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ISCHEMIC PRECONDITIONING PRIOR TO INTERMITTENT PRINGLES MANEUVER IN LIVER RESECTIONS

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Category: Prospective clinical trial

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Abstract

Background: Continuous inflow vascular occlusion during liver resections causes less severe ischemia and reperfusion injury (IRI) if it is preceded by ischemic preconditioning (IP) or if intermittent inflow occlusion is used during the resection. No previous clinical trial has studied the effects of adding IP to intermittent inflow occlusion.

Methods: Consecutive patients (n=32) with suspicion of malignant liver disease had liver resections (minimum 2 segments) performed with inflow occlusion 15/5. Half of the patients were randomized to receive IP (10/10). The patients were stratified according to volume of resection and none had chronic liver disease. The patients were followed for 5 days with microdialysis (μ D).

Results: All patients completed the study and there were no deaths. No differences were seen between the groups regarding demographics or perioperative parameters (bleeding, duration of ischemia, resection volume, complications and serum lab tests). There were no differences in ALT, AST, Bilirubin or PT-INR levels, but μ D revealed lower levels of lactate, pyruvate and glucose in the IP group having major liver resections (ANOVA). Nitrite and nitrate levels in μ D decreased postoperatively but no differences were seen between the groups. In one patient an elevated μ D-glycerol curve was seen before the diagnosis of a stroke was made.

Conclusions: IP before intermittent vascular occlusion does not reduce the serum parameters used to assess IRI. IP seems to improve aerobic glucose metabolism as the levels of glucose, pyruvate and lactate locally in the liver were reduced compared to controls in patients having resected >3 segments. μ D may be used to monitor metabolism locally.

INTRODUCTION

Clamping of the portal pedicle, *i.e.*, the Pringles Maneuver (PM) is used to reduce bleeding during liver transection [1, 2]. The liver suffers from ischemia and reperfusion injury (IRI) when the portal pedicle is clamped [3-5]. Intermittent clamping in a manner of 15 minutes of ischemia and 5 minutes of reperfusion repetitively (15/5) has been shown to reduce the IRI compared to continuous PM [6, 7].

In 1986 it was shown that ischemic preconditioning (IP) reduced ATP depletion in the heart during the subsequent ischemic insult [8]. IP also protects the liver from IRI if it is employed before continuous PM [2, 9-11]. IP in liver surgery is typically performed as 10 minutes of PM followed by 10 to 15 minutes of reperfusion prior to continuous PM. There are two studies comparing 15/5 with IP followed by continuous clamping and one of these could show less bleeding after IP [12]. The other showed lower AST levels and less apoptosis in the intermittent clamping group when ischemia exceeded 40 minutes [13]. Even so, 15/5 intermittent clamping causes IRI and in patients at risk of postoperative liver failure it is necessary to reduce this damage further. Experimental studies indicate that the protective effect of IP may be mediated by metabolites of ATP degradation (e.g. adenosine) or NO [14, 15]. It is possible that these metabolites “prepare” the liver cells for a second ischemic event making them more resistant to IRI. No clinical trial has studied the effect of IP in the liver when it is employed together with 15/5 intermittent vascular clamping.

The interpretations of the effects of liver IRI in humans have concerned changes in the systemic blood levels of AST, ALT, bilirubin and prothrombin time (PT). For practical and ethical reasons, observations made from tissue analyses in human liver IRI spans only over a few hours, typically at the start and at the end of surgery. In humans these analyses have shown that IP reduces endothelial cell apoptosis [2] and ATP degradation [12] in the immediate postischemic period.

Microdialysis (μ D), however, offers the opportunity to clinically follow various local metabolic changes in the liver for several days [16, 17].

Hypothesis:

Does IP (10/10) before 15/5 Pringles maneuver reduce the ischemia and reperfusion injury or change the glucose metabolism compared to 15/5 Pringles maneuver alone in surgical liver resections?

The IRI was evaluated using postoperative serum analyses of bilirubin, aminotransferases and PT-INR.

Glucose metabolism was evaluated by the blood levels of glucose and lactate as well as the recovered levels of glucose, pyruvate, lactate and glycerol in microdialysate.

This prospective clinical trial has been registered at the web site 'www.controlled-trials.com',

ISRCTN29593599.

MATERIALS AND METHODS

The study protocol was approved by the Regional Ethics Committee at the University of Linköping, University Hospital, Linköping, Sweden.

Study design and patient selection

This prospective single-blinded clinical trial was conducted between January 16th 2008 and June 8th 2009 at the Department of Surgery, University Hospital, Linköping, Sweden. Patients intended for resection of two or more segments of the liver due to metastatic liver disease or suspicion of T1-2 gallbladder cancer

were asked to participate in the study. No patients intended for multiorgan resection, portal ligature or with a suspicion of chronic liver disease were included.

