Improved right ventricular function after intracoronary administration of a C1 esterase inhibitor in a right heart transplantation model

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Abstract

Objective: Myocardial injury from ischemia can be augmented after reperfusion due to proinflammatory events including complement activation, leukocyte adhesion, and release of various chemical mediators. It has been shown that intracoronary administration of a C1 esterase inhibitor (C1 INH) significantly reduces myocardial necrosis in an experimental model of ischemia. Our study addresses the question whether the most susceptible region of the heart for ischemic injury, the right ventricle (RV), can benefit from the protective effects of C1 esterase inhibition after transplantation.

Methods: To precisely control RV volume in vivo an isovolumic model was used in which the RV volume was regulated using an intracavity high compliance balloon inserted into donor hearts of domestic pigs (34 ± 4 kg). After 4 h of ischemia, donor hearts were transplanted into recipient pigs (44 ± 4 kg). Treatment groups, each with six animals, consisted of C1 INH treatment or control. After opening the cross clamp, the C1 INH group animals received 20 IU/kg body weight of C1 INH intracoronary over a 5 min period. The control animals received no drug therapy. The hearts were reperfused for 60 min, and thereafter the RV balloon volume was increased in 10 ml increments until RV failure occurred. These measurements were repeated after 120 min of reperfusion.

Results: There was no significant difference in maximal RV developed pressure between the two groups (after 1 h, 35.7 ± 5.9 vs. 40.6 ± 12.7 mmHg; after 2 h, 41.5 ± 10.7 vs. 46.3 ± 15.2 mmHg; for C1 INH and control animals, respectively). However, the RV could be loaded with a significantly higher volume after both 1 h (60.0 ± 20.0 ml (C1 INH) vs. 46.7 ± 13.7 ml (control) balloon volume, P < 0.05), and 2 h of reperfusion (70.0 ± 8.9 ml vs. 60.0 ± 6.3 ml; C1 INH and control animals, respectively; P < 0.05). Conclusions: Intracoronary administration of a C1 INH significantly improves right ventricular function in an experimental transplant model. Thus, inhibition of the classic complement cascade may be a promising therapeutic approach for effective protection of myocardium from reperfusion injury after transplantation.

Keywords: Myocardial protection; Heart transplantation; Right ventricular function; C1 esterase inhibitor

1. Introduction

Activation of the complement system involves a complex cascade of reactions containing more than 30 various glycoproteins present in blood in the form of components, factors, or other regulators and/or on the surface of different cells in the form of receptors. These reactants are activated from an inactive state by immune complexes (classical pathway), by carbohydrates (lectin pathway), or by other substances, mainly of bacterial origin (alternative pathway). Fig. 1 illustrates the different pathways of the activation cascade and its potential inhibiting mechanisms.

Tissue injury following ischemic myocardial infarction may also cause complement activation. Abundant deposition of membrane attack complex C5b–9 may be readily observed in tissue following ischemic injury [1]. A possible pathophysiological role for complement activation following tissue ischemia has been demonstrated in experimental models of myocardial infarction in which complement depletion reduced the size of tissue injury and local infusion of a soluble C1 INH into the left anterior descending artery had a similar beneficial effect [2]. C1 INH prevents proteolytic activation of C1 and hence blocks the classical pathway of complement activation. Thus, the formation of the pro-inflammatory peptides C3α, C5α and the terminal complement complex C5b–9 are decreased [3].
The right ventricle is the most susceptible region of the heart for injury after both isolated heart transplantation [4] and after combined heart and lung transplantation [5]. Ischemia-induced injury appears to be the principal underlying cause, and in addition a deleterious effect due to reperfusion of still viable myocytes after storage must also be considered. Accordingly, we developed an isovolumic right heart transplantation model, which allowed total control of RV function after transplantation under conditions of constant, controlled left ventricular (LV) hemodynamics. Our study investigated whether the right ventricle can benefit from the protective effects of C1 esterase inhibition and blocking of the classic complement cascade after transplantation.

2. Materials and methods

The following protocol was reviewed and approved by the Sub-committee on Research Animal Care, Hannover Medical School. All animals received humane care in compliance with the European Convention on Animal Care.

2.1. Right heart transplantation model

2.1.1. Donor heart preparation

Twelve domestic pigs with a mean weight of 34 ± 4 kg were endotracheally intubated after sedation with pancuronium (0.1 mg/kg i.v.) and induction of anesthesia with pentobarbital (30–50 mg/kg i.v.). Animals were mechanically ventilated at an FiO₂ of 0.4 at a rate of 15 ventilations per min and tidal volume of 20 cc/kg. Intravenous access was established via a line in an ear and jugular vein. Arterial blood pressure was measured via the left common carotid artery and was monitored continuously in combination with the ECG.

