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Continuous Venous Infusion of Adenosine in Patients Undergoing Partial Liver-Resection - a Potential Method to Reduce Ischemia-Reperfusion Damage?

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Abstract

Purpose: Hepatic ischemia-reperfusion injury following Pringle maneuver during liver surgery has significant impact on postoperative hepatic function. The purpose of this study was to determine if continuously infused adenosine can prevent/reduce the hepatic ischemia-reperfusion injury.

Method: This study focused on the pharmacological pre- and/or post-conditioning by intra venous adenosine infusion in 23 humans scheduled for liver surgery including Pringle's maneuver.

Result: Plasma adenosine concentrations measured in central venous and arterial samples revealed a clearance of adenosine of almost 94% after lung passage in the arterial samples. Adenosine plasma concentrations in portal venous samples were not affected by adenosine infusion. Inflammatory, hepatocellular and ischemic markers were not different between the control group, the preconditioning group or the pre- and post-conditioning group.

Conclusion: This preliminary study shows that high doses of central-venous infused adenosine could neither affect systemic inflammation nor the degree of effector organ (liver) damage as a result of surgical trauma and ischemia and reperfusion. Because of the impossibility of sufficient systematic metabolic tissue monitoring, the absence of hepatic effects might be best attributed to non-sufficiently high concentrations of adenosine at the target organ.

Keywords: Pre-conditioning; Post-conditioning; Adenosine infusion; Partial liver resection; Pringle maneuver; Humans

Abbreviations

ADO: Adenosine; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; Ctrl: Control Group; ECG: Electrocardiogram; IRI: Ischemia-Reperfusion Injury; LRA: Liver Resection Area; LRV: Liver Resection Volume; PC: Preconditioning Group; PoC: Pre- and Post-Conditioning Group

Background

Surgical treatment of distinct liver diseases is still a challenging field to the clinician. Approximately 25% of circulating blood volume runs through the liver, consecutively, hemorrhages and hemodynamic disturbances are the leading problems during surgeries, accordingly, a temporary clamping of blood supplying vessels (Pringle maneuver) is sometimes necessary to avoid excessive hemorrhage. The unavoidable consequence of Pringle maneuver is an Ischemia-Reperfusion Injury (IRI) [1]. Consequences of IRI are tissue edema and malperfusion, hepatocellular dysfunction and inflammation, altogether resulting in liver dysfunction and in worst cases, total liver failure [2,3].

There are promising approaches to attenuate the negative consequences of IRI in the context of Pringle maneuver, like pre- and post-conditioning. Pre-conditioning is a procedure in which the organs' tolerance to a harmful and anticipatable stimulus is simulated to induce adaption to it. Hepatic preconditioning has been successfully realized by temporary decreasing the oxygen content of circulating blood (´hypoxic preconditioning´, e.g. [4]) or short periods of interrupted blood supply (´ischemic preconditioning´, e.g. [5]). Post-conditioning is done by applying the conditioning stimulus after the damaging event, for example by brief ischemic periods [6]

One of the key mediators of hypoxic/ischemic pre- and post-conditioning of the liver in animal models is Adenosine (ADO) [7]. In man, intravenous bolus application of ADO is approved for the termination of supraventricular tachycardia, due to the drug's short half-time (seconds). This property demands for a continuous infusion in order to simulate the beneficial downstream effects of ADO as a signal transductor of pre-/post-conditioning. With respect to recent human studies in ischemic heart disease [8] and from promising results in man and in murine models [9,10], similar amounts of ADO might be necessary to achieve hepatic pharmaceutical pre- and post-conditioning effects. The purpose of this pilot study was to investigate possible protective effects of adenosine continuously infused in patients before and after hepatic resections

Materials and Methods

Study design

After approval by the Institutional Review Board of the Ludwig-Maximillians-University (Munich, Germany) (protocol number 089/04) every patient gave informed consent before enrolment. The study and its proceedings were registered at the national institute of health's clinical study register (Clinicaltrials.gov registration number: NCT00845689) and met the CONSORT criteria.

Patients were eligibly randomized into one of three groups prior to surgical resection of a liver tumor including Pringle maneuver:

-    Placebo infusion before and after resection (control group: Ctrl)

-    ADO infusion before resection and placebo infusion after resection (preconditioning group: PC)

-    ADO infusion before and after resection (pre- and post-conditioning group: PoC)

To increase reliability, the same surgical and anesthesiologic teams treated the patients in a blinded fashion.

