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Original Research

Comparison of hepatoprotective effect from ischemia-reperfusion injury of remote ischemic preconditioning of the liver vs local ischemic preconditioning of the liver during human liver resections

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ABSTRACT

Aim: To compare and evaluate the hepatoprotective effect of remote ischemic preconditioning (RIPC) with local ischemic preconditioning (LIPC) of the liver during human liver resections.
Methods: A prospective, single-centre, randomised control trial was conducted in the Clinical Hospital "***" from April 2017 to January 2018. A total of 60 patients, who underwent liver resection due to colorectal cancer liver metastasis, were randomised to one of three study arms: 1) a RIPC group, 2) an LIPC group and 3) a control group (CG) in which no ischemic preconditioning was done before liver resection. The hepatoprotective effect was evaluated by comparing serum transaminase levels, bilirubin levels, albumin, and protein levels, coagulograms and through pathohistological analysis. The trial was registered on ClinicalTrials.gov (NCT****).
Results: Significant differences were found in serum levels of liver transaminases and bilirubin levels between thegroups, the highest level in the CG and the lowest level in the LIPC group. Levels of cholinesterase were also significantly higher in the LIPC group. Pathohistological findings graded by the Rodriguez score showed favourable changes in the LIPC and RIPC groups versus the CG.
Conclusion: Strong evidence supports the hepatoprotective effect of RIPC and LIPC preconditioning from an ischemia-reperfusion injury of the liver. Better synthetic liver function preservation in these two groups supports this conclusion.

1. Introduction

Liver resection is the first line of treatment for primary and secondary liver malignancies [1,2]. The major surgical problem during liver resections is intraoperative blood loss. Intermittent portal triad clamping associated with low central venous pressure achieved during the procedure decreases intraoperative blood loss during liver resection [3,4]. The sequence of hepatic ischemia and reperfusion has been associated with ischemia/reperfusion (IR) injury of the liver. After major liver resection under partial or total vascular exclusion, IR injury of the remnant liver may be a serious complication, leading to postoperative liver dysfunction and increased morbidity and mortality [5]. IR results in reduced perfusion of the liver and the induction of the inflammatory cascade involving the adhesion of leukocytes to endothelial cells and transmigration into the sinusoids. The IR injury correlates with the severity and duration of ischemia [6]. Hepatic IR injury has an early and late phase. In the early phase, the Kupffer cells are most responsible for the activation of the inflammatory cascade, the release of free radicals and cytokines and endothelial injury. In the late phase of IR, liver injury neutrophils release free radicals and cause parenchymal injury [7].

The benefit of preconditioning in liver surgery has been well-known. Experimental and clinical evidence suggests that preconditioning can prevent or decrease IR injury, especially after long ischemic periods [8]. There are several preconditioning techniques (mechanical and pharmacological), neither of which has been established as a "gold standard". In this trial, two different mechanical techniques of ischemic preconditioning were analysed.

Local ischemic preconditioning (LIPC) is a process during which a short period of ischemia is followed by a period of reperfusionbefore the prolonged ischemia, which seems to render organs more tolerant to IR injury [9]. Local ischemic preconditioning is protective for different tissues, including skeletal muscles [9], brain [10], retina [11], spinal cord [12], kidney [13], intestine [14] and liver [15]. The precise

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mechanism by which LIPC confers hepatoprotection is not fully understood yet. It is postulated that LIPC suppresses cytokine release, enhances the production of hepatoprotective adenosine, and increases adenosine triphosphate (ATP) availability by slowing the rate of ATP depletion, thus leading to up regulation of the process of cellular ATP production and liver regeneration, and also reduction of the liver apoptotic response [15–17].

Remote ischemic preconditioning (RIPC) involves the protection of an organ from prolonged ischemia by brief periods of ischemia and reperfusion to a remote organ. Previous studies have shown that RIPC improved parenchymal perfusion and oxygenation that reduced hepatocellular injury in the early phase of IR injury [18,19]. Protective effects of RIPC are achieved owing to interactions between neural, humoral, and systemic pathways. These interactions lead to inhibition of the inflammatory response and activation of various hepatoprotective subcellular cascades [20]. However, most of these studies have been performed in animals. There are not many studies conducted in humans which evaluate these methods of preconditioning.

Considering the results from published studies, we decided to carry out a prospective randomised control trial, which would clarify the effectiveness of RIPC or LIPC in preventing IR injury of the liver during liver resection, by evaluating the postoperative synthetic function of the liver remnant. Our hypothesis was that RIPC is effective in preventing IR injury during human liver resections. The aim of the study was to evaluate hepatoprotective effect from IR injury of RIPC of the liver against LIPC of the liver during human liver resections.

