

The Influence of Aprotinin on Myocardial Function after Liver Ischemia-Reperfusion

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Key words: aprotinin, ischemia, reperfusion, isolated heart, isolated liver

Abstract

Background: The beneficial effect of aprotinin, a naturally occurring protease inhibitor, on preservation of organs such as the liver, kidney and lung has been documented.

Objective: To explore the effects of hepatic ischemia and reperfusion on both liver and myocardial function, using a dual isolated perfused organ model with and without aprotinin.

Methods: Isolated rat livers were stabilized for 30 minutes with oxygenated modified Krebs-Henseleit solution at 37°C. Livers were then perfused continuously with KH or KH + aprotinin 10⁶ KIU/L for an additional 135 min. Livers of two other groups were made globally ischemic for 120 min, then perfused for 15 min with KH or with KH + aprotinin. Isolated hearts (Langendorff preparation) were stabilized for 30 min and then reperfused with KH or KH + aprotinin exiting the liver for 15 min. The liver's circuit was disconnected, and hearts were re-circulated with the accumulated liver + heart effluent for an additional 50 min.

Results: In the ischemia and ischemia + aprotinin groups, portal vein pressure (1 and 15 min reperfusion) was 331±99% and 339±61% vs. 308±81% and 193±35% of baseline, respectively ($P < 0.03$ vs. ischemia). There were no other differences in the enzyme leakage between aprotinin-treated or untreated ischemic livers. Left ventricular pressure was stable in the controls. However, LV pressure in groups perfused with ischemic liver effluent declined within 65 min reperfusion, whether aprotinin treated or not (84±8% and 73±5% of baseline, respectively, $P < 0.004$ only for ischemia vs. control)

Conclusion: When aprotinin was used, LV pressure was inclined to be higher while liver portal vein pressure was lower, thus providing protection against liver and heart reperfusion injury.

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The human liver exhibits considerable tolerance to severe ischemic episodes. Complete vascular occlusion of the normothermic liver has been extended for up to 30 minutes [1,2]. This may be attributed to the unique regulatory features of the hepatic vasculature and the high oxygen extraction capacity of the liver. Vascular interactions between the hepatic artery and portal vein insure a constant rate of perfusion of the sinusoids even if systemic arterial pressure falls below 60 mmHg [3]. Experimental studies indicate that after 15 minutes of hemorrhagic shock, blood flow is reduced in all visceral organs except the liver [4,5]. The liver normally extracts less than 40% of oxygen at normal blood flow, but its oxygen extraction capacity approaches 100% during ischemia or hypoxemia [3]. The high efficiency of oxygen extraction is sufficient to maintain hepatic oxygen consumption within 10% of normal, even if blood flow is reduced by more than 50% [6].

The recent surge of interest in human liver transplantation has focused more attention on the mechanism of injury during hepatic ischemia and on methods for improving liver preservation. Although the human liver can be successfully preserved under hypothermic conditions for up to 10 hours, remote organ injury due to ischemia-reperfusion remains a potential obstacle [7]. The etiology of hepatic injury-induced myocardial dysfunction is still unclear, but there is growing evidence that oxygen-derived free radical-generated injury plays a major role in ischemia-reperfusion injury of the liver [8,9]. Hemodynamic instability during liver transplantation in the reperfusion period has been described [10,11], and attributed to hypovolemia, acute left ventricular failure due to the release of myocardial depressants from the post-ischemic donor liver, or to citrate intoxication with concomitant decreases in LV contractility [10-12]. Post-perfusion syndrome is characterized by hemodynamic changes in the form of bradyarrhythmias, decreased mean arterial pressure and systemic vascular resistance, and increases in mean pulmonary artery and central venous pressure [10-13].

