Strategies to improve outcome after partial liver resection

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Chapter 12

Hypothermic *in situ* perfusion of the porcine liver using Celsior or Ringer-lactate solution

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Abstract

**Introduction.** Hypothermic perfusion (HP) of the liver is applied during total vascular exclusion (TVE) to reduce ischemic injury during liver resection. Ringer-lactate solution is commonly used for *in situ* HP. Celsior solution is used for organ preservation but might also be suitable for *in situ* HP. The aim of this study was to compare Ringer-lactate solution (RL) with Celsior solution (Cs) for HP in a pig model of 60 min TVE.

**Animals and Methods.** Twenty pigs underwent 60 min TVE of the liver followed by 24h reperfusion. HP was performed via the portal vein using ice cold Cs or RL (4°C). Pigs were assigned to three groups; group 1: TVE without HP (no-HP, n=9), group 2: TVE with HP using RL (n=6) and group 3: TVE with HP using Cs (n=5). Outcome parameters were plasma aspartate aminotransferase (AST), arterial pH and base excess directly after TVE, vascular inflow and bile production, tissue pO₂ levels, liver tissue glutathione content, coagulation parameters prothrombin time (PT) and thrombin-antithrombin (TAT) complexes.

**Results.** In the no-HP group, plasma AST values were significantly increased during reperfusion (p<0.05) while liver tissue pO₂ levels (p<0.01) were decreased when compared to the RL and Cs groups. Post-TVE metabolic acidosis was significantly reduced in the Cs group (p<0.05). After 24 hours reperfusion, bile production and liver tissue glutathione content were significantly higher (p<0.05) in the Cs group (42.0±1.7 mL/hour and 44.9±2.2 nmol/mg, resp.) as compared to the RL group (31.5±3.5 mL/hour and 19.6±1.8 nmol/mg, resp.), while prothrombin time (PT) was more prolonged in the RL group and the no-HP group as compared to the Cs group (p<0.05).

**Conclusion.** The protective effect of HP during TVE was confirmed in this study. HP with Cs was more effective in reducing metabolic acidosis, restoring radical scavenging capacity and bile production and maintaining coagulation capacity as compared to HP with RL.
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Introduction

Total vascular exclusion (TVE) consists of clamping of the portal vein, the hepatic artery and the supra-and infrahepatic caval vein. It is applied during extended liver resection when backflow from the caval vein causes significant blood loss or when part of the caval vein is involved in the tumour. TVE leads to hepatic ischemia and subsequent reperfusion (I/R) injury when the clamps are removed and normal blood flow to the liver is restored. Especially in patients with underlying liver disease, hepatic ischemia for periods longer than 60 minutes leads to increased risk of liver failure and death. Liver I/R injury is caused by the release of reactive oxygen species and the onset of an inflammatory response by Kupffer cells. This leads to further damage of the parenchymal and sinusoidal endothelial cells, leading to necrosis, apoptosis and microcirculatory failure.

In situ hypothermic perfusion (HP) of the liver during TVE is an option to reduce hepatic I/R injury during extensive liver resections. The protective effect of HP is based on the concept that hypothermia leads to diminished metabolism and hence, decreased energy and oxygen demand. Ringer-lactate (RL) is one of the most often used solutions for HP. It is a crystalloid solution without any additives and without buffering capacity. The aim of HP with Ringer-lactate solution is solely to cool the liver and thereby to decrease metabolism and the need for oxygen. At 28°C, however, cellular metabolism and oxygen demand is still around 50%. Next to RL solution, organ preservation solutions have also been applied during HP. The advantage of organ preservation solutions lies in their ability to prevent tissue acidosis, cell swelling, free radical damage and energy depletion, all phenomena which occur during ischemia and subsequent reperfusion. A relatively new organ preservation solution, called Celsior (Cs) solution, has also shown to be effective in both experimental and clinical organ preservation. It was originally designed for donor heart and lung preservation, but has also proven to be suitable for the preservation of liver grafts. Cs contains reduced glutathione which has a strong anti-oxidative effect. Also, glutathione plays a role in preserving endothelial function by enhancing endothelium-dependent NO release, as has been demonstrated in pulmonary grafts. Cs also contains mannitol and lactobionate to prevent cell swelling and glutamate for energy supply. Histidine buffer has been added to prevent tissue acidosis and magnesium sulphate acts as a membrane stabilizer.

The aim of this study was to assess whether in situ HP during TVE using Cs shows a benefit above RL solution in terms of further diminishing liver damage and preserving endothelial cell integrity and microcirculation. TVE with HP using Cs or RL was also compared with TVE without HP.

