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ORIGINAL ARTICLE

Effect of Ischemic Preconditioning in Whole Liver Transplantation From Deceased Donors. A Pilot Study

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The effect of ischemic preconditioning (IPC) in orthotopic liver transplantation (OLT) has not yet been clarified. We performed a pilot study to evaluate the effects of IPC in OLT by comparing the outcomes of recipients of grafts from deceased donors randomly assigned to receive (IPC+ group, n = 23) or not (IPC− group, n = 24) IPC (10-min ischemia + 15-min reperfusion). In 10 cases in the IPC+ group and in 12 in the IPC− group, the expression of inducible nitric oxide synthase (iNOS), neutrophil infiltration, and hepatocellular apoptosis were tested by immunohistochemistry in prereperfusion and postreperfusion biopsies. Median aspartate aminotransferase (AST) levels were lower in the IPC+ group vs. the IPC− group on postoperative days 1 and 2 (398 vs. 1,234 U/L, $P = 0.002$; and 283 vs. 685 U/L, $P = 0.009$). Alanine aminotransferases were lower in the IPC+ vs. the IPC− group on postoperative days 1, 2, and 3 (333 vs. 934 U/L, $P = 0.016$; 492 vs. 1,040 U/L, $P = 0.008$; and 386 vs. 735 U/L, $P = 0.022$). Bilirubin levels and prothrombin activity throughout the first 3 postoperative weeks, incidence of graft nonfunction and graft and patient survival rates were similar between groups. Prereperfusion and postreperfusion immunohistochemical parameters did not differ between groups. iNOS was higher postreperfusion vs. prereperfusion in the IPC− group ($P = 0.008$). Neutrophil infiltration was higher postreperfusion vs. prereperfusion in both groups (IPC+, $P = 0.007$; IPC−, $P = 0.003$). Prereperfusion and postreperfusion apoptosis was minimal in both groups. In conclusion, IPC reduced ischemia/reperfusion injury through a decrease of hepatocellular necrosis, but it showed no clinical benefits. ***Liver Transpl 12:628-635, 2006.*** © **2006 AASLD.**

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The ischemia/reperfusion (I/R) injury occurring after cold ischemia in the setting of liver transplantation is a substantially unavoidable phenomenon, whose exact mechanisms have not been fully clarified.[1,2] Primary graft nonfunction (PGNF) and initial poor function (IPF) nowadays occur in about 2-4% and 5-15% of cases, respectively, and are probably linked to I/R injury, being more frequently observed with the use of marginal donors and with prolonged cold ischemia.[3-9] Typical features of I/R injury are a marked inflammatory response and cell death principally through apoptosis of sinusoidal endothelial cells following reperfusion.[1,2,10]

Protection from I/R can be achieved by preexposure to one or more brief periods of ischemia followed by reperfusion.[11-15] This phenomenon, called ischemic preconditioning (IPC), was first described in the heart by Murry and colleagues[11] and then observed in other organs, including the liver.[12-14] In particular, 5-10 min-

Abbreviations: I/R, ischemia/reperfusion; PGNF, primary graft nonfunction; IPF, initial poor function; IPC, ischemic preconditioning; OLT, orthotopic whole liver transplantation; IHC, immunohistochemical; AST, aspartate aminotransferase; MPO, myeloperoxidase; iNOS, inducible nitric oxide synthase.

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utes of ischemia followed by 10-15 minutes of reperfusion before either warm or cold ischemia significantly improves survival and liver injury in rat and mouse experimental models.[12-14] Studies on the effect of IPC in large-size animals (e.g., pigs) are less numerous and with more contrasting results.[15-17] The clinical efficacy of IPC has been assessed in patients undergoing major hepatectomy.[18] The mechanisms involved in the protective effect of IPC are only partially known, but they seem to improve microcirculation, to attenuate the inflammatory injury, and to prevent energy depletion.[12,14,19-22]

To date, there are few data available on the effect of IPC in orthotopic whole liver transplantation (OLT) in humans.[5,23-25] We undertook a pilot study with an analysis of postoperative biochemical, clinical, and immunohistochemical (IHC) parameters in recipients of grafts subjected or not to IPC.

