Research Article

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Randomized controlled trial analyzing the effect of 15 or 30 min intermittent Pringle maneuver on hepatocellular damage during liver surgery

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Background & Aims: Aminotransferases are commonly used to determine the optimal duration of ischemic intervals during intermittent Pringle maneuver (IPM). However, they might not be responsive enough to detect small differences in hepatocellular damage. Liver fatty acid-binding protein (L-FABP) has been suggested as a more sensitive marker. This randomized trial aimed to compare hepatocellular injury reflected by L-FABP in patients undergoing liver resection with IPM using 15 or 30 min ischemic intervals.

Methods: Twenty patients undergoing liver surgery were randomly assigned to IPM with 15 (15IPM) or 30 (30IPM) minutes ischemic intervals. Ten patients not requiring IPM (noIPM) served as controls. Primary endpoint was hepatocellular injury during liver surgery reflected by systemic L-FABP plasma levels. Between group comparisons were performed using area under the curve and repeated measures two-way ANOVA.

Results: The IPM groups had similar characteristics. Aminotransferases did not differ significantly between 15IPM and 30IPM at any time point. L-FABP levels rose up to 1853 ± 708 ng/ml in the 15IPM and 3662 ± 1355 ng/ml in the 30IPM group after finishing liver transection and decreased rapidly thereafter. There were no significant differences between 15IPM and 30IPM in cumulative L-FABP level ($p = 0.378$) or L-FABP level at any time point ($p = 0.149$). Blood loss, remnant liver function and morbidity were comparable.

Conclusions: IPM with 15 or 30 min ischemic intervals induced similar hepatocellular injury measured by the sensitive marker L-FABP. The present study confirms the results of earlier trials, suggesting that IPM with 30 min ischemic intervals may be used.

Keywords: Ischemia–reperfusion injury; Intermittent Pringle maneuver; Liver surgery; Liver fatty acid-binding protein; Aminotransferase; Hepatocellular damage; Splanchnic congestion.

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Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; ASA, American Society of Anaesthesiologists; AUC, area under the curve; ΔAV, arteriovenous difference; BMI, body mass index; F, flux; IPM, intermittent Pringle maneuver; L-FABP, liver fatty acid-binding protein; NRH, nodular regenerative hyperplasia; PDV, portal drained viscera.

Introduction

Hepatic inflow occlusion (Pringle maneuver) is used to minimize blood loss during liver surgery as excessive intra-operative blood loss and red blood cell transfusions adversely affect short- and long-term outcomes [1–3]. Different clamping techniques can be applied, such as partial or complete hepatic inflow occlusion using either continuous or intermittent pedicle clamping [4]. Generally, intermittent clamping is regarded superior to continuous clamping as it results in a better preserved remnant liver function [5]. The optimal duration of the ischemic intervals during intermittent Pringle maneuver (IPM) is unknown and depends on the balance between ischemia-induced hepatocellular damage and blood loss. As each period of reperfusion is associated with increased blood loss [5–7], prolonged ischemic intervals might be preferable. Indeed, two randomized trials showed that complete IPM using 30 min ischemic intervals resulted in similar remnant liver function and hepatocellular damage compared with IPM using 15 min ischemic intervals, while intra-operative blood loss was lower after 30 min ischemic intervals [6,8].

Hepatocellular damage after pedicle clamping is commonly evaluated using alanine and aspartate aminotransferase (ALAT and ASAT) levels on consecutive post-operative days [7–9]. However, it remains uncertain if the assay of aminotransferases is sufficiently sensitive enough to detect small differences in hepatocellular injury [9]. Due to the relatively large molecular mass (96 kDa) and long half-life of aminotransferases, their plasma levels react slowly to acute tissue damage. In addition, aminotransferase levels on the first post-operative days may not only reflect ischemia-induced hepatocellular damage but also effects of post-operative care such as drug-induced hepatotoxicity or blood transfusions. Furthermore, aminotransferases, and especially ASAT, have modest organ specificity [10]. More

