Transpl Int (2003) 16: 730–735
DOI 10.1007/s00147-003-0621-3

ORIGINAL ARTICLE

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A single-center experience with retrograde reperfusion in liver transplantation

Received: 4 September 2002
Revised: 18 January 2003
Accepted: 11 March 2003
Published online: 18 June 2003

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Abstract Poor graft function secondary to injury by ischemia and reperfusion remains a major problem with regard to morbidity and mortality in clinical liver transplantation (LTX). Up to one fifth of patients suffer from poor initial liver function due to severe damage to hepatocytes. This situation leads either to primary nonfunction described in approximately 6% of LTX or to slow recovery. We present a new method of reperfusion during LTX. From July 1998 to July 2002, 42 LTX in 39 recipients, (10 female, 52 years old (26–70) were performed. LTX was carried out in piggy-back technique. After completing the piggy-back anastomosis, the caval vein was declamped immediately, and retrograde low pressure reperfusion of the graft with low oxygenated venous blood was established. Portal anastomosis was performed using a running suture. In order to provide optimal retrograde liver perfusion, no clamping of the donor portal vein was done. After completing portal anastomosis, the recipient portal vein was declamped immediately. During arterial anastomosis, the transplanted liver was antegradely perfused via the portal vein. After completing hepatic artery anastomosis, declamping of the hepatic artery was done and arterial perfusion started. No backtable or in-situ-flushing except the described reperfusion technique was performed. Forty-two LTX in 39 recipients using piggy-back technique and retrograde reperfusion via the caval vein followed by antegrade reperfusion via the portal vein were performed; 38 out of 39 patients (97.44%) were alive and well at day 8 after LTX. One patient (2.56%) died of a pre-existing portal vein thrombosis on day 2 after LTX. Three patients had to undergo retransplantation for hepatic artery thrombosis (7.14%). Liver enzymes, bilirubine, prothrombine time and AT III on day 1, 3, 5 and 8 after LTX showed favourable values. Median aspartate aminotransferase (ASAT) was 219 U/l on day 1 after LTX. One-month survival rate was 95.23%, and 1-year survival rate 87.88%. Two patients died of liver-associated causes (5.12%). One patient died of a late hepatic artery thrombosis, and one more of rejection. No other severe case of rejection appeared. We can conclude that retrograde reperfusion might be highly sufficient method of removing perfusion fluid from the transplanted liver. Low pressure perfusion with low oxygenated blood might reduce the production of free oxygen radicals. Retrograde reperfusion via the caval vein and

antegrade reperfusion via the portal vein seemed to lower postoperative liver enzyme values and to improve initial liver function after LTX.

Keywords Liver transplantation · Reperfusion injury · Retrograde reperfusion · Primary nonfunction

Introduction

Liver transplantation (LTX) is the treatment of choice for patients with end-stage liver disease. Although the techniques for LTX have become standardized since Starzl described the first series of successful liver transplantations [1], controversy continues. One area which has been investigated with several studies, is the technique of flushing and/or reperfusing the liver during transplantation [2, 3, 4]. Nevertheless, liver retrieval and implantation are invariably associated with graft damage due to cold and warm ischemia and reperfusion. These processes result in cell damage, which may lead to non-function of an otherwise viable organ. The development and use of Belzer's University of Wisconsin (UW) solution resulted in the decrease of endothelial cell death after reperfusion of livers stored in UW solution [5]. Despite these improvements, primary graft dysfunction secondary to injury by ischemia and reperfusion remains a major problem with regard to morbidity and mortality in clinical LTX [6]. Up to one fifth of the patients [7] suffer from poor initial liver function due to severe hepatocyte damage, with concomitant borderline synthetic and metabolic activity. This situation leads either to primary nonfunction described in approximately 6% of LTX [8] or to slow recovery [9].

