

Twenty-Four Hour Steroid Avoidance Immunosuppressive Regimen in Liver Transplant Recipients

Wei-qiang Ju, Zhi-yong Guo, Xiaoting Ling, Xiao-shun He, Lin-wei Wu,
Qiang Tai, An-bin Hu, Ming Han, Xiao-feng Zhu

Abstract

Objectives: To investigate the efficacy and safety of an immunosuppressive regimen of steroid avoidance in combination with induction therapy and tacrolimus in liver transplant recipients.

Materials and Methods: Eighty-two adult liver transplant recipients were randomized into 2 groups: standard protocol group (n=41) in which steroids were withdrawn 3 months after the operation, and a 24-hour steroid avoidance group (n=41) in which steroids were eliminated within 24-hours. The incidence of acute rejections, infections (bacterial, fungal, and cytomegalovirus), and metabolic complications were analyzed between the groups.

Results: The incidence of early posttransplant diabetes mellitus and the average dosage of insulin consumption among diabetic recipients were significantly higher in recipients in the standard protocol group than in the 24-hour avoidance group ($P < .05$). In addition, the incidence of hypertension and infection during the follow-up were also higher in patients of the standard protocol group ($P < .05$). The incidence of hypertension in the early posttransplant period, hyperlipemia, and acute rejection during the follow-up were comparable between the groups ($P > .05$).

Conclusions: Twenty-four hour steroid avoidance combined with induction therapy and tacrolimus maintenance is a safe and efficient immunosuppression strategy that can significantly reduce posttransplant infections and other complications owing to long-term use of steroids, without increasing the risk of acute rejection.

Key words: Liver transplant, Immunosuppression, Steroids, Basiliximab, Tacrolimus

Introduction

With the wide application of powerful immunosuppressive agents, conventional immunosuppression is facing challenges. Steroids have long been a mainstay in immunosuppression protocols for organ transplant, whereas, long-term use of steroids leads to a high incidence of metabolic disorders and cardiovascular complications. More and more clinicians support the early withdrawal of steroids aiming to minimize steroid-related morbidity.¹⁻³ Based on our primary success in steroid withdrawal 3 months posttransplant, we attempted to evaluate the safety and efficacy of immunosuppressive regimen with steroid elimination within 24 hours in a cohort of Chinese liver transplant recipients.

Materials and Methods

Patients

Between September 2006 and September 2008, three hundred seven adult liver transplants were performed in our center. To avoid intrinsic bias, patients with any 1 of the following characteristics were excluded: (1) pretransplant infections (except chronic HBV/HCV infection); (2) receiving marginal grafts including those from donors with moderate to

From the Organ Transplant Center, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Grant support: The study is supported by the National High Technology Research and Development Program of China (863 Program) (2012AA021008), the Key Clinical Project from the Ministry of Health (2010159), National Natural Science Foundation of China (30972951 and 81170448), special fund for science research by Ministry of Health (201002004), the China Medical Board in New York (06837), the Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-Year Plan Period of China (2008BA160B02 and 2008BA160B06) and Science and Technology Planning Project of Guangdong Province (2008B030301308).

Acknowledgements: This work was not supported by any grants or financial support.

Corresponding author: Dr. Xiaoshun He, Organ transplant center of the first affiliated hospital of Sun Yat-sen University, No. 58 Zhongshan Er Road, Guangzhou, 510080, China
Phone: +86 20 8730 6082 Fax: +86 20 8730 6082 E-mail: gdtrc@126.com

Experimental and Clinical Transplantation (2012) 3: 258-262

severe fatty liver, HBV infection, age older than 60 years, or grafts with a cold ischemia time longer than 14 hours; (3) combined transplant or retransplant; (4) partial liver transplant, including living-donor liver transplant and split liver transplant; or (5) unwillingness to participate in the study.