The study had to be discontinued due to new regulations that prevented the in house pharmaceutical department providing randomized medication placebo/adenosine. In total, 23 patients were included in this study for elective hepatic resection.

Anesthesia and surgery

All patients were anesthetized and cardio-circulatory stabilized in a standardized fashion (details see supplementary information).

After laparotomy, the arteria hepatica and vena portae were exposed for preparing the vascular inflow occlusion, i.e. Pringle maneuver. The first continuous intravenous infusion of adenosine was administered during a stable condition while preparing the liver/liver vessels, immediately before Pringle maneuver (preconditioning). Patients received an adenosine-solution via a central-venous line in incremental dosage starting at a rate of 30 μg/kg/min. In the absence of adverse side effects, the infusion rate was raised by 30 μg/kg/min steps until a maximum rate of 150 μg/kg/min. If hypotension or significant changes in ECG became apparent, the infusion rate was lowered until the disappearance of the symptoms and continued at the highest rate tolerated. Total infusion time was on average 10 min, followed by 10 min of recovery. Hepatic resection was then carried out under Pringle maneuver. The liver was kept ischemic for at least 30 min or the end of resection.

After the termination of ischemia and hemodynamic stabilization during reperfusion a subgroup of patients received a second infusion of adenosine in the same manner as before (post-conditioning). The adenosine infusion was continued until the end of the surgical procedure.

Arterial and central venous blood samples were obtained at corresponding time points. For portal venous blood samples, the portal vein was directly punctured during surgery.

After surgery, patients were monitored for at least 24 h at the anesthesia intensive care unit.

Plasma concentrations of adenosine

Central venous, arterial and portal venous blood samples were drawn intraoperatively. Plasma concentrations of adenosine were analyzed by dual column switching high affinity   performance/reversed phase high performance liquid chromatography technique using an internal surface nitrophenylboronic acid precolumn as described elsewhere [2,11].

Inflammatory, Ischemic and hepatocellular function measurements

Cytokines were measured in a blinded fashion using Luminex xMap® technology (Bio-Plex®) with commercially available reagents from Bio-Rad-Laboratories Inc. (California, USA). Data were analyzed with Bio-Plex®-Software, and assays for cytokines Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Interleukin-10 (IL-10) were performed.

Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Prothrombin time and complete blood count were measured by routine procedures at the Laboratory of the Clinical Chemistry at the University Hospital, LMU Munich, Germany. To determine lactate levels an on-site blood gas analysis was performed using an ABL625 blood gas analyzer (Radiometer, Copenhagen, Denmark).
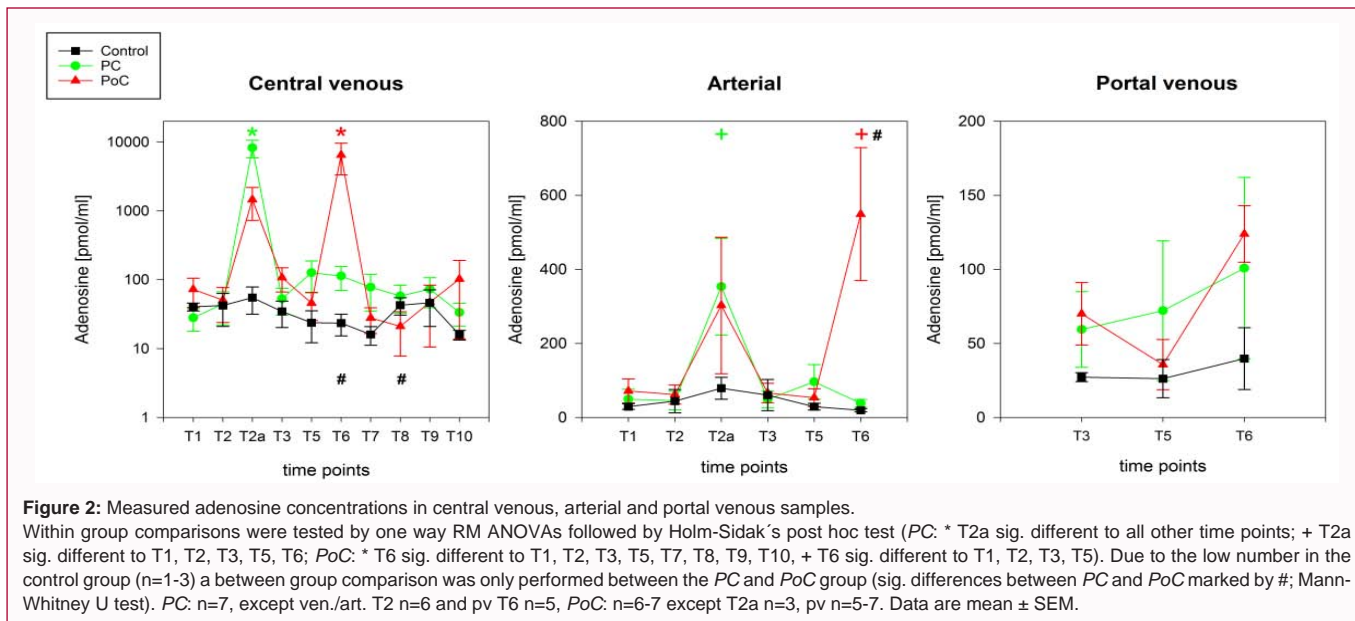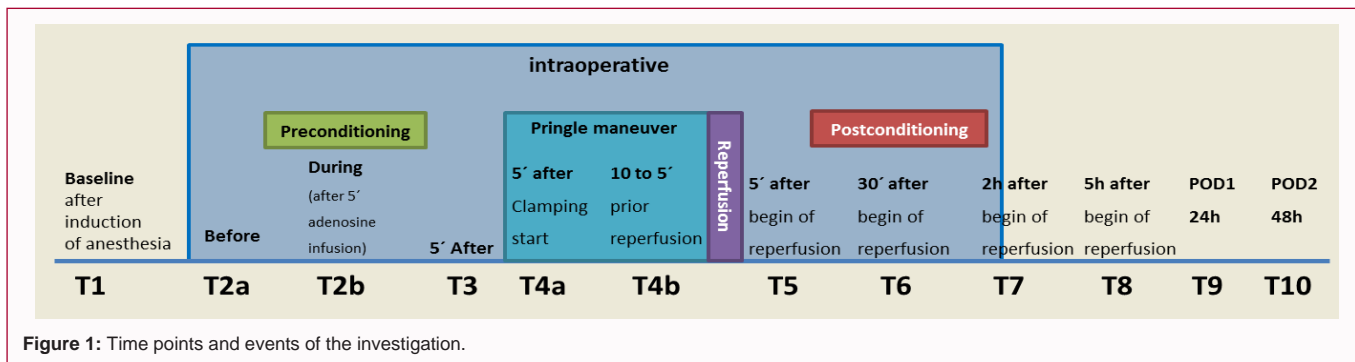
Statistics

All data were tested for normal distribution using the Shapiro-Wilk test. Within group comparisons were executed by one-way repeated measure analysis of variances (one-way RM-ANOVA) followed by the post hoc Holm-Sidak test. Between group comparisons were performed by Kruskal-Wallis tests and followed by the Mann-Whitney U test. Data were considered statistically different if the p-value was <0.05. Significant different data are marked bold and with * in each figure. Calculations were done using IBM SPSS Statistics V.25 (Armonk, NY, USA) and SigmaPlot® 12.5 (Systat, Software, Chicago, IL, USA)

Results

Demographic data and procedural characteristics

Due to the discontinuation of this randomized trial, study groups differ in size and some characteristics. The control group consists of 5 patients (m/w 3/2) with a mean age of 57.2 yrs (range 35 to 74)

Figure 1: Time points and events of the investigation.

Figure 2: Measured adenosine concentrations in central venous, arterial and portal venous samples.
Within group comparisons were tested by one way RM ANOVAs followed by Holm-Sidak´s post hoc test (*PC*: * T2a sig. different to all other time points; + T2a sig. different to T1, T2, T3, T5, T6; *PoC*: * T6 sig. different to T1, T2, T3, T5, T7, T8, T9, T10, + T6 sig. different to T1, T2, T3, T5). Due to the low number in the control group (n=1-3) a between group comparison was only performed between the *PC* and *PoC* group (sig. differences between *PC* and *PoC* marked by #; Mann-Whitney U test). *PC*: n=7, except ven./art. T2 n=6 and pv T6 n=5, *PoC*: n=6-7 except T2a n=3, pv n=5-7. Data are mean ± SEM.

