Effects of 10 Minutes of Ischemic Preconditioning of the Cadaveric Liver on the Graft's Preservation and Function

The Ying and the Yang

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Summary Background Data: Although extensively studied in animal models, ischemic preconditioning has not yet been studied in clinical transplantation.

Objective: To compare the results of cadaveric liver transplantation with and without ischemic liver preconditioning in the donor.

Patients and Methods: Alternate patients were transplanted with liver grafts that had (n = 46, $Group_{Precond}$) or had not (n = 45, $Group_{Control}$) been subjected to ischemic preconditioning. Liver ischemia-reperfusion injury, liver and kidney function, morbidity, and in-hospital mortality rates were compared in the 2 groups. Initial poor function was defined as a minimal prothrombin time within 10 days of transplantation <30% of normal and/or bilirubin >200 μmol/L.

Results: The postoperative peaks of ASAT (IU/L) and ALAT (IU/L) were significantly lower in $Group_{Precond}$ (556 ± 968 and 461 ± 495, respectively) than in the $Group_{Control}$ (1073 ± 1112 and 997 ± 1071, respectively). The rate of technical morbidity and the incidence of acute rejection were similar in both groups. Initial poor function was significantly more frequent in the $Group_{Precond}$ (10 of 46 cases) than in the $Group_{Control}$ (3 of 45 cases). Hospital mortality rates were similar in the 2 groups. In multivariate analysis, body mass index of the donor, graft steatosis, and ischemic preconditioning were significantly predictive of the posttransplant peak of ASAT. In univariate analysis, only preconditioning was significantly associated with initial poor function.

Conclusions: Compared with standard orthotopic liver transplant, ischemic preconditioning of the liver graft in the donor is associated with better tolerance to ischemia. However, this is at the price of decreased early function. Until further studies are available, the clinical value of preconditioning liver grafts remains uncertain.

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Ischemia-reperfusion injury is the main cause of liver graft failure.[1–3] Several strategies[4] have been designed to limit this injury and its consequences. These include discarding grafts with severe steatosis,[5,6] optimizing the preservation solution,[7] minimizing the ischemia time,[8] and matching the quality of the graft to the status of the recipient.[4] Several recent animal studies have shown that ischemic preconditioning, during which brief exposure to warm ischemia provides robust protection against injury during long periods of ischemia, increases tolerance to reperfusion injury. This phenomenon was first described for the heart[9] and mainly for normothermic ischemia (or warm ischemia) injury in many experimental models.[10,11] It has also been described for several tissues and organs including the liver.[2,12–17]

One preliminary study[18] and 2 randomized studies[19,20] in humans showed that, when livers were subjected to ischemic preconditioning (by transient portal triad clamping) before partial hepatectomy under continuous portal triad clamping, patients suffered from less postoperative liver injury as indicated by lower transaminase levels and endothelial cell injury. These 3 studies failed to demonstrate any advantage of preconditioning over the respective control groups in terms of postoperative liver function (similar prothrombine time and bilirubin levels), morbidity, or mortality rate. In addition, the protective effect of preconditioning on ischemia-reperfusion injury was lost for the patients that *a priori* need it most, namely, those >60 years and those with liver steatosis.[19] Several experimental studies have reported that ischemic preconditioning has a beneficial effect on cold ischemia-reperfusion injury for different organs, including heart, intes-

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tine, lung, and kidney.[21,22] The study by Arai et al in a rat model of liver transplantation[23] showed that ischemic preconditioning increases survival. This is consistent with other studies[24–27] with the exception of one.[28] Few studies have tested this concept in humans, and those that have been done gave discordant data. Totsuka et al[29] showed that livers from human donors who sustained cardiopulmonary arrest and were resuscitated had similar survival and function to those from other donors. In addition, the serum concentration of transaminases after transplantation appears to be lower in patients who receive organs from donors with prior cardiopulmonary arrest compared with those without. Conversely, Wilson et al[30] showed that reversible cardiac arrest prior to graft harvesting did not trigger any preconditioning benefit in liver transplantation. Our aim was to evaluate the effects of ischemic preconditioning of the liver graft in the cadaveric donor on ischemia-reperfusion injury in the recipient. Alternate patients were transplanted with grafts that had or had not been preconditioned in the donor. Preservation injury, early graft function, mortality, morbidity, and patient survival after transplantation were compared in the 2 groups.