The definitive decision to include a patient in the study was taken after the laparotomy and the patient was considered to have resectable disease. Before randomization, patients were stratified according to the size of the resection. Sixteen patients had a resection of 2-3 segments and another 16 patients had a resection volume of >3 segments. Closed envelopes were prepared from a random table generated at the web site 'www.random.org'. Half of the patients in each of the strata were randomized to have IP prior to the Pringles' maneuver making a control group (n=8) within each stratum (**figure 1**). The patients were blinded to their group allocation whereas the surgeons were not.

Figure 1: Schematic illustration of the randomization. The study population was set at 32 subjects. When the size of the resection was decided, an envelope of that stratum was opened to reveal IP or no IP.

Power

In this trial we introduce a novel method of assessing postoperative liver metabolism after IRI. No reliable calculations of power could thus be made; instead we looked at clinical significance. IP takes 20 minutes to perform and the benefit from this maneuver must justify the extra time spent in the operating room (OR). It was therefore decided that if no differences were seen in 16 patients having IP (i.e. 5 hours in the OR), the results would not be of clinical interest.

Operative procedure

Anaesthesia was induced with fentanyl together with either propofol or sodium thiopental. Rocuronium was used for relaxation and anaesthesia was then maintained with sevoflurane. To minimize bleeding during the liver transection, an intravenous (i.v.) infusion of nitroglycerine 0.1 mg/ml was given to keep a low central venous pressure (CVP). Five patients had patient controlled analgesia with i.v. morphine pumps postoperatively and 27 patients had epidural catheters activated postoperatively. All operations started between 8.30 and 9:30 a.m. and all patients were operated through a right subcostal incision angled and extended to the sternum. Resectability and the size of the resection was decided after the abdomen was palpated, the liver mobilized and an intraoperative ultra sound had been performed. Definitive inclusion, stratification (2-3 or >3 segments) and randomization (IP or no IP) were made at this point. A cotton tape was placed around the hepatoduodenal ligament. In all patients the liver was transected with the Cavitron Ultrasonic Surgical Aspirator (CUSA Excel, Integra, Lyon, France) device. Blood samples were taken preoperatively, after ischemia, at wound closure, at 8 p.m. on the day of operation and then on the POD 1-4 at 7 a.m. All blood and serum samples (lactate, AST, ALT, Bilirubin, PT-INR, albumin, Hb and WBC), were analyzed at the department of clinical chemistry, University Hospital, Linköping using the standard methods of this department. Bed-side analyses of capillary blood glucose levels were performed using the Accu-Check® Inform II (Roche, Mannheim, Germany). Just after

the abdomen had been opened, a liver biopsy was taken and immediately frozen in liquid nitrogen. It was then freeze-dried and stored in -20°C until analysis.

Microdialysis

The μ D-catheter consists of two concentric plastic tubes, one inside the other. The tubes communicate at the distal end of the catheter where there is a semi-permeable membrane. The outer tube is perfused slowly with a physiologic solution and equilibrates with the interstitial fluid before it is recovered through the inner tube (**figure 2a**). Two 0.9 mm thick μ D-catheters (CMA 63, CMA, Solna, Sweden) with 30 mm long semi-permeable membranes and a pore-size of 20 kDalton were tunneled through the skin and the abdominal wall. They were introduced close to each other in the anticipated residual liver and secured to the capsule of Glisson with a 4/0 absorbable suture. Both μ D-catheters were anchored to the skin with adhesive tape (**figure 2b**). Perfusion of the μ D-catheters with Ringers acetate (Braun, Stockholm, Sweden) was kept at 2.0 μ l/min during the surgical procedure and the postoperative ICU stay, and then decreased to 0.3 μ l/min at the ward, using CMA 107 micropumps (CMA). Steady state was considered to have been achieved after 20 minutes of μ D-catheter perfusion. The microvials were collected every 30 minutes in the OR, every hour at the ICU and every fourth hour at the ward. The catheters remained in place until the afternoon of the postoperative day (POD) 4 (**figure 3**). One of the microvials was immediately analyzed in the bed-side analyzer ISCUS (CMA) for lactate, glucose, glycerol and pyruvate levels. The other microvial was frozen at -20 °C for later analysis of NOx (see below) levels.

Figure 2: Schematic illustration of the microdialysis catheter where diffusion of molecules over the semi-permeable 20 kDa membrane is driven by concentration gradients. The micropump perfuses the catheter and the dialysate is recovered in the microvial **(a)**, *presented with kind permission of CMA microdialysis*. Schematic illustration showing the positioning of the catheter tips within the liver parenchyma. The micropumps are kept in a waist belt **(b)**.

Classification of complications and clinical status

The most severe complication was registered for each patient according to the Dindo-Clavien classification [18]. The modified early warning score (MEWS) [19] was used to assess the clinical status of the patients every fourth hour. MEWS includes the following parameters: pulse, blood pressure, respiratory frequency, temperature, urinary output and patient consciousness.

