A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic

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Abstract

OBJECTIVE: To evaluate the effects of pharmacological preconditioning with a volatile anesthetic in patients undergoing liver resection with inflow occlusion. BACKGROUND: In liver surgery, ischemic preconditioning and intermittent clamping are the only established protective strategies to reduce tissue damage due to ischemia during inflow occlusion. Preconditioning with volatile anesthetics has provided protection against cardiac and renal ischemic injury in several animal models through NO and HO-1 pathways. But pharmacological preconditioning has never been tested in patients undergoing liver surgery in a randomized trial. METHODS: Sixty-four patients undergoing liver surgery with inflow occlusion were randomized intraoperatively for preconditioning with sevoflurane or not (ClinicalTrials.gov NCT00516711). Anesthesia was performed intravenously with propofol. Thirty minutes before inflow occlusion propofol was replaced by sevoflurane in the preconditioning group. Primary endpoint was postoperative liver injury assessed by peak values of liver transaminases. Postoperative complications were recorded according to an established scoring system. RESULTS: Sevoflurane preconditioning significantly limited the postoperative increase of serum transaminase levels by 261 U/L (95% CI, 65 to 458; P = 0.01) for the ALT and by 239 (95% CI, -2 to 480; P = 0.05) for the AST corresponding to decreases of baseline levels of 35% and 31%, respectively. Patients with steatosis had an even better benefit than patients without steatosis. The rates of any complication (risk ratio 0.46; 95% CI, 0.25 to 0.85; P = 0.006) and of severe complications requiring invasive procedures (risk ratio 0.25; 95% CI, 0.06 to 1.08; P = 0.05) were also lowered by preconditioning. CONCLUSION: This first randomized trial of pharmacological preconditioning in liver surgery in humans showed a protective effect of preconditioning with volatile anesthetics. This strategy may provide a new and easily applicable therapeutic option to protect the liver and to lower complication rates.
A Randomized Controlled Trial on Pharmacological Preconditioning in Liver Surgery using a Volatile Anesthetic

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**Running head:** Pharmacological preconditioning in liver surgery

**Abbreviations:** ASA: American Society of Anesthesiologists, Alanine-Aminotransferase (ALT), Alkaline Phosphatase (ALP), Aspartate-Aminotransferase (AST), Central Venous Pressure (CVP), Inducible Nitric Oxide Synthase (iNOS), Intensive Care Unit (ICU), Nitric Oxide (NO), Randomized Controlled Trial (RCT), Toll-Like Receptor4 (TLR4), White Blood Cells (WBC),

24 pages, 3 figures, 4 tables
MINIABSTRACT

Pharmacological preconditioning with volatile anesthetic in liver surgery is protective regarding the postoperative increase of transaminases as well as the clinical outcome. The protective effect is pronounced in patients with liver steatosis. The study indicates that NO may play a role in the protective pathway of volatile anesthetics.
ABSTRACT

Objective: To evaluate the effects of pharmacological preconditioning with a volatile anesthetic in patients undergoing liver resection with inflow occlusion.

Background: In liver surgery, ischemic preconditioning and intermittent clamping are the only established protective strategies to reduce tissue damage due to ischemia during inflow occlusion. Preconditioning with volatile anesthetics has provided protection against cardiac and renal ischemic injury in several animal models through NO and HO-1 pathways. But pharmacological preconditioning has never been tested in patients undergoing liver surgery in a randomized trial.

Methods: 64 patients undergoing liver surgery with inflow occlusion were randomized intraoperatively for preconditioning with sevoflurane or not (ClinicalTrials.gov NCT00516711). Anesthesia was performed intravenously with propofol. 30 minutes before inflow occlusion propofol was replaced by sevoflurane in the preconditioning group. Primary endpoint was postoperative liver injury assessed by peak values of liver transaminases. Postoperative complications were recorded according to an established scoring system.

