Recombinant hirudin enhances cardiac output and decreases systemic vascular resistance during reperfusion after cardiopulmonary bypass in a porcine model

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Objective: Cardiopulmonary bypass and surgical stress are accompanied by a systemic inflammatory response and activation of coagulation. Thrombin forms fibrin and activates platelets and neutrophils. Consequently, disseminated microthrombosis might increase capillary vascular resistance and thus impair reperfusion. We hypothesized that recombinant hirudin, a direct inhibitor of thrombin, could attenuate coagulation and enhance microvascular flow during reperfusion.

Methods: Twenty pigs undergoing 60 minutes of aortic clamping and 75 minutes of normothermic perfusion were randomized in a blinded setting to receive an intravenous bolus of recombinant hirudin (10 mg, 0.4 mg/kg; $n = 10$) or placebo ($n = 10$) 15 minutes before aortic declamping and then continued with an intravenous 135-minute infusion of recombinant hirudin (3.75 mg/h, 0.15 mg/kg) or placebo. Thrombin-antithrombin complexes, activated clotting times, and several hemodynamic parameters were measured before cardiopulmonary bypass, after weaning from cardiopulmonary bypass, and at 30, 60, 90, and 120 minutes after aortic declamping. Intramucosal pH and P_{CO_2} were measured from the luminal surface of ileum simultaneously with arterial gas analysis at 30-minute intervals.

Results: Recombinant hirudin inhibited thrombin formation after aortic declamping; at 120 minutes, thrombin-antithrombin complexes levels ($\mu\text{g/L}$, mean $\pm$ SD) were 75 ± 21 and 29 ± 44 ($P < .001$) for placebo and pigs receiving recombinant hirudin, respectively. When compared with the placebo group, pigs receiving recombinant hirudin showed significantly higher stroke volume, cardiac output, and lower systemic vascular resistance at 60 and 90 minutes after aortic declamping ($P < .05$). Based on arteriomucosal P_{CO_2} and pH differences, progressive worsening of intestinal microcirculatory perfusion occurred in the placebo group but not in the recombinant hirudin group.

Conclusion: Infusion of thrombin inhibitor recombinant hirudin during reperfusion was associated with attenuated postischemia left ventricular dysfunction and decreased vascular resistance. Consequently microvascular flow was improved during ischemia-reperfusion injury. Control of thrombin formation during reperfusion may be a feasible approach to improve oxygen delivery to reperfused vascular beds.

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In cardiac surgery cardiopulmonary bypass (CPB) and surgical stress are accompanied by a systemic inflammatory response,¹⁻³ which results in activation and interaction of several humoral amplification systems. Aortic clamping causes global myocardial ischemia but CPB can also cause hypoperfusion of various organs. Although CPB maintains splanchnic circulation, low pumping pressure, nonpulsatile perfusion, and hypothermia lead to splanchnic hypoperfusion and ischemia.⁴⁻⁶ Restoration of myocardial blood flow and normal circulation conditions to hypoperfused areas results in ischemia-reperfusion injury.

Ischemia-reperfusion injury is initiated by overlapping cascades of inflammatory mediators at systemic and local levels causing an acute inflammatory response and activation of coagulation in postischemic tissues.⁷⁻¹⁰ Postischemic endothelium becomes activated and expresses proinflammatory and procoagulatory properties contributing to the no-reflow phenomenon by promoting endothelial edema, neutrophil and platelet plugging, microthrombosis, and increased vasomotor tone.¹¹ Inflammatory mediators¹² and also surgical trauma^{13,14} activate endothelium to express tissue factor, thus promoting generation of thrombin throughout the intravascular space but especially in the reperfused tissues.^{9,10} Thrombin, the key enzyme of coagulation, plays a central role between inflammatory and coagulation systems. Cytokines enhance thrombin formation and thrombin activates platelets, endothelium, and leukocytes and forms fibrin from fibrinogen.^{15,16} All these events may potentially promote microthrombosis, potentiate reperfusion-induced capillary occlusion, and lead to ischemic organ dysfunction.