Nitrite/Nitrate in microdialysate

The instructions in the commercial “Nitrite/Nitrate Fluorometric Assay Kit” (Cayman Chemical Company, Ann Arbor, MI 48108, USA) were followed when the sum of NO_2^- and NO_3^- was analysed in the μD samples. The fluorometric intensity was measured with the “1420 Multilabel Counter Victor³_{TM} V” instrument (PerkinElmer, Väsby, Sweden), using an excitation wavelength of 355 nm and emission wavelength of 460 nm, with a filter tolerance of ± 20 nm. The included standard curve was used when calculating the results (given in μM). The *in vitro* μD recovery of $\text{NO}_2^-/\text{NO}_3^-$ was tested and found to be $70 \pm 10\%$ at the perfusion rate of $1.0 \mu\text{L}/\text{min}$ using a 30 mm 20 kDa μD -catheter.

Glycogen

Glycogen in the liver tissue was quantified with the “Biovision Glycogen Assay” kit (BioVision Research products, CA 94043 USA). Freeze-dried tissue was put in iced Eppendorf tubes, mortared and extracted into sterile water. Enzymes were then inactivated by boiling for 5 minutes and thereafter the tubes were centrifuged at 13000 rpm for 5 minutes. The supernatant was put in 96-well plates. As recommended in the assay kit, the glucose levels in the extracts (corresponding to $50 \mu\text{g}$ freeze-dried tissues) were measured with and without hydrolyzing the glycogen by glucoamylase. After reaction with OxiRed, the absorbance was finally colorimetrically measured in an ELISA reader at 550 nm. The glycogen level was calculated against a standard curve included in the kit, and the result is shown as μg glycogen per mg freeze-dried tissue.

Statistics

Patients having IP (n=16) were compared with the controls (n=16) and then the patients having IP (n=8) were compared to the controls (n=8) within the same stratum. The results are given for these stratified groups, unless otherwise indicated in the text. A secondary comparison was made between minor (2-3 segments, n=16) and major (>3 segments, n=16) resections to control for confounding changes depending on resection size.

The microdialysate samples were retrieved at the time points specified above. The μ D samples were then grouped in 7 different phases: baseline (sample 1), peroperatively (samples 2-7), postoperatively the day of surgery (8-24), POD 1 (25-30), POD 2 (31-36), POD 3 (37-42) and POD 4 (43-46). Individuals were nested in the IP and control groups, respectively, and assigned as the random factor in an ANOVA. A Tukey Post Hoc analysis was performed if there were significant statistical differences found between the groups in the ANOVA.

Non-parametric values are given as ranges (median) and analysed with the Mann-Whitney U-test and parametric values given as mean (SEM) (except in table 1 where mean (SD) is used) and analysed using the Students t-test. STASTICA 8.0 software (StatSoft Inc Tulsa, Oklahoma, USA) was used for all statistical calculations. A p-value < 0.05 was considered statistically significant.

Results:

All randomized patients completed the study according to intention to treat and there was no mortality within the study. All patients during the study period (n= 79) having liver surgery at the department of

surgery, Linköping, were retrospectively reviewed. Two patients had declined participation in the study and 45 did not meet the study criteria.

Demographics

There were no differences between the whole IP group (n=16) and the controls (n=16) or between the stratified groups regarding demographics (sex, age and neoadjuvant chemotherapy) or intraoperative data (bleeding, transfusions, resection volume, duration of the ischemia or the glycogen levels), see

Table 1. Subjects having the large volume (>3 segments, n=16) resections proved to have had a larger liver volume resected (700(349) vs. 184(105) ml, $p<0.001$), a greater transectional area (122(29) vs.

93(37) cm^2 , $p<0.05$) and a longer duration of the operation (328(95) vs. 251(50) minutes, $p<0.05$), than those having resected 2-3 segments (n=16).

Table 1: Demographic and perioperative data. Results given as mean (SD) for parametric data, whereas non-parametric data are given as range (median). Student T-test and Mann-Whitneys U-test are used, respectively and Fisher exact test is used for categorical variables. IP-group (n=8) and controls (n=8) are compared within each stratum, 2-3 segments and >3 segments resected, respectively. Fdt=freeze dried tissue