Aprotinin, a 6,000 MW trypsin and kallikrein inhibitor isolated from bovine organs, has been used for over three

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KH = Krebs-Henseleit

LV = left ventricular

decades as an intensive care drug for acute pancreatitis [14]. This inhibitor forms very tight complexes with human pancreatic trypsin 1 and 2, but it neither inhibits pancreatic chemotrypsin A nor binds pancreatic elastase. In recent years aprotinin has routinely been used during cardiopulmonary bypass where it appears to exert a hemostatic effect by several mechanisms, including the inhibition of fibrinolysis and preservation of platelet function [15,16]. Aprotinin has been reported to have an anti-ischemic effect on LV myocardium of different species when added to the blood during cardiopulmonary bypass surgery [16,17]. Because proteolytic enzymes play a role in ischemic damage, aprotinin is expected to be protective in this process. Besides its anti-fibrinolytic and inhibitory effect on the kallikrein-bradykinin system, other groups and ours have found that aprotinin is beneficial for the isolated rat heart perfused with a solution without blood components [18,19]. Aprotinin was shown to depress the release of myocardial lysosomal enzymes and preserve ATP tissue and myocardial ultrastructure post-ischemia [20]. This protease inhibitor was also effective in reducing liver or lung injury after ischemic storage [21].

In a previous study, we used an isolated perfused organ model to demonstrate injury in a remote organ — heart or lung — following liver ischemia and reperfusion [13]. The present study was designed to investigate the effects of aprotinin on the functional recovery of the ischemic liver and heart, using a dual isolated perfused double organ model.

Materials and Methods

Chemicals

All chemicals used in the experiments were of reagent grade or better, and were purchased from Sigma Chemical Company (St. Louis, MO, USA). Aprotinin was purchased from Bayer AG, (Leverkusen, Germany).

General animal surgery

Experiments were conducted in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the Rabin Medical Center's Felsenstein Medical Research Center.

Isolated perfused liver preparation

Adult male Wistar rats ($n=39$) weighing 340 ± 9 g were anesthetized by intraperitoneal injection of phenobarbital 50 mg/kg. During laparotomy the portal vein and the supra-diaphragmatic inferior vena cava were cannulated with 16G and 13G cannulas respectively. The infrahepatic inferior vena cava, the gastroepiploic vein and the hepatic artery were ligated and the liver was left intact, attached to the animal carcass. Livers were kept warm by a heating lamp and kept moistened. A thermometer was placed under the right hepatic lobe and temperature was maintained constant at 37°C .

The liver was perfused for 30 min via the portal vein with oxygenated modified Krebs-Henseleit solution in mM: 118 NaCl, 4.7 KCl, 25 NaHCO_3 , 2.5 CaCl_2 , 1.2 MgSO_4 , 1.2 KH_2PO_4 , 11 α -D-glucose, at a rate of 0.1 ml/min/g rat weight (3 ml/min/g liver weight). Liver outflow pressure was maintained at 0 cmH₂O. The perfusate was maintained at a constant temperature (37°C) and equilibrated with 95% O_2 /5% CO_2 to achieve an influent PO_2 of ≥ 450 mmHg, PCO_2 about 30 mm Hg, and a pH between 7.30 and 7.50. The perfusate was pumped to the liver via the portal cannula with a peristaltic pump (Watson Marlow 505 U).

Isolated perfused heart preparation

Adult male Wistar rats ($n=39$) weighing 270 ± 4 g were injected with 500 IU of heparin intraperitoneally and anesthetized with diethyl ether. The anterior chest wall was opened longitudinally and the heart was quickly excised and placed in a beaker containing cold (4°C) heparinized saline solution. It was then mounted on a stainless steel cannula (non-working Langendorff preparation) and perfused with fresh KH. The solution was oxygenated with 95% O_2 -5% CO_2 using a membrane oxygenator and passed through a $5\ \mu$ filter (Schliecher + Schull FP-050 Dassel, Germany) into a warmed reservoir that directed the perfusate to the heart at a constant pressure of 75 ± 5 mmHg. Hearts were paced by electrical stimulation (5 V, 10 msec duration) at 300 beats/minute using an external Harvard stimulator (Edenbridge, Kent, UK) ensuring constant heart rate. A latex balloon (Hugh Sacks Electronics, Germany) filled with water was inserted into the LV cavity through a small incision in the left atrium and was connected to a Statham Medical (Mennen Medical Inc., New York, USA) PI32284 pressure transducer. The balloon was tied and inflated to obtain 0–5 mmHg end-diastolic pressure, and LV isovolumic pressure was monitored throughout the experiment using an AT-CODAS (Dataq Instr. Inc., Arkon, Ohio, USA) on an Olivetti M2905 microcomputer. The pulmonary artery was cannulated to obtain perfusate samples of pH, PO_2 and PCO_2 . Coronary flow was measured by collecting the effluent flow into a calibrated beaker for 1 min.