and mean Body Weight (BW) of 69.2 kg (44 to 90). Four patients had malignant tumors, leading in two cases to a hemi hepatectomy, whereas three were treated by segment resection. Liver Resection Area (LRA) was 153 cm^2 (SD170), and Liver Resection Volume (LRV) 360 ml (SD302). Eight patients (m/w 4/4, age 48.5 yrs (25 to 67), BW 78.8 kg (57 to 95)) were assigned to the preconditioning group. Six patients suffered of malignant cancer, overall, seven cases were operated by segment resection, one by hemi hepatectomy with a total LRA of 129 cm^2 (SD60) and LRV of 200 ml (SD95). The post-conditioning group included 11 patients (m/w 9/2) at an average age of 60.9 years (36 to 73) with malignant tumors. BW was 73.6 kg (58 to 95). Four were operated by hemihepatectomy, the others by segment resection. LRA was 138 cm^2 (SD119), LRV was 275 ml (SD232). Pringle maneuver duration was comparable in all groups (Ctrl: 31.7 min (SD3.7), PC: 31.0 min (SD4.2), PoC: 34.3 min (SD 4.3)).

Concentrations of adenosine

Central venous adenosine concentrations increased at the time points of adenosine infusion in the respective groups (significant at T2a PC and T6 PoC, Figure 1 and 2). Due to the low n (3) at T2a in the PoC a statistically significant difference could not be calculated. An elevation of arterial adenosine levels was also seen at T2a for the PC and PoC group and T6 for the POC group but at a 16-fold lower level. After begin of reperfusion portal venous concentrations of adenosine were slightly elevated in the PC and PoC group irrespective of the adenosine infusion.
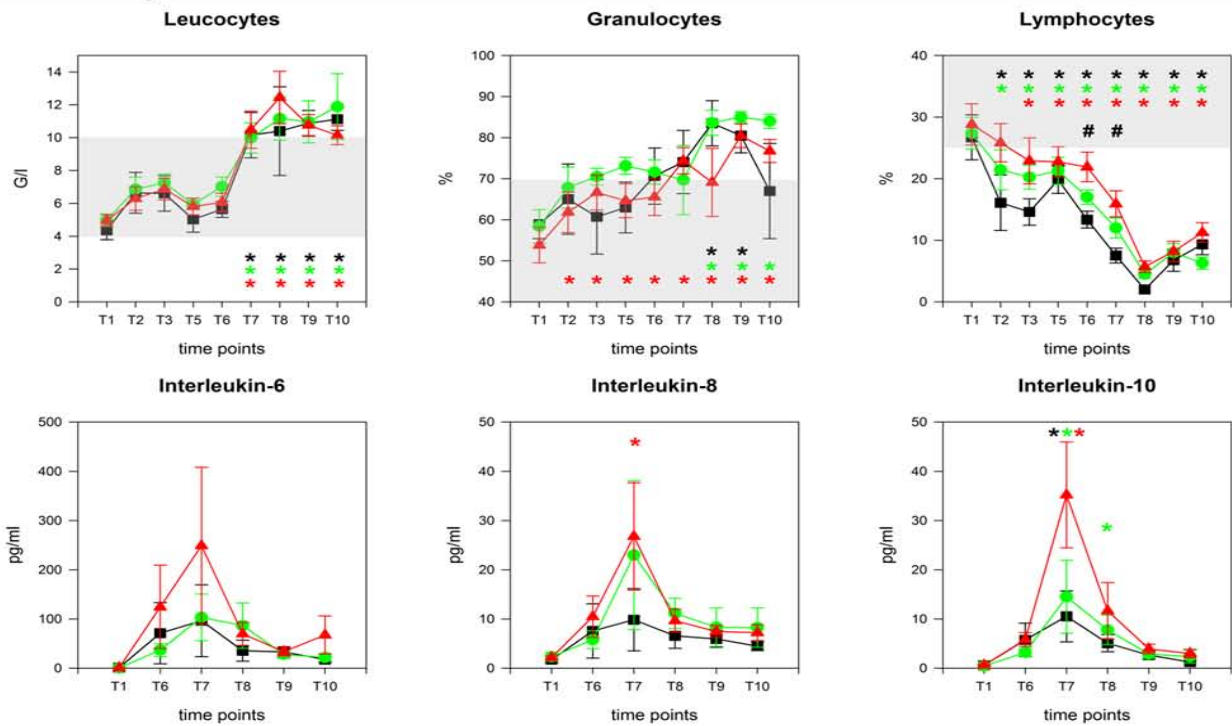
The total amount of ADO infused was during preconditioning on average 107 mg (SD40) and during post-conditioning 434 mg (SD200).