The thorax was opened via median sternotomy, and the precordial thymus tissue removed. The pericardium was opened in a T-shaped manner, and the heart suspended with sutures building a pericardial cradle. The pericardial border was prepared cranially to allow adequate exposure to the aortic arch and the pulmonary artery. The superior vena cava was surrounded with a polypropylene suture and the azygos vein ligated. The aortopulmonary tissue was dissected to allow safe and complete cross clamping of the aorta. Heparin (300 IE/kg i.v.) was administered, and the cardioplegia cannula secured with a pursestring suture in the ascending aorta. After completing the surgical preparation, a 10 min stabilization phase was allowed. Subsequently, the inferior vena cava was transected, the left atrial appendage cut and the ascending aorta cross clamped. Administration of a cardioplegic solution (ViaSpan® (Belzer UW) Du Pont Pharma; electrolyte (mEq/l) Na⁺, 1,29; K⁺, 1,25; calculated osmolarity: 320 mosM) under controlled conditions (1000 ml in 5 min at perfusion pressure of 40 mmHg) was then initiated. The hearts were also topically cooled with crushed ice. After completion of cardioplegia, the hearts were excised in standard fashion, and placed on crushed ice consisting of normal saline.

To precisely control RV volume in vivo, an isovolumetric model was used in which RV volume was regulated using an intracavity balloon. A high-compliance latex balloon was

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**Fig. 1. Complement activation and its possible inhibition.** Different activation pathways of the complement system. The components of the classical and lectin pathway are numbered C1–9. C1 is composed of three subcomponents Clq, C1r, and C1s. Clq is omitted in the lectin pathway. The early components of the alternative pathway are known as factors, and each molecule is named by a letter, i.e., B, D, P. All three pathways use in the later stages of activation the same terminal components C5b–9 that form the membrane attack complex. *The early inhibition of the classical pathway by the C1 esterase inhibitor (C1 INH). MASP, mannan-binding protein associated serine protease.*
inserted into the RV through the transected pulmonary artery. The tricuspid valve was closed via the right atrium by sutures to prevent balloon herniation and thus provide absolute volume control in this model. To ensure that the balloon could fill the entire RV cavity and conform maximally to its internal contour, the tricuspid valve chordae tendineae were cut, and the Thebesian venous blood was drained by a 14-gauge cannula inserted into the RV apex. The balloon was ligated at the pulmonary valve level around a 2 cm diameter polyurethane tubing. Measured amounts of saline solution were added or withdrawn through a port at the end of the tubing. The compliance of the latex balloon system was tested for each balloon volume used in the experimental protocol.

2.1.2. Recipient preparation

Twelve domestic pigs with a mean weight of 44 ± 4 kg were anesthetized and ventilated as described above. The heart was approached via median sternotomy. After administration of 300 IU mg/kg heparin i.v., total cardiopulmonary bypass was implemented via bicaval and bifemoral (arterial) cannulation. The animals were kept normothermic.

In this model, the right ventricle was isolated from the circulation by draining systemic venous return and coronary sinus effluent to a pump oxygenator. Oxygenated blood was returned to the systemic arterial circulation via the femoral arteries and the left atrium via separate calibrated roller pumps. The coronary arteries remained directly perfused from the ascending aorta.

During the experiments, known amounts of saline were introduced in 10 ml increments into the RV balloon-tubing system. RV developed pressure and RV dP/dt could be measured via a pressure transducer attached to a separate port at the polyurethane tubing (Micron Miniature Transducer MP 15, volume displacement: 9 × 10⁻⁵ mm³/mmHg). The RV cavity balloon volume was determined as the total volume in the balloon-tubing system minus the volume in the tubing.

LA pressure was measured via a fluid-filled catheter inserted into a pulmonary vein and connected to a strain-gauge transducer (Spectramed Inc., Oxnard, CA). LV pressure was measured with a micromanometer-tipped catheter placed in the LV cavity. Systemic blood pressure was measured with a fluid-filled catheter in the right internal mammary artery, and was adjusted and controlled mechanically by either pumping blood into or out of the femoral arteries.