Results: Sevoflurane preconditioning significantly limited the postoperative increase of serum transaminase levels by 261 U/L (95% CI 65 - 458, p=0.01) for the ALT and by 239 (95% CI -2 - 480, p=0.05) for the AST corresponding to decreases of baseline levels of 35% and 31%, respectively. Patients with steatosis had an even better benefit than patients without steatosis. The rates of any complication (risk ratio 0.46, 95% CI 0.25 - 0.85, p=0.006) and of severe complications requiring invasive procedures (risk ratio 0.25, 95% CI 0.06 - 1.08, p=0.05) were also lowered by preconditioning.

Conclusion: This first randomized trial of pharmacological preconditioning in liver surgery in humans showed a protective effect of preconditioning with volatile anesthetics. This strategy may provide a new and easily applicable therapeutic option to protect the liver and to lower complication rates.
Hemorrhage remains a significant concern during major liver resection influencing postoperative recovery and long-term survival.\textsuperscript{1,2} Inflow occlusion by clamping of the portal triad (Pringle maneuver), as routinely used in many centers\textsuperscript{3} prevents blood loss during liver transsection,\textsuperscript{4-6} particularly when associated with low central venous pressure (CVP).\textsuperscript{7} However Pringle maneuver induces ischemic injury in the remnant liver, which is associated with increased morbidity and mortality.\textsuperscript{8} Diseased livers such as steatotic or fibrotic livers may be the most vulnerable to temporary interruption of blood flow.\textsuperscript{9-11}

Surgical techniques are known to protect liver cells against subsequent sustained ischemia in cases of ischemic-reperfusion injury in animal models\textsuperscript{12} as well as in humans.\textsuperscript{11,13} Currently, ischemic preconditioning with continuous clamping, but also intermittent portal triad clamping (cycles of 15 minutes of ischemia followed by 5 minutes of reperfusion) are the only clinically established protective strategies against liver injury due to prolonged ischemia.\textsuperscript{14,15} The underlying protective principle of ischemic preconditioning and intermittent clamping is a limitation of stress exposure of the liver that triggers natural defense mechanisms against subsequent ischemic insults.\textsuperscript{16}

Pharmacological preconditioning as a hepatoprotective strategy in humans has not yet been described, while volatile anesthetic agents such as isoflurane or sevoflurane have been widely studied to attenuate cardiac mechanical dysfunction and limit ultrastructural abnormality on reperfusion after ischemia in the myocyte.\textsuperscript{17,18} Intravenous anesthetics such as propofol do not seem to have comparable protective properties.\textsuperscript{19} It has been shown in a rat model that the application of isoflurane before induction of hepatic ischemia protected the liver from ischemia-reperfusion injury.\textsuperscript{20}

Therefore we designed a randomized controlled trial to evaluate the protective effects of sevoflurane preconditioning in patients undergoing hepatectomy under inflow occlusion. In the presence of sevoflurane, an attenuation of liver injury is hypothesized on the basis of a diminished increase of liver transaminases. Our secondary endpoint was to evaluate the
impact of sevoflurane preconditioning in reducing postoperative complications. Finally, we evaluated inducible nitric oxide synthase (iNOS) expression in liver tissue as a putative protective pathway related to the use of sevoflurane.

**MATERIAL AND METHODS**

*Experimental Design*

Consecutive patients undergoing elective liver resection with inflow occlusion between April 2006 and November 2007 were assessed for study eligibility. Exclusion criteria for the present study were age < 18 years, liver cirrhosis, additional ablation therapies (cryosurgery or radiofrequency), living donors, as well as liver resections without inflow occlusion. Enrolled patients were randomized at the beginning of the operation into an intervention group (preconditioning with sevoflurane/sevoflurane group) or a control group (propofol group). The randomization sequence without any stratification was generated by computer and sealed, consecutively numbered envelopes provided concealment of random allocation. Each patient was operated under the supervision of one of two blinded, experienced hepatobiliary surgeons. The time of continuous inflow occlusion was adapted as needed, but had to exceed 30 minutes.

The study was approved by the institutional review board for human studies and internationally registered at ClinicalTrials.gov NCT00516711. The manuscript complies with the CONSORT checklist. Written informed patient consent was obtained from all participants.