PATIENTS AND METHODS

Study Population and Experimental Design

The study was conducted from January 2000 to January 2003. The study population included 91 consecutive patients who underwent a liver transplant: 1) in an elective situation, 2) with a whole cadaveric liver, 3) from a donor without cardiac arrest or severe hemodynamical instability prior to harvesting. Alternate patients were assigned to each of the study groups. Forty-six patients received a graft that had been preconditioned in the donor (10 minutes of portal triad clamping followed by 10 minutes of reperfusion followed by multiorgan harvesting, $Group_{Precond}$) and 45 received a graft that had not been preconditioned ($Group_{Control}$). This protocol of preconditioning was the same than the one used in the 3 clinical studies reported so far.[18,19] During the study period, 292 transplantations not fulfilling all the abovementioned conditions were not included in the study. The protocol was approved by the investigation and review board of our center and was always accepted by the teams harvesting other organs.

Surgical Procedures

Liver Harvesting

The rapid procurement technique of Starzl et al was used in both groups.[8,31] The graft was perfused with cold University of Wisconsin solution.[8] A wedge biopsy was performed at the beginning of the harvesting procedure to evaluate steatosis (baseline biopsy). This biopsy was available in 37 of 45 (82%) and 41 of 46 (89%) of $Group_{Control}$ and $Group_{Precond}$ donors, respectively, ($P = 0.3$). Grafts were classified as steatotic (versus nonsteatotic) when macrovacuolar steatosis was observed in >20% of hepatocytes.

OLT Technique

OLT was performed as reported elsewhere. In brief, the native liver was totally removed with caval preservation.[32] Temporary portacaval shunt was performed according to the transplant surgeon's preference.[33] The whole liver graft was then implanted. Cold ischemia time was considered as the time elapsed from devascularization in the donor until portal reperfusion in the recipient. A liver biopsy was performed before closure of the abdomen to evaluate ischemia-reperfusion injury (postreperfusion biopsy). This was done in 32 of 45 (71%) and 38 of 46 (83%) of $Group_{Control}$ and $Group_{Precond}$ subjects, respectively ($P = 0.2$). Ischemia-reperfusion injury was classified as moderate to severe (versus absent) when at least 10% of hepatocytes were necrotic, mainly in the center of the lobule or disseminated throughout.[5]

Postoperative Management

Transplanted patients received a standard immunosuppression regimen of tacrolimus and methylprednisolone. Early outcome was assessed by measuring ischemia-reperfusion liver injury, measured by the peak ASAT concentration; liver function tests, including the minimum prothombin time; and the peak bilirubin concentration and kidney function measured by the peak creatinine concentration. All peak levels and minimum values were recorded within 10 days of transplantation, primary nonfunction (immediate absence of graft function leading to retransplantation or death), and initial poor function (minimal prothrombin value <30% of normal level and/or maximum bilirubin concentration >200 μmol/L after ruling out hemolysis and biliary obstruction), and clinical outcome. For the latter, the following data were recorded: technical complications including hemoperitoneum needing surgery, arterial, portal, outflow and biliary complications. Histologically proven acute rejection was recorded, provided it occurred within 6 weeks of transplantation and needed increased immunosuppression. Postoperative mortality was defined as death occurring during the primary hospitalization period following transplantation.

Data Analysis

All quantitative data are expressed as mean ± SD. A P value <0.05 was considered significant. To be clinically relevant, only data available before transplantation and potentially predictive of the maximum value of ASAT in the recipient within 10 days of transplantation were assessed by univariate analysis. The assessed data included: age and liver function tests of donors and recipients, the length of donors stay in ICU prior to harvesting, the application of ischemic preconditioning, the presence of graft steatosis, and body mass index of the donors. All the patients had a complete data

set. If significantly correlated with the end-point, factors were evaluated by regression multivariate analysis. Likewise, only data available before transplantation and potentially predictive of initial poor function were assessed by univariate analysis. As only one factor was found to be significantly correlated with this end-point (see Results), logistical regression was not carried out. Despite the study design, correlation of end-points to specific perioperative data (namely, cold ischemia time, duration of operation, and transfusion requirements) are reported in Results. Survival rates were calculated using the Kaplan-Meier method, and groups were compared with the log-rank test. All statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC).

Transplant surgeons, biologists, intensive care specialists, and pathologists were not informed whether the graft had been subjected to ischemic preconditioning in the donor.

RESULTS

Donors, Recipients, and Intraoperative Data

There were no significant differences between the donors, the recipients, and operative data in the 2 groups (Table 1).