2.2. Transplantation

After completion of surgery on the donors, prepared hearts were placed in an ice chest and stored at 4°C. Total ischemic time was 4 h (including transplantation). After excising recipient hearts, donor hearts were transplanted with a left atrial running suture anastomosis (polypropylene 3-0) and an anastomosis between donor and recipient aorta (polypropylene 4-0). After de-airing, the aortic cross clamp was removed, and the heart defibrillated and paced at a rate of 120 beats/min. Thirty minutes after opening the cross clamp, an arterial line from the extracorporeal circulation was inserted into the left atrium. Left heart cardiac output was controlled by infusing oxygenated blood from the cardiopulmonary bypass circuit into the left atrium at a low rate of 200 ml/min to prevent any influence of left ventricular contraction on right ventricular function. As seen in echo-pilot studies this LV volume load is sufficient to physiologically open the mitral and aortic valve and minimizing the influence of left ventricular function to right ventricular performance. The mean arterial pressure was kept constant at 60 mmHg via heart lung machine flow adjustment.

2.3. Experimental protocol

After transplantation the animals were randomly assigned to either the C1 INH (n = 6) or control groups (n = 6). After opening the cross clamp the C1 INH group animals received 20 IU/kg body weight of C1 esterase inhibitor (Berinert®, Centeon Pharma; Marburg, Germany) administered into the right coronary artery over a 5 min period. The control animals received no drug therapy. The hearts were reperfused for 1 hour, and thereafter the RV balloon volume was increased by 10 ml increments until RV failure occurred. The point of RV failure was defined as a decrease in RV developed pressure (RVDp), which occurred with the final administered RV balloon volume increment. RVDp was defined as \( P_{\text{systol}} - P_{\text{diastol}} - P_{\text{compliance}} \). The measurements were repeated after 120 min of reperfusion. Left heart hemodynamics were kept constant under the conditions described above. The latex balloon and tubing system may influence measured RV pressure (Pcompliance). The compliance of the RV balloon and tubing system was characterized with RV balloon volumes from 0 to 100 ml. At maximum volume, a balloon compliance of 8 mmHg was obtained. The compliance relationship was linear over the range of balloon volumes used. The compliance test was performed for each balloon used and yielded identical curves. As previously described, the result was used to correct measured values for developed RV pressure [6].

Blood samples for measurement of plasma levels of the terminal complement complex (TCC, C5b–9) and increase (%) of the C1 INH levels were obtained at the following intervals: after implementation of cardiopulmonary bypass, before opening of the aortic cross clamp (after transplantation), within the first 5 min of reperfusion, 30 and 60 min after opening of the aortic cross clamp and after the second hemodynamic measurement. Samples were collected directly from the coronary sinus and jugular vein. Creatine kinase (CK) samples were taken 30 and 60 min after opening the cross clamp and immediately before cessation of the experiment. Additional monitoring of troponin was not performed as a significant difference in the increase of
troponin after transplantation was not expected due to the small muscle mass of the RV.

2.4. Postmortem studies

To calculate the proportion of right ventricular free wall perfused by the right coronary artery, the RCA was perfused with Monastral® blue B suspension (Sigma Chemical Company, St. Louis, MO) at a pressure of 100 mmHg. The perfused hearts were incubated at 37°C in normal saline for 30 min, and were then fixed by immersion in phosphate-buffered formalin (pH 7.0). After fixation of the heart, the right ventricular free wall, and the septum were separated and weighed. Subsequently, the macroscopically undyed areas of each region were separated and weighted. The proportion of RV free wall supplied by the RCA was calculated by this technique and expressed as a percentage of the total regional weight.

2.5. Statistical analysis

Data are expressed as mean ± SD. The paired Student’s t-test was used to determine statistical significance of intragroup comparisons of RVDP, RV dP/dt and the maximal RV balloon volume after 1 vs. 2 h of reperfusion. Inter-group comparisons were performed by analysis of variance and a subsequent Student–Newman–Keuls test. Statistical significance was defined as P < 0.05.

3. Results

The mean donor and recipient weight was comparable among the two groups: 35 ± 2 vs. 33 ± 5 kg for donors and 44 ± 2 vs. 46 ± 4 kg for recipients (C1 INH vs. controls respectively; P = n.s.). The approximately 10 kg higher mean body weight of recipient animals was chosen in order to produce a more stable hemodynamic situation after transplantation. Animals with increased weight demonstrated neither a reduction in blood pressure nor an occurrence of arrhythmias after transplantation. The selected blood pressure could be achieved by pump flow adjustment alone. No drug therapy was necessary. Results of pig heart morphometric analyses are summarized in Table 1. C1 INH and control pigs did not differ with respect to their morphometric data, hence pooled data are presented in Table 1.