Hirudin is a selective and effective inhibitor of thrombin. In previous studies of inflammation-associated coagulopathy, recombinant hirudin (r-hirudin; lepirudin) blunted endotoxin-induced activation of coagulation in humans¹⁷ and attenuated liver injury in rats.¹⁸ In rabbits, r-hirudin significantly reduced renal fibrin deposits in a model of endotoxin-induced disseminated intravascular coagulation.¹⁹ Effects of hirudin on CPB-induced ischemia-reperfusion model have not been studied. We wanted to test in a porcine model whether r-hirudin could attenuate reperfusion-induced formation of thrombin and whether this inhibition might have any effect on general hemodynamics or intestinal microcirculation.

Materials and Methods

Animals

Twenty pigs of either sex weighing approximately 26 kg were used as experimental animals. They were allowed at least 3 days of in-house acclimatization with ad libitum access to standard laboratory food and water. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide

for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). All experiments were approved by the Local Ethical Committee of Helsinki University Central Hospital.

Anesthesia

General anesthesia was induced by giving ketamine hydrochloride 500 mg intramuscularly. Two milliliters of pentobarbital sodium, 3 mL of pancuronium bromide, and 0.1 mg of fentanyl citrate were given intravenously. The pigs were intubated and ventilated with Siemens Servo Ventilator 900 C (Siemens-Elema AB, Solna, Sweden) under continuous electrocardiogram (ECG) monitoring (S/5 Anesthesia Monitor, Datex-Ohmeda, Helsinki, Finland). General anesthesia was maintained with continuous pancuronium promide (0.25 mg/kg/h), pentobarbital (5 mg/kg/h), and fentanyl (0.015 mg/kg/h) infusions.

Operative Technique

The left femoral artery and vein were catheterized. Heparin (500 IU/kg) was given just after the vein was catheterized. Arterial blood pressure was continuously monitored and the femoral vein was used for intravenous anesthesia. The right femoral vein was catheterized for infusion of drug or placebo. A small midline laparotomy was performed and intestinal air-automated 14F tonometry catheter (Tonometrics Catheter, Datex-Ohmeda) was inserted into the lumen of ileum 50 cm proximal from the ileocecal junction and secured with a 5-0 polypropylene purse-string suture. A midline sternotomy was performed. After sternotomy, the pulmonary artery was catheterized with a pediatric 5F Swan-Ganz catheter (Baxter Health Corporation, Santa Ana, Calif) via superior caval vein. Venous drainage was provided by a cannula in the right atrium and an aortic perfusion catheter was placed in the aorta. The left ventricle was catheterized through the apex of the heart for pressure measurements. After the initial dose of heparin activated coagulation time (ACT) was measured before CPB and at 30-minute intervals until 90 minutes post-CPB and an additional dose (100-200 IU/kg) of heparin was given during perfusion whenever ACT was below 400 seconds. As a result, 4 animals in the placebo group and 3 animals in the r-hirudin group received additional doses of heparin during CPB. Protamine was not given.

A Gambro 2-roller pump (Gambro, Lund, Sweden) and a D 705 miniflow pediatric hollow fiber oxygenator (Dideco, Mirandola, Italy) were used in all experiments. CPB circuit was primed with 1000 mL Ringersteril (Baxter; Vantaa, Finland) and 5000 IU of heparin. Hemodynamic, biochemical, and intestinal tonometric baseline values were determined before normothermic perfusion (2 L/min) was started. The oxygenator was heated with a heat exchanger (Heater, Amer Group LTD, Tekamer, Helsinki, Finland), and temperature was kept at +37.2°C. After the initiation of perfusion, the aorta was clamped and the heart was arrested with +4°C (15 mL/kg) Plegisol (Abbot Laboratories, North Chicago, Ill). Myocardial temperature was continuously monitored and a pericardial insulation pad was used during the crossclamping time.