Analysis of Baseline and Postreperfusion Biopsies

The baseline biopsy revealed steatosis in 5 of 37 (13.5%) and 7 of 41 (17%) available cases in the $Group_{Control}$ and

TABLE 1. Characteristics of Donors and Recipients, and Intraoperative Data

Characteristic	Without Ischemic Preconditioning (n = 45 cases)	With Ischemic Preconditioning (n = 46 cases)	P
Donor data			
Male-to-female ratio	18/27	17/29	0.8
Age (yr)	49.7 ± 13.2	46.9 ± 16.9	0.4
No. (%) of deaths due to:			0.6
Central nervous system disease	29 (64%)	34 (74%)	
Central nervous system trauma	13 (29%)	9 (20%)	
Other	3 (7%)	3 (6%)	
Hospital stay (days)	2.8 ± 3.0	3.7 ± 4.7	0.3
Total bilirubin (μmol/L)	12 ± 5	11 ± 7	0.7
Gamma-glutamyl transpeptidase (IU/L)	50 ± 58	62 ± 55	0.3
ASAT (IU/L)	61 ± 115	70 ± 87	0.7
ALAT (IU/L)	45 ± 58	58 ± 75	0.3
Prothrombin time (% of normal)	70 ± 21	74 ± 18	0.4
No. of grafts with steatosis	5/37	7/41	0.8
Recipient data			
Male-to-female ratio	34/11	32/14	0.5
Age (yr)	46.1 ± 11.0	49.4 ± 11.9	0.2
No. (%) transplanted for:			0.8
Cirrhosis	23 (51%)	24 (52%)	
Cancer	10 (22%)	8 (17%)	
Other	12 (27%)	14 (31%)	
Total bilirubin (μmol/L)	63 ± 182	95 ± 179	0.4
ASAT (IU/L)	116 ± 193	75 ± 112	0.2
ALAT (IU/L)	61 ± 78	57 ± 92	0.8
Prothrombin time (% of normal)	64 ± 21	63 ± 24	0.9
Intraoperative data			
Cold ischemia time (min)	461 ± 96	436 + 116	0.3
Transfusion			
No. of blood units	7.3 ± 6.8	9.1 ± 10.8	0.3
No. of fresh frozen plasma units	16.5 ± 12.8	16.8 ± 15.3	0.9
Duration of operation (min)	462 ± 98	441 ± 119	0.4

$Group_{Precond}$, respectively ($P = 0.8$). The postreperfusion biopsy detected ischemia-reperfusion injury, as defined in the methods section, in 26 of 32 (81%) and 21 of 38 (55%) available cases of $Group_{Control}$ and $Group_{Precond}$, respectively ($P = 0.02$).

Analysis of Hospital Mortality, Technical Morbidity, Acute Rejection, and Length of Stay in Intensive Care Unit and Hospital

One of the patients in the $Group_{Control}$ (2%, due to sepsis) and one of those in the $Group_{Precond}$ (2%, due to disseminated toxoplasmosis, $P = 0.98$) died while in the hospital.

The mean number of technical complications per patient was similar in $Group_{Control}$ and $Group_{Precond}$ (0.2 ± 0.6 versus 0.3 ± 0.6, respectively, $P = 0.5$). The incidence of hemoperitoneum needing surgery (3 of 45 versus 6 of 46, respectively, $P = 0.3$), arterial thrombosis (2 of 45 versus 1 of 46, respectively, $P = 0.5$) and biliary complications (5 of 45 versus 7 of 46, respectively, $P = 0.6$) were similar in both groups. There were no cases of portal vein thrombosis or outflow block in our series.

Acute rejection rates were similar in the 2 groups (12 of 45 versus 9 of 46 for $Group_{Control}$ and $Group_{Precond}$, respectively, $P = 0.4$).

A trend toward a longer hospital stay and a longer stay in intensive care was observed for the $Group_{Precond}$ compared with the $Group_{Control}$ (38 ± 25 versus 31 ± 12 days and 15 ± 14 versus 12 ± 6, respectively); however, this did not reach statistical significance ($P = 0.1$, and $P = 0.1$, respectively).

Survival

Four patients (2 from each group) died 6, 10, 15, and 21 months after transplantation. No difference in patient survival was found between the groups (98% and 93% at 1 year in $Group_{Control}$ and $Group_{Precond}$, respectively, $P = 0.2$, log-rank). The mean follow-up period was 25 ± 13 months; no cases of retransplantation occurred.