3.1. Hemodynamic data

There was no significant difference in maximal RV developed pressure between the two groups (after 1 h, 35.7 ± 5.9 vs. 40.6 ± 12.7 mmHg and after 2 h, 41.5 ± 10.7 vs. 46.3 ± 15.2 mmHg; for C1 INH and control animals, respectively; P = 0.803). RV dP/dt, obtained at maximal RV developed pressure, increased in both groups between 1 and 2 h of reperfusion. This increase was not significant in the control group (4580 ± 550 mmHg/s² after 1 h vs.

### Table 1

<table>
<thead>
<tr>
<th>Morphometric Analysis</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor weight (kg)</td>
<td>34.1 ± 3.8</td>
<td>27.0 – 40.0</td>
</tr>
<tr>
<td>Recipient weight (kg)</td>
<td>43.6 ± 4.1</td>
<td>37.1 – 52.0</td>
</tr>
<tr>
<td>Total heart weight (g)</td>
<td>135.1 ± 30.7</td>
<td>101 – 171</td>
</tr>
<tr>
<td>Right ventricular free wall weight (g)</td>
<td>35.9 ± 3.1</td>
<td>26.0 – 37.8</td>
</tr>
<tr>
<td>Left ventricular free wall weight (g)</td>
<td>61.6 ± 15.7</td>
<td>48.0 – 68.5</td>
</tr>
<tr>
<td>Septum weight (g)</td>
<td>37.4 ± 6.4</td>
<td>30.9 – 41</td>
</tr>
<tr>
<td>Proportion of RV free wall supplied by RCA (%)</td>
<td>83 ± 10</td>
<td></td>
</tr>
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</table>

* Mean ± SD; (n = 12).

4916 ± 600 mmHg/s² after 2 h reperfusion, but was significant in the C1 INH group (4082 ± 450 vs. 5709 ± 650 mmHg/s²; P = 0.025). As illustrated in Fig. 2, the RV could be loaded with a significantly greater volume in the C1 INH group as compared to the control group after 1 h of reperfusion before the onset of RV failure (60.0 ± 20.0 vs. 46.7 ± 13.7 ml; C1 INH vs. control, respectively; P < 0.05). The same observations could be seen after 2 h of reperfusion (70.0 ± 8.9 vs. 60.0 ± 6.3 ml; C1 INH vs. control respectively; P < 0.05). After reperfusion, the RV volume, measured at maximal RVDP, increased in both the control (42 ± 15 vs. 58 ± 10 ml: 1 vs. 2 h reperfusion respectively; P < 0.05) and C1 INH groups (57 ± 12 vs. 70 ± 15 ml: 1 vs. 2 h reperfusion; P < 0.05).

There was no difference in LV function between the two groups, after either 1 or 2 h of reperfusion (LV pressure after 1 h: control vs. C1 INH: 29.5 vs. 29.5 mmHg, P = n.s.; after 2 h, 31.1 ± 33.6 mmHg, P = n.s.). Statistical analysis of LV dP/dt revealed similar results: control vs. C1 INH: after

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**Fig. 2.** Volume load of the right ventricle at maximum developed right ventricular pressure. After intracoronary administration of a C1 INH the right ventricle could be loaded with significantly more volume at the maximal developed right ventricular pressure. Observations after 1 and 2 h of reperfusion.
4754 before termination of the experiment, 6287
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treatment. The plasma concentration of the terminal
onset of RV failure was significantly higher after C1 INH
However, the maximal volume load of the RV before the
administration of a C1 INH. In the isovolumic right
transplantation was significantly improved after intracoronary-

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myocardium against reperfusion injury after prolonged
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Previous studies have shown that myocardial injury after
ischemia followed by reperfusion is related to leukocyte
activation with subsequent release of cytokines and
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[7] which progresses to completion in ischemic and
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To control the effects of confounding variables for right
heart function, an isovolumic model was selected for this
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the clinical milieu, the complexity of RV hemodynamics
and associated determinants provided the rationale for its
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range of compensation mechanisms that may obscure data
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Furthermore, in experiments where normal right ventricles
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The inlet and outlet of the right ventricle were appropriately
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1 h, 8897 ± 1210 vs. 8950 ± 1110 mmHg/s²; after 2 h,
10403 ± 2150 vs. 11220 ± 1990 mmHg/s² (P = n.s.).

3.2. Laboratory data

C1 INH levels in the coronary sinus effluent were signifi-
cantly increased in all animals after intracoronary adminis-
tration (P < 0.001). There was no significant difference in
terminal complement complex C5b–9 levels between the
control group and the C1 INH group. The absolute plasma
C5b–9 levels are shown in Fig. 3. No differences between
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respect to the CK levels (30 min, 397 ± 175 vs.
477 ± 142; 60 min, 1751 ± 824 vs. 1453 ± 584; and
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volume. Furthermore, left-sided hemodynamics were
precisely controlled to maintain constancy of this important
influence on RV function.