The characteristic injury from cold preservation and reperfusion that becomes manifest in liver allografts following transplantation has been focus of intensive research [10, 11]. Several studies have identified the sinusoidal endothelial cells as the primary targets of this process, while hepatocytes are significantly less vulnerable to the effects of cold preservation and reperfusion [12]. Injury of the sinusoidal lining is characterized by a loss of cell integrity, Kupffer cell activation and the subsequent release of lysosomal enzymes and potent mediators [13], which may lead to aggravation of post-transplant liver injury, including secondary damage of hepatocytes [6]. During the perioperative phase of LTX, cytokines play an important role in the regulatory process. Among others, tumor necrosis factor-α (TNF-α) and interleukin-6 interact with blood-cells and non-parenchymal liver-cells, such as Kupffer-cells and sinusoidal endothelial cells [14, 15].

Although numerous studies have investigated the mechanisms of preservation and reperfusion injury, these processes are not completely understood. Nevertheless, much work has been done to find possibilities to decrease preservation and reperfusion injury. For example, the destruction of Kupffer cells is very effective in preventing ischemia-reperfusion injury [16]. Treatment with gadolinium chloride destroys more than 80% of Kupffer cells, diminishes the production of reactive oxygen species and the liberation of inflammatory mediators. Interleukin-10 (IL-10) was found to suppress the production of cytokine, free oxygen radical and nitric oxide derivates. These functions of IL-10 suggest that its administration might inhibit reperfusion injury [17].

Much work has been done to improve the effectiveness of UW solution by simplifying its composition or adding other compounds. One of these numerous studies showed that high-Na^+ low K^+ UW cold storage solution reduces reperfusion injuries of the rat liver graft [18]. Other results indicate that supplementation of UW solution with the bile salt taurourodeoxycholic acid (TUDCA) is associated with less frequent and less severe ultrastructural damage of hepatocytes and endothelial cells and lower release of cytolytic enzymes [19].

Although numerous studies have investigated methods of reducing reperfusion injury, no generally accepted protocol to minimize liver dysfunction after the ischemic periods exists. We present a new method of reperfusion in LTX, leading to reduction of reperfusion injury. Retrograde reperfusion via the caval vein and antegrade reperfusion via the portal vein lowered postoperative liver enzyme values and improved initial liver function after LTX.

Materials and methods

In order to evaluate retrograde reperfusion during LTX, a retrospective study over the last 4 years was performed. To monitor the integrity of hepatocytes after liver transplantation, aspartate aminotransferase (ASAT) values on day 1, 3, 5 and 8 have been selected. ASAT is located within hepatocytes (32% cytosol and 68% mitochondrial), has a short half-life time (17 h, versus 47 h of ALAT) and is increased after severe cell damage. ASAT elevations due to skeletal or myocardial muscle damage can occur, but are not likely to influence the results. Poor early graft function was defined as peak ASAT > 2500 U/L. Poor early graft function causing reLTX or death within 14 days was called primary nonfunction.

Patients

From July 1998 to July 2002, 42 consecutive LTX in 39 recipients, 10 female, 52 years old (26–70) were performed. Indications for liver transplantation were hepatitis B and/or C (13), alcoholic cirrhosis (12), hepatocellular carcinoma (8), primary biliary cirrhosis

(2), α-1-antitrypsin deficiency (2), autoimmune hepatitis (1) and Wilson's syndrome (1).