Materials and methods

Animals

Twenty male pigs (Vendrig, Amsterdam, The Netherlands) were used, weighing 40-50 kg. All pigs were allowed to acclimatize to the laboratory environment for 7 days with free access to standard laboratory food (Blok, Woerden, The Netherlands) and water. Pigs were fasted
overnight with free access to water before the experiments. The experiments were approved by the Animal Ethics Committee of the Academic Medical Center, University of Amsterdam, The Netherlands.

**Anesthesia**

Methods concerning premedication and anaesthesia were described previously\(^\text{12}\). Blood temperature, measured in the brachiocephalic vein, was continuously monitored and a physiological body temperature of 37-38°C was maintained by means of a heating lamp and a heating mat. Arterial blood gas analysis was performed before and at regular intervals after TVE (ABL 505/OSM 3 HEMOXIMETER\(^\text{®}\), Copenhagen, Denmark). Metabolic acidosis was corrected with 8.4% \(\text{HCO}_3^-\) (Fresenius Kabi Nederland B.V., ’s Hertogenbosch, The Netherlands).

**Surgical procedure**

A midline laparotomy was performed followed by dissection (Force\(^\text{TM}\) 20, Valleylab, Boulder, USA) and cannulation of the common bile duct for continuous measurement of bile production. Another cannula was placed into the distal common bile duct and the duodenum to return the bile to the intestine of the animal during the experiment. The portal vein, the hepatic artery and the caval vein were dissected free and the right gastric and the gastroduodenal artery were divided together with the right gastric vein in order to cut off any accessory blood supply to the liver. TVE was achieved by clamping of the infra and suprahepatic caval vein, the common hepatic artery and the portal vein. To prevent splanchnic congestion and venous congestion in the lower limbs, a situation that is lethal in pigs, a polyethylene prosthesis with one side port was used to bypass blood from the infrahepatic caval vein and portal vein to the suprahepatic caval vein through a transverse incision in the infrahepatic caval vein.

The prosthesis was inserted and guided intralumenally in cranial direction. It was fixed into the caval vein using two slings, which were placed in suprahepatic as well as in infrahepatic position. Then, the portal vein was divided and the proximal end was connected to the side port of the prosthesis after which the clamps were removed from the caval vein and portal vein. The distal end of the portal vein was used to perfuse the liver with cold RL or Cs solution. The perfusate was allowed to drain through the transverse incision of the caval vein into the abdominal cavity, from where it was removed.

After 60 minutes of TVE, the perfusion was stopped and the proximal portal vein was reconnected to the distal end. A ligature was placed cranial from the side port around the infrahepatic caval vein (to secure the intraluminal prosthesis and to prevent blood from entering the abdominal cavity after reperfusion of the liver) and the sling around the suprahepatic caval vein was removed. The liver was then reperfused by releasing the clamps from the portal vein and the common hepatic artery and the abdomen was closed.

**Experimental design**

All 20 pigs underwent 60 minutes of TVE followed by 24 hours of reperfusion. The animals were randomly divided into three groups. In the first group, TVE was performed without
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HP (no-HP group; n=9) In the second group, livers were perfused with ice cold RL solution with an average core liver temperature of 28°C (RL group; n=6). In the third group, livers were perfused with ice cold Cs solution also maintaining an average core liver temperature of 28°C (Cs group; n=5). The components of the two perfusion solutions are shown in table 1. Core liver temperature was measured by two temperature probes (Metatemp, Industrial Temperature Measurement, Steenbergen, The Netherlands) and monitored continuously (Thermistor 4070, Mallinckrodt, St. Louis, USA). The probes were placed in the right lateral and the left medial liver lobes. The tip was placed 4-5 cm within the liver tissue.

The average temperature was used for adjusting the flow of the perfusion solution. Perfusion was powered by a roller pump (Gambro Instrumenta AB, Lund, Sweden) and the flow was adjusted from 50 to 500 ml/hour depending on the core liver temperature. Flow was started at 100-200 ml/hour and when the target core liver temperature was attained, flow was adjusted to 50 ml/hour, to maintain core liver temperature at the desired level. In the group without perfusion, liver temperature was maintained at 37-38°C. After TVE and reperfusion, the pigs remained under anesthesia for 24 hours after which they were sacrificed.

Hepatocellular injury

To assess hepatocellular injury, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were measured in arterial blood samples taken before TVE and after 1, 6, 12 and 24 hours of reperfusion using routine laboratory techniques.