Because left heart hemodynamics also influence maximal
RVDP [11], observations in this study were made under
conditions of controlled, constant left heart hemodynamics,
with constant left heart output and systolic left ventricular
(and hence, aortic) pressure. Thus, the present model
allowed optimal control of potential variables that may
influence data obtained on right ventricular function. While such a well controlled and hemodynamically defined model might not correspond to native physiology and hence the clinical situation, the use of such a highly defined model nevertheless represented a logical and necessary approach to control various determinants of RV function.

As expected there were no differences in post-treatment LV function between the C1 INH-treated animals and the control group. This finding is most likely due to the fact that the C1 INH was administered only in the right coronary artery which supplied flow to more than 80% of the RV free wall. Hence, the LV was most likely exposed to only a nominal amount of C1 INH during intracoronary infusion. Thus, the integrity of data interpretation was maintained as the left side of the heart was ’kept constant’ by mechanical adjustment of the heart lung machine and flow into the left atrium. In previous studies [12] it has been shown that increased RV function does not necessarily have to be expressed as increased RV pressure, but as greater volume load which the RV can pump before the onset of RV failure. In this experimental model there was also no increase in maximal developed RV pressure after C1 INH application. The slight but not significant increase in RV dP/dt in the control group between 1 and 2 h reperfusion may have resulted from the general recovery of the heart after reperfusion. In the C1 INH group however, the RV dP/dt was significantly improved after 2 h versus 1 h of reperfusion. In concordance with this observation the RV balloon volume, measured at maximal developed RV pressure increased significantly after the application of C1 INH. A 10 ml increase in RV volume would mean that the RV, paced at 130 min/min, could ’pump’ 1.3 l more per min after C1 INH protection. Hence, notwithstanding the general recovery of the ventricle due to reperfusion, the presence of an additional stimulating or protective effect to the myocardium may be hypothesized. As treatment with C1 INH was the only different parameter between the two groups, C1 fixation by C1 INH may have beneficially protected the ischemic myocardium from reperfusion injury. These results indicate that a similar pathophysiologic mechanism leads to post-reperfusion myocardial injury after transplantation or acute myocardial infarction.

As previously described by Radke [13], the efficacy of the applied human C1 INH was proven by pretesting in a thermal trauma study in pigs. Plasma levels of terminal complement complex C5b–9 increased as a function of time (Fig. 3). Interestingly, however, there was no statistical difference in C5b–9 levels between the C1 INH and control group at any defined measure point. C1 INH is regulated and used in various systems [3,14]. However, there was only an analysis of C1 INH in one specific system in our experimental model. Even though we could not measure a difference in C1 INH levels between the two groups, we cannot exclude the possibility that C1 INH was utilized in a different system than measured, leading to the improved clinical function. It has been speculated that not only the substitution of decreased C1 INH to normal levels, but the increase to higher than normal levels are responsible for a clinical benefit [15]. This fact may explain the beneficial clinical outcome after C1 INH application in our experimental model.

In our experimental model the CK levels increased continuously with time, and no differences were observed between the two groups. However, it still may be possible that the RV produced less CK after C1 INH protection, but due the small muscle mass of the RV the data demonstrated no significant differences. In addition, there was an increased CK release from the pigs lower extremities due to the ligation of the femoral arteries for cannulation.

Future studies will be needed to investigate whether C1 esterase inhibition has a protective effect on the left ventricle after transplantation. In addition, studies in a chronic model with biopsy will be required to substantiate if the protective effect of C1 INH produces less myocardial necrosis. For this purpose either an orthotopic or biventricular working, heterotopic transplant model must be used.

In conclusion, our acute study demonstrates that RV function after transplantation is significantly improved after intracoronary administration of a C1 INH. Using an isovolumic right heart transplant preparation with total control of LV function, the maximal volume load of the RV before the onset of RV failure was significantly higher after C1 INH application as compared to the control group with an absence of reperfusion protection. There was no decrease of the terminal complement complex plasma levels in the coronary sinus effluent and jugular vein after C1 INH application during reperfusion. However, the clinical situation with respect to increased RV function, improved significantly. Thus, intracoronary administration of a C1 INH may protect the prolonged ischemic myocardium from reperfusion injury after transplantation. The mechanism of ischemic injury after transplantation may be similar to myocardial injury during ischemic infarction. Thus, C1 esterase inhibition of the classic complement cascade may represent a promising therapeutic approach for effective protection of the myocardium from reperfusion injury after transplantation.

References