General experimental protocols

The study was designed for four groups and each experiment involved a liver and a heart. All organs were allowed to stabilize for 30 min each. The livers were then perfused for 2 hours or made globally ischemic. This was followed by 15 min liver reperfusion in which the effluent was directed to the heart through a pump, an oxygenator, a filter and a reservoir, as described above. At the end of this phase, the liver was removed from the circuit and the heart was left to re-circulate with the accumulated effluent for an additional 50 min. In all experiments the hearts were never subjected to ischemia.

Specific experimental protocols

- **Group 1: Control (n=8)**

The control liver was stabilized and perfused for an additional 2 hours with oxygenated KH solution, the time being equivalent to that of the ischemic period. The heart of another animal was prepared and stabilized with oxygenated KH for 30 min before the termination of the 2 hour perfusion of liver. Liver effluent was directed into the heart circulation for 15 min. Hepatic perfusion was then stopped and hearts continued to reperfuse with the hepatic heart perfusate for an additional 50 min (total reperfusion time 65 min).

- **Group 2: Control with aprotinin (n=8)**

Liver and heart were perfused as in group 1 with a perfusion solution of KH + aprotinin 10^6 KIU/L. This group was designed in order to check the direct effect of aprotinin on normally perfused liver and then heart without any period of ischemia. The concentration of aprotinin was the same as we used in our previous study [26]. Aprotinin was added to the KH solution during all perfusion periods.

- **Group 3: Ischemia (n=15)**

The liver was stabilized and subjected to global ischemia of 120 min, after which the liver and the heart were perfused, as described above.

- **Group 4: Ischemia with aprotinin (n=8)**

The liver was stabilized with KH + aprotinin followed by 120 min of global ischemia. The heart was stabilized with KH + aprotinin. The liver was reperfused with KH + aprotinin, following the same time protocol as in group 2.

Data collection and determination of organ function

During the experiments the tests were assessed and executed at the following time points: liver and heart at the end of stabilization periods; liver at 1 and 15 min reperfusion; heart at 5, 10 and 15 min reperfusion; and heart at the re-circulation period with liver-heart effluent at 10, 20, 30, 40 and 50 min.

Direct measurements

Portal vein, cardiac systolic and diastolic pressures were determined. For the wet-to-dry weight ratio, organ specimens were weighed and dried at a temperature of 80°C for 24 hours.

Specific laboratory tests

The effluent of the liver and heart were also collected for analysis of aspartate aminotransferase levels, using commercial kits.

Statistical analysis

Results were expressed by mean \pm standard error of the mean. To avoid differences in baseline values, the value at the stabilization period was used as the individual control value of the liver and heart. All control values were

considered as 100% for portal vein pressure, LV pressure, and coronary flow. The results are presented as percent change from baseline values. Statistical significance of the differences between the groups was assessed by multi-variable analysis of variance with repeated measurements using the multiple comparison option of Duncan. $P < 0.05$ was considered significant.

Results

Assessment of liver function

- **Perfusion pressure**

Baseline perfusion pressure in the portal vein of the control group was 11 ± 1 mmHg and remained stable throughout the entire experiment. The baseline portal pressures in the ischemia and ischemia + aprotinin groups were 12 ± 2 and 10 ± 1 mmHg, respectively. In the ischemia-reperfusion livers, perfusion pressure increased to 305 ± 60 and $193 \pm 37\%$ ($P < 0.03$ versus ischemia) in the ischemia and ischemia + aprotinin groups, respectively, at 15 min reperfusion [Figure 1].

- **AST level**

AST levels of the non-ischemic liver effluent during all perfusion periods were much lower than the ischemic reperfused livers ($P < 0.0001$) [Figure 2]. The maximal AST level of the ischemic liver effluent was at 1 min of reperfusion and was similar in both non-treated and treated livers (465 ± 118 vs. 447 ± 130 IU/L, respectively).

- **Wet-to-dry weight ratio**

Wet liver weight and the wet-to-dry ratio were significantly higher ($P < 0.05$) in the ischemic reperfused livers than in control groups (4.5 ± 0.33 vs. 3.57 ± 0.09 , $P < 0.05$). The addition of aprotinin to KH did not influence the wet-to-dry ratio (4.9 ± 0.38).