Surgical technique

The donor liver was harvested using standard techniques. University of Wisconsin (UW) solution was used in 32 cases and Histidine-Tryptophan-Ketoglutarat (HTK) solution in 10 cases. Recipient hepatectomy included preservation of the caval vein for piggy-back technique. The hepatic veins were closed with running monofile sutures. Partial clamping of the caval vein enabled a midline, longitudinal caval incision of about 4–5 cm and preservation of the caval blood flow. After completing piggy-back anastomosis, the caval vein was declamped immediately, and retrograde low pressure reperfusion of the graft with low oxygenated venous blood was established. Central venous pressure was intended to be higher than 8 mm Hg to enable appropriate retrograde reperfusion of the transplanted liver. Significant venous backflow via the portal vein appears immediately after declamping the piggy-back anastomosis. Venous bleeding from the liver except portal backflow was stopped immediately after declamping the venous anastomosis, as appropriate. Portal anastomosis was performed using a running suture. Venous backflow via the portal vein was sucked into a cell saver device. In order to provide optimal retrograde liver perfusion, no clamping of the donor liver portal vein was done. After completing portal anastomosis, the recipient portal vein was declamped immediately. Venous bleeding from the transplanted liver was stopped before hepatic artery anastomosis was performed. During arterial anastomosis, the transplanted liver was antegradely perfused via the portal vein. After completing hepatic artery anastomosis, the hepatic artery was declamped, and arterial perfusion started. No backtable or in-situ-flushing except the described reperfusion technique was performed.

Definition of ischemic and anastomosis times

Cold ischemic time is defined as the time from the beginning of donor liver perfusion to insertion of the liver into the recipient. Warm ischemic time is defined as the time from insertion of the liver into the recipient to opening the hepatic artery anastomosis. Time from the insertion of the liver to declamping of the caval vein was defined as cavo-caval anastomosis time; time from declamping the caval vein to declamping the portal vein was defined as portal anastomosis time, and from this point on, to declamping of the hepatic artery counted as hepatic artery anastomosis time.

Immunosuppression

During the first week after LTX, all patients received horse ATG 1,5 to 3,3 mg/kg per day (lymphoglobulin, Pasteur Merieux) and a methylprednisolone taper starting with 70 mg every 6 h. Calcineurin inhibitors were initiated according to the kidney function between day 1 and 3, and standard trough levels were intended to be reached on day 7. From day 7, aprednisolon was given at 15 mg/d and tapered after the first month. Not later than 3 months after LTX, all patients were off steroids. MMF was started orally between days 2 and 7 with target MPA through levels about 1 μg/ml after day 7.

Patient monitoring

Liver enzymes, bilirubine, prothrombine time and AT III were observed simultaneously during the first week after LTX. Furthermore, graft and patient survival, rejection rate, adaptation of immunosuppression and complications of all patients are monitored up to the present.

Statistical analysis

Data are presented as mean/standard deviation or mean/range, as appropriate. Laboratory values are shown as box and whiskers plots with descriptive statistics. The horizontal lines from bottom to top meaning the lower limit, the 25th quartile, the median (MED), the 75th quartile and the upper limit. "+" indicate outliners.

Results

Of 39 patients, 38 (97,4%) were alive and well at day 8 after LTX. One patient died of a pre-existing portal vein thrombosis on day 2 after LTX. Retransplantation was indicated and performed in 3 patients due to hepatic artery thrombosis. Times for ischemia, anastomosis and reperfusion are displayed in Table 1. During LTX 6,1 (1–19) packed red cells, 10,7 (1–20) fresh frozen plasma and 1,9 (1–6) packed platelets were used. A total of 3248,3 ml (1077–8500) were vacuumed off with the cellsaver device.

In contradiction to the literature [20], circulatory problems or electrolyte imbalance after declamping any of the anastomoses were very uncommon. A postreperfusion syndrom, defined as a drop of arterial blood pressure > 30% pulmonary hypertension or low cardiac output, was observed in 2 patients. No or poor venous perfusion and highest liver enzymes in this series was observed in these patients.