After screening, 91 patients were randomized to receive standard immunosuppressive protocol (SP group) or 24-hour steroid avoidance protocol (24-h SA group) according to random sequence generated by SPSS software (SPSS: An IBM Company, version 13.0, IBM Corporation, Armonk, New York, USA). Nine patients were excluded from the analysis owing to ABO blood type incompatibility (4/9) and perioperative death (5/9). Among the patients who died perioperatively, 3 patients in the SP group died of acute heart failure (1/5), renal failure (1/5), and massive intraperitoneal bleeding (1/5), and 2 patients in the 24-h SA group died of renal failure (1/5) and primary allograft nonfunction (1/5). The medical records of the remaining 82 adult liver transplant recipients were retrospectively analyzed. There were 64 men and 18 women (aged, 26 to 68 y; mean age, 45.7 ± 7.8 y). The indications for liver transplant included hepatocellular carcinoma (36/82), liver cirrhosis secondary to hepatitis B (33/82), liver cirrhosis secondary to hepatitis C (3/82), alcoholic cirrhosis (3/82), severe hepatitis (6/82), and polycystic liver (1/82). The study protocol was approved by the ethical board of our institute, and the study was conducted in accord with the Helsinki Declaration. Written, informed consent was obtained from all recipients.

Immunosuppressive protocols

In the SP group, patients received induction therapy with 2 doses of 20 mg IL-2 receptor antibody (basiliximab) intraoperatively and on day 4 posttransplant. Tacrolimus was added on day 4 at an initial dosage of 0.04 mg/kg/d, and the dosage was adjusted to maintain a trough level of 8 to 12 ng/mL (6 to 10 ng/mL within 3 months posttransplant, and 5 to 8 ng/mL from 3 to 6 months posttransplant). A dose of 500 mg methylprednisolone was given intraoperatively on day 1 posttransplant, then 240 mg on day 2, then tapered 10 mg every day. At the end of the intravenous steroid reduction, intravenous steroids were converted to methylprednisolone tablets with an

initial dosage of 48 mg on postoperative day 9, which was reduced by 8 mg every 3 days and maintained at 4 mg/d thereafter, until it was completely stopped 3 months posttransplant. Some patients received a short-term treatment of mycophenolate mofetil (MMF) or rapamycin.

In the 24-h SA group, use of basiliximab and tacrolimus was equivalent to the standard protocol group, while only 2 doses of 500 mg were given intraoperatively and on day 1 posttransplant. Mycophenolate mofetil or rapamycin also was administered in some patients if necessary.

Follow-up

Liver function (alanine aminotransferase [ALT], total bilirubin), renal function (creatinine, blood urea nitrogen [BUN]), blood pressure, serum glucose, insulin used, serum lipid, infections (including bacterial, fungal and *cytomegalovirus* infections), and acute rejection episodes, were monitored postoperatively.

New-onset diabetes mellitus was diagnosed as fasting serum glucose levels ≥ 7.0 mmol/L for 3 consecutive days. Hypertension was diagnosed as systolic pressure ≥ 140 mm Hg and (or) diastolic pressure ≥ 90 mm Hg. Hypercholesterolemia was diagnosed when the fasting serum cholesterol level was > 5.72 mmol/L and (or) the fasting serum triglyceride level was > 1.7 mmol/L. Confirmative diagnosis of acute rejection in each patient required clinical manifestation, liver function tests, and a liver biopsy. Clinical presentations, laboratory tests, and causative studies were required for infection diagnosis.

Statistical Analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 13.0, IBM Corporation, Armonk, New York, USA). Continuous variables were tested for normal distribution and expressed as means \pm standard deviation (SD) or median (range) as appropriate. Comparison in quantitative data between the groups was performed using an independent samples *t* test; the chi-square test was applied to compare qualitative data. Univariate survival curves were estimated using the Kaplan-Meier method and compared by the log-rank test. A *P* value less than .05 was considered statistically significant in all analyses.

