of other studies demonstrated several cardioprotective effects of milrinone.

First, the Ca^{2+} overload and the myocadial stunning caused by CIRI could be prevented by an improved Ca^{2+} metabolism with faster calcium uptake to the Sarcoplasmatic Reticulum (SR) induced by the inhibition of a SR associated phosphodiesterase III and the consecutive cAMP dependent activation of the SR Ca^{2+} ATPase [8,15]. Second, milrinone prevents the opening of Mitochondrial Permeability Transition Pore (mPTP). The opening of mPTP after ischemia usually

Parameter	Baseline CON	Baseline MIL	Unit
-dLVP/dt max	1245 ± 158	1112 ± 52	mmHg/ms
LVSP	102 ± 12	107 ± 3	mmHg
CO	1.04 ± 0.23	0.85 ± 0.07	l/min
+dLVP/dt max	1327 ± 231	1717 ± 108	mmHg/ms
LVEDP	4.1 ± 1.2	2.8 ± 0.7	mmHg
SWI	9.9 ± 2.5	9.2 ± 0.5	[10^3× dynes ×cm ×beats×1/g]
CF	129 ± 17	115 ± 9	ml/min
HR	148 ± 11	136 ± 7	1/min

Table 1: Average preischemic baseline values (± SEM) of the CON and MIL group measured before the cold ischemia for -dLVP/dt max, LVSP, CO, + dLVP/dt max, LVEDP, SWI, CF and HR.

Figure 2: H-FABP concentrations (in ng/ml with SEM) in study groups MIL and CON after the second working heart period.
* statistically significant p<0.01

Figure 3: Oxygen consumption (in ml per heartbeat) of 100 g myocardium (with SEM) in the groups MIL and CON after 1, 20, 30 and 45 minutes of the second working heart period.

appears in the early reperfusion phase and is regarded as the most important event leading to cell death in ischemia-reperfusion injury [3]. Prevention of the opening of mPTPs was proved to reduce infarction size after milrinone application [16]. Third, milrinone was showed to reduce apoptosis after simulated ex-vivo cold ischemia and reperfusion in human myocardial biopsies [17].

The cardioprotective effect seen in our study was an improved relaxation in the milrinone treated hearts and which can be explained by an improved intracellular Ca^{2+} reuptake and storage in the SR preventing Ca^{2+} overload. Furthermore the lower h-FABP values in the milrinone-treated group indicate less myocardial necrosis probably by the described mechanism of mPTP regulation.

Beside those cardioprotective effects, milrinone use is already well established in the therapeutical setting of the Low Cardiac Output Syndrome (LCOS) after pediatric and adult cardiac surgery [7]. LCOS may also occur after cardiac transplantation causing higher morbidity and mortality rates. In our study milrinone was showed to improve cardiac output without increasing the oxygen consumption or the heart rate. The improved cardiac output was due to a better relaxation and the subsequent better left ventricular filling while the changes in contractility between the two groups were non-significant. In consideration of our results we can state that milrinone has a very favourable effect on cardiac performance after cold ischemia reperfusion injury, when applied during the reperfusion phase on

cardiopulmonary bypass. The major benefit in an isolated left heart perfusion model was an improved relaxation resulting in a higher cardiac output.

There are three mechanisms that can lead to a better myocardial perfusion measured by higher coronary flow in our study. First, the coronary autoregulation reacts to higher oxygen demand and causes vasodilatation. Second, the higher coronary sinus flow is due to a better myocardial perfusion during the diastole induced by improved relaxation. And finally the effect can also be assigned to a direct vasodilative effect of milrinone [18]. As there was no difference between the groups in terms of oxygen consumption, the autoregulation is unlikely to be responsible for the higher myocardial perfusion of the milrinone-treated hearts. Consequently the observed effect is a result of a better relaxation in combination with milrinone-induced direct coronary vasodilation.

As our results show, the described cardioprotective property of milrinone is mainly due to an improved coronary perfusion supporting cardiac recovery after cold ischemia.

We applied milrinone after the cold ischemia period and during the reperfusion, while Besirli et al. added milrinone already to the cardioplegic solution and described its beneficial effects in terms of cardioprotection. Further studies are needed to evaluate the optimal point of time for the drug application [19]. We consider the systemic application during reperfusion as a measure that should be considered by the surgeon in order to achieve cardioprotection and a stable hemodynamic situation after reimplantation of the organ.

In conclusion, this study has proved cardioprotective effects of milrinone used in a setting imitating cardiac transplantation procedure with cold ischemia and reperfusion. We showed improved diastolic cardiac performance with positive influence on cardiac output and coronary blood flow after cold ischemia with neither an increase in oxygen consumption nor the positive chronotropic effects described in previous studies. The milrinone use during cardiac transplantation may reduce myocardial damage, improve cardiac performance and provide better hemodynamic conditions for the donor heart and consequently reduce morbidity and mortality after cardiac transplantation.

Acknowledgments

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