accurate markers for detection and monitoring of hepatocellular injury in man are available. One of these markers is liver fatty acid-binding protein (L-FABP) [11,12]. L-FABP is a cytosolic protein that is abundantly present in liver tissue. Its biological function involves facilitation of intracellular fatty acid transport and participation in lipid metabolism [12]. After hepatocyte damage, it diffuses quickly into the circulation because of its small mass (≈13–14 kDa). Circulating L-FABP, released from damaged cells, is cleared by the kidneys with a half-life of 11 min and as a result, plasma levels rapidly return to normal [13].

The present randomized controlled trial aimed to assess the effect of complete IPM using either 15 or 30 min ischemic intervals on hepatocellular injury reflected by L-FABP as opposed to the less sensitive damage markers ALAT and ASAT.

Materials and methods

Experimental design

Consecutive patients scheduled to undergo liver surgery at Maastricht University Medical Centre and willing to participate were enrolled in this prospective randomized controlled trial. Exclusion criteria were (1) presence of liver cirrhosis confirmed by biopsy, (2) concomitant extra-hepatic procedures or bilio-enteric anastomosis, (3) steroid hormone medication, (4) renal dysfunction defined as serum creatinine >137 μmol/l in men and >104 μmol/l in women [14], and (5) laparoscopic liver resection.

Immediately after the surgeon decided complete IPM would be required during liver transection, patients were randomized in a 1:1 ratio to receive either IPM with 15 min ischemic intervals (15IPM) or 30 min ischemic intervals (30IPM), both followed by 5 min reperfusion. Randomization was performed in the operating theater by a researcher using numbered, sealed, opaque envelopes. An independent researcher generated the randomization sequence. Patients were blinded to the allocated intervention. Patients who did not require IPM (noIPM) served as controls.

The study protocol was approved by the Medical Ethics Committee of Maastricht University Medical Centre and registered at ClinicalTrials.gov NCT01099475. The manuscript complies with the updated CONSORT guidelines [15]. Informed consent was obtained prior to surgery.

Surgical procedure

Patients were anesthetized using isoflurane and propofol. They routinely had an epidural catheter, urinary catheter, two peripheral venous catheters and indwelling catheters in a jugular vein and radial artery. Body temperature was maintained using a Bair Hugger system (Arizant Healthcare Inc., Eden Prairie, MN).

The surgical procedure was performed using a subcostal bilateral incision and Olivier retractors to improve exposure [16]. After dissection of the teres hepatis ligament, the liver was mobilized. Thereafter, an intra-operative ultrasound was performed to define the position of the tumor in relation to vascular and biliary structures. As IPM was not routinely applied, a patient was randomized for 15IPM or 30IPM only after the surgeon had decided a complete Pringle maneuver would be required. During 15IPM or 30IPM, the complete portal triad was clamped using a rubber sling. The time of inflow occlusion was adapted to the need according to the randomization protocol. Occasionally, the left or right pedicle was ligated after protocolled IPM. Five minute reperfusion intervals were applied during which transection was stopped and cut surfaces were gently compressed to ensure hemostasis. A Cavitron Ultrasonic Surgical Aspirator (CUSA system 200 macrodissector, Cavitron Surgical Systems, Stamford, CT) and Argon beam coagulation (Force GSU System, Valleylab, Boulder, CO) were used for liver transection. A stapler device or clamps were used for transection of the hepatic veins. Central venous pressure was maintained below 5 cm H_2O during transection to reduce venous back-bleeding. After surgery, the weight of the resection specimen was recorded. Perioperative care was protocolled, as described earlier [17].

Blood sampling

Arterial blood samples were drawn from the radial artery catheter according to a fixed protocol (Fig. 1). Before and after parenchymal transection, blood was sampled from the portal vein and one of the hepatic veins from the non-tumorous side of the liver by direct puncture. Blood samples were transferred to prechilled EDTA-containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The tubes were centrifuged at 4 °C at 3500*g* for 15 min to separate plasma and stored in aliquots at −80 °C till batch analysis.