Table 1 Times for ischemia, anastomosis and reperfusion

Times	Minutes
Cold ischemic time	294 ± 51
Warm ischemic time	90 ± 23
Cavo-caval anastomosis time	22 ± 7
Portal vein anastomosis time	23 ± 9
Hepatic artery anastomosis time	36 ± 12
Time of retrograde reperfusion	23 ± 9
Time of antegrade reperfusion	36 ± 12

Table 2 Liver enzymes, bilirubin, prothrombine time and AT III on day 1, 3, 5 and 8 after LTX, mean values

	POD 1	POD 3	POD 5	POD 8
ASA U/L	219	72	32	13
ALAT U/L	177	125	89	55
GGT U/L	37	88	125	103
Bilirubin mg/dl	2,7	1,7	2	1,8
PT %	72	91	88	92
AT III %	61	78	89	90

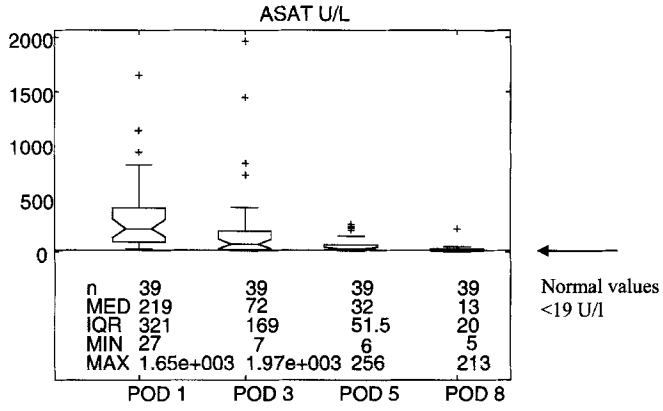

Fig. 1 The course of ASAT during the first week after LTX

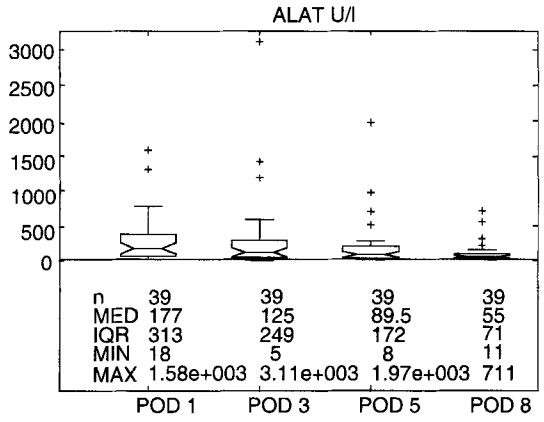

Fig. 2 The course of ALAT during the first week after LTX

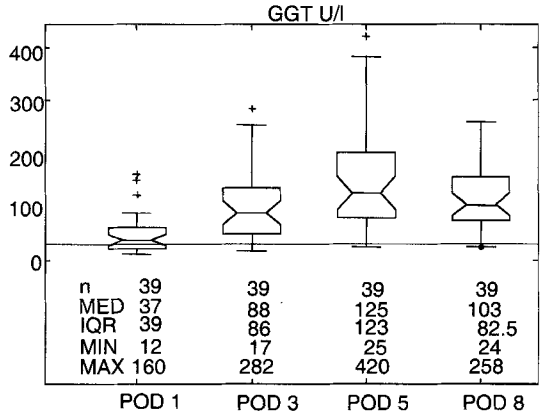

Fig. 3 The course of GGT during the first week after LTX

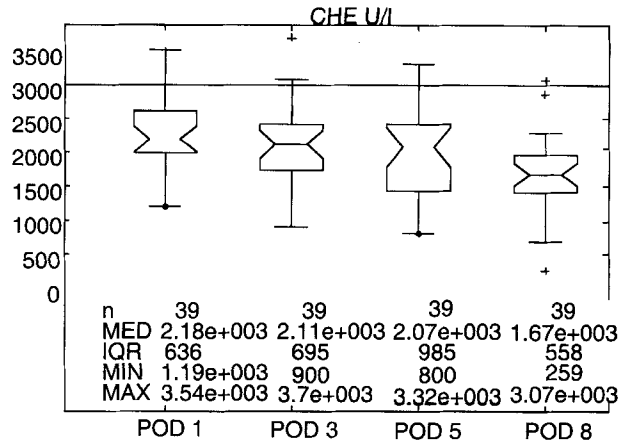

Fig. 4 The course of CHE during the first week after LTX

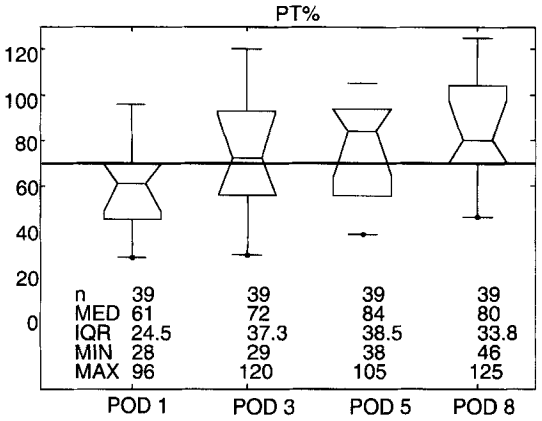

Fig. 5 The course of Quick during the first week after LTX

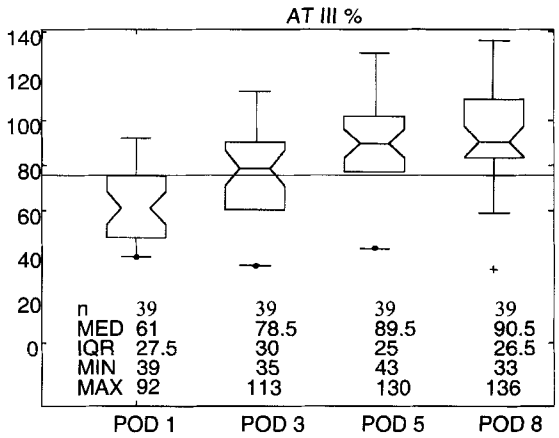

Fig. 6 The course of AT III during the first week after LTX

Liver enzymes, bilirubine, prothrombine time and AT III on day 1, 3, 5 and 8 after LTX showed favourable values and are displayed in Table 2. The course of ASAT, ALAT, GGT, CHE, Quick and AT III during the first week after LTX is presented in box-blots (Figs. 1, 2, 3, 4, 5, 6). Except in the 3 patients presenting with hepatic artery thrombosis, primary nonfunction or poor early graft function did not occur.