METHODS

Study Design

The effect of IPC applied during liver graft procurement from deceased donors was investigated in recipients of OLT from May 2002 to October 2004 at the Liver and Multiorgan Transplant Unit, Department of Surgery and Transplantation, University of Bologna, Italy.

Male and female subjects aged >18 years and undergoing primary, isolated, nonurgent OLT were eligible to participate in the study. Patients requiring retransplantation, patients with fulminant hepatic failure, and recipients of split liver transplantation and of combined or domino transplantation were excluded from this study.

When two of the authors of the present study (M.C. and G.E.) were the surgeons in charge of organ procurement, a 1:1 randomization was performed through blind extraction of computer-generated sealed envelopes. Under the condition of hemodynamic stability of donors during intensive care unit stay (absence of cardiac arrests and/or of hypotension episodes with systolic arterial blood pressure <70 mm Hg for >1 hour), and excluding cases of multivisceral, intestinal, or pancreas donation, liver graft procurement was preceded (group 1, IPC+) or not (group 2, IPC−) by hepatic pedicle clamping.

In the IPC− group, after abdominal inspection, confirmation of viability of the graft and accomplishment of surgical maneuvers preceding the hypothermic perfusion of the donor, 500 IU/kg of body weight of heparin were administered intravenously. The infrarenal abdominal aorta was cannulated and hypothermic perfusion was carried out with 5 L of Celsior solution (Sangstat Europe, Lyon, France) through the aorta and 1 L liter through the portal vein. The liver was then harvested according to the conventional technique.[26]

In the IPC+ group, a 10-minute Pringle maneuver was performed by means of a vascular clamp, followed by 15 minutes of warm reperfusion before starting cold ischemia. After initiation of cold perfusion, the procedure was the same as in the IPC− group.

A drop in systolic arterial blood pressure below 75 mm Hg was considered the only criteria for discontinuing IPC, while severe hypotension (systolic arterial blood pressure <70 mm Hg for some minutes) or donor cardiac arrest were the criteria for excluding the case from recruitment. This pilot study was conducted under approval by the local Ethics Committee.

Baseline Characteristics–Evaluation of Postoperative Biochemical and Clinical parameters

Recipient and donor demographic, clinical and biochemical profiles, and operative parameters were analyzed and compared between the IPC+ and IPC− groups. The primary end point of the study was the comparison between the IPC+ and IPC− groups of the serum levels of aspartate aminotransferase (AST), alanine aminotransferase, and total bilirubin and prothrombin activity measured 24 hours after graft reperfusion and on postoperative days 2 to 7, 14, and 21.

Secondarily, we analyzed the incidence of PGNF and of IPF, the need for retransplantation, and patient and graft survivals in the 2 study groups. PGNF was defined as a condition of liver failure that occurred in the absence of technical or immunological problems, which led to retransplantation or death within the first week after surgery.[4] Minimal prothrombin activity value <30% with respect to normal levels and/or maximum total bilirubin value >15 mg/dL in the absence of hemolysis or biliary obstruction were defined as IPF.

Evaluation of Liver Biopsies

We planned to obtain paired ≥10 mm deep wedge or Tru-cut biopsies from the left hepatic lobe at the opening of the peritoneal cavity in the donor, and 2 to 5 hours after portal revascularization in the recipient from both groups. Liver specimens from the first biopsy were divided into two fragments. The first, providing at least 20 mm^2 of tissue section, was snap-frozen in n-methylbutane precooled in liquid nitrogen and stored at −80°C for subsequent IHC staining. The second sample was formalin fixed and paraffin embedded for hematoxylin & eosin staining. Biopsies after graft reperfusion were entirely snap-frozen and stored at −80°C for subsequent IHC staining.