2. Patients and methods

Sixty patients with colorectal cancer liver metastasis who underwent liver resection in the Clinical Hospital "***" from April 2017 until January 2018 were included in this study. The Clinical protocol was approved by Clinical hospital "***" Ethics Committee (no.25022016). All patients included in this study signed informed consent for participating in the study. The patients included in the study underwent resection of at least one liver segment under intermittent portal triad clamping. Hepatic tumours were detected preoperatively with multislice computed tomography (MSCT), magnetic resonance imaging (MRI) or positron tomography (PET–CT). Patients excluded from this study were those having any other underlying liver disease, or preoperative increased liver transaminase or bilirubin. Patients with ASA classification score higher than three, chronic cardiac, pulmonary and/or renal disease were also excluded from the study (Fig. 1). All patients were preoperatively classified by the guidelines of the American Society of Anaesthesiologists (ASA). All anaesthetic and operative procedures were performed by the same team of two experienced hepatic surgeons (M.R., L.P.) and two anaesthesiologists (S.B., D.J.).

On the evening before their operation, all patients received antithrombotic premedication with low molecular weight heparin. The same standardised anaesthetic protocol was used to manage all patients included in the study. The therapeutic strategy was fluid restriction strategy aimed to maintain a mean arterial pressure (MAP) over 65 mmHg and central venous pressure about 5 mmHg. Blood transfusions were administrated when haemoglobin was below 8.5 g/dL and fresh frozen plasma when the INR was more than 1.5. If the MAP was below 60 mmHg norepinephrine was administrated. After the operation, patients were transferred to the intensive care unit and extubated.

After anaesthesia induction the patients were randomly assigned by a computer program to three groups:

1 a group of patients which we preconditioned with RIPC of the right upper limb (three cycles of 5 min ischemia of right upper limb by tourniquet up to 200 mmHg followed by 5 min of reperfusion) [21,22];
2 a group of patients who were preconditioned with LIPC of the liver (15 min of portal triad clamping followed 10 min of reperfusion) [23,24];
3 a control group (CG) of patients which was not ischemically preconditioned.

The type of laparotomy was a right subcostal "J" laparotomy. After laparotomy, we implemented one of the preconditioning protocols [21–24] and started to mobilise the liver. Intraoperative ultrasonography (US) was used to identify the exact localisation of the liver tumour and its precise relationship with the liver vasculature. Next, the type of resection required was specified. Liver transection was performed with a blunt-clamp dissection technique, which allows visualisation of intrahepatic vessels and individual ligation of major blood or bile vessels. It was performed with the use of the LigaSure device (Valleylab) [25]. In all patients, the Pringle manoeuvre was used to avoid blood loss during liver transection.

The liver synthetic function was assessed by measurement of the laboratory liver tests preoperatively, on the first, third, and seventh day after resection. Residual synthetic liver function and liver ischemic-reperfusion injury were determined by levels of bilirubin, total proteins, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamic aminotransferase (GGT), cholinesterase, alkaline phosphatase (AP), prothrombin time (PV), activated partial thromboplastin time (APTV). We also recorded patients' clinical conditions, the type of liver resection, operative time, total warm liver ischemia time, blood loss and the need for transfusion. Doppler US of the hepatic artery and portal vein was performed intraoperatively before and after preconditioning in the RIPC and LIPC groups.

Patohistologic evaluation of all resected liver specimens was done postoperatively. Liver paraffin-embedded, standard haematoxylin-eosin stained sections were analysed. According to Rodriguez four elements of the liver histology were analysed: steatosis (microvesicular and macrovesicular) in 4 grades, the degree of sinusoidal congestion and dilatation in 3 grades, leukocyte infiltration in 3 grades and necrosis (focal, confluent, or zonal) in 3 grades [26]. Each biopsy was evaluated by a single pathologist blinded to the treatment allocation.

3. Statistical analysis

3.1. Sample and instruments

Data collection for this study is

- 20 patients in each of the three groups with all values for:AST, ALT, GGT, PV, APTV, leukocyte, erythrocyte, haemoglobin, hematocrit, urea, creatinine, serum proteins, albumins, cholinesterase, bilirubin at all periods of measurement
- 20 patients from RIPC and LIPC group of patients exposed to ischemic preconditioning for US assessment of flow through the hepatic artery and portal vein before and after preconditioning
- 20 patients from each group of patients exposed to ischemic preconditioning for patohistologic analysis (leukocyte infiltration, sinusoidal congestion, steatosis, hepatocyte necrosis).

3.2. Data analysis

Results are based on analysed scores due to homogeneity. Although a relatively small sample, the distribution is in a normal range. According to *Kolmogorov-Smirnov and Shapiro Wilks* tests, the assumption of normality has been met for this sample, and parametric tests could be used.

ANOVA and *Tukey HSD* tests were used to detect differences between the three groups of in all points of measurement for values of: AST, ALT, GGT, PV, APTV, leukocyte, erythrocyte, haemoglobin, hematocrit, urea, creatinine, serum proteins, albumins, cholinesterase and bilirubin.

Differences between the two groups of patients exposed to ischemic