Assessment of myocardial performance

- **Systolic pressure**

During the stabilization period, the LV pressure was similar in all four groups (control 100 ± 7 , control +

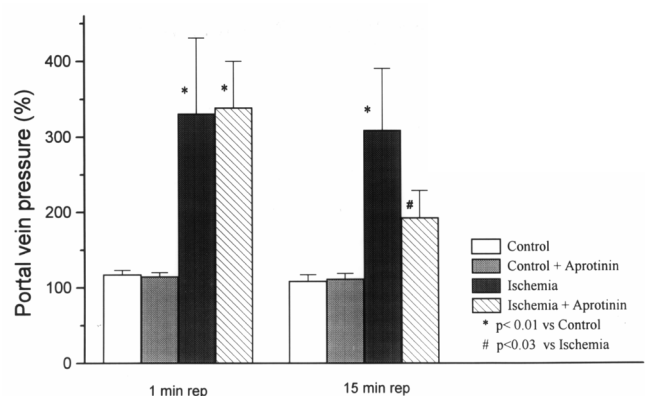


Figure 1. Portal vein pressure presented as percentage of baseline values.

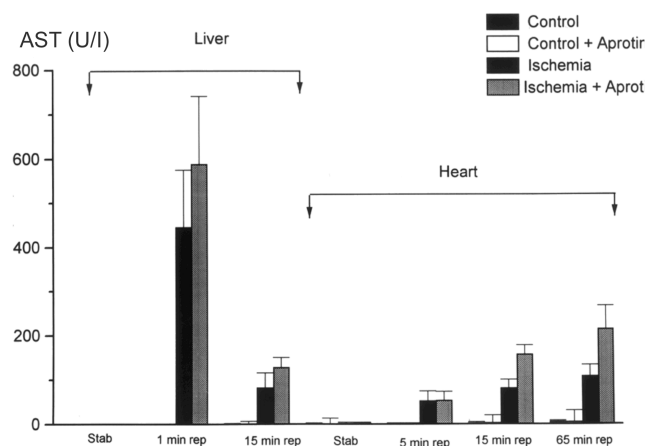


Figure 2. AST levels in the liver and heart effluent.

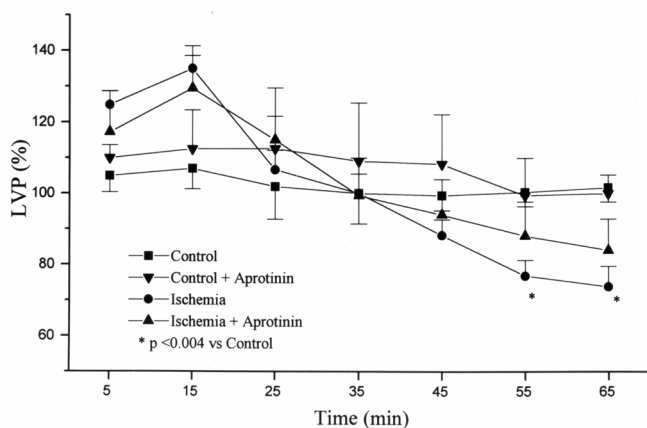


Figure 3. Changes in left ventricular pressure presented as a percentage of baseline values.

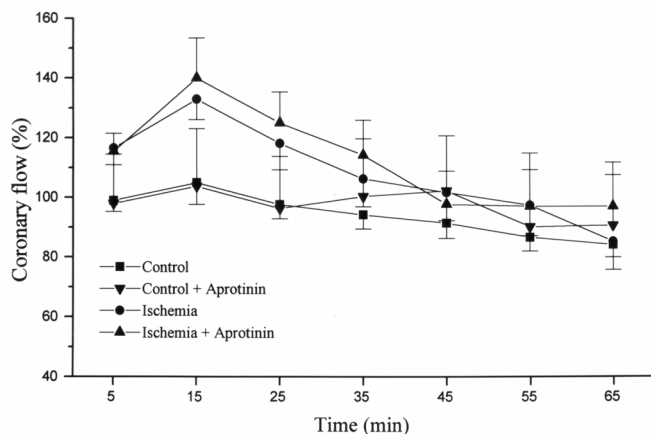


Figure 4. Changes in coronary flow rate presented as a percentage of baseline values.