Cardioplegic solution (2 mL/kg) was added every 15 minutes and if ventricular fibrillation occurred. Arterial acid-base balance was monitored during operation at 30-minute intervals (ABL System 615, Radiometer Medical A/S, Copenhagen, Denmark).

Study Setting

In a randomized and blinded manner an intravenous bolus (10 mg, 0.4 mg/kg) of lepirudin (Refludan, Aventis Behring, Marburg, Germany, and called from hereon “r-hirudin”; $n=10$) or placebo (20 mL NaCl; $n = 10$) was given 15 minutes before aortic clamp was released and then continued with a 135-minute intravenous infusion of r-hirudin (3.75 mg, 0.15 mg/kg) or placebo.

Aortic clamping was released after 1 hour. After clamp release, ventilation was begun at 7 minutes and the heart was supported by continuing CPB for 15 minutes. If ventricular fibrillation was present after 4 minutes, defibrillation was used repeatedly in 1-minute intervals until hemodynamically effective sinus rhythm was achieved.

Heart rate (HR), ECG, arterial blood pressure (AP), central venous pressure (CVP), and pulmonary artery pressure were monitored continuously. CVP was standardized before each measurement of the hemodynamic profile. Cardiac output (CO) was obtained simultaneously with HR, pulmonary arterial diastolic pressure (PADP), pulmonary capillary wedge pressure (PCWP), AP, left ventricular pressure (LVP), and systemic vascular resistance (SVR) measurements. Inotropic medication was not used. Blood from mediastinal and pleural spaces was collected and returned into the CPB system. Blood from the abdomen was collected into a separate reservoir. Experimental animals were killed during anesthesia by exsanguination after a surveillance period of 105 minutes after cessation of CPB.

Hemodynamic Profile

Hemodynamic measurements were obtained before perfusion and after weaning from CPB and at 15, 30, 60, 90, and 120 minutes after clamp release. ECG, AP, PADP, CVP, and LVP were monitored continuously. CO measurements were obtained using a thermodilution method.²⁰ A thermodilution probe was directed into the main pulmonary artery and connected to a cardiac computer (S/5 Anesthesia Monitor, Datex-Ohmeda). The recorded value was taken as an average from 3 acceptable measurements. SVR was calculated using the equation $SVR = (\text{mean arterial pressure} - \text{CVP}) \cdot 80/\text{CO}$.

Blood Samples

Blood samples from the catheter inserted through the femoral vein into the inferior caval vein were obtained before perfusion, just before aortic declamping, and at 5, 10, 30, and 120 minutes after aortic clamp was released. Thrombin antithrombin complexes (TAT) were measured using commercial reagents (Enzygnost TAT micro, Dade Behring, Liederbach, Germany). ACT was measured from blood samples obtained from the femoral artery using a 2-channel Automated Coagulation Timer (Medtronic Blood Management, Parker, Colo).

Tonometric and Blood Gas Measurements

An air-automated tonometry catheter inserted into the ileum included a semipermeable silicone balloon at the distal end of the catheter. Four milliliters of room air was pumped into the silicon balloon. CO₂ freely equilibrated between the intestinal mucosa, intestinal lumen, and the balloon. The proximal end of the catheter was connected to a tonometry module (S/5 Tonometry Module,

Datex-Ohmeda). The system drew a gas sample from the balloon and intestinal PCO₂ (PICO₂) was automatically measured every 10 minutes. Intramucosal pH (pHi) was automatically calculated by the monitor from PACO₂ and pHa values obtained from arterial gas analysis, entered by user, and PICO₂ measured. pHi was calculated by using the equation $pHi = pHa + \log_{10} \cdot (PACO_2/PICO_2)$. Tonometric and arterial acid-base values were obtained before perfusion (0), after 30 minutes of perfusion, just before aortic declamping, and at 30, 60, 90, and 120 minutes after aortic clamp release.