Hepatic pedicle blood flow

Portal vein and hepatic artery blood flow were monitored continuously (Transonic Systems, A.B. Medical B.V., Roermond, The Netherlands).

Microvascular perfusion

Microvascular perfusion was assessed by performing intrahepatocellular tissue pO₂ measurements using a polarographic pO₂ needle electrode (SIGMA pO₂-Histogram KIMOC, Eppendorf, Hamburg, Germany), as described previously. A pO₂ histogram was constructed from 100 consecutive pO₂ values obtained by stepwise withdrawal of the needle over a 2 cm tract (pilgrim step method) in the right median liver lobe. Intrahepatic pO₂ measurements were performed before TVE and after TVE at 10 min, 1 and 24 hours.

Glutathione concentration

Glutathione concentrations were measured in liver tissue, bile and blood plasma according to the method of Tietze. Liver biopsies were taken from the rim of the right medial liver lobe. The biopsies were homogenized in 1 mL ice cold phosphate buffered saline. After protein determination (BCA protein assay, Pierce) in liver tissue homogenates, 3.6 mL metaphosphoric acid (MPA, 50 g/L) was added to the homogenates, bile and plasma samples and the suspensions were vortexed and centrifuged (4,500 rpm, 10 min). Supernatants were used for glutathione measurements. Total (oxidized and reduced) glutathione concentrations were measured before, and 10 min, 1, 6 and 24 hours after TVE.
Coagulation parameters

Prothrombin time (PT) was determined using a one stage clotting assay and Thromborel-S thromboplastin (Dade Behring, Leusden, The Netherlands). The plasma concentration of thrombin-antithrombin complexes (TAT) were measured by ELISA (Enzygnost TAT, Dade Behring, Leusden, The Netherlands).

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago, Ill, USA). One-way ANOVA, followed by Bonferroni post-test was used for differences between groups. Repeated measures ANOVA was used for measurements at multiple time-points followed by Bonferroni post-test for differences between groups or paired T-test for differences within groups at different time points. A p-value <0.05 was considered significant.

Results

Survival

Three animals (33%) in the no-HP group died shortly after TVE. Death in these animals was caused by massive congestion of the liver leading to non-controllable hypotensive shock, highly elevated heart rate and eventually cardiac arrhythmia and arrest. No animals died during the experiment in the other groups.

Arterial blood gas analysis

After reperfusion, the first arterial blood gas analysis was performed at 10 min. Abnormal values found on these analyses were immediately corrected by adjusting ventilation (for pO₂ or pCO₂ abnormalities) and/or infusion of HCO₃⁻ (for metabolic acidosis). On these analyses, significantly lower arterial pH and base excess values were found in the no-HP group and the RL group as compared to the CE group (Table 2). In all groups, arterial pH and base excess values were significantly lower after 10 min. reperfusion as compared to before TVE.

Parenchymal injury

AST was significantly elevated in the no-HP group as compared to the RL group and the Cs group after TVE (Fig 1a). No statistical differences were found between the RL group and the CE group. ALT values showed a similar pattern (data not shown).

Vascular inflow into the liver

Decrease d flow through the liver was found immediately after reperfusion in animals that did not undergo HP. Blood flow in the hepatic artery and the portal vein was significantly lower in the no-HP group as compared to the RL and Cs groups, 10 min. after reperfusion (Fig 1b). Flow subsequently normalized in all groups and no significant differences were found anymore.
Bile production

Baseline bile production was 35-40 mL/hour. During TVE, bile production decreased to almost zero (Fig 1c). After release of the clamps, bile production gradually increased in all groups. After 1, 6 and 12 hours reperfusion, bile production was significantly higher in the RL and Cs groups as compared to the no-HP group. After 24 hours reperfusion, bile production was significantly higher in the Cs group as compared to the no-HP and RL groups.

Figure 1. Aspartate aminotransferase (AST) levels (a), hepatic artery and portal venous blood flow (b) and bile production (c), measured before TVE, during TVE (T), 10 min after TVE (R) up to 24 hours after TVE

1a: AST values were significantly lower in the RL group and the Cs group compared to the no-HP group (** repeated measures ANOVA, p<0.05). No differences in AST were found between the RL group and the Cs group. 1b: After 10 min reperfusion, blood flow was significantly decreased in no-HP as compared to the other groups (** P<0.02). No significant differences were found in blood flow on the other time points. 1c: Bile production was significantly higher in the RL group and the Cs group as compared to the no-HP group at all time points after TVE (** p<0.05). After 24 hours reperfusion, bile production was significantly higher in the Cs group as compared to the no-HP group and the RL group (* p<0.05).
Microvascular perfusion

Microvascular perfusion, as measured by intrahepatic tissue pO2 levels, decreased significantly 10 min after reperfusion as compared to pre-TVE values in the no-HP group (Fig 2). After 1 hour reperfusion, microvascular perfusion was significantly increased in the Cs group as compared to before TVE. This relative hyperperfusion also occurred to a lesser extent in the RL group, but not in the no-HP group. After 24 hours reperfusion, intrahepatic tissue pO2 levels had normalized in all groups.

