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Tacrolimus as a Liver Flush Solution to Ameliorate the Effects of Ischemia/Reperfusion Injury Following Liver Transplantation

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The goal of this report is to evaluate in a prospective randomized fashion the effect of flushing hepatic allografts with tacrolimus before transplantation. A prospective, double-blinded, randomized trial was performed. Twenty patients receiving orthotopic liver transplants from October 2000 to October 2001 were randomized into two groups. Group 1 (active) was administered tacrolimus, 20 ng/mL, plus Plasma-lyte A (Baxter Healthcare Corp, Deerfield, IL) liver flush solution; and group 2 (placebo) was administered only Plasma-lyte A. Ischemia/reperfusion injury was assessed in both groups after transplantation by means of serum laboratory values to assess hepatocellular damage, synthetic function, and ion transport capacity. Peak values were recorded for each parameter, and their distributions were compared. There were no statistically significant differences between groups for age, sex, total ischemia time, or cause of liver disease. Global multivariate comparison of peak changes in all measures of liver function indicated liver injury was significantly lower with tacrolimus treatment than placebo ($P = .01$). The sample median for group 1 was less than for group 2 in all parameters measured. Individual statistical comparison showed that peak changes from baseline aspartate aminotransferase and activated partial thromboplastin time values were significantly improved ($P \leq .05$) with tacrolimus treatment than placebo treatment. In this prospective, double-blinded, randomized trial, we show that flushing the liver before transplantation with Plasma-lyte A containing tacrolimus results in superior early graft function and decreased hepatocellular injury after reperfusion compared with flushing with Plasma-lyte A alone. (*Liver Transpl 2003;9:144-149.*)

In the current era of cold storage, the limiting factor of organ viability is the injury incurred from ischemia and subsequent reperfusion. The issue is becoming of increasing significance as the quality of donors is trending downward. This trend of accepting lower quality organs is driven by the ever-expanding discrepancy between patients in need of a transplant and available donors. The United Network for Organ Sharing (UNOS) currently reports a waiting list of 18,000 patients, whereas the number of donors per year has become static in the past 5 years at approximately 5,000 per year. Improved vehicle safety standards, public awareness of head protection, and acute trauma care continue to decrease the number of young healthy organ donors. Of necessity, programs are now accepting donors with more dire clinical conditions, including patients on vasopressor therapy and those with positive viral serological test results, which would have been contraindications in the past. The average cadaveric organ donor currently is 45 years old, a striking 20 years older than the average donor only a decade ago (UNOS data).

Overall, the changing quality of donor livers demands an optimal method of preservation to maximize function after revascularization in the recipient. Although decades of scientific investigation have focused on the solution within the graft during the preservation period, there has been little consideration of the solution used to flush the organ before transplantation. Studies of a flush solution called Carolina rinse, containing vasoactive and antioxidant compounds, have shown a protective effect with its use.[1,2]

Investigations with cultured hepatocytes from human samples have documented membrane-stabilizing effects with intravenous tacrolimus, a calcineurin inhibitor used in transplantation for its powerful immunosuppressive effects.[3] Work in animal models has shown decreased cellular injury from ischemia and reperfusion when tacrolimus was administered before ischemia.[4-7]

Based on such evidence, this pilot study was developed to examine the hepatotropic and protective effects of tacrolimus placed in a liver flush solution, with the hypothesis that it may decrease hepatocellular injury on reperfusion of the preserved hepatic allograft, thus enhancing early graft function in orthotopic liver transplantation.

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Methods

Patients

After approval of the study proposal by the Mayo Foundation Institutional Review Board, all patients who met criteria for orthotopic liver transplantation at Mayo Clinic Hospital were considered. Informed consent was obtained from all potential recipients on the waiting list during the study period.

Study Design

On activation for transplantation, each patient was randomly assigned to one of two groups: group 1 was administered tacrolimus and Plasma-lyte A (Baxter Healthcare Corp, Deerfield, IL) flush solution, and group 2 was administered Plasma-lyte A flush solution only.

Flush Composition

In group 1, the flush contained tacrolimus at a concentration of 20 ng/mL. This was accomplished by adding 5 mg of tacrolimus to a 250-mL container filled with normal saline, which produced a solution with a 20-μg/mL concentration of tacrolimus. Then 1 mL of this solution was added to a 1-L bag of Plasma-lyte A to provide the final flush solution administered to group 1 with a 20-ng/mL concentration of tacrolimus. In group 2, standard Plasmalyte A without additives was the flush solution used.