Bleeding during study drug infusion from the laparotomy wound and from the intestinal biopsy sites was measured by collecting blood from abdomen into a separate reservoir. Blood from the mediastinal and pleural spaces was also measured.

Statistical Methods

Differences between the 2 experimental groups in hemodynamic, tonometric, and blood gas measurements were statistically evaluated using the repeated measure analysis of covariance model, which included the baseline value as a covariant. Baseline values between the 2 experimental groups were compared with 2-tailed Student *t* test. Data are presented as mean $\pm$ standard deviation (SD).

Results

In the placebo group 1 animal died in the beginning of the experiment because of a rupture in the aortic root. One animal died in the placebo group and 1 in the r-hirudin group at 30 minutes after aortic declamping and 2 in the r-hirudin group at 90 minutes after aortic declamping because of a hemodynamic collapse. Eight animals in the placebo group and 7 animals in the hirudin group survived until the end of surveillance period.

There were no differences between the placebo and r-hirudin groups in the time (6.8 ± 2.6 minutes vs 6.2 ± 3.0 minutes, $P = .66$) needed to achieve regular hemodynamically effective rhythm nor in the number of defibrillations needed.

Blood Samples

R-hirudin effectively inhibited coagulation as assessed by ACT. Fifteen minutes after the bolus of r-hirudin ACT was significantly prolonged in the r-hirudin group compared with the placebo group (834 ± 231 seconds vs 515 ± 260 seconds, $P = .008$). Also 15 minutes after weaning from bypass ACT was significantly longer in the r-hirudin group when compared with the placebo group (658 ± 335 seconds vs 274 ± 75 seconds, $P = .005$). Plasma levels of TAT increased in both groups during CPB (Figure 1). Notably, in the placebo group there was a pronounced reperfusion-associated increase in TAT levels from 30 to 120 minutes after aortic declamping and the difference between the experimental groups was significant at 120 minutes (placebo 75.4 ± 15.6 $\mu\text{g/L}$ vs r-hirudin 28.9 ± 7.1 $\mu\text{g/L}$, $P < .001$) after aortic declamping (Figure 1).

Figure 1. The effect of r-hirudin on thrombin-antithrombin (TAT) complexes during CPB and during reperfusion. Both experimental groups consisted of 10 animals. *P* value for comparison between the 2 experimental groups was calculated with analysis of covariance. Values are given as mean $\pm$ SD. Pre CPB, Before CPB; 0, just before aortic declamping.

Hemodynamics

Hemodynamic parameters are presented in Table 1 and in Figure 2. HR increased slowly but progressively in both groups toward the end of the surveillance period (Table 1). CO dropped similarly in both groups but the pigs receiving r-hirudin showed significantly higher stroke volume (SV) and CO at 60 minutes and at 90 minutes after aortic declamping (Figure 2). Importantly, in the r-hirudin group SV and CO showed recovery toward the preperfusion situation while in the controls no such trend was observed (Figure 2). In the placebo group SVR increased markedly during reperfusion (Figure 2). This reperfusion-induced increase in SVR was effectively abolished in the r-hirudin group (Figure 2). The difference between the r-hirudin and placebo groups reached statistical significance at 60 and 90 minutes after aortic declamping (Figure 2). Pulmonary vascular resistance (PVR) was recorded only for some of the animals. PVR showed a qualitatively similar pattern of changes as SVR. PVR was significantly higher in the placebo group at 60 minutes after aortic declamping (990 ± 337 dyne $\cdot$ s/cm⁵, $n = 4$, vs 526 ± 191 dyne $\cdot$ s/cm⁵, $n = 8$; $P = .01$).

In both groups, after CPB there were lower systolic, mean, and diastolic arterial pressures compared with values before CPB (Table 1). Arterial pressures slowly returned toward baseline values thereafter but no significant difference was seen between the 2 groups. In LVP a similar trend was seen but there was no significant difference between the 2 groups (Table 1).