Hematoxylin & eosin evaluation was performed by an experienced pathologist (A.D.) without knowledge of the study group assignment. The main focus was on the degree of steatosis, which was semiquantitatively graded as a percentage of involved hepatocytes by using a 40× magnification light microscope. As the presence of microvesicular steatosis is not a fundamental determinant for graft viability,[27] only the percentage of macrovesicular steatosis was considered.

On frozen sections, by means of IHC, we studied (1) neutrophil infiltration (expressed as the number of myeloperoxidase [MPO]-positive cells/mm^2), (2) CD8 lymphocyte infiltration (expressed as the number of CD8-positive cells/mm^2), (3) expression of the inducible form

TABLE 1. Details on Monoclonal Antibodies Used for Immunohistochemistry

Antibody Specificity	Clone/Code	Dilution	Source
iNOS	6	1:400	Biosciences Pharmingen, San Diego, CA
MPO	M748	1:100	Dako AS, Glostrup, Denmark
CD8	UCHT	1:40	Dako AS, Glostrup, Denmark
Apoptosis (CytoDEATH)	M30	1:80	Roche Applied Science, Indianapolis, IN

of nitric oxide synthase (iNOS), and (4) amount of hepatocellular apoptosis (expressed as the number of M30-positive hepatocytes/mm2). Details on monoclonal antibodies used for IHC are reported in Table 1.

Twenty serial sections (5-μm thick) were obtained from each frozen liver specimen; they were air dried overnight, fixed for 5 minutes with acetone, wrapped in aluminum foil, and stored at −20°C until used. Before staining, sections were fixed for 5 minutes in acetone and for 10 minutes in chloroform at room temperature. Sections were incubated overnight at 4°C when stained for iNOS and for 30 minutes at room temperature when stained with the other reagents, then washed in phosphate-buffered saline for 15 minutes. The reactions were detected by an immunoperoxidase technique, as previously described.[28,29]

Each section was evaluated blindly by 2 observers (A.G. and G.B.). The number of positive reactions was evaluated by using an ocular grid, which allowed estimation of section surface area, and expressed as number per square area. Neutrophils and CD8 lymphocytes were quantified by counting 10 fields using an original magnification ×40 objective. Because of the low number of positive reactions, cells with cytoplasmic positivity for M30 antibody were counted on the whole section. Expression of iNOS was scored according to a semiquantitative scale from focal faint positivity (score, 0.5) to diffuse staining with different intensity from low (score, 1) to very strong (score, 4).

The median lobular area evaluated in each biopsy was 35 mm^2 (range, 20-50 mm^2). Data were expressed as median number per square millimeter.

Statistical Analysis

Results were expressed as median and range. Differences between continuous variables were evaluated with the Mann-Whitney *U* test for intergroup comparisons and with the Wilcoxon test for related samples for intragroup comparisons. Differences between categorical variables were calculated with the chi-square test or Fisher exact test. Actuarial survivals were computed with the Kaplan-Meier method and the differences between groups were compared by the log-rank test. A P value <0.05 was considered statistically significant. Statistical analysis was carried out with the SPSS software packaging (SPSS, Inc., Chicago, IL).

RESULTS

Surgical Procedures and Tolerance to IPC

Twenty-six donors were randomized to receive IPC, while conventional organ procurement was performed in 27 donors. Only dopamine and/or norepinephrine were used as vasopressors in the entire series. In 3 cases in the IPC+ group (11.5%), hypotension occurred before initiation of IPC procedures; the procedure was therefore abandoned and the cases excluded from analysis. In the IPC− group, 2 cases (7.4%) were excluded from the study because of severe hypotension occurring during organ harvesting, while in 1 case (3.7%) the liver graft recipient experienced caval stenosis with immediate worsening of graft function, determining exclusion from the study.