		2-3 segments			>3 segments		
		IP	Controls	p	IP	Controls	p
Sex	Males (%)	4 (50)	5 (62)	0.72	4 (50)	5 (62)	0.72
Age	Years	67 (15)	62 (9)	0.44	60 (13)	66 (10)	0.27
Preop glycogen levels	$\mu\text{g}/\text{mg}$ fdt	40 (6.0)	42 (9.8)	0.74	41 (7.4)	37 (7.7)	0.41
Preop chemotherapy	# (%)	4 (50)	3 (38)	0.72	7 (88)	6 (75)	0.72
Units of insulin given	Total # (range)	51 (0-47)	24 (0-20)	0.72	84 (0-60)	60 (0-60)	0.72
LOS	days	7-13 (9)	6-11 (6)	0.51	8-13 (10)	7-51 (11)	0.51
Blood loss	mL	100-1700 (359)	50-2400 (300)	1.0	100-1500 (625)	200-1800 (750)	0.38
Units of blood transfused	Total # (range)	6 (0-4)	4 (0-2)	0.96	10 (0-5)	7 (0-3)	0.88
Units of plasma transfused	Total # (range)	9 (0-7)	7 (0-5)	0.96	13 (0-6)	18 (0-7)	0.57
Resected volume	mL	55-340 (190)	60-400 (165)	0.57	225-1150 (698)	300-1200 (550)	0.96
Transected area	cm^2	91 (36)	95 (40)	0.84	132 (33)	113 (23)	0.20
Bleeding/ cm^2	mL/cm^2	1.0-11 (5.2)	0.5-15 (5.9)	0.85	0.9-25 (4.4)	2.3-21 (6.6)	0.44
Duration of ischemia	min	44 (10)	44 (13)	0.98	35 (11)	44 (8.0)	0.08
Transection time	min	64 (22)	77 (40)	0.46	62 (32)	60 (11)	0.86
Duration of Surgery	min	242 (57)	261 (42)	0.52	292 (60)	359 (112)	0.21
Bleeding/min (Transection)	mL/min	1.8-19 (5.7)	0.9-20 (6.5)	0.88	2.6-36 (9.2)	3.9-42 (11.2)	0.65
Bleeding/min (Total)	mL/min	0.5-5.2 (1.8)	0.6-2.9 (1.1)	0.45	0.4-5.2 (2.0)	0.9-4.7 (3.6)	0.76
Complications	n (%)	3 (38)	2 (25)	0.72	4 (50)	3 (38)	0.96
Diagnosis:							
Metastases	n (%)	7 (88)	4 (50)	0.28	8 (100)	7 (88)	1.0
Benign	n (%)	1 (12)	3 (38)		0	0	
Carcinoma (HCC?)	n (%)	0	0		0	1 (12)	
Gall bladder cancer	n (%)	0	1 (12)		0	0	
Steatosis (moderate)	n (%)	1(12)	0		0	3 (38)	0.20
Portal fibrosis (moderate)	n (%)	1 (12)	0		0	0	

Two patients in the control group had non-insulin dependent diabetes mellitus. Seven patients received insulin i.v. perioperatively, 4 in the IP group and 3 in the control group. No insulin whatsoever was administered in the study after the day of surgery as glucose levels remained lower than 12 mM in all patients during the study period. The glucose levels in the blood showed no differences between the groups (**Table 2 and 3**). The overall median length of stay (LOS) was 9 days with no differences between the groups, see **Table 1**. Patients having major resections had longer LOS than those having small resections (9 vs. 10 days, $p = 0.014$).

Table 2: Analyses of blood samples in the patients having resected 2-3 segments. IP-group (n=8) compared to controls (n=8). ANOVA.