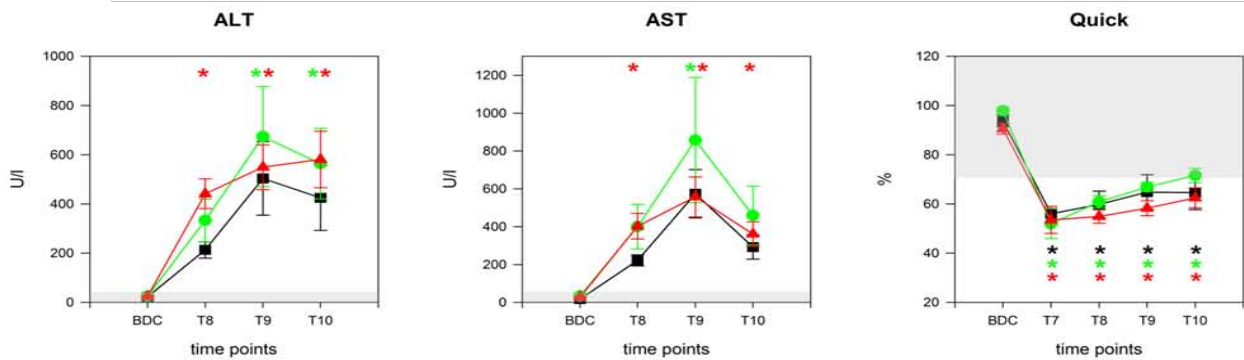### Changes in inflammatory, Ischemic and hepatocellular function

Irrespective of the groups, leukocytes significantly increased at T7 and stayed elevated for the remaining time points. Granulocyte percentage changed over time with a continuous increase and highest values after surgery (T8-T10, Figure 3). In contrast, percentages of lymphocytes decreased after surgery with lowest values at T8. Though all groups followed the same trend, a statistically significant difference was detected between the Ctrl and PoC at T6 and T7. Cytokine concentrations increased during surgery and peaked 2 h after begin of reperfusion (T7) in all groups. The highest levels of all cytokines (T7) were found in the PoC group. Due to the high variability and the lowest n at T7 a statistical significance between groups could not be observed.

Hepatocellular functions as reflected by the transaminases ALT and AST were significantly elevated after surgery peaking on post operative day 1 (POD1) in the PC and PoC group (BDC *vs.* T9/10). Due to the low n at baseline in the control group statistics could not be performed. Nevertheless, the course was the same compared to the other groups and high above normal values (5 to 20 times). The Quick value as a marker of the extrinsic coagulation system, relying on coagulation factors produced in the liver, was significantly reduced
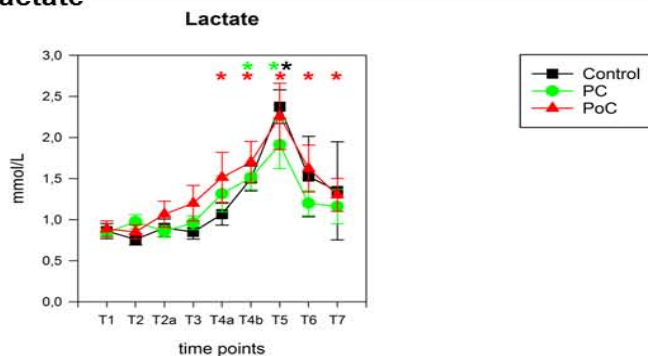
Figure 3: Inflammatory, Hepatocellular Function and Lactate measurements.
A one-way RM ANOVA was performed followed by Holm-Sidak post hoc testing. Comparisons were done versus BDC/T1, significant differences are marked by * and the respective color for the group. Between groups comparisons were calculated using the Kruskal Wallis test followed by the Mann-Whitney U test. Sig. Differences are marked by #. Data are mean ± SEM., *grey background:* Laboratory normal range. Data sets were in part not complete (**A. Inflammatory Parameters: White cell count:** *Ctrl:* n=5 except T8 n=2, T9 n=4, T10 n=3-5; *PC*: n=8 except T2/5/9/10 n=7; *PoC:* n=11 except T2/8 n=8-10; **Cytokines**: *Ctrl*: n=5 except T7 n=4, *PC*: n=8 except T7 **n=3**, *PoC*: n=10 except T7 n=6, T9/10 n=9; **B. Hepatocellular Parameters: ALT:** *Ctrl:* n=5 except BDC n=2, *PC:* n=8 except BDC n=6, T10 n=7, *PoC:* n=9-11; **AST:** *Ctrl:* n=5 except BDC n=1, T8 n=4, *PC:* n=8 except BDC n=2, T10 n=7, *PoC:* n=9-11 except BDC n=6; **Quick:** *Ctrl:* n=5 except BDC n=4, T7 n=1, *PC:* n=7-8 except T7 n=3, *PoC:* n=10-11 except T7 n=4; **C. Lactate:** *Ctrl:* n=4-5 except T4a n=3, *PC:* n=7-8 except T7 n=5, *PoC:* n=8-9 except T4a/5 n=7, T7 n=3).

after 2 h after the begin of reperfusion when compared to baseline. On POD2 (T10) levels were still out of the normal range. Arterial lactate values increased during Pringle maneuver and peaked 5 min after begin of reperfusion before returning to almost baseline values independently of the study groups.