IPC was well tolerated in all cases where it was applied, with no significant deterioration of hemodynamic parameters of the donor (data not reported). IPC did not jeopardize the viability of other abdominal or thoracic organs.

Forty-one (87%) OLTs were performed with preservation of the retrohepatic inferior vena cava and 6 (4 in the IPC+ group and 2 in the IPC− group; 13%) with the conventional technique. All grafts underwent sequential portal and arterial reperfusion. No induction immunosuppression was used.

Cases Excluded from Randomization

A total of 197 OLTs were performed during the study period. Seventy procurements and subsequent OLTs (35.5%) were performed by other harvesting surgeons within the above reported criteria of eligibility for this study. In 35 cases (17.8%), donor cardiac arrest or severe hypotension had been documented before procurement. Other causes of exclusion were retransplantations (18 cases, 9.1%), combined transplants (10 cases, 5.1%), fulminant hepatic failure (5 cases, 2.5%), split-liver transplantation (3 cases, 1.5%), transplants with grafts procured by other centers (2 cases, 1%), and domino OLT (1 case, 0.5%).

Baseline Characteristics

Recipient profiles, donor characteristics and operative parameters of the IPC+ and IPC− groups are reported in Table 2. The 2 groups were comparable as regards all the parameters examined. No graft had steatosis >30%.

Due to technical difficulties in performing immediate

TABLE 2. Profile of IPC+ and IPC− Groups

	IPC+ (n = 23)	IPC− (n = 24)	*P*
Patient Profiles			
Sex (M/F)	20/3	18/6	0.4
Age (years)	54 (32-66)	53.5 (27-66)	0.8
Indication for OLT			
Postnecrotic cirrhosis	8 (34.8%)	11 (45.8%)	0.3
HCC on cirrhosis	10 (43.5%)	4 (16.7%)	
Cholestatic cirrhosis	2 (8.7%)	1 (4.2%)	
Alcoholic cirrhosis	1 (4.3%)	3 (12.5%)	
Other	2 (8.7%)	5 (20.8%)	
MELD score	19 (10-28)	19 (8-43)	0.9
Donor Characteristics			
Sex (M/F)	9/14	16/8	0.06
Age (years)	68 (20-80)	63 (34-82)	0.2
Cause of death			
Cerebral hemorrhage	19 (82.6%)	19 (79.1%)	0.6
Cranial trauma	2 (8.7%)	4 (16.7%)	
Other	2 (8.7%)	1 (4.2%)	
Dopamine (μg/kg/min)	4.0 (0-11)	4.0 (0-20)	0.7
Use of norepinephrine (y/n)	9/14	7/17	0.4
ICU stay (days)	3.0 (1-12)	4.0 (1-31)	0.1
AST (U/L)	37 (9-294)	29 (15-80)	0.4
ALT (U/L)	23 (7-566)	22.5 (13-165)	0.7
Total bilirubin (mg/dL)	0.6 (0-1.8)	0.6 (0-2.9)	0.6
γ-GT (U/L)	22 (7-172)	34 (4-193)	0.7
Na^+ (mEq/L)	148 (134-170)	149.5 (134-164)	0.5
Macrovesicular steatosis >10%	3 (13%)	2 (8.3%)	1.0
Operative Parameters			
Operation time (min)	440 (225-725)	465 (280-1015)	0.7
Total ischemia time (min)	388 (259-830)	383 (279-695)	0.7
PRBC transfusion (mL)	3,600 (0-15,313)	3,180 (600-16,233)	0.9

Abbreviations: M, male; F, female; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; ICU, intensive care unit; ALT, alanine aminotransferase; γ-GT, gamma-glutamyl transpeptidase; Na^+; serum sodium level; PRBC, packed red blood cells.

cold preservation for both prereperfusion and postreperfusion tissue samples, paired liver biopsies were obtained from 10 grafts in the IPC+ group (43.5%) and from 12 grafts in the IPC− group (50%). The same clinical and biochemical variables evaluated in the whole study population were analyzed in cases with available prereperfusion and postreperfusion biopsies, revealing no significant differences between groups (Table 3).