Results

Patient survival

The median duration of follow-up in the SP group and 24-h SA group was 23 months (range, 12-36 mo) and 21 months (range, 12-36 mo). Five recipients died during the follow-up. Three cases were in the SP group (7.3%), including 2 cases owing to neoplasm recurrence at 13 months and 18 months postoperatively, and 1 case died owing to liver function failure caused by bile duct ischemia and necrosis at 33 months after the transplant. Two cases (4.9%) died in the 24-h SA group, including 1 case owing to neoplasm recurrence at 14 months posttransplant, and 1 case owing to liver function failure caused by bile duct ischemia and necrosis at 29 months after the transplant. There was no significant difference in cumulative 2-year survival between the groups (92.7% vs 95.1%, $\chi^2 = 0.194$; $P = .660$). Figure 1 shows the survival curve generated by a Kaplan-Meier analysis.

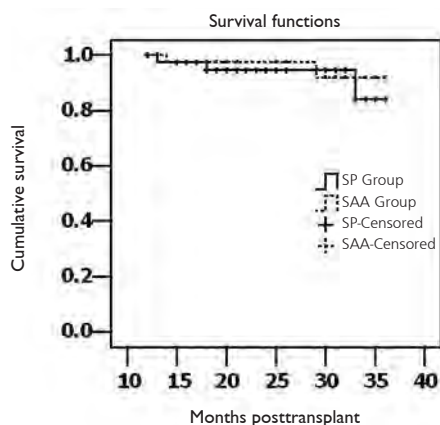


Figure 1. Kaplan-Meier survival curve of patients in the SP group and the 24-h SA group (log-rank test, $\chi^2 = 0.194$; $P = .660$).

Use of mycophenolate mofetil or rapamycin

Eight patients in the SP group and 10 patients in 24-h SA group experienced fluctuations in their liver function tests within 3 months. Insufficient immunosuppression was suspected in these patients, and short-term (7-21 d) use of MMF (750 mg twice daily) or sirolimus (1 mg once daily) was withdrawn after liver function tests became stable again. There was no significant difference between the groups when comparing the number of cases and duration of MMF or sirolimus use postoperatively (Table 1).

Table 1. Mycophenolate mofetil or rapamycin use in patients of the 2 groups.

Group	MMF/rapamycin use (cases, %)	ALT peak value (U/L)	Mean drug combination duration (d)
SP group	19.5(8/41)	186 ± 39	11 ± 5
24-h SA group	24.4 (10/41)	165 ± 40	13 ± 4
Statistical value	$\chi^2 = 0.285$	$t = 1.63$	$t = 1.38$
P value	.594	> .05	> .05

Abbreviations: 24-h SA group, steroid almost-avoidance; ALT, alanine aminotransferase; MMF, mycophenolate mofetil; SP, standard protocol

Acute rejection episodes

Seven recipients had biopsy-proven acute rejection, including 3 patients (3/41) in the SP group and 4 patients in 24-h SA group (4/41) ($P > .05$). Patients with acute rejection were treated by steroid bolus therapy as follows: 1000 mg methylprednisolone was infused intravenously on the day of confirmed diagnosis, tapered gradually, and discontinued on day 10. If necessary, steroid bolus therapy could be used repeatedly. All patients with acute rejection in 24-h SA group were cured by 1 course of steroid bolus therapy, while 2 patients with acute rejection in the SP group were cured by 1 course of steroid bolus therapy; the remaining one needed 2 courses of steroid bolus therapy.

Graft function and posttransplant metabolic complications

There was no significant difference between the groups in terms of liver function tests (ALT, TBIL) and renal function (Cr, BUN) after surgery. Within the first week posttransplant, the incidence of hyperglycemia was higher in the SP group than in the 24-h SA group (87.8% vs 36.6%; $P < .05$), and mean insulin used also was higher in the SP group than in the 24-h SA group (39.6 ± 7.9 vs 20.5 ± 6.7 IU; $P < .05$). More importantly, at the end of the follow-up, 11 patients out of 82 recipients developed new-onset diabetes mellitus, with 9 cases (9/41) in the SP group and 2 cases (2/41) in the 24-h SA group. The incidence of new-onset diabetes mellitus was lower in the 24-h SA group than in the SP group (4.9% vs 21.9%; $P < .05$). And all diabetic recipients required insulin therapy on varying dosages (24-48 U/d).