Glutathione levels

After reperfusion, liver tissue glutathione significantly decreased in the no-HP group as compared to pre-TVE (Fig 3a). This did not occur in the other groups. After 10 min and 6 hours reperfusion, liver tissue glutathione was significantly higher in the Cs group as compared to the no-HP group. After 24 hours reperfusion, liver tissue glutathione was significantly higher

Figure 3. Liver tissue (a) and bile (b) glutathione concentration, measured before TVE and during reperfusion

3a: After 10 min and 6 hours reperfusion, liver tissue glutathione was significantly higher in the Cs group as compared to no-HP (* p<0.05). After 24 hours reperfusion, liver tissue glutathione was significantly higher in the RL as compared to no-HP (* p<0.05) and also in the Cs group as compared to RL and no-HP (** p<0.02). 3b: After 6 and 24 hours reperfusion, bile glutathione was significantly higher in the RL and Cs groups as compared to the no-HP group (* p<0.05). Bile glutathione was also significantly higher in the Cs group as compared to the RL group after 24 hours reperfusion (** p<0.05). * Significantly different from pre-TVE (p<0.05).
in the RL and Cs groups as compared to the no-HP group. Liver tissue glutathione was also higher in the Cs group as compared to the RL group after 24 hours reperfusion. Liver tissue glutathione was significantly higher in the Cs group after 24 hours reperfusion as compared to pre-TVE. During reperfusion, bile glutathione significantly increased in the RL and Cs groups as compared to pre-TVE (Fig 3b). After 6 and 24 hours reperfusion, bile glutathione was significantly higher in the RL and Cs groups as compared to the no-HP group. Bile glutathione was also significantly higher in the Cs group as compared to the RL group after 24 hours reperfusion. Plasma glutathione levels during reperfusion showed no differences between groups, nor when compared to pre-TVE values (data not shown).

**Coagulation parameters**

The function of the coagulation system, as assessed by PT, significantly decreased at all time points after TVE in all groups (Fig 4a). After 12 and 24 hours reperfusion, PT was significantly prolonged in the no-HP group as compared to the RL and Cs groups. After 24 hours reperfusion, PT was also significantly higher in the RL group as compared to the Cs group. Only in the Cs group, PT values almost returned to baseline values after 24 hours reperfusion. Activation of coagulation, leading to thrombin generation, as measured by plasma TAT complexes, was significantly increased at all time points during reperfusion as compared to before TVE in all groups (Fig 4b). After TVE, TAT complexes were significantly higher in the no-HP group as compared to the RL and Cs groups. No statistical differences in TAT complexes were found between RL and Cs groups.

Figure 4. Prothrombin time (PT) (a) and thrombin-antithrombin (TAT) complexes (b) measured before TVE and during reperfusion

4a: PT was significantly higher at all time points after reperfusion as compared to before TVE in all groups (p<0.05). After 12 and 24 hours reperfusion, PT was significantly higher in the no-HP group as compared to the RL and Cs groups (** p<0.05). After 24 hours reperfusion, PT also was significantly higher in the RL group as compared to the Cs group (* p<0.05). 4b: TAT complexes were significantly higher at all time points during reperfusion as compared to before TVE in all groups (p<0.05). After 1, 6, 12 and 24 hours reperfusion, TAT complexes were significantly higher in the no-HP group as compared to the RL and Cs groups (** p<0.05).
Chapter 12

Discussion

The effect of in situ HP using Celsior solution, a relatively new organ preservation solution, was compared with HP using Ringer-lactate and no-HP during 60 min TVE of porcine liver. The advantage of HP during TVE was confirmed in this study and is in agreement with other reports 7-12. Although no differences in parenchymal damage and microcirculatory perfusion were found, HP with Celsior solution resulted in less decrease of arterial pH and base loss, better restoration of bile production, more radical scavenging capacity and better maintenance of coagulation capacity as compared to HP with Ringer-lactate solution. Hence, HP with Celsior solution is advantageous over HP with Ringer-lactate solution. The arterial pH drop and base loss observed directly after reperfusion was probably caused by the onset of anaerobic glycolysis due to the lack of oxygen. This leads to metabolic acidosis secondary to the formation of lactate. Whereas acidic pH can protect against anoxic cell death, reperfusion of ischemic cells at acidic pH leads to more cell killing (pH paradox) 27, 28. The minor decrease in arterial pH and base loss in the Celsior group as compared to the Ringer-lactate group is most likely caused by the histidine buffer contained in Celsior. Ringer-lactate solution does not have a buffer capacity and has a low pH (6.0) by itself.