Postoperative Biochemical Parameters

AST levels 24 hours after graft reperfusion and on postoperative day 2 were significantly lower in the IPC+ group than in the IPC− group (Fig. 1). Alanine aminotransferase levels 24 hours after graft reperfusion and on postoperative days 2, 3, and 7 were significantly lower in the IPC+ group than in the IPC− group (Fig. 2). No other significant differences were found in serum levels of AST, alanine aminotransferase, total bilirubin and prothrombin activity between groups throughout the first 21 postoperative days.

Considering only cases with available biopsies, the median AST level 24 hours after graft reperfusion was the only biochemical parameter significantly lower in the IPC+ group vs. the IPC− group throughout the first 21 postoperative days: 481 U/L (160-849) vs. 1,130 U/L (173-2,390) (P = 0.03).

Clinical Outcomes

No patient in the IPC+ group and only 1 (4.2%) in the IPC− group experienced PGNF (*P* = 1.0). Two patients (8.7%) in the IPC+ group and 2 (8.3%) in the IPC− group had IPF (*P* = 1.0). Two patients (8.7%) in the IPC+ group and 2 (8.3%) in the IPC− group required retransplantation (*P* = 1.0). Causes of retransplantation were IPF (2 cases) in the IPC+ group, and PGNF (1 case) and IPF (1 case) in the IPC− group. The median follow-up of the entire population was 10 months (range, 1-30). No patient in the IPC+ group and 2 patients (8.3%) in the IPC− group died (*P* = 0.4), in both cases due to infections.

By limiting the above analysis to cases with available prereperfusion and postreperfusion biopsies, we had no cases of PGNF, 1 case of IPF resulting in graft loss and retransplantation in the IPC+ group, and 2 deaths in the IPC− group (*P* = not significant for all comparisons).

TABLE 3. Profile of IPC+ and IPC− With Available Prereperfusion and Postreperfusion Biopsies

	IPC+ (n = 10)	IPC− (n = 12)	*P*
Patient Profiles			
Sex (M/F)	9/1	8/4	0.3
Age (years)	51 (41-66)	54 (27-65)	0.6
Indication for OLT			
Postnecrotic cirrhosis	4 (40%)	5 (41.3%)	0.4
HCC on cirrhosis	5 (50%)	2 (16.7%)	
Cholestatic diseases		1 (8.3%)	
Alcoholic cirrhosis	1 (10%)	2 (16.7%)	
Other		2 (16.7%)	
MELD score	20 (10-28)	17 (8-43)	0.4
Donor Characteristics			
Sex (M/F)	6/4	7/5	1.0
Age (years)	68 (27-78)	65 (34-82)	0.3
Cause of death			
Cerebral hemorrhage	9 (90%)	10 (83.3%)	0.6
Cranial trauma	1 (10%)	1 (8.3%)	
Other		1 (8.3%)	
Dopamine (μg/kg/min)	1.5 (0-10)	4 (0-12)	0.2
Use of norepinephrine (y/n)	3/7	2/10	0.6
ICU stay (days)	2 (1-4)	3.5 (1-13)	0.1
AST (U/L)	30 (9-189)	23.5 (15-80)	0.3
ALT (U/L)	18 (13-326)	21 (14-69)	0.4
Total bilirubin (mg/dL)	0.7 (0.3-1.8)	0.7 (0.2-2.9)	0.6
γ-GT (U/L)	21.5 (13-53)	34 (11-135)	0.3
Na^+ (mEq/L)	147 (134-158)	148 (134-157)	0.8
Macrovescicular steatosis >10%	2 (20%)	1 (8.3%)	0.5
Operative Parameters			
Operation time (min)	425 (225-665)	477 (315-1,015)	0.8
Total ischemia time (min)	393 (259-830)	380 (310-569)	0.8
PRBC transfusion (mL)	4650 (1,800-11,500)	3180 (600-13,770)	0.2

Abbreviations: M, male; F, female; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; ICU, intensive care unit; ALT, alanine aminotransferase; γ-GT, gamma-glutamyl transpeptidase; Na^+; serum sodium level; PRBC, packed red blood cells.