			preop	pre-T	post-T	6 h	PoD 1	PoD 2	PoD 3	PoD 4
ALT	IU/L	IP	19 (4.5)	88 (73)	153 (122)	290 (170)	408 (271)	479 (339)	334 (212)	232 (131)
		Controls	37 (25)	98 (37)	187 (58)	268 (131)	481 (260)	549 (405)	410 (333)	286 (202)
AST	IU/L	IP	23 (5.8)	95 (82)	155 (108)	298 (159)	402 (248)	365 (274)	164 (111)	81 (42)
		Controls	29 (15)	99 (43)	196 (49)	281 (121)	472 (261)	361 (235)	160 (94)	80 (18)
Bilirubin	mg/dL	IP	0.69 (0.47)	0.84 (0.33)	1.07 (0.45)	1.39 (1.13)	1.96 (2.05)	1.72 (1.47)	1.22 (0.88)	1.05 (0.81)
		Controls	0.54 (0.14)	0.78 (0.23)	0.95 (0.34)	1.02 (0.37)	1.40 (0.92)	1.59 (1.21)	1.00 (0.34)	0.86 (0.30)
LDH	IU/L	IP	170 (30)	255 (161)	313 (148)	489 (264)	406 (167)	263 (144)	186 (39)	166 (31)
		Controls	177 (24)	252 (89)	357 (92)	375 (139)	478 (288)	313 (162)	206 (41)	211 (73)
Albumin	g/L	IP	38 (4.2)	29 (6.2)	25 (3.6)	26 (5.4)	28 (4.2)	28 (3.2)	28 (4.1)	26 (3.5)
		Controls	40 (3.3)	31 (3.5)	26 (3.6)	28 (6.0)	28 (4.5)	29 (2.7)	28 (3.9)	27 (4.2)
PT	INR	IP	1.1 (0.2)	1.2 (0.1)	1.3 (0.2)	1.4 (0.2)	1.5 (0.2)	1.2 (0.1)	1.1 (0.1)	1.1 (0.1)
		Controls	1.0 (0.1)	1.1 (0.1)	1.2 (0.1)	1.3 (0.1)	1.4 (0.2)	1.4 (0.3)	1.2 (0.3)	1.1 (0.2)
Lactate	μM	IP	1.3 (0.4)	2.2 (2.1)	3.3 (0.8)	1.8 (1.2)	1.4 (0.3)	1.3 (0.4)	1.6 (0.7)	1.3 (0.4)
		Controls	1.6 (0.4)	2.0 (0.6)	3.2 (1.3)	1.6 (0.8)	1.6 (0.5)	1.5 (0.6)	1.3 (0.5)	1.5 (0.4)
Glucose	mM	IP	4.3 (0.8)	7.7 (1.2)	8.5 (2.6)	9.0 (1.3)	8.8 (2.1)	8.5 (1.7)	8.0 (1.9)	7.2 (0.9)
		Controls	4.4 (0.6)	7.9 (2.3)	10 (2.7)	10 (2.0)	9.6 (1.9)	9.0 (1.8)	8.8 (1.9)	8.4 (2.0)
Hb	g/L	IP	138 (11)			123 (10)	121 (14)	119 (10)	116 (11)	115 (12)
		Controls	140 (16)			115 (18)	119 (18)	116 (17)	112 (20)	113 (14)
WBC	x10 ⁹ /L	IP	6.7 (1.4)			11 (2.2)	10 (2.9)	12 (3.1)	9.4 (2.2)	7.4 (2.3)
		Controls	6.7 (0.7)			8.6 (1.8)	10 (2.0)	12 (1.8)	10 (1.8)	8.3 (1.7)
Trc	x10 ⁹ /L	IP	218 (68)			170 (60)	170 (64)	162 (50)	172 (64)	191 (61)
		Controls	269 (89)			192 (78)	178 (42)	200 (55)	193 (64)	244 (49)
CRP	mg/L	IP	14 (9.4)	13 (9.1)	13 (7.9)	14 (8.0)	44 (18)	142 (58)	142 (66)	101 (49)
		Controls	14 (6.8)	12 (4.8)	12 (4.2)	14 (5.2)	55 (26)	151 (49)	147 (33)	98 (26)
MEWS		IP		0.7 (0.6)	1.0 (1.5)	0.7 (0.8)	1.0 (1.0)	0.6 (0.6)	0.4 (0.6)	0.3 (0.5)
		Controls		1.3 (1.0)	1.2 (1.1)	2.1 (1.4)	1.6 (1.2)	1.5 (1.2)	0.7 (0.9)	1.0 (0.9)

Table 3: Analyses of blood samples in the patients having resected >3 segments. IP-group (n=8) compared to controls (n=8). ANOVA and Tukey post hoc analyses. * = p<0.05, ** = p<0.01, *** = p<0.001.