Discussion

This study was designed to determine possible protective effects by continuous adenosine infusion during liver surgery including Pringle maneuver. The major finding of this study is that the high amounts (up to 150 µg/kg/min) of continuously infused adenosine were well tolerated and no major critical incident was observed, irrespective of the age and the surgical scale of intervention. However, protection by adenosine could not be observed.

Centrally infused adenosine was almost completely cleared and hence adenosine concentrations were not further increased in the arterial or portal venous vessels at the target organ (liver). This observation might be related also to the unexpected lack of effect of adenosine on systemic inflammation or tissue protection as mirrored by liver enzymes and the quick value.

Studies by Utterback investigating the *in vivo* effects of adenosine in patients [12] are showing mean pulmonary extraction rates of adenosine at a body temperature of 35.1°C to be 70% to 80% pending on the infusion rates. However, it was not described in their report for how long the infusion occurred. In this study we observed a degradation rate exceeding 90%. The infusion regimen and the stepwise increase of the doses might have resulted in this higher extraction, as well as the higher cardiac index (here at T2a 3.5 ± 0.6 l/min/m^2 *vs.* Utterback 2.0 ± 0.1/min/m^2) and a slightly higher body temperature (mean temp: Min. 35.5ºC ± 0.55; max. 36.2ºC ± 0.62). In the light of these high extraction rates, either a moderately lower body temperature, or the local infusion directly into the portal vein might have been more effective, though both approaches are offering limits in the surgical procedures. However Zhu et al. [13] have directly infused adenosine to increase hepatic artery flow in orthotopic liver transplantation. They were able to show that an increase of hepatic blood flow was achieved by low rates of a continuous adenosine infusion (0.7 to 2.8 µg/kg/min). Interestingly, the adenosine infusion did also not improve the liver function, though one cannot fully compare these results as our study here applied adenosine infusion as preconditioning in which the liver´s tolerance to the Pringle maneuver should have been primed and improved. Adenosine is a powerful agent and besides its cardio-protective properties during IRI [8,14,15], it can decrease systemic vascular resistance during continuous infusion [16] and preserve vascular functions during Systemic Inflammatory Response Syndrome (SIRS) [6,16,17]. Hence, the authors expected adenosine to be an appropriate candidate also in humans for hepatoprotection before and/or after Pringle maneuver. From an investigation in rabbits in a hepatic ischemia/reperfusion preconditioning model [18], it is known, based on biochemical and tissue/histopathological markers, that the best protection can be achieved by Ischemic Preconditioning (IPC). Intra portly applied adenosine or prostaglandin E1 prior to Pringle maneuver had some beneficial effects, but the best hepatic protection was achieved by IPC. These authors concluded that IPC induces a broader spectrum of mechanisms including the reduction of oxidative stress, inflammation and hepatic energy metabolism. In a previous study in humans, we showed that IPC was able to increase adenosine formation and to attenuate the Pringle maneuver induced degradation of adenine nucleotides resulting in purines. In

conclusion, IPC can reduce disturbances in hepatic energy metabolism [19] but protection could not be induced by infusing adenosine as a sole drug with the methods applied in our study.

In summary, we conclude from this controlled study in 23 patients undergoing liver surgery that adenosine was not effective to protect from systemic inflammatory reaction or to improve liver cell integrity or function in the frame of the clinical outcome parameters assessed. Further investigation might be needed to optimize the application protocol.

Limitations

Due to discontinuation of the delivery of the certified study medication this study was not completed with the planned patient number.

Authors Contribution

A,C., M.T. and I.K., designed the work; A.M., I.K., M.T. and A.C. acquired the study data; M.F. and Q.Z. analyzed the data, M.F. and A.C. interpreted the results and drafted the work. Q.Z., A.M., I.K., M.T. and A.C. substantively revised it.

All authors have approved the submitted version and any substantially modified version that involves the author's contribution to the study.

Furthermore, every author has agreed to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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