*Anesthesia*

All patients received oral midazolam (7.5 mg) as a premedication. Electrocardiogram, radial arterial pressure, arterial oxygen saturation (SaO₂) and depth of anesthesia (measured by bispectral index) were monitored routinely. If necessary, thoracic epidural anesthesia was
performed using continuous application of 0.33% ropivacaine (4 - 8 ml/hour with a bolus application of 4 ml after induction of anesthesia).

General anesthesia was induced with 3 µg/kg fentanyl, target-controlled infusion of propofol, set at a plasma target concentration of 4 - 6 µg/ml, and 0.5 mg/kg atracurium. Anesthesia was maintained with target-controlled infusion of propofol (plasma target concentration of 2 - 4 µg/ml), fentanyl 1 - 2 µg/kg, and atracurium 5 - 10 mg boluses according to clinical needs, as well as remifentanil 0.3 - 0.6 µg/kg/min.

In patients with preconditioning propofol anesthesia was replaced by sevoflurane (Fig. 1): 30 minutes before induction of ischemia, propofol anesthesia was stopped and replaced by sevoflurane (induction of 5 minutes). Pharmacological preconditioning with endexpiratory sevoflurane of 3.2 Vol % was performed for 10 minutes. The following 5 minutes were utilized to stop sevoflurane application and to replace it by propofol (washout of 5 minutes). After a further 10 minutes, ischemia was started for at least 30 minutes.

**Surgical Procedure**

Surgical procedures were performed in a standardized manner under the supervision of two experienced HPB surgeons. A first liver specimen was taken after laparotomy (baseline biopsy). During mobilization of the liver, the time point of 30 minutes before clamping of the portal triad was defined by the surgeon and communicated to the anesthesiologist. According to the randomization, a pharmacological preconditioning with sevoflurane was performed or not, whereby the surgeon was kept blinded for the whole operation. Selective devascularisation of the resected specimen was carried out in anatomical resections,¹¹ but not in atypical hepatectomies. During resections a low CVP (0 – 5 mm Hg) was required. Liver transsection was performed by parenchyma crushing using a small Kelly clamp (3 mm diameter tip).⁶ Small vessels (<2mm) were coagulated with the irrigated bipolar forceps set at 120 W, while all other structures including major intrahepatic bile ducts
were ligated or clipped. A stapler device was only used for the transsection of the hepatic veins. The time of continuous inflow occlusion was adapted as needed, but had to exceed 30 minutes, as this is the minimal ischemic period with detectable postoperative liver injury. Inflow occlusion was achieved by the tourniquet technique around the portal triad with a 4-mm mersilene tape. Separate clamping of aberrant left hepatic arteries was carefully performed when present. 30 minutes after reperfusion a second biopsy was performed.

Endpoints

Each patient was followed for the entire hospitalization. The primary endpoint was postoperative hepatocyte injury defined by peak alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) levels over 7 postoperative days. The secondary endpoint was postoperative complications as assessed by our treatment-oriented complication score. Grades I, II and IIIa were assessed as minor, while grades IIIb, IV and V were major complications, requiring interventions with general anesthesia and/or intensive care management. Additional endpoints were peak values of white blood cells (WBC), bilirubin, alkaline phosphatase (ALP) and creatinine levels as well as length of hospital stay, length of intensive care unit (ICU) stay, and operating time. Additionally, histological evaluation of steatosis and fibrosis, as well as the quantification of iNOS gene expression were determined.

Histological Evaluation

Liver resection specimens or intraoperative liver biopsies (baseline biopsy) were evaluated by a single pathologist (WJ) for the presence of steatosis and fibrosis. Using haematoxylin and eosin (H&E) stained sections, the degree of total steatosis was graded as absent (< 10%), mild (10 - 30%), moderate (> 30 - 60%), or severe (> 60%) based on the percentage of hepatocytes with fat droplets. Liver fibrosis was quantified according to the METAVIR score using sirius red stained sections: absent (F0), portal fibrosis without septa
(F1), portal fibrosis with rare septa (F2), numerous septa (F3) and cirrhosis (F4).\textsuperscript{22}

Histological analysis was performed without knowledge of the postoperative outcome of patients.