Patients receiving grafts procured far away had a significantly longer ischemic time. No statistically significant differences between these subgroups were found with respect to liver enzymes, bilirubine, prothrombine time and AT III on day 1, 3, 5 and 8 after LTX. Furthermore, there were no differences between livers perfused with UW and HTK solution. No correlation could be found between any parameter and the immunosuppressive regimen (cyclosporine/mycophenolate mofetil/cortisone or tacrolimus/mycophenolate mofetil/cortisone).

The 1-month survival rate was 95,23%, the 1-year survival rate to 87,88% and the 3-year survival rate to 76,92%. Among the 8 patients who died, 4 had a fully functioning graft. Liver-associated causes of death were pre-existing portal vein thrombosis on day 2 after LTX (1), rejection (1), late hepatic artery thrombosis (1) and recurrence of hepatocellular carcinoma (1). Non-liver associated causes of death occurred in 4 patients (sepsis 2, cerebral apoplexy 1, suicide 1). In one patient, sepsis developed after retransplantation and Friedländer's pneumonia. The other patient came with perforated appendicitis exacerbating to sepsis.

Rejection rate was 2,56%. Severe rejection was found only in 1 patient. Rejection therapy with ATG was necessary in 1 patient, 3 patients received pulsed steroids. Mild rejection as an increase of respective laboratory values was in general successfully managed by adaption of immunosuppressive therapy.