			preop	pre-T	post-T	6 h	PoD 1	PoD 2	PoD 3	PoD 4
ALT	IU/L	IP	32 (7.5)	145 (116)	191 (159)	490 (211)	616 (513)	609 (658)	412 (451)	288 (287)
		Controls	28 (16)	177 (175)	373 (349)	472 (387)	433 (308)	412 (333)	307 (260)	219 (172)
AST	IU/L	IP	59 (70)	176 (189)	245 (206)	569 (303)	748 (728)	422 (438)	187 (149)	62 (23)
		Controls	66 (100)	243 (244)	395 (259)	529 (334)	432 (250)	294 (231)	144 (118)	76 (41)
Bilirubin	mg/dL	IP	0.44 (0.20)	0.61 (0.18)	0.50 (0.29)	1.17 (0.45)	1.77 (0.76)	1.73 (0.97)	2.29 (2.30)	1.14 (0.63)
		Controls	0.44 (0.14)	0.77 (0.33)	0.85 (0.34)	1.39 (0.38)	1.68 (0.67)	1.73 (0.85)	1.70 (1.01)	1.46 (1.00)
LDH	IU/L	IP	195 (34)	311 (146)	424 (192)	741 (351)	664 (450)	328 (154)	244 (61)	215 (38)
		Controls	182 (34)	365 (200)	733 (415)	616 (334)	388 (111)	264 (79)	212 (33)	196 (24)
Albumin	g/L	IP	38 (3.5)	29 (3.1)	20 (3.7)	27 (3.5)	30 (4.3)	28 (3.8)	27 (2.7)	26 (2.1)
		Controls	37 (4.7)	26 (4.3)	23 (5.5)	28 (1.8)	29 (7.0)	31 (4.3)	29 (3.9)	28 (4.5)
PT	INR	IP	1.0 (0.1)	1.1 (0.1)	1.2 (0.1)	1.2 (0.1)	1.5 (0.2)	1.6 (0.4)	1.4 (0.3)	1.3 (0.3)
		Controls	1.1 (0.1)	1.1 (0.1)	1.3 (0.1)	1.5 (0.1)*	1.6 (0.2)	1.6 (0.2)	1.4 (0.1)	1.3 (0.2)
Lactate	μM	IP	1.5 (0.3)	2.4 (1.1)	2.4 (0.4)	2.2 (0.9)	1.7 (0.7)	1.8 (0.8)	1.6 (0.5)	1.5 (0.2)
		Controls	2.0 (1.0)	2.2 (1.0)	4.4 (1.4)**	2.8 (1.0)	1.9 (0.6)	2.1 (0.8)	1.8 (0.9)	1.7 (0.4)
Glucose	mM	IP	4.8 (1.0)	8.5 (2.0)	7.9 (1.8)	8.7 (1.5)	7.7 (2.1)	8.0 (2.3)	7.9 (1.7)	7.2 (1.0)
		Controls	4.6 (0.9)	9.2 (3.3)	8.3 (1.8)	8.7 (1.8)	8.6 (1.9)	8.1 (2.2)	7.6 (2.5)	7.1 (1.7)
Hb	g/L	IP	123 (10)			109 (11)	112 (9.2)	111 (16)	105 (12)	104 (12)
		Controls	134 (17)			110 (13)	112 (13)	114 (12)	104 (11)	109 (11)
WBC	x10 ⁹ /L	IP	7.3 (1.9)			9.0 (2.7)	11 (4.3)	14 (5.5)	11 (3.5)	8.4 (3.1)
		Controls	7.6 (1.0)			12 (4.2)	13 (4.2)	17 (3.7)	12 (2.9)	11 (3.1)
Trc	x10 ⁹ /L	IP	267 (143)			237 (129)	240 (140)	203 (99)	209 (97)	247 (113)
		Controls	270 (57)			177 (41)	194 (33)	200 (27)	175 (28)	229 (38)
CRP	mg/L	IP	10 (0)	10 (0)	10 (0)	14 (4.7)	59 (50)	134 (87)	132 (76)	80 (38)
		Controls	30 (49)	27 (42)	11 (3.1)	12 (3.5)	52 (45)	121 (53)	107 (47)	81 (36)
MEWS		IP		1.5 (1.3)	1.6 (0.9)	2.2 (1.2)	2.1 (1.1)	1.5 (1.0)	1.2 (0.8)	1.5 (0.9)
		Controls		1.2 (1.0)	1.3 (1.3)	1.9 (1.3)	1.6 (1.1)	1.1 (0.9)	1.0 (1.1)	0.5 (0.6)

Figure 3: Treatment protocol of the IP-group and the control group. The black arrows shows when the parenchymal transection started. Microdialysate (μD) was collected at the given intervals (minutes). The first sample (10 min.) was discarded and the second (30 min.) was used as baseline data. IP = Ischemic Preconditioning.

Complications and diagnosis

Complications occurred in 12 patients (37.5 %) and these were scored according to Dindo-Clavien [20].

No differences were seen between the groups. There was one stroke due to carotid artery stenosis (grade 4), one reoperation due to wound dehiscence (3b), one biliary leakage, one abscess and 4 pleural effusions, all drained percutaneously (3a) and 4 antibiotic or conservatively treated infections (grade 2 or 1), **table 1**.

Twenty-six patients were diagnosed with metastases (23 colorectal, 1 anal, 1 ovarian and 1 malignant melanoma), 4 patients had benign lesions (1 Haemangioma, 1 cyst, 1 gallbladder polyp and 1 with chronic cholecystitis), 1 gallbladder cancer and 1 with HCC in a non-fibrotic liver. No patient was preoperatively suspected of having parenchymal liver disease. Histological examination of the parenchyma revealed that 4 patients had grade 2 (10-29%) moderate macrovesicular steatosis (post chemotherapy) and one patient had moderate portal fibrosis (Ludwig & Batts), see **table 1**. The rest had no or mild fatty changes or portal fibrosis. One patient had early primary sclerosing cholangitis.

Serum and blood analyses

No differences were seen between the IP group (n=16) and the controls (n=16) when serum and blood analyses were compared. Neither was any such differences seen between the groups when the patients having minor resections (**Table 2**) were compared. The patients that had IP prior to a major resection (**Table 3**) had lower levels of lactate and PT-INR immediately postoperatively (2.4(0.4) vs. 4.4 (1.4), p=0.003 and 1.2(0.1) vs. 1.5 (0.1), p=0.041, respectively). Serum and blood analyses of

Liver microdialysis

After surgery and up to POD 1 there were lower levels of μ D-glucose, μ D-pyruvate and μ D-lactate in the IP group (n=16) compared to the controls (n=16) (data not shown). When subgroup analyses were performed, these differences disappeared in the minor resections subgroups (2-3 segments, IP (n=8) vs.

controls, n=8), **figure 4 a-d**, except for a lower level of glucose on POD 2 in the IP treated patients (6.3 (0.39) vs .8.4 (0.46), p=0.045).