Regarding hypertension, within the first week postoperatively, the incidence of hypertension was comparable between the SP group and the 24-h SA group (46.3% vs 41.5%; $P > .05$). However, at the end of the follow-up, the incidence of hypertension was lower in the 24-h SA group than in

the SP group (4.9% vs 21.9%; $P < .05$). All recipients with hypertension needed antihypertensive therapy. In addition, at the end of the follow-up, the incidence of bacterial infections was much higher in the SP group than in the 24-h SA group (39.0% vs 14.6%; $P < .05$). Nonetheless, the incidence of hyperlipidemia was not significantly different between the 2 groups (12.2% vs 9.8%; $P > .05$). Table 2 summarizes the incidence of hyperglycemia status, insulin use, and hypertension within the first week posttransplant, while Table 3 summarizes the incidence of diabetes mellitus, hypertension, bacterial infection, hyperlipidemia, and acute rejection at the end of the follow-up.

Discussion

Steroids have been a cornerstone of immunosuppressive therapy in solid-organ transplant for half a century. To date, at most transplant centers, it is common to use steroids to induce and maintain immunosuppression. While effectively preventing acute rejection, there is particular concern about the adverse effects of steroids. With wide application of novel and powerful immunosuppressive agents, attempts are made to withdraw steroids as early as possible, posttransplant, in numerous transplant centers.

Steroid withdrawal protocols include long-term withdrawal (6-12 mo), early withdrawal (within 3 mo), very early withdrawal (2 wk), steroid almost-avoidance (elimination within 1 wk), and steroid complete-avoidance protocol. Several studies have demonstrated the safety of long-term and early withdrawal protocols.^{4, 5} Moreover, our experience shows that an immunosuppressive regimen with very early steroid withdrawal is safe and effective.⁶ Regarding steroid almost-avoidance protocol, most studies have shown promising outcomes when combined with basiliximab plus tacrolimus. Nonetheless, whether combined with MMF or

rapamycin, different centers adopted their own practices.^{3, 7} In a recent study, a steroid-free regimen with tacrolimus and MMF in liver transplants yielded good outcomes, with 86.7% and 83.9% 2-year patient and graft survival, and a 26.2% rejection rate, suggesting that it may be beneficial to use steroid-free immunosuppression combined with tacrolimus and MMF.⁸

This study shows that steroid almost-avoidance could lead to minimization of immunosuppressive agents use, and prevention of steroid-related complications. There is no need to adopt a drug combination when a single agent could provide satisfactory immunosuppression. A short-term combination would be advantageous in some high-risk patients. In this study, all recipients received basiliximab induction therapy, followed by tacrolimus maintenance. Short-term addition of MMF or rapamycin was used in 8 patients of the SP group and 10 patients of the 24-h SA group when they experienced impaired liver function. There was no significant difference between the groups when comparing incidence of acute rejection episode ($P > .05$). These results again confirm that tacrolimus alone is sufficient for immunosuppressive maintenance.

Recipients in the 24-h SA group yielded a lower incidence of new-onset diabetes and reduced use of insulin among hyperglycemic recipients ($P < .05$) in the first week posttransplant, suggesting that steroid elimination within 24 hours can lead to an encouraging trend toward a lower incidence of new-onset diabetes and less-severe diabetes, which may translate into a better recovery of patients postoperatively. At the end of the follow-up, the incidence of diabetes and hypertension were significantly lower in patients in the 24-h SA group versus the SP group ($P < .05$), proving the anticipated benefits of steroid minimization in lowering the risks of metabolic diseases. There was no significant difference in incidence of hyperlipidemia between the

Table 2. Hyperglycemia, insulin used, and hypertension within 7 days postoperation.

Group	Diabetes (cases, %)	Mean insulin consumed (U/d)	Hypertension (cases, %)
SP group	87.8 (36/41)	39.6 ± 7.9	46.3 (19/41)
SAA group	36.6 (15/41)	20.5 ± 6.7	41.5 (17/