**Determination of Inducible Nitric Oxide Synthase (iNOS)**

Total RNA was extracted from both biopsies and reverse transcribed using a special kit (Taqman® Reverse Transcription Reagents). Quantitative PCR (QPCR) was carried out with a 7500 Fast Real Time PCR System. The following primers were used for the QPCR: human iNOS (Hs00167257-m1), and human 18S (Hs99999901-s1). QPCR was performed using Taqman® Universal PCR Mastermix (Nr. 4304437) according to the manufacturer’s protocol. Gene expression was normalized to 18S, and increase in iNOS gene expression was calculated with the comparative $C_T$ method of gene expression before and after ischemia, respectively. iNOS gene expression from baseline biopsy was assigned as value of 1. All products were purchased from Applied Biosystems, Foster City, CA, USA.

**Statistical Analysis**

In an intention-to-treat analysis we first compared primary and secondary continuous endpoints between groups using two-sample t-tests. In addition, we adjusted these analyses for baseline transaminases and bilirubin levels, for preoperative chemotherapy (yes/no) and pringle time in multivariable linear regression analyses. We calculated risk ratios for binary outcomes (complications) and tested for statistical significance using Fisher’s exact test.

We conducted a limited number of subgroup analyses with three prespecified predictors that may modify the effects of pharmacological preconditioning on postoperative hepatocyte injury (ALT/AST). We assessed the effect of steatosis (yes/no, defined by the presence of at least 10% of hepatocytes containing fat droplets), preoperative chemotherapy (yes/no) and age (< 60 years/ ≥ 60 years) by introducing interaction terms into the regression
analyses, which is the most rigorous and widely recommended approach for subgroup analyses. Because of the low power of interaction testing to detect subgroup effects we considered subgroup effects to be significant if \( p \leq 0.10 \). All analyses were conducted using STATA (STATA for Windows, version 9.2, Stata Corp; College Station, TX).

RESULTS

What was the Patient Selection for Study Participation?

Figure 2 shows the study flow from screening of potential participants to the final assessment. The main reason for non-inclusion of a number of patients, was the concomitant availability of a second randomized controlled trial focusing on liver regeneration after major liver resection. Furthermore, after randomization, 6 patients had to be excluded because of violation of the study protocol due to the intraoperative decision of an approach without Pringle maneuver (2 patients in the control group) or because of a delay of more than 30 minutes between preconditioning and hepatic inflow occlusion (4 patients in the preconditioning group).

Was the Preconditioning Group Comparable with the Control Group?

30 patients were included in the preconditioning group (sevoflurane group) and 34 patients in the control group (propofol group). Table 1 shows the patients characteristics and baseline values of the outcome parameters. The mean age was slightly different, 54.2 years in the sevoflurane group versus 57.8 years in the control group, with 16 and 19 males, respectively. American Society of Anesthesiologists (ASA) physical status classification, type of disease, histology of liver parenchyma, and the use of chemotherapy prior resection were distributed similarly between both groups. None of the parameters were statistically different.

Thirty-three patients were operated for liver metastasis from colorectal cancer, 6 for echinococcosis, 3 for hepatocellular carcinoma, 3 for cholangiocarcinoma, 1 for a
neuroendocrine tumor, 11 for other benign lesions such as liver adenoma, focal nodular
hyperplasia, hemangioma or cysts, and 7 for other malign tumors (various kinds of metastatic
diseases) (Tab. 1). Twenty-six patients underwent a major hepatectomy, of whom 8 had an
anatomic right hemi-hepatectomy (SV – VIII), 5 had a left hemi-hepatectomy (SI – IV), 7 had
extended right hemi-hepatectomy (SIV – VIII), and 6 had an atypical major resection (Tab.
2). Intraoperative parameters as shown in Table 2 were similar in both groups. The mean time
of inflow occlusion was 36 minutes (sevoflurane group) and 35 minutes (control group)
respectively, while the operating time was around 4 hours.

Did Pharmacological Preconditioning Prevent Postoperative Liver Injury?