Method of Flush

After the recipient total hepatectomy, the donor allograft was removed from the cold University of Wisconsin solution on the back table, and the suprahepatic inferior vena cava anastomosis subsequently was performed. During anastomosis, the liver allograft was flushed with one of the two room-temperature solutions as described. Both the hepatic artery and portal vein were flushed with 500 mL each. The solution was run for approximately 15 minutes, and the operating team was blinded to the contents of the solution (placebo versus active). The efflux of this solution mixed with the preservation solution originally in the allograft was evacuated simultaneously from the peritoneal cavity by continuous suction placed in the retrohepatic fossa. After infusion, portal vein anastomosis was completed, and the liver was reperfused. The hepatic arterial anastomosis was performed to complete the hepatic vascular circuit. Finally, the biliary anastomosis was concluded to complete the operation.

Management

All patients enrolled in this study were treated with the same medical management postoperatively. The protocol includes tacrolimus and corticosteroids for immunosuppression.

Data Collection

Donor and recipient demographics were compiled for all participants in the study. In evaluating the donor pool, UNOS criteria for defining marginal donors was applied, which consists of at least one of the following: use of vasopressors, liver transaminase levels greater than three times baseline, longer than 5 days in the hospital before donation, or greater than 30% macrovesicular steatosis. Total ischemia time from cross-clamp of the aorta during the donor operation to reperfusion in the recipient was recorded in each case.

After transplantation, serum measurements for aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, prothrombin time (PT) with international ratio, and activated partial thromboplastin time (aPTT) were obtained postoperative days 1 and 2.

Table 1. Donor and Recipient Demographics

	Group 1	Group 2
Donor		
Age (yr)	44.2	48.8
Gender (% male)	60	60
Marginal donors (%)	80	70
Recipient		
Cause of liver disease (% hepatitis C)	70	50
Age (yr)	52.7	47.3
Gender (% male)	80	50
Race (% white)	80	80

Statistical Method

Distributions of peak changes in the tacrolimus group were compared with those of the placebo group. For each individual measure, statistical significance was calculated by means of exact Wilcoxon's rank-sum test. The margin of error for the difference between groups was obtained by calculating an exact 95% confidence interval for the Hodges-Lehmann shift parameter. The statistical significance of a global comparison of all six measures simultaneously was calculated using the O'Brien multivariate rank test. Computations were performed using SAS, version 8.2 (SAS Institute, Cary, NC) and StatXact4 software (Cytel Software Corp, Cambridge, MA). All *P* values are two-sided.

Results

Demographics

Average donor age was 44.2 years (range, 13 to 68 years) in group 1 and 48.8 years (range, 22 to 70 years) in group 2; this difference was not significant. Donors for both groups were composed of 60% men and 40% women. The donor pool for both groups contained a similar number of marginal donors, with the active group (group 1) having one more marginal donor than the placebo group (Table 1).

Mean recipient age was 52.7 years (range, 47 to 64 years) in group 1 compared with a mean age of 47.3

Table 2. Comparison of Measures of Liver Reperfusion Injury

	Group 1	Group 2	Difference	
			*P**	95% Confidence Interval†
AST (IU/L)				
Peak change‡	371 (66-3,521)	1330 (376-2,999)	.02	−1,217 - −96
Day 2 change	124 (−13-3,521)	561 (29-2,999)	.09	−885 - 46
Day 1 change	371 (66-1,636)	1144 (376-2,986)	.01	−1,195 - −106
Baseline	85 (36-208)	106 (50-191)	.63	−66 - 47
ALT (IU/L)				
Peak change	409 (48-1,482)	702 (334-2,559)	.18	−811 - 81
Day 2 change	315 (28-1,482)	562 (290-2,559)	.14	−816 - 89
Day 1 change	387 (42-809)	589 (334-1,232)	.12	−565 - 44
Baseline	52 (10-118)	44 (27-170)	.84	−39 - 31
Total bilirubin (mg/dL)				
Peak change	−1.0 (−8.6-3.2)	−1.4 (−23.7-4.1)	.74	−2.5 - 5.7
Day 2 change	−1.7 (−9.3-3.2)	−1.7 (−33.5-1.1)	.91	−2.3 - 7.0
Day 1 change	−1.0 (−8.6-1.5)	−1.4 (−23.7-4.1)	.78	−2.6 - 4.5
Baseline	4.0 (0.9-14.0)	3.5 (0.9-40.7)	.90	−8.9 - 2.7
PT (s)				
Peak change	−2.2 (−6.1-2.4)	0.1 (−4.9-10.7)	.11	−7.1 - 1.2
Day 2 change	−5.5 (−12.5-0.2)	−2.6 (−7.9-6.6)	.09	−7.8 - 0.6
Day 1 change	−2.2 (−6.1-2.4)	0.1 (−4.9-10.7)	.12	−6.7 - 1.1
Baseline	22.1 (16.5-34.9)	18.8 (16.2-26.2)	.21	−1.6 - 5.6
INR				
Peak change	−0.2 (−0.6-0.3)	0.0 (−0.5-1.2)	.12	−0.8 - 0.1
Day 2 change	−0.6 (−1.4-0.0)	−0.3 (−0.9-0.7)	.10	−0.8 - 0.1
Day 1 change	−0.2 (−0.6-0.3)	0.0 (−0.5-1.2)	.12	−0.8 - 0.1
Baseline	1.9 (1.3-3.3)	1.5 (1.3-2.3)	.31	−0.2 - 0.6
PTT (s)				
Peak change	−9.0 (−17.4-1.1)	−1.3 (−14.5-26.9)	.04	−14.5 - −1.1
Day 2 change	−13.9 (−23.6-−6.6)	−11.7 (−33.5-1.4)	.44	−9.7 - 4.7
Day 1 change	−9.1 (−17.4-1.1)	−1.3 (−14.5-26.9)	.04	−14.7 - −1.1
Baseline	43.8 (34.4-58.3)	40.0 (33.2-66.6)	.57	−5.0 - 7.7