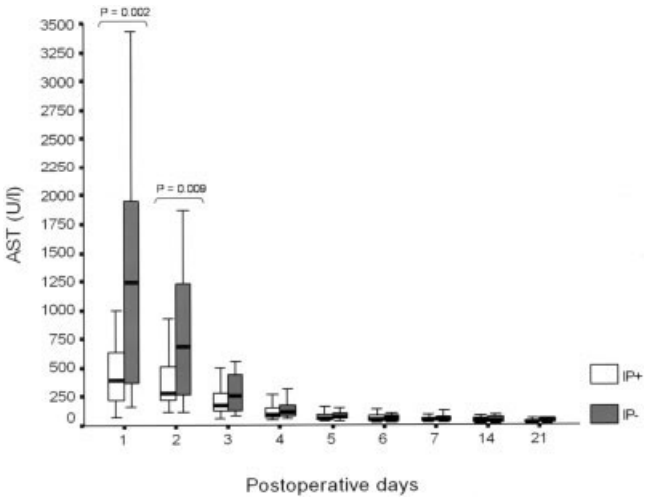

Figure 1. Postoperative course of AST in the IPC+ (white bars) and IPC− (gray bars) groups. Intra-bar lines represent median values. Bar edges represent 25% and 75% confidence intervals. Error bars represent 5% and 95% confidence intervals.

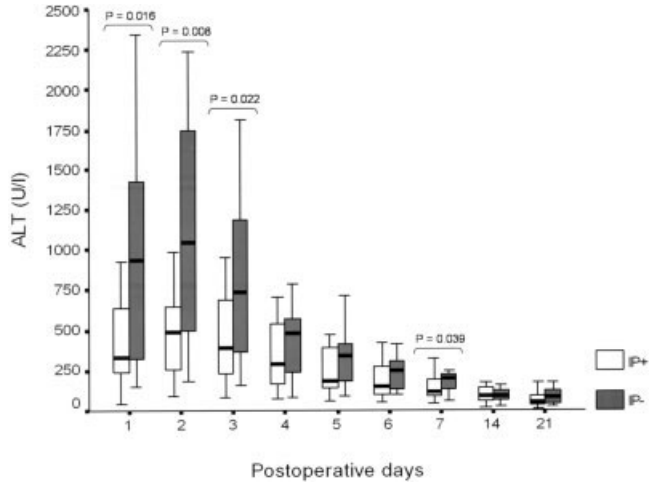

Figure 2. Postoperative course of alanine aminotransferase in the IPC+ (white bars) and IPC− (gray bars) groups. Intra-bar lines represent median values. Bar edges represent 25% and 75% confidence intervals. Error bars represent 5% and 95% confidence intervals. ALT, alanine aminotransferase.

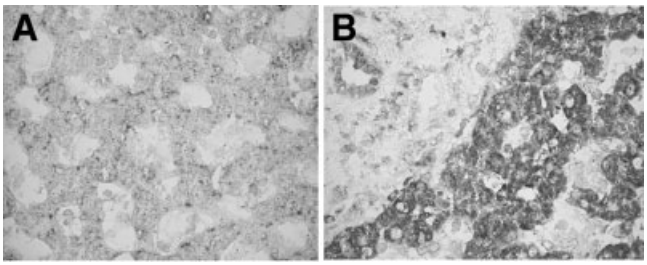

Figure 3. Prereperfusion and postreperfusion staining for iNOS in an IPC− case. Hepatocellular expression of iNOS turns from low expression (score 1) in (A) prereperfusion biopsy to a very high expression (score, 4) in (B) postreperfusion biopsy, with absence of staining in the portal area, but positivity in the bile duct.