The degree of ischemia and reperfusion injury of the liver was assessed by
postoperative peak serum ALT and AST levels. The peak of the transaminases occurred
between 6 hours after the end of surgery and the third postoperative day. In most of the
patients, ALT and AST levels returned to normal within 7 days. Sevoflurane preconditioning
significantly lowered peak ALT levels by 261 U/L (95% CI 65 - 458, p=0.01) and AST levels
by 239 U/L (95% CI 2 - 480, p=0.05) corresponding to decreases of baseline levels of 35%
and 31%, respectively (Tab. 3). Unadjusted and adjusted results were almost identical. Other
liver parameters such as bilirubin and ALP, but also peak values of WBC and creatinine did
not reach statistical differences.

Did Pharmacological Preconditioning Influence the Clinical Outcome after Liver
Surgery?

One patient in each group (both underwent extended right hemihepatectomy for hilar
cholangiocarcinoma) died within 1 week following major hepatectomy. Death occurred due
to sepsis. The complication rate was significantly lower in the sevoflurane group compared
with the in the propofol group (30% vs 65%, risk ratio 0.46, 95% CI 0.25 - 0.85, p=0.006)
There was no significant differences regarding minor complications among grade I, II and IIIa. However, major complications (grade IIIb, IV, and V) differed significantly between groups with 6.7% vs 26.5% (p=0.05). The mean hospital stay was 2 days shorter in the sevoflurane group (11 vs 13 days), but without statistical significance. ICU stay was comparable between groups.

**Is Inducible Nitric Oxide Synthase (iNOS) Involved in Cellular Signaling in Pharmacological Preconditioning in Liver?**

Nitric Oxide (NO) has been proposed to mediate the beneficial effects of ischemic preconditioning in the liver. But it also seems to play an essential role in pharmacological preconditioning with isoflurane in myocytes. Therefore the expression of iNOS mRNA was compared between pre- and postresection liver biopsies. iNOS mRNA was significantly upregulated in the preconditioning group (5.96 ± 7.12) compared with the control group (1.3 ± 1.15, p=0.001) in (Tab. 3).

**Does Liver Steatosis, Preoperative Chemotherapy or Age of Patients Influence the Protective Effect of Pharmacological Preconditioning?**

30 out of 64 patients (47%) had steatosis with > 10% of hepatocytes containing fat droplets (9 of these patients had more than 30%). As shown in Fig. 3a & b pharmacological preconditioning demonstrated strong protective effects in steatotic patients in terms of postoperative serum peak ALT und AST levels compared with non-steatotic patients. Subgroup effects were significant for both peak ALT (p=0.03) und AST levels (p=0.08). Although preoperative chemotherapy favored preconditioning with differences in peak transaminases between control and preconditioning group in patients with chemotherapy of 361 U/L (ALT) and 430 U/L (AST) compared to 174 U/L and 16 U/L without chemotherapy, results were not significantly different (Fig. 3a & b). Also no significant difference was found
for the effect regarding age. The difference in peak ALT between control and preconditioning group was 481 U/L (554 U/L for AST) in patients ≥ 60 years and 207 U/L (94 U/L for AST) in patients < 60 years (Fig. 3a & b).

**DISCUSSION**

This randomized controlled trial demonstrates for the first time the protective effects of pharmacological preconditioning in patients undergoing liver resection with inflow occlusion. Not only was postoperative liver injury attenuated, as measured by serial serum levels of transaminases, but also clinical outcome was significantly improved by pharmacological preconditioning. The observed protective effects were more pronounced in patients with liver steatosis. This study additionally suggests that NO may mediate the protective pathway of volatile anesthetics.

Numerous strategies have been designed to reduce ischemia-reperfusion injury after liver resection. Basically, inflow occlusion is less harmful than total vascular exclusion. Two protective strategies to prevent ischemic-reperfusion injury have been clinically accepted: ischemic preconditioning and intermittent clamping of the portal triad. Ischemic preconditioning consists of a short period of inflow occlusion, usually 5-10 minutes, followed by 5 minutes of reperfusion prior to the actual inflow occlusion for the operation. Intermittent clamping consists of 10 minute intervals of reperfusion during the whole period of the operation, and although apparently more protective, may lead to significant blood loss. Both procedures require a surgical intervention and prolong the overall time of the surgical procedure. Hence, a pharmacological approach not requiring additional surgical procedures is a more attractive alternative than the established surgical strategies.