In the r-hirudin group PADP was higher before CPB, mainly because 2 animals had exceptionally high (30 mm Hg) values. During the follow-up PADP increased in both groups but no significant difference was observed between the 2 groups.

Intestinal Tonometry and Blood Gas Analysis

Data on intestinal tonometry and blood gas analysis are shown in Figure 3 and in Table 2. Reperfusion was associated with progressive local mucosal hypoperfusion in both groups as evidenced by significantly increased Pico₂ and decreased intestinal pH values in both groups. In the placebo group but not in the r-hirudin group, there were changes in curve trends of Pico₂ and pH_i at 30 minutes after aortic declamping but this did not result in significant intergroup differences (Figure 3).

Progressive systemic acidosis observed in both groups was not due to inefficient oxygenation as arterial O₂ increased during CPB but returned to and remained at near pre-CPB values after CPB in both groups (Table 2).

To evaluate local microcirculatory perfusion in intestinal mucosa, the gaps between pH_a and pH_i and P_aCO₂ and Pico₂ were calculated (Figure 3). Progressive intestinal hypercapnia and acidosis developed between 30 and 120 minutes after the clamp release in the placebo group. Remarkably, no such local increments in acidosis or hypercapnia were observed in the r-hirudin group. The rise in P(i-a)CO₂ from 30 to 120 minutes postreperfusion was 5.82 ± 6.65 kPa and 0.79 ± 1.7 kPa in the placebo and r-hirudin groups, respectively ($P = .044$). Corresponding values for pH(a-i) were 0.21 ± 0.21 versus 0.018 ± 0.08 ($P = .024$).

Hemoglobin significantly decreased in both groups over time ($P < .001$) and it was significantly lower in r-hirudin group at 90 minutes after aortic declamping (Table 2). Enhanced bleeding was seen in the r-hirudin group (1753 ± 671 mL vs 850 ± 376 mL, $P = .007$). Bleeding from mediastinal and pleural spaces, which was returned to CPB circuit, was 500 ± 236 mL in the placebo group and 1200 ± 615 mL in the r-hirudin group ($P = .011$). However, only blood collected from abdominal cavity into a separate reservoir, which was not returned to the animal, presented net blood loss. This was not significantly different between the 2 groups (530 ± 296 mL vs 325 ± 223 mL, $P = .126$, r-hirudin vs controls, respectively).

Discussion

This study tested the hypothesis that direct inhibition of thrombin could attenuate adverse functional consequences of ischemia-reperfusion injury. Thrombin is an enzyme with pleiotrophic effects on cells, including platelets, leukocytes, endothelial cells, and cardiomyocytes, as well as on several circulating coagulation factors. On the other hand, clinically available anticoagulants, including heparin, possess non-thrombin-related pharmacological effects on plasmatic clotting factors and circulating cells. This makes it most difficult to study potential pathophysiological significance of thrombin per se during clinical ischemia-reperfusion injury. Therefore, we chose to study r-hirudin, the most thrombin-specific clinical anticoagulant that is not known to possess