Liver Graft Tolerance to Ischemia-Reperfusion

At day 5, patients in $Group_{Precond}$ had significantly lower serum ASAT concentrations (50 ± 29 versus 88 ± 90 IU/L, respectively, $P = 0.007$) and ALAT concentrations (138 ± 106 versus 365 ± 393 IU/L, respectively, $P = 0.0003$) than those in $Group_{Control}$ (Table 2). Patients in $Group_{Precond}$ had a significantly lower peak ASAT concentration (556 ± 968 versus 1073 ± 1112 IU/L, respectively, $P = 0.02$) and a significantly lower peak ALAT concentration (461 ± 495 versus 997 ± 1071 IU/L, $P = 0.003$) than those in $Group_{Control}$.

TABLE 2. Postoperative Liver and Kidney Function Tests in 45 Versus 46 Cases Without Versus With Graft Ischemic Preconditioning

Tests	Without Ischemic Preconditioning ($n = 45$ patients)	With Ischemic Preconditioning ($n = 46$ patients)	*P*
ASAT (IU/L)			
Day 5	88 ± 90	50 ± 29	0.007
Maximum value within 10 days	1073 ± 1112	556 ± 968	0.02
ALAT (IU/L)			
Day 5	365 ± 393	138 ± 106	0.0003
Maximum value within 10 days	997 ± 1071	461 ± 495	0.003
Prothrombin time (% of normal)			
Day 3	64 ± 10	59 ± 16	0.06
Day 5	65 ± 10	60 ± 18	0.1
Day 7	68 ± 9	64 ± 18	0.2
Day 15	76 ± 11	71 ± 15	0.1
Minimum value within 10 days	47 ± 11	44 ± 14	0.3
Bilirubin (μmol/L)			
Day 7	62 ± 76	76 ± 93	0.4
Day 15	42 ± 50	59 ± 96	0.3
Maximum value within 10 days	102 ± 97	122 ± 134	0.4
Creatinine (μmol/L)			
Maximum value within 10 days	173 ± 143	173 ± 124	0.9

Factors Associated With the Maximum ASAT Concentration Within 10 Days of Transplantation

To be clinically relevant, only data available before transplantation were included in univariate and multivariate analyses to identify independent factors affecting the peak ASAT concentration. Three factors were identified in univariate analysis, including ischemic preconditioning (peak value 1073 ± 1112 versus 556 ± 968 IU/L for $Group_{Control}$ and $Group_{Precond}$, respectively, $P = 0.02$), presence of graft steatosis (peak value 2132 ± 1716 versus 633 ± 812 IU/L for grafts with and without steatosis respectively, $P < 10^{-3}$), and the body mass index of the donor ($P < 10^{-3}$). None of the other factors evaluated were significant; these included age, liver function tests of both the donor and the recipient, and the length of donor's hospital stay prior to harvesting (data not shown). In the multivariate analysis, the same 3 factors remained independently associated with a higher peak of ASAT: preconditioning ($P = 0.01$), presence of graft steatosis ($P < 10^{-3}$), and the body mass index of the donor ($P < 10^{-3}$).

The peak levels of ASAT and ALAT were not significantly associated with cold ischemia time ($P = 0.07$, and $P = 0.4$, respectively), duration of operation ($P = 0.09$ and $P = 0.3$, respectively), or intraoperative transfusion need ($P = 0.8$ and $P = 0.6$, respectively).

Effect of Liver Graft Preconditioning on Liver and Renal Function

We found no statistically significant difference in serum levels of bilirubin and prothombin time between the 2 groups at any time point (Table 2). However, at all time points, a trend toward a lower prothrombin time and a higher bilirubin level was found in $Group_{Precond}$ compared with $Group_{Control}$. No cases of primary nonfunction occurred. Initial poor function occurred in 6 (13%) and 15 (33%) patients from $Group_{Control}$ and $Group_{Precond}$, respectively ($P = 0.03$).

The maximum creatinine levels within 10 days of transplantation were similar in the 2 groups ($P = 0.9$).

Factors Associated With Initial Poor Function

Univariate analysis showed that preconditioning was the only pretransplantation factor associated with initial poor function *(*$P = 0.04$). None of the other factors tested was significant, including donor's age ($P = 0.6$), body mass index ($P = 0.8$), duration of hospital stay ($P = 0.5$), and liver steatosis ($P = 0.3$). Likewise, the age ($P = 0.7$) and creatinine level of the recipient prior to transplantation ($P = 0.1$) were not significantly associated with initial poor function.