aprotinin 96 ± 8 , ischemia 95 ± 4 , and ischemia and aprotinin 97 ± 5 mmHg). The LV pressure in the control groups was stable throughout the experimental protocol. LV pressure of the hearts perfused with the ischemic non-treated liver effluent decreased gradually to $73 \pm 5\%$ of the baseline at the end of the

reperfusion period (65 min, $P < 0.004$ versus control). The addition of aprotinin was associated with a reduction of LV pressure to $84 \pm 8\%$ of baseline values ($P 0.05$ versus control) [Figure 3]. No statistical difference was observed between the two ischemic groups.

Coronary flow

The measured coronary flow was stable and similar in the baseline levels of all groups and ranged within accepted values (13–16 ml/min). No statistical differences were observed between all the tested groups in coronary flow [Figure 4] whether treated with aprotinin or not.

Discussion

Aprotinin has been used to reduce intraoperative and postoperative blood loss in large numbers of patients. This hemostatic effect appears to be due to interference with contact activation of the intrinsic coagulation pathway that occurs during exposure of blood to the cardiopulmonary bypass circuit, to preservation of platelet function, and to inhibition of fibrinolysis. Inhibition of kallikrein, which normally accelerates factor XII activation, may decelerate the intrinsic cascade. Aprotinin may reduce fibrinolysis by the inhibition of plasmin and activated protein C [15,16]. Besides its anti-fibrinolytic and inhibitory action on the kallikrein-bradykinin system, aprotinin also has anti-ischemic properties. The effect of aprotinin on organ preservation has been reported to be beneficial in different organs (kidney, liver, heart and lung) [17–25]. This proteinase inhibitor was shown to protect dog myocardium from ischemic injury in terms of diminished area of infarction after coronary artery ligation [17]. Premedication with high doses of aprotinin provided protective effects against warm and cold ischemic damage of the liver [21–24]. It was effective in reducing the damage to myocardial contractility and coronary flow as well as post-ischemic troponin release in a non-blood perfused isolated heart model [18,19].

In a previous study, we observed an induction of acute lung and myocardial dysfunction by liver ischemia reperfusion [13]. In the present work, we used the same protocol and investigated the net effect of aprotinin on remote organ damage, without examining its known hemostatic effect. The medium we used did not contain the cellular components of blood.

The lack of the drug's toxicity while perfusing the normal non-ischemic hearts was demonstrated previously by our group [18]. In this model we perfused both liver and heart with the same concentration of aprotinin (10^6 KIU/L). No differences in liver and myocardial performance were observed in the control non-ischemic perfused liver and heart. These results offer additional evidence of the safety of the drug.

In the ischemic livers, we found that only the portal pressure at 15 minutes of reperfusion was statistically lower in the treated livers, which served as indirect proof

AST = aspartate aminotransferase

of the possible protection of aprotinin since the enzyme leakage in both ischemic groups was similar. There was no difference in AST levels in the ischemic livers, whether treated with aprotinin or not. These findings are in contrast to those in pigs and rats, suggesting that treatment with aprotinin may reduce the liver injury during and after transplantation, as reflected by the enzyme profile [21,24]. AST levels in coronary flow were always lower than in the liver effluent levels, indicating that the source of this leakage was in the liver, since the hearts were never subjected to ischemia. In the present study the only significant difference was the reduced portal pressure, measured 15 min after reperfusion in the aprotinin-treated ischemic group.

Our study appears to be the only one investigating the effect of aprotinin in an isolated dual organ model. Although LV pressure of the hearts perfused with ischemic liver effluent supplemented with aprotinin was better than in those not supplemented, no statistical difference between them was observed. In our previous report on an isolated rat heart model we demonstrated protection of aprotinin on myocardial contractility and coronary flow as well as creatine protein kinase leakage [18]. In that study the heart was subjected to 30 minutes of ischemia at 31°C. The current study involves two different organs, a longer ischemic period (2 hours) for the liver, and a higher temperature (37°C).

In conclusion, aprotinin administration before and after the induction of liver ischemia demonstrated only mild beneficial effects, attenuating the ischemia-reperfusion injury of the isolated rat liver or heart.

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Life must be lived forwards, but it can only be understood backwards.

Soren Kierkegaard