Infections occurred in 25 cases. Beside recurrence of pretransplant existing infection (hepatitis C recurrence 6, hepatitis B recurrence 2) viral infections happened in 8 patients (zoster 3, cytomegalovirus infection 5). No death was caused by viral infection. Bacterial infections appeared in 7 cases (pneumonia 3, erysipelas 2, urinary tract infection 2). 2 patients died of bacterial infections (described under sepsis). Fungal infections were found in 2 cases (aspergillus pneumonia, candida esophagitis). No patient died of fungal infection.

Discussion

Experimental studies have demonstrated, that the main part of the liver injury caused by low or no perfusion does not occur at the time of hypoxia, but during reperfusion [21]. In the first description of human liver transplantation by Starzl et al. [22] in 1963, the hepatic artery was used for initial reperfusion of the liver before portal venous flow was restored. Another approach was to reperfuse the liver via the portal vein. This is a low resistance circulation that carries a greater blood supply than the hepatic arterial system. Oxygen saturation in the portal venous system is low, but due to its greater blood flow, oxygen supply to the liver is comparable to that provided by the hepatic artery. No clinically significant differences in early graft function between hepatic artery and portal vein reperfusion during LTX have been found [3]. Two recent human studies suggest that simultaneous portal vein and hepatic artery reperfusion may reduce biliary complications [4, 23], and animal studies indicate that simultaneous [24] or primary hepatic artery [25] reperfusion may reduce ischemia reperfusion injury. We present a new method of reperfusion in LTX leading to reduction of reperfusion injury. Retrograde reperfusion via the caval vein and antegrade reperfusion via the portal vein lowered postoperative liver enzyme values and improved initial liver function after LTX. In the literature, standard reperfusion peak levels of ASAT about 638 ± 156 U/l after LTX were described [26]. In our patients, we reduced postoperative liver enzyme values (ASAT) of about mean 219 U/l.

According to our experience, retrograde perfusion efficiently removes perfusion fluid from the transplanted liver. Furthermore, we hypothesize that low pressure perfusion with low-oxygenated blood reduces the production of free oxygen radicals. Improvement of initial graft liver function by retrograde reperfusion during LTX might provide substantial benefits for the graft recipient: immediate clotting, rapid elimination of metabolites and toxic agents and earlier recovery.

No or poor venous reperfusion was observed in patients with the highest liver enzymes. Thus we suppose, the better the reperfusion, the lower are the postoperative liver enzyme values. Graft reperfusion during LTX is generally associated with a variety of transient hemodynamic disturbances. Systemic hypotension and pulmonary hypertension are the predominant physiologic changes. About 1 of 3 patients undergoing LTX develop postreperfusion syndrome in which severe hypotension occurs [20]. We noticed a markedly reduced incidence of postreperfusion syndrome (4,76%). In general, we do not observe hyperkaliemia, pulmonary hypertension, right heart failure, or low cardiac output after declamping any of the anastomosis.

A discrepancy exists worldwide between the number of suitable liver donors and the increasing demand for organs. Therefore, many centers have considered widening their liver donor acceptance criteria, and this may increase the incidence of primary dysfunction with negative effects on the results of transplantation. Donor age, steatosis, ischemia time, hypotension and ICU stay were risk factors associated with higher incidence of primary dysfunction [27]. Combination of risk factors should be avoided, and ischemia time, as the only variable that can be controlled, should be kept as short as possible. With the technique of retrograde reperfusion, more marginal donor livers could be accepted for LTX, even with expanding of ischemia time. The number of liver transplantations could be increased, and mortality on the waiting list could be decreased.

The results of our retrospective study demonstrate that retrograde reperfusion via the caval vein and antegrade reperfusion via the portal vein lowered postoperative liver enzyme values and improved initial liver function after LTX. Reperfusion injury and the incidence of primary nonfunction and dysfunction could be decreased. Hemodynamic disturbances during LTX were uncommon, therefore we suppose that the incidence of postreperfusion syndrom could be diminished, when retrograde reperfusion is performed. To evaluate the previously obtained retrospective single center results, a prospective, randomized, open multicenter trial was proposed.

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