The occurrence of initial poor function was not significantly associated with cold ischemia time ($P = 0.7$) or duration of operation ($P = 0.1$). On the contrary, it was significantly associated with intraoperative requirement for transfusion ($P = 0.01$).

DISCUSSION

This study using the model of cadaveric whole liver transplantation is the first to evaluate the effect of ischemic preconditioning of the graft in humans. In accordance with most animal studies, our results show that ischemic preconditioning protects against ischemia-reperfusion injury as indicated by lower ASAT levels.[34] Multivariate analysis showed that ischemic preconditioning was independently predictive of a lower peak ASAT concentration posttransplantation in association with already recognized factors, namely, steatosis of the graft[1,5] and the body mass index of the donor.[4,35] Ischemic preconditioning did not have the same positive impact on liver function. Indeed, ischemic preconditioning was the only factor significantly associated with initial poor function. There is no universally accepted definition of initial poor function.[1,4–6,35–39] Like others, we consider that transaminases reflect the ischemia-reperfusion injury of the liver graft, whereas PT and bilirubin (even though the latter is multifactorial) are the best markers of graft function in clinical practice. The fact that initial poor function was more common in the $Group_{Precond}$ than in the $Group_{Control}$ had no deleterious consequences on patient or graft survival rates in our series. However, it is reasonable to speculate that differences would be found with a much larger sample size as poor initial function is a major pronostic factor following liver transplantation.[1,4,8] This is supported by longer stays in intensive care and hospital in $Group_{Precond}$ compared with in $Group_{Control}$.

The contradictory effect of ischemic preconditioning cannot be confirmed in animal studies as none of them explored the coagulation factors and bilirubin levels after ischemic preconditioning of the liver. However, Adam et al[28] showed that preconditioning of the liver graft was associated with altered liver function compared with a control group in a rat model. In the 2 human studies that evaluated the preconditioning-like effect of reversible cardiac arrest in the cadaveric donor, posttransplantation prothrombin time and bilirubin level were similar regardless of whether the donor had sustained temporary cardiac arrest prior to harvesting.[29,30] According to the authors, numerous biases, including the duration of cardiac arrest and the delay from cardiac arrest to graft harvesting, preclude the transposition of their observations to the clinical situation of ischemic preconditioning.

It is now established that the occurence of ischemic preconditioning differs between different tissues within a given species and in the same tissue in different species.[9,40–43] For example, 2 studies, one using porcine kidneys[44] and one using dog kidneys,[45] failed to identify renal ischemic preconditioning. These results differ strongly from those obtained in small animal species.[40,41,46–48] The many

mechanisms of ischemic preconditioning might explain the abovementioned discrepancies;[10,11,49] however, this is beyond the scope of our study. We are conscious that biases in our study might explain the negative effect of ischemic preconditioning on liver graft function. These biases include the preconditioning protocol and the choice of study design. Pharmacologic preconditioning protocols that do not include a warm ischemia step (with portal triad clamping) might prevent this negative effect.[50,51]

Although factors associated with initial poor function have previously been reported, most studies concentrate on donor and perioperative prognostic criteria, including cold ischemia time, duration of operation, and intraoperative transfusion.[4] In clinical practice, these data are not available when deciding to transplant a patient with a given liver graft. The objective of this report was to identify factors of prognostic value available at the time when the decision to transplant is taken. Indeed, no correlation was found between the cold ischemia time, the duration of operation, or the transfusion need on the one hand and the peak levels of ASAT and ALAT on the other hand.

In summary, ischemic preconditioning of cadaveric liver grafts protects against ischemia-reperfusion injury. This beneficial effect is counterbalanced by a deleterious effect on the early graft function, with an increased incidence of initial poor function. Our study suggests that warm ischemia triggers the positive effect of preconditioning on ischemia-reperfusion injury but adds its deleterious effect on the liver function to that of cold ischemia.[7]

CONCLUSION

This first clinical application of ischemic preconditioning of a graft confirms its protective effect against ischemia-reperfusion injury. Preconditioning as performed in our protocol did neither improve nor compromise the outcome of cadaveric liver transpantation. As immediate and sufficient function of the graft is the primary goal of transplantation, the use of ischemic preconditioning, via 10 minutes of warm ischemia, is not appropriate for liver transplantation in clinical practice.

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