Figure 4: Glucose (a), pyruvate (b), lactate (c) and glycerol (d) levels in the microdialysate (μD) in the group having resected 2-3 segments. The IP group (n=8) and the control group (n=8). Shaded fields separate the phases used in the ANOVA and the significant differences found in the Tukey post hoc analysis are noted: * = p<0.05. POD = Post Operative Day.

IP treatment before a major resection, however, resulted in lower lactate levels already during the transection phase (4.25 (0.34) vs .5.8 (0.37), p<0.001). This decrease in the lactate levels continued during the rest of the day of the operation (2.6 (0.17) vs .4.0 (0.22), p<0.001) and included lower levels of glucose (4.9 (0.32) vs .6.6 (0.35), p<0.001) and pyruvate (147 (9.1) vs .198 (9.8), p<0.001) as well. Also on

the POD 2 the levels of glucose (6.1 (0.38) vs .8.3 (0.52), $p=0.022$) and lactate (1.8 (0.13) vs. 2.3 (0.16), $p=0.047$) were lower in the IP treated patients having had >3 segments resected (**Figure 5 a-d**).

No differences were seen whatsoever in the μ D-glycerol levels. We also compared all patients having major resections (n=16) with those having minor ones (2-3 segments, n=16), but could not detect any differences in the μ D analyses. Glucose and lactate levels in the blood did not show any differences between the groups, except a lower s-lactate immediately after the operation in the IP treated patients having >3 segments resected (**table 2 and 3**).

Figure 5: Glucose (a), pyruvate (a), lactate (c) and glycerol (d) levels in the microdialysate (μ D) in the group having resected >3 segments. The IP group (n=8) and the control group (n=8). Shaded fields separate the phases used in the ANOVA and the significant differences found in the Tukey post hoc analyses are noted: * = $p<0.05$, *** = $p<0.001$. POD = Post Operative Day.

NO_x

NO has been investigated as a possible mediator of the IP effect and therefore the NO_x levels were analyzed. These continuously decreased postoperatively in the IP group from 31 (11) to 22 (11) μ M ($p=0.0018$) until POD 3, where they started to return to the original levels. A similar decrease was seen in the control group, from 31 (12) to 22 (10) μ M, $p=0.0092$. There were no differences between the groups, nor did the volume of the resection affect the NO_x levels in the μ D.

Glycogen

The preoperative glycogen levels were similar in both groups and also in both strata. The glycogen levels were not different when the younger (<67 years) half of the patients was compared to the older half. The female patients, however, had lower glycogen levels than males (36.7 (2.0) vs. 45.0 (2.1), $p=0.0097$).

DISCUSSION

This is the first trial to study the effects of IP in a surgical cohort having liver resections performed with standardized intermittent inflow control 15 minutes of ischemia and 5 minutes of reperfusion. The levels of the parenchymal glucose, pyruvate and lactate were lower in the IP-treated patients having major liver resections, indicating that IP modulates glucose metabolism in livers where the parenchymal mass is significantly reduced.

The harmful effects of IRI should be avoided [4, 21], but still the PM was shown to be used by 93% of the surgeons in a Japanese survey 2002 [22]. With the introduction of new technical devices of liver transection the amount of bleeding has been reduced and it is our impression that the need of vascular

occlusion has diminished. Despite its reduced use, PM remains a powerful adjunct utilized in difficult resections and, indeed, IRI remains a problem for those patients with marginal postoperative liver function.

Ischemic preconditioning enhance the glucose metabolism

The lactate and pyruvate levels seen in this study is coherent with findings in porcine liver IRI studies [17, 23, 24] and with findings in human venous blood analyses [25]. Anaerobic glycogenolysis explains the high glucose levels seen during ischemia [16]. The end product of the glycolysis, pyruvate, is transported into the mitochondria and there irreversibly decarboxylated by pyruvate dehydrogenase [26]. Acetyl CoA is formed in the reaction, but cannot be further metabolized in the citric acid cycle when there is lack of oxygen. Accumulation of acetyl CoA inhibits pyruvate dehydrogenase competitively and pyruvate therefore accumulates in the cytosol and is metabolised to lactate for energy. In the mitochondrion, Acetyl CoA is metabolised to other, less effective, energy substrates such as ketone bodies, acetoacetate and β -hydroxybutyrate [27]. The lack of energy reduces the activity in the Cori cycle and the accumulation of lactate persists.