TABLE 1. Hemodynamic data

Parameter	Time after aortic declamping					
	Pre-CPB	15 minutes	30 minutes	60 minutes	90 minutes	120 minutes
HR (beats/min)						
Hirudin	134 ± 20	136 ± 16	136 ± 14	136 ± 15	143 ± 20	156 ± 26
Placebo	118 ± 13	136 ± 27	151 ± 20	151 ± 18	155 ± 25	153 ± 22
<i>P</i> value	.06	>.2	.06	.08	.14	>.2
SAP (mm Hg)						
Hirudin	120 ± 18	100 ± 15	112 ± 11	121 ± 21	125 ± 29	134 ± 20
Placebo	117 ± 15	96 ± 25	110 ± 23	113 ± 33	123 ± 24	115 ± 14
<i>P</i> value	>.2	>.2	>.2	>.2	>.2	.20
MAP (mm Hg)						
Hirudin	83 ± 13	58 ± 10	64 ± 17	70 ± 25	70 ± 27	76 ± 19
Placebo	80 ± 13	55 ± 8	64 ± 9	67 ± 19	74 ± 19	66 ± 17
<i>P</i> value	>.2	>.2	>.2	>.2	>.2	>.2
PAPD (mm Hg)						
Hirudin	18 ± 10	19 ± 5	20 ± 8	21 ± 7	24 ± 8	26 ± 5
Placebo	12 ± 4	17 ± 6	17 ± 6	23 ± 7	25 ± 5	25 ± 6
<i>P</i> value	.11	>.2	>.2	>.2	>.2	>.2
LVP (mm Hg)						
Hirudin	131 ± 20	114 ± 16	124 ± 19	124 ± 29	127 ± 39	133 ± 28
Placebo	131 ± 13	103 ± 27	109 ± 23	108 ± 39	122 ± 24	117 ± 21
<i>P</i> value	>.2	>.2	>.2	.14	>.2	>.2
PCWP (mm Hg)						
Hirudin	5 ± 2	5 ± 1	5 ± 2	6 ± 2	5 ± 2	5 ± 1
Placebo	4 ± 1	5 ± 2	4 ± 2	5 ± 2	5 ± 2	5 ± 2
<i>P</i> value	>.2	>.2	>.2	>.2	.11	.02

HR, Heart rate; SAP, systemic arterial pressure; MAP, mean arterial pressure; PAPD, pulmonary artery diastolic pressure; LVP, left ventricular pressure; PCWP, pulmonary capillary wedge pressure; Pre-CPB, before cardiopulmonary bypass (*P* value was calculated with Student *t* test).

P value for comparison between the experimental groups was calculated with analysis of covariance.

significant unspecific hemodynamic effects. However, the complex interacting cellular and enzymatic mechanisms of ischemia-reperfusion injury may prevent interpreting the current hemodynamic results as being exclusively due to isolated thrombin inhibition, leaving open a possibility of yet unknown non-thrombin-related effects of r-hirudin. Bearing these reservations in mind, we feel the current approach to be a feasible effort to better understand the potential pathophysiological significance of thrombin activity after CPB.

Transient left ventricular dysfunction due to myocardial reperfusion injury is a significant and common problem after cardiac surgery.²¹ In the control group SV decreased by 44% after CPB and showed no improvement thereafter. In remarkable contrast, during early reperfusion animals receiving r-hirudin could regenerate a significant part of the lost left ventricular function. At 60 minutes after clamp release, animals receiving r-hirudin showed 86% of the preperfusion SV while the corresponding average figure for control animals was 49%. The mechanisms by which thrombin inhibition may improve ventricular function remain to be determined. However, besides possible attenuation of microcirculatory occlusions by r-hirudin, the current data from the in vivo setting offer logical support to the

previous in vitro demonstration that thrombin may directly inhibit myocyte contractile function.^{22,23} Thus, improved thrombin inhibition may be a feasible approach to reduce myocardial reperfusion injury.

Effects of isolated thrombin inhibition on reperfusion-induced changes on peripheral microcirculation after CPB have not been reported previously. In a feline model of ischemia-reperfusion injury of mesenteric vascular bed, infusion of antithrombin abolished the postischemia increase in vascular resistance that was observed in control animals.²⁴ However, as antithrombin may exhibit specific anti-inflammatory properties distinct from its inhibiting effect on thrombin,^{24,25} the contribution of thrombin inhibition to the improved microvascular flow remained undetermined. In the current study, infusion of r-hirudin prevented the increase in SVR after CPB that was observed in the control group. Accordingly, reperfusion seems, indeed, to be associated with progressive but transient increase in SVR.²⁴ More importantly, the current data demonstrate that this phenomenon could effectively be abolished by thrombin inhibition. This effect could theoretically either improve or worsen peripheral circulation depending on the compensatory capacity of the heart. Therefore, considering therapeutic applications of thrombin inhibition during reperfusion, it