Pharmacological preconditioning with a volatile anesthetic is a new approach in liver surgery. Beneficial results in clinical trials, particularly of preconditioning with sevoflurane, have been shown in cardiac surgery, while in liver surgery only one animal study is
This study established for the first time that volatile anesthetics confer protection to livers exposed to ischemia and reperfusion in patients requiring major liver surgery.

The study design, particularly regarding the endpoints, was based on our previous clinical and experimental experience with such studies. Patients with cirrhosis were excluded from the study because there might have been different and more severe effects of inflow occlusion. Additionally, some patients were not included because they were already participating in another ongoing randomized controlled trial (RCT), focused on regeneration after major liver resection. At our center, more than 95% of the patients with liver resections are included in prospective studies. This high inclusion rate is not normally attainable by one single RCT, due to predefined inclusion and exclusion criteria. At our center, we aim to have at least two parallel ongoing trials to make participation in a RCT possible for almost all of our patients. On the other hand, some interferences concerning inclusion of patients between patients can occur. As shown in Fig 2, patient collection was consecutive and unselective aside of the parallel RCT.

Considering the entire series, we found statistically significant protection from preconditioning with sevoflurane in terms of postoperative peak ALT and AST levels. These results highlight the overall protective effects against reperfusion injury considering the heterogeneous groups of patients with ischemic times of 30 – 50 minutes, the often use of additional procedures, and patients with or without hepatic steatosis. The results were adjusted according to potential confounders (Table 3) to exclude any influence of minimal differences between the patient groups, which occur despite the randomization of patients. The decrease of peak transaminases due to pharmacological preconditioning of around 30% appears comparable to the effect of ischemic preconditioning.

Postoperative morbidity and mortality were evaluated using a standardized classification system enabling stratification of complications by severity. The overall morbidity rate of 45%, the mortality rate of 3% and the incidence of severe complications
(grades IIIb – V) of 17% were comparable to previously published data after liver resection.\textsuperscript{11, 14, 36, 37} The present study demonstrated significantly improved clinical outcome after pharmacological preconditioning: on the one hand regarding overall complication rates (65% vs. 30%, \(p=0.006\)) and on the other regarding major complication (Grade IIIb to V) (27% vs 7%, \(p=0.05\)). This finding underscores the potentially powerful protective effects of volatile anesthetics, as none of the previous trials on surgical preconditioning or intermittent clamping could show significant differences regarding clinical outcome. A possible explanation for this phenomenon might be the release of inflammatory mediators upon hepatic ischemia-reperfusion, which could also trigger a proinflammatory cascade in organs other than the liver. Several studies have stressed the importance of toll-like receptor\textsuperscript{4} (TLR4) in the pathophysiology of ischemia-reperfusion in the heart,\textsuperscript{38} kidney,\textsuperscript{39} and liver.\textsuperscript{40} Theoretically, mediators released upon reperfusion might also induce TLR4 expression in other organs, where the systemic application of the volatile anesthetic could interfere, either by decreasing enhanced TLR4 expression or by blocking these receptors. Further studies have to be performed to gain more insight into these signaling pathways.

The mechanisms of protection of hepatocytes due to pharmacological preconditioning remain unclear and may involve several pathways. Barrier et al. demonstrated the modulation of gene expression due to ischemic preconditioning\textsuperscript{26}. Particularly the upregulation of iNOS confirms the hypothesis that preconditioning has been linked to NO production. In our study, the expression of iNOS upon reperfusion significantly increased compared to the baseline value in the preconditioning group, although the second liver biopsy was performed after only 30 minutes of reperfusion. This indicates a potential protective role of NO in pharmacological preconditioning. NO is also a key signaling component involved in preconditioning elicited by volatile anesthetics in the myocyte, activating protein kinase C,\textsuperscript{27} which ultimately activates sarcolemmal and mitochondrial K\textsubscript{ATP} channels.
There is growing evidence that liver steatosis is associated with impaired outcome after hepatic resection. Subgroup analysis of previous studies has demonstrated a higher protective effect of ischemic preconditioning as well as of intermittent clamping in steatotic livers, an effect possibly associated with the preservation of ATP during ischemia. Our data confirm higher degree of protection in steatotic livers, also by pharmacological preconditioning (Figures 3a & b). Although this study was small for subgroup analyses and despite the fact that interaction testing has low power to detect subgroup effects we found strong effect modification by steatosis (p for subgroup analysis = 0.03 for ALT and 0.08 for AST). We considered the subgroup effects to be significant if p values for the interaction term was >0.10 because of the low power of this test as did others. Previous studies have demonstrated this effect in liver steatosis of >25% and >30%, respectively.