Formation of reactive oxygen species is an important step in the onset of I/R damage 5, 6. Reduced glutathione is known to be a potent radical scavenger and has shown the ability to reduce I/R damage in different models 29, 30. Increased radical scavenging capacity in HP with Celsior solution was most pronounced after 24 hours reperfusion but was already visible after 10 min reperfusion. It is hypothesized that the reduced glutathione in Celsior solution was directly oxidized in the liver during TVE. Accordingly, excretion of oxidized glutathione into the bile was also most pronounced in the Celsior group. It also was seen earlier after reperfusion and peaked after 24 hours reperfusion. Peak values of liver and bile glutathione after 24 hours reperfusion probably better reflect the restorative capacity of glutathione. Leakage of glutathione from damaged cells into the systemic circulation did not appear to be a major factor in this study, as no differences were found in plasma glutathione levels after TVE as compared to pre-TVE in all groups.

Microcirculatory disturbances within the liver were most apparent directly after reperfusion. Hepatic artery and portal venous flow as well as intrahepatic pO₂ levels were decreased after 10 min reperfusion and subsequently recovered in the no-HP group. In the animals that did not survive the experiments, this no-reflow state persisted and was accompanied by massive congestion of the liver and non-correctable systemic shock. Activation of coagulation is also known to occur as part of I/R injury 11, 32. The formation of TAT complexes was significantly increased in the no-HP group. HP with Celsior solution not only resulted in reduction of the formation of TAT complexes, but also PT was almost normalized within 24 hours of reperfusion. So HP with Celsior solution leads to faster restoration of liver synthesis function as compared to HP with Ringer-lactate, while coagulation activation is equally reduced. The restoration of liver synthesis function by Celsior, measured with PT, is remarkable and has not been described previously.

From organ preservation studies it is known that additives such as energy supplements, pH buffer and membrane stabilizers can decrease cold ischemic damage and prolong preservation periods 23. Although organ preservation solutions such as University of
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Wisconsin (UW) solution have been used for HP, no comparative studies with Ringer-lactate have been performed. Currently, many consider UW solution as the gold standard in organ preservation. In our experience, however, UW has some disadvantages. Due to the colloid hydroxyethylstarch, UW solution has a relatively high viscosity, rendering it difficult to use as a wash-out solution. Furthermore, it is an intracellular type solution containing a high potassium concentration (120 mmol/L). Therefore, it is potentially dangerous to use in situ because of the risk of cardiac arrhythmias, when UW is spilled into the systemic circulation. Celsior solution is a relatively new organ preservation solution with a lower potassium concentration (15 mmol/L) and lower viscosity than UW solution. We therefore decided to use Celsior solution in this study.

In humans, in situ HP of the liver during TVE resulted in a drastic reduction of parenchymal damage during liver resection and decreased post-operative complications. Although TVE may only be needed in complex liver resections, it is advised to use HP during TVE, even when the expected duration of TVE is below 60 min. The safe limit for vascular inflow occlusion during partial liver resections has not been defined. A period of 60 min is considered to be relatively safe in humans, depending on quality of the liver parenchyma. In our pig model, however, TVE for 60 min without HP resulted in a 33% mortality rate (3/9). When, during pilot experiments, TVE duration was extended to 90 min, no animals survived (0/3). These results confirm the fact that the pig liver is more susceptible to I/R damage than the human liver, and in order to test different perfusion solutions, a period of 60 min TVE suffices in the pig model.

In conclusion, the protective effect of in situ HP during TVE was confirmed in this study. Furthermore, whereas no difference in parenchymal damage or microcirculatory disturbances were found, HP with Celsior solution was more effective in reducing metabolic acidosis, restoring radical scavenging capacity and bile production and maintaining coagulation capacity as compared to HP with Ringer-lactate. Therefore, when applying HP in humans, Celsior is considered the preferred perfusion solution.

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Reference list


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