Figure 2. The effect of r-hirudin on stroke volume (SV) (A), cardiac output (CO) (B), and systemic vascular resistance (SVR) (C) during CPB and during reperfusion. *P* value for comparison between the 2 experimental groups was calculated with analysis of covariance. Values are given as mean $\pm$ SD. *Pre CPB*, Before CPB.

is encouraging that instead of possible systemic hypotension, myocardium was able to generate better SV and CO and so compensate peripheral potentially hypotension-promoting effects of r-hirudin. Thus, a net effect most probably was enhanced flow in various microcirculatory vascular beds.

CPB results in reduced intestinal intramucosal blood flow, mucosal acidosis, and dysfunction.^{4,5,26,27} In animal studies jejunal and ileal mucosal flow decreased by 38% to 73%^{4,6} during CPB. Ischemia-reperfusion injury in the intestine is characterized by inflammation and capillary plugging.²⁸ In the current study, intestinal tonometry indirectly assessing tissue oxygenation²⁹ showed progressive intramucosal acidosis during and after CPB, indicating reduced microvascular blood flow to the intestinal mucosa. However, because of several confounding factors, quantitating

Figure 3. Intestinal pH (pHi) (A) and the gradients for P_{CO}₂ [P(a-i)CO₂] (B) and pH [pH(a-i)] (C) between arterial and intestinal values during CPB and during reperfusion. A significant decrease during CPB and during reperfusion in pHi was seen over time (*P* < .001, analysis of covariance) but no intergroup differences were observed. Of note, a change in the curve trends of cross-mucosal P_{CO}₂ and pH gradients at 30 minutes after aortic declamping was observed in the placebo group but not in the r-hirudin group. None of the intergroup differences were statistically significant. Values are given as mean $\pm$ SD. *Pre CPB*, Before CPB; *CPB*, 30 minutes after the start of CPB; *0*, just before aortic declamping.

intestinal perfusion should be based on the gap between arterial and intestinal P_{CO}₂ rather than on the intramucosal pH alone.³⁰⁻³² In our tonometric measurements the gap between the intestinal and arterial P_{CO}₂ indicated severe splanchnic hypoperfusion. In the previous studies pHi fell

TABLE 2. Arterial gas analysis

Parameter	Time after aortic declamping						
	Pre-CPB	CPB	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
pHa							
Hirudin	7.45 ± 0.08	7.35 ± 0.09	7.33 ± 0.08	7.31 ± 0.10	7.30 ± 0.12	7.27 ± 0.17	7.22 ± 0.2
Placebo	7.42 ± 0.06	7.36 ± 0.09	7.33 ± 0.10	7.32 ± 0.08	7.28 ± 0.08	7.27 ± 0.08	7.26 ± 0.1
P value	>.2	>.2	>.2	>.2	>.2	>.2	>.2
Pao ₂ (kPa)							
Hirudin	25.4 ± 7.0	45.5 ± 10.7	44.5 ± 8.5	20.0 ± 7.1	18.7 ± 6.9	19.3 ± 6.3	18.8 ± 9.2
Placebo	24.6 ± 6.9	46.1 ± 13.2	40.5 ± 11.7	18.5 ± 7.5	16.0 ± 2.3	14.4 ± 3.8	16.9 ± 5.4
P value	>.2	>.2	>.2	>.2	>.2	>.2	>.2
Paco ₂ (kPa)							
Hirudin	5.2 ± 1.1	5.6 ± 0.5	5.8 ± 0.4	5.6 ± 0.6	5.5 ± 0.5	5.7 ± 0.7	5.9 ± 1.1
Placebo	5.4 ± 0.3	5.5 ± 0.6	5.7 ± 0.6	5.7 ± 0.4	5.8 ± 0.7	5.6 ± 0.8	5.2 ± 1.3
P value	>.2	>.2	>.2	>.2	>.2	>.2	.11
Hb (g/L)							
Hirudin	83 ± 11	59 ± 11	65 ± 13	67 ± 12	60 ± 11	53 ± 13	46 ± 16
Placebo	87 ± 14	70 ± 9	75 ± 9	72 ± 8	71 ± 12	68 ± 14	63 ± 18
P value	>.2	.08	.14	>.2	.03	.006	.003

Pre-CPB, Before cardiopulmonary bypass (P value was calculated with Student *t* test); CPB, during cardiopulmonary bypass.