An increasing number of patients with tumors undergo extensive chemotherapy with multiple drugs prior to surgery, impairing the postoperative outcome. Drugs such as irinotecan (Campto, Pfizer®) and, to a lesser degree, oxaliplatin (Eloxatin, Sanofi, Aventis®) have been associated with the development of steatohepatitis, while bevacizumab (Avastin, Hoffmann-LaRoche®), a monoclonal antibody, impairs liver regeneration and wound healing through its regulation of angiogenesis. The present study did not show a significant improvement of the protective effect in chemotherapeutic livers (Figures 3a & b). The sample size to negate this effect definitively might have been too small, and needs to be addressed in larger studies.

In contrast to previous observations in ischemic preconditioning, age had no significant influence on the protective effect of pharmacological preconditioning (Fig 3a & b). However the beneficial effect was higher in patients above 60 years. We previously identified age as a factor influencing the effect of ischemic preconditioning in a multivariate analysis, and suggested that tolerance of hepatocytes against ischemic injury might be different in
younger compared to older patients. The age limit for ischemic preconditioning was approximately 60 years, and only patients with age below benefited from this strategy.

Although the results of the primary endpoint showed a number of significant effects, the sample size is relatively small. Therefore interpretation of subgroup analysis, in particular, needs to be done carefully. This trial is focused on blood parameters and clinical outcome, while a pathway of the protective effect can be hypothesized. Investigations concerning the mechanism in animal models are requested. Further trials on the protective effect of different applications of sevoflurane would be of major interest.

In conclusion, this trial provides evidence of the protective effect of preconditioning with volatile anesthetics on ischemic/reperfusion injury in patients with liver resection. Pharmacological preconditioning prevents hepatic injury defined by low levels of transaminases but also improves the clinical outcome, particularly in patients with an increased risk of postoperative liver failure such as those with steatosis. This strategy, although needing further investigations, may provide a new and easily applicable therapeutic option to protect the liver.
REFERENCES


FIGURES

Fig. 1

Treatment Protocol of Preconditioning and Control Group.

Fig. 2

Flow of Participants through Study.
Fig. 3a
Subgroup Effects on ALT.

- Steatosis: Yes, 491 (238-745); No, 99 (-183-381); p = 0.03
- Preop-chemotherapy: Yes, 361 (48-770); No, 174 (-110-458); p = 0.42
- Age (years): ≥60, 481 (196-765); <60, 207 (-115-529); p = 0.30

Fig. 3b
Subgroup Effects on AST.

- Steatosis: Yes, 487 (123-852); No, 81 (-208-370); p = 0.08
- Preop-chemotherapy: Yes, 430 (-192-993); No, 16 (-237-268); p = 0.21
- Age (years): ≥60, 554 (170-937); <60, 94 (-273-462); p = 0.33
# TABLES

## Table 1: Baseline Characteristics

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<tr>
<td><strong>Charlson Index: mean (SD)</strong></td>
<td>5.17 (3.13)</td>
<td>4.88 (2.86)</td>
</tr>
<tr>
<td><strong>Bilirubin mean (SD) mmol/L</strong></td>
<td>12.77 (10.67)</td>
<td>11.74 (11.11)</td>
</tr>
<tr>
<td><strong>Preop. chemotherapy yes/no</strong></td>
<td>15/15</td>
<td>19/15</td>
</tr>
</tbody>
</table>