NOTE. N = 10 per group. Values expressed as median (range) unless noted differently.
Abbreviation: INR, international normalized ratio.
**P* are from exact Wilcoxon's rank-sum test and are two-sided.
†Exact 95% confidence intervals for the Hodges-Lehmann shift parameter.
‡Change from baseline to the worst of day 1 or day 2.

years (range, 33 to 53 years) in group 2. Demographic comparison of recipients showed no statistically significant difference between groups 1 and 2 in age, sex, race, or cause of liver disease (Table 1).

Mean total ischemia time from cross-clamp of the aorta during the donor operation to reperfusion in the recipient was 384.5 minutes in group 1 (range, 270 to 600 minutes). These values did not differ significantly from group 2, in which mean ischemia time was 378.1 minutes (range, 240 to 538 minutes; $P = .89$).

Postoperatively, all 20 patients were taken to the intensive care unit and extubated within 24 hours. All patients remained hemodynamically stable during the 48-hour study period; no patient required further surgery or major intervention during this time. Postoperative day 1, ultrasound showed normal flow through the anastomoses in all cases.

Graft Quality

The complete data set for all measured parameters is listed in Table 2.

In the experimental group, group 1, mean AST levels were 604.3 and 638.3 IU/L postoperative days 1 and 2 compared with a median baseline level of 85 IU/L, respectively. The increase and change from baseline were less than values recorded for group 2, which increased to 1,294.3 and 934.8 IU/L postoperative days 1 and 2 from a baseline of 106 IU/L, respectively. Similarly, ALT levels were 499.6 IU/L day 1 and 511.7 IU/L day 2 from a baseline of 52 IU/L in group 1, in

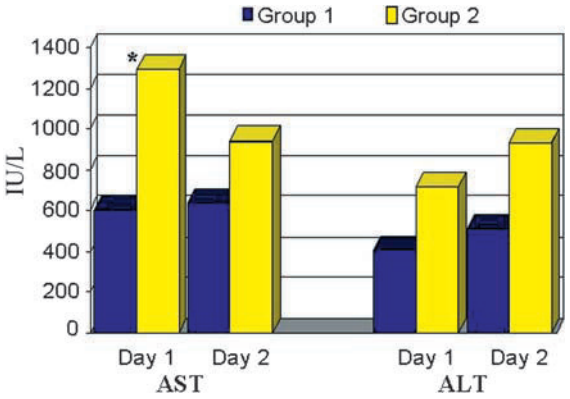

Figure 1. Comparison of average peak AST and ALT levels postoperative days 1 and 2 between groups 1 and 2. Each bar represents the mean for 10 patients. *$P = .02$.

contrast to an increase to 714.6 IU/L day 1 and 930.7 IU/L day 2 from a baseline of 44 IU/L for group 2. AST and ALT results are shown in Figure 1.

Synthetic Function

Average total bilirubin levels the day of transplantation were 5.04 mg/dL in group 1 and 9.50 mg/dL in group 2, skewed by a single patient with a preoperative value of 40.7 mg/dL, which was 25 mg/dL greater than that of any other patient in the study. Mean total bilirubin levels recorded for group 1 were 3.42 mg/dL day 1 and 2.68 mg/dL day 2 compared with 5.63 and 3.32 mg/dL for group 2, respectively. Median changes from baseline total bilirubin levels by postoperative day 2 were 1.9 and 1.65 mg/dL for groups 1 and 2, respectively.