P value for comparison between the 2 experimental groups was calculated with analysis of covariance.

during CPB but after CPB pHi was relatively constant or slowly began to return back to baseline values.^{4,6} In contrast, we observed a progressive mucosal acidosis throughout the follow-up. One possible explanation is bleeding seen in both groups, resulting in decrease in hemoglobin and relative hypovolemia, which has been shown to induce splanchnic vasoconstriction and redistribution of the peripheral blood flow.³³ It has also been shown that during progressive hemorrhage regional critical hypoperfusion in the gut (ie, inadequate oxygen supply to meet tissue requirements and subsequent metabolic acidosis) develops before whole-body perfusion is compromised.³⁴ This is consistent with our observation that although during reperfusion hemodynamics were well compensated, hypoperfusion of the gut occurred.

Although r-hirudin evidently did not protect the animals from intestinal hypoperfusion, significant beneficial effect could still be demonstrated when compared with the control animals. The increasing difference between arterial and intestinal pH during 30 to 120 minutes of reperfusion indicates that microcapillary perfusion significantly deteriorated in the placebo group while in the r-hirudin group this phenomenon was not observed. This effect was probably mostly due to an enhanced cardiac output seen in the r-hirudin group. In addition, since low hemoglobin reduces oxygen transport and there was a hemoglobin difference in favor of the control group, the significance of CO may further increase.

Although r-hirudin has proven to be a safe and effective anticoagulant in patients undergoing CPB, difficulties in monitoring, rather long half-life, possible enhanced bleed-

ing tendency, and lack of specific antidote are clear disadvantages of its use in clinical practice. Therefore the current positive hemodynamic effects of r-hirudin should be taken as demonstration of potential of improved control of thrombin rather than a practical solution for the problem. Specifically, no recommendation for human use of hirudin combined with heparin can be derived from the current study. Further studies are being conducted to test whether other thrombin modulators might have the same beneficial effect with less bleeding.

R-hirudin directly inhibits the active site pocket and fibrinogen binding site of free and clot-bound thrombin.^{35,36} R-hirudin, in relative difference to heparin, primarily inhibits thrombin activity instead of thrombin generation.^{37,38} However, thrombin activates several clotting factors and amplifies its own formation. Thus, blocking thrombin activity by r-hirudin might secondarily inhibit further thrombin formation.¹⁷ Accordingly, in our ischemia-reperfusion model, direct thrombin inhibition throughout the drug infusion was evidenced by highly prolonged ACT values in the r-hirudin group. Instead, a statistically significant difference in the TAT levels developed only at 120 minutes after aortic declamping when the animals in the placebo group showed rapid escalation of thrombin formation but animals receiving r-hirudin did not. Although the more extensive hemodilution in the r-hirudin group may have contributed to the observed difference in TAT concentration, the data suggest that r-hirudin may be an effective inhibitor of reperfusion-induced thrombin formation in addition to being a direct inhibitor of preformed thrombin.

In conclusion, infusion of thrombin inhibitor r-hirudin during reperfusion was associated with attenuated postischemia left ventricular dysfunction and decreased vascular resistance. Further studies are needed to clarify whether these phenomena were extensively thrombin-related or involved other, yet unidentified secondary effects of r-hirudin. Overall, however, the results suggest that control of coagulation at the level of thrombin activity during ischemia-reperfusion injury may potentially improve oxygen delivery to reperfused vascular beds.

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