Outcome measures

The primary endpoint was hepatocellular damage reflected by systemic L-FABP plasma levels during and after liver surgery. Secondary endpoints were hepatocellular damage reflected by ALAT and ASAT, hepatic function reflected by total bilirubin level and prothrombin time, cumulative ischemia and reperfusion time, duration of operation, amount of intra-operative blood loss, blood loss per gram resected liver weight, need for red blood cell transfusion during surgery or within 2 days after the operation, and morbidity and 30-day mortality. Resections were divided into major (⩾3 segments) or minor (<3 segments or non-anatomical resections) [18]. The clinical course of the participants was studied prospectively. Post-operative complications were graded according to the Clavien-Dindo score [19]. Grades I and II were regarded as minor and grades III, IV and V as major morbidity. Post-resectional liver failure was defined according to the "50–50 criteria" on post-operative day 5 [20]. Bile leakage was considered in case of (1) leakage of any quantity of bile via the abdominal wound or drain at least 48 h post-operatively; (2) intra-abdominal collection of bile at the time of re-operation or percutaneous drainage; or (3) cholangiographic evidence of contrast leakage. Intra-abdominal abscess was present if there was (1) leakage of any quantity of purulent fluid via the abdominal drain or (2) intra-abdominal collection of pus at the time of re-operation or percutaneous drainage, both combined with a positive microbiological culture.

Determination of hepatocellular damage

L-FABP was used as a marker of liver tissue damage. L-FABP plasma levels were determined using a commercially available enzyme-linked immunosorbent assay (Hycult Biotechnology, Uden, The Netherlands). According to the manufacturer's manual, L-FABP plasma levels in healthy individuals were approximately 12 ng/ml. ALAT and ASAT levels were determined by the clinical chemistry laboratory of Maastricht University Medical Centre. The upper limit of normal was 35 IU/L for ALAT and 30 IU/L for ASAT.

Source of L-FABP before and after IPM

L-FABP is highly expressed in the liver, intestine, and kidneys [12]. As a result, systemic L-FABP plasma levels during liver surgery can reflect either hepatic, intestinal, or renal damage [21]. The source of systemic L-FABP plasma levels was therefore determined by sampling blood from the portal and hepatic vein simultaneously with an arterial sample and then by calculation of arteriovenous differences (ΔAV) and net organ fluxes (F; flow × ΔAV) across the splanchnic area, portal drained viscera (PDV) and liver. Plasma flow was measured in a previous similar series of patients and amounted to 320 ± 42 ml/min in the portal vein and 110 ± 23 ml/min in the hepatic artery [16]. Splanchnic flow was calculated as portal vein plus hepatic artery plasma flow. Fluxes were calculated as F_{PDV} = portal plasma flow × ([portal vein] − [artery]), $F_{splanchnic}$ = splanchnic plasma flow × ([hepatic vein] − [artery]) and $F_{liver} = F_{splanchnic} - F_{PDV}$ and corrected for body weight. Positive fluxes indicate release, whilst negative fluxes indicate uptake.

Determination of liver histology

The presence of underlying disease in the non-tumorous liver was assessed by an experienced HPB pathologist in the resection specimen using H&E staining. The presence of >30% hepatic steatosis, grades 1–3 fibrosis and nodular regenerative hyperplasia was evaluated in liver tissue distant from the tumor.

Statistical analysis

Based on previous observations, mean L-FABP plasma level after transection using IPM with 15 min ischemic and 5 min reperfusion intervals was 775 ± 210 ng/ml [13]. The present study was powered to detect a 100% relative difference in L-FABP levels between the groups with 15 or 30 min ischemic intervals, favouring the 15 min group. The 100% difference was chosen because it was regarded clinically relevant and precluded large influences of analytical variation.