Ischemia causes ATP breakdown to ADP, AMP and finally adenosine. Several experimental studies indicate that it is the two last metabolites that mediate the protective effect of IP [28-31]. AMP activates adenosine monophosphate kinase (AMPK). It is possible that AMPK activation conserves energy by preservation of ATP, resulting in less lactate accumulation [32]. Adenosine activates its G-protein coupled receptor. This eventually increases the intracellular cAMP concentration, which improves the glucose uptake and activates mitochondrial ATP-dependent K^+ -channels, thereby improving mitochondrial respiration and reducing ATP depletion [33]. Coherent with this hypothesis, IP has been shown to reduce the ATP degradation in both in the ischemic rat liver[31] as well as in humans [9]. In

the rat, the accumulation of lactate was also reduced at the same time [31]. It is also known that preservation of ATP and glycogen improves graft survival after liver transplantation [34, 35].

The blood glucose and baseline glycogen levels were equal in both groups, but the postischemic μ D-glucose, μ D-pyruvate and μ D-lactate levels were lower in the IP group. This indicates that more pyruvate enters the citric acid cycle after IP, possibly explained by a higher number of viable hepatocytes and/or hepatocyte mitochondria. There were, however, no differences seen between the groups in ALT or AST levels, indicating that the hepatocyte injury from ischemia was similar in both groups.

So, protection from IRI by IP is multifactorial, but whatever the mechanisms, energy preservation is critical for cell survival. Although not known at this point, the reduced μ D-lactate levels during ischemia may indicate a better mitochondrial function after IP treatment. If this is true, microdialysis provides a tool to observe the energy status of the liver in the clinical setting. The clinical impact of IP at this stage is doubtful, but interventional investigations aiming at improving liver function can thus use microdialysis to follow metabolism postoperatively.

Resection size

The differences seen in the μ D analyses occurred in patients having major resections indicating that this subgroup metabolically benefits from IP treatment. It is logic that patients with a smaller remnant liver are more susceptible of IRI and thus may have a worse postoperative liver function. Future studies using μ D to study postoperative liver function should therefore primarily be undertaken in patients having major resections. The patients having resections larger than three segments (n=16) had as expected higher levels of AST, ALT, lactate, bilirubin and PT-INR in the blood than those that had minor resections.

Technical aspects

The placement of the μ D-catheters was estimated to prolong the duration of the operation about 10 minutes and the steady state and sampling of the base-line microdialysis sample prolonged the operation an additional 30 minutes. Patients randomized to have IP had another 20 minutes of open abdomen, i.e. the study protocol prolonged the duration of surgery with 40-60 minutes.

The baseline data should be interpreted with caution as a true steady state is not achieved after the 10 minutes used in this study. Trying to reach a true steady state would have increased the duration of surgery with at least another 20 minutes and was abandoned for ethical reasons.

No catheter dislocations occurred and all catheters could be removed with a slight jerk and pull on POD 4 with no more than a slight discomfort for the patient. Two catheters stopped functioning on POD 3, possibly due to clotting of the semi-permeable membrane.

Microdialysis

μ D offers a unique ability to observe parenchymal metabolism over time. The μ D results should, however, not be viewed as absolute figures as the recovery of metabolites depends on several factors. Local blood flow, changes in catheter perfusion velocity, the level of the micropump in relation to the catheter tip (e.g. supine or standing patient) and the integrity of the semi-permeable membrane due to blood clotting or local inflammatory reactions may influence the final results. Rather, the results are to be viewed as relative changes over time [36-38].

During the operation and in the ICU the micropump flow velocity was higher to ensure adequate collected volumes of the dialysate. When the patients were moved to the ward, the perfusion velocity was reduced, possibly enhancing recovery of metabolites from POD1 and onwards. This reduction was

made at about the same time for all patients and the possible changes in recovery therefore have no practical implication when groups are compared.

The findings in our μ D analyses have doubtful clinical implication at this point, but the study shows that postoperative sampling is feasible and the method can be used with ease, both for patients and investigators. Academically, however, μ D have promising venues of postoperative metabolic surveillances in the clinical setting. For instances, pharmacologic interventions can be studied almost in real-time at the parenchymal level in postoperative patients. Studying the target organ without the systemic dilution seen in venous blood samples helps establishing optimal therapeutic concentrations of drugs.

Individual patient

One patient had a stroke diagnosed on the morning of POD 3. The μ D-glycerol curve was dramatically elevated already during the previous night (**figure 6**) and it is possible that the stroke had occurred several hours earlier. This raises the question if μ D monitoring may be used to detect complications early.

Conclusion:

The use of microdialysis in studying postoperative liver metabolism is feasible and carries little, if any, risk for the patients. This study was not powered for studying morbidity (or mortality) after IP and clinically we cannot show any benefits of IP in the setting of intermittent PM. The results herein, however, indicate that IP-treated patients having major resections improve their aerobic metabolism with less accumulation of lactate in the liver. Theoretically this may be beneficial for patients with marginalized postoperative liver function after major liver resections.

Figure 6: The microdialysis glycerol levels in a patient that was diagnosed with a stroke on post operative day 3 (POD 3).

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