Overall 1-year patient and graft survival rates were 100% and 91% in the IPC+ group, and 92% and 82% in the IPC− group ($P = 0.7$).

Evaluation of IHC Hepatic Tissue Staining

Postreperfusion biopsies were performed 204 minutes (range, 150-283) after portal revascularization in the IPC+ group and 198 minutes (range, 131-299) after portal revascularization in the IPC− group ($P = 0.8$).

iNOS was usually homogeneously expressed in the whole section, mainly in the hepatocytes, but also in biliary cells and, occasionally, in the sinusoidal area. The reaction pattern was granular-like with a wide range of intensity (Fig. 3). Neutrophils were mainly detected widespread in the lobular areas and, less frequently, in the portal areas, while an opposite distribution was observed for CD8. Apoptosis was detected with a pattern of cytoplasmic expression in the hepatocytes.

A summary of IHC data is reported in Table 4. No significant differences emerged between the IPC+ and IPC− groups in prereperfusion, postreperfusion and differential expression (from prereperfusion to postreperfusion) of MPO, CD8, iNOS and M30.

In the IPC+ group, postreperfusion expression of iNOS and M30 did not significantly change compared to prereperfusion values ($P = 0.1$ and $P = 0.3$, respectively). Conversely, MPO expression significantly increased ($P = 0.007$) and CD8 expression significantly decreased ($P = 0.005$) with respect to baseline levels.

In the IPC− group, the expression of M30 did not significantly vary from prereperfusion to postreperfusion biopsies ($P = 0.1$), whereas a significant increase was observed in iNOS and in MPO expression, and a significant decrease of CD8 ($P = 0.008$, $P = 0.003$, and $P = 0.02$, respectively).

DISCUSSION

The interest in IPC as a simple strategy for improving hepatic functional recovery encouraged our group to design a pilot study in human liver transplantation. Only 2 clinical reports on the impact of IPC in preventing I/R injury in deceased donor liver transplantation have been published so far.[5,25]

In our study, IPC was accomplished on the basis of evidence of its efficacy in animal studies with 10 minutes of ischemia + 10-15 minutes of reperfusion,[12-14] this procedure being well tolerated by all our donors. Our study design was substantially different from that of Koneru et al., in which a Pringle maneuver of 5 minutes only was performed, with nonstandardized warm reperfusion periods before initiation of cold ischemia.[25] Our approach was similar to the one reported by Azoulay et al.,[5] who used an interval of 10 minutes between IPC and initiation of cold ischemia.

The IPC+ and IPC− groups were comparable for main demographic, clinical, pathological, and operative parameters. No major differences in clinical outcomes emerged between treated and control subjects. IPC was not harmful for the functional recovery of grafts, whereas a beneficial effect was observed for a higher tolerance to cold ischemia, as expressed by the lower level of aminotransferases in the first postoperative days in the IPC+ group. This result was similar to that observed by other authors.[5,24] Conversely, the postoperative course of bilirubin and prothrombin activity did not differ between the 2 groups. These latter findings contrast with those reported by Azoulay et al., who described a more pronounced cholestasis and a higher incidence of IPF in recipients of grafts receiving IPC.[5]

We investigated tissue changes in the early postreperfusion phase by immunohistochemistry in a proportion of cases from the IPC+ and IPC− groups. Postreperfusion biopsies were obtained between 2 and 5 hours following portal revascularization. Unlike a previous preliminary report,[24] where biopsies were routinely obtained 2 hours after reperfusion, we could not perform them at fixed intervals because satisfactory hemostasis was not definitively achieved within that time span in some cases. This might constitute a bias for our analysis. However, there was no difference between the 2 study groups in the median interval between graft reperfusion and the second biopsy. Immunohistochemistry revealed that the baseline and postreperfusion conditions of grafts in our 2 study groups were comparable for the expression of MPO, CD8, iNOS, and M30.