SD: standard deviation
Preop.: preoperativ
### TABLE 2: INTRAOPERATIVE PARAMETERS

<table>
<thead>
<tr>
<th></th>
<th>PRECONDITIONING</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major/minor resection, number</td>
<td>12/18</td>
<td>14/20</td>
</tr>
<tr>
<td>Additional surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None/colorectal/other</td>
<td>26/2/2</td>
<td>26/2/4</td>
</tr>
<tr>
<td>Hepatectomy, number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First/second/third/&gt;third</td>
<td>23/6/1/0</td>
<td>31/1/1/1</td>
</tr>
<tr>
<td>Pringle time mean (SD)</td>
<td>36.03 (5.62)</td>
<td>35.12 (6.32)</td>
</tr>
<tr>
<td>Operation time mean (SD)</td>
<td>259.83 (87.81)</td>
<td>267.65 (95.72)</td>
</tr>
</tbody>
</table>

Major resection: ≥4 segments  
Minor resection: < 4 segments  
SD: standard deviation

### TABLE 3: POSTOPERATIVE VALUES

<table>
<thead>
<tr>
<th></th>
<th>PRECONDITIONING</th>
<th>CONTROL</th>
<th>ADJUSTED DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ALT mean (SD) U/L</td>
<td>463.53 (287.95)</td>
<td>717.71 (497.47)</td>
<td>261.48 (65.43 - 457.54, p = 0.01)</td>
</tr>
<tr>
<td>Peak AST mean (SD) U/L</td>
<td>507.53 (291.76)</td>
<td>733.35 (636.51)</td>
<td>239.10 (-1.84 - 480.04, p = 0.05)</td>
</tr>
<tr>
<td>Peak bilirubin mean (SD) mmol/L</td>
<td>33.67 (26.89)</td>
<td>44.47 (63.93)</td>
<td>12.91 (-16.92 - 42.74, p = 0.39)</td>
</tr>
<tr>
<td>Peak ALP mean (SD) mmol/L</td>
<td>154.77 (104.08)</td>
<td>162.12 (109.35)</td>
<td>2.00 (-58.83 - 62.84, p = 0.95)</td>
</tr>
<tr>
<td>Peak WBC mean (SD) x10^3/mL</td>
<td>11.48 (3.34)</td>
<td>13.03 (3.54)</td>
<td>1.11 (-0.59 - 2.80, p = 0.20)</td>
</tr>
<tr>
<td>Peak creatinine mean (SD)</td>
<td>93.67 (63.99)</td>
<td>95.62 (47.71)</td>
<td>-4.03 (-37.77 - 29.72, p = 0.81)</td>
</tr>
<tr>
<td>mRNA for iNOS</td>
<td>5.96 (7.12)</td>
<td>1.3 (1.15)</td>
<td>-4.41 (-7.50 - -1.33, p = 0.001)</td>
</tr>
</tbody>
</table>

Adjusted for age, baseline bilirubin, steatosis, preoperative chemotherapy, pringle time, baseline ALT/AST
<table>
<thead>
<tr>
<th>Complications</th>
<th><strong>Preconditioning</strong></th>
<th><strong>Control</strong></th>
<th>Risk ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any, n (%)</td>
<td>9 (30.0)</td>
<td>22 (64.7)</td>
<td>0.46 (0.25-0.85, p=0.006)</td>
</tr>
<tr>
<td>Major (IIIb-V)</td>
<td>2 (6.7)</td>
<td>9 (26.5)</td>
<td>0.25 (0.06-1.08, p=0.05)</td>
</tr>
<tr>
<td>ICU stay, number (%)</td>
<td>4 (13.3)</td>
<td>9 (26.5)</td>
<td>0.50 (0.17-1.47, p=0.23)</td>
</tr>
<tr>
<td>Hospital stay (days): mean (SD)</td>
<td>10.93 (4.40)</td>
<td>12.79 (8.86)</td>
<td>2.00 (-1.70 - 5.70, p=0.28)</td>
</tr>
</tbody>
</table>

Adjusted for age, baseline bilirubin, steatosis, preoperative chemotherapy, pringle time, baseline ALT/AST