Average preoperative PTs were 22.75 seconds for group 1 and 20.26 seconds for group 2, and by postoperative day 2, average PT was 17.30 seconds in group1 compared with 18.55 seconds in group 2. Median change from baseline was 5.50 seconds in group 1 in contrast to 2.60 seconds in group 2. Similarly, mean preoperative aPTTs were 43.56 seconds for group 1 and 42.84 seconds for group 2, which changed by postoperative day 2 to 29.29 seconds for group 1 and 30.20 seconds for group 2. The resultant median improvement during the 2-day study period was 14.27 seconds for group 1 compared with 11.70 seconds for group 2, also significant.

Statistical Analysis

The sample median for group 1 was lower than that for group 2 for all measures except total bilirubin level. In individual comparisons, peak changes from baseline AST and aPTT values were superior for group 1 by a statistically significant margin ($P < .05$). Both these parameters reached the peak change postoperative day 1 (Table 2).

Multivariate Analysis

Global multivariate comparison of peak changes in all six measures of liver function indicated that liver injury was statistically significantly less with tacrolimus treatment than placebo treatment ($P = .01$).

Discussion

Results of this study document a beneficial effect of tacrolimus in attenuating ischemia-reperfusion (I/R) injury when supplemented to the flush solution used to evacuate the preservation solution from the hepatic allograft just before reperfusion.

The entire story behind the capacity of tacrolimus for ischemic protection has yet to be completely illuminated, although we continue to gain insight. Evident from data published to date is that I/R protection conferred by tacrolimus is the result of multifaceted actions in the vastly complex cascades of ischemia and reperfusion.

Early work discovered that tacrolimus alters hepatic microcirculation, which conveyed protection from I/R injury.[4] Subsequently, it was shown that tacrolimus may be able to promote maintenance of microcirculation in the face of the normally deleterious reperfusion by suppressing endothelial expression of the potent vasoconstrictor endothelin-1.[8] Further evidence suggested that tacrolimus influences the nitric oxide pathway, as well.[9]

Intraparenchymal distribution of blood flow may be a contributing factor to the injury-preventing benefits of tacrolimus or a consequence of its action within the myriad of I/R pathways.

During ischemia, rapid depletion of adenosine triphosphate (ATP) renders ATP-driven membrane pumps ineffective, resulting in cellular edema as ions move into the intracellular space.[10,11] Changes in intracellular ion concentrations, particularly calcium, are of critical significance because calcium is a secondary messenger involved in the activation of phospholipases and has been described as a final common pathway for cellular death.[12] Tacrolimus has been shown to decrease calcium concentrations within the mitochondria during ischemia and reperfusion, which resulted in maintenance of mitochondrial function and regulation of the enzymatic systems responsible for initiation of inflammatory pathways.[13]

Free radical production after reperfusion from the products of ATP breakdown that accumulate during

ischemia is an important mechanism for cellular injury.[14,15] In a rat model of hepatic I/R injury, improved survival was found after two thirds partial hepatectomy with restoration of hepatic ATP contents when tacrolimus pretreatment was used.[16] More specifically, tacrolimus has been shown to have a favorable impact on the hepatocyte oxidation/reduction state with improved ketone body ratios, which prevented ATP content deprivation under hypoxic conditions.[17] Interestingly, the same model failed to show a similar effect with cyclosporine, another calcineurin inhibitor.

The release of free radicals is not only a product of substrate variation, as described, but also secondary to the inflammatory response.[11] As reperfusion injury ensues, endothelial disruption, expression of adhesion molecules, and leukocyte infiltration with subsequent activation of the entire inflammatory armamentarium occurs.[11,18] Some investigators have suggested that neutrophil infiltration and cumulative free radical release are the most important mediators of I/R injury.[19,20]

Tacrolimus, as an immunomodulatory agent, is likely to have a profound effect on the inflammation avenue of I/R injury. It has been shown to block early activation of the transcription factor nuclear factor-κB.[21,22] This ubiquitous transcription factor mediates early gene expression of cytokines, chemokines, growth factors, immunoreceptors, and cell adhesion molecules during I/R injury.[22] In the cardiac model, tacrolimus has been shown to suppress nuclear factor-κB and subsequently reduce leukocyte accumulation.[23] Investigations of hepatic ischemia found that tacrolimus administered before ischemia decreased interleukin-1 (IL-1) and tumor necrosis factor-α levels, as well as neutrophil migration.[23] Other studies have shown that tacrolimus pretreatment results in decreased expression of tumor necrosis factor-α, IL-1, and IL-6.[23-25] More recently, it was documented to reduce expression of adhesion molecules, with a demonstrated reduction in leukocyte rolling and adhesion.[19]

In conclusion, although complete elucidation of the role of tacrolimus in hepatic I/R injury deserves further investigation, in this trial, the first prospective randomized trial conducted in the clinical arena, we show that use of a flush solution containing tacrolimus results in superior early graft function and decreased hepatocellular injury.

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