In the early postreperfusion phase, a significant increase of neutrophil infiltration and a decrease of CD8 accumulation were observed in both IPC+ and IPC− groups. While confirming the role of neutrophils in I/R injury, these data are in contrast with most observations on reduced inflammatory changes by applying IPC in experimental and clinical liver transplantation.[21,24]

Cell death has been shown to occur mainly through apoptosis in the early postreperfusion phases, and especially involving sinusoidal endothelial elements.[10] However, a recent study on a model of cold ischemia–warm reperfusion in rats showed that the early postreperfusion period is characterized by endothelial cell necrosis, followed by delayed hepatocyte apoptosis, neither of the 2 processes being of major importance for graft viability, which is inversely correlated with the late hepatocyte necrosis.[30]

We investigated the impact of apoptotic phenomena

TABLE 4. Immunohistochemical Parameters in IPC+ and IPC− Groups

	Prerereperfusion			Postreperfusion		
IHC parameters	IPC+ (n = 10)	IPC− (n = 12)	*P*	IPC+ (n = 10)	IPC− (n = 12)	*P*
iNOS (score)	1 (0.5-3.5)	1 (0.5-2)	0.2	1.25 (0.5-4)	2 (0.5-5)	0.6
Neutrophils/mm^2	38 (11-79)	42 (24-162)	0.5	123 (35-228)	166 (55-331)	0.1
CD8/mm^2	27 (7-50)	27 (14-112)	0.7	13 (4-31)	17 (4-77)	0.3
Apoptosis/mm^2	0.4 (0-1.2)	0.2 (0-1.1)	0.4	0.4 (0-10.4)	0.4 (0-1.3)	0.5

NOTE: Results are expressed as median values and ranges.

using M30 monoclonal antibody, a specific marker for epithelial cells, whose target is a neoepitope appearing in early stages of apoptosis and not present in cells dying due to necrosis.[31,32] We previously validated this method for the evaluation of epithelial apoptosis in the liver.[29] In the present study, hepatocellular apoptosis was scarcely represented in both groups. This suggests that, at least in the initial phase, this phenomenon is not relevant.

Taken together, the similarity of neutrophil infiltration between treated and nontreated grafts and the apparent negligible importance of apoptosis in the early postreperfusion period cannot provide a pathological basis for the differences in the postoperative course of aminotransferases.

The role of nitric oxide in I/R injury is still controversial, but the positive effect of IPC has been recently linked to nitric oxide production.[12,19,20] The constitutive or endothelial form of nitric oxide synthase seems pivotal in maintaining an immediate source of nitric oxide.[33-35]

The action played by iNOS is less clear. Several recent studies on hepatic ischemia showed that the morphologic and biochemical expression of I/R injury is reduced by using an iNOS inhibitor and by inducing ischemia in iNOS knockout animals, suggesting that iNOS may trigger postreperfusion damage after ischemia.[33,35,36]

In our study the postreperfusion expression of iNOS significantly increased compared to baseline values in the IPC− group, but not in the IPC+ group. However, since the postreperfusion expression of iNOS was comparable between groups, we could not definitely ascribe the lower aminotransferase levels in the IPC+ group in the early postoperative period to a limitation of iNOS expression in preconditioned grafts.

In conclusion, this study showed that IPC with 10 minutes of ischemia + 15 minutes of reperfusion applied to deceased donors was not detrimental for graft viability. IPC had a positive impact on postoperative levels of aminotransferases, but did not modify other clinical parameters. The similar prereperfusion and postreperfusion expression of iNOS, neutrophil infiltration, and hepatocellular apoptosis between the IPC+ and IPC− groups could not provide a pathological basis for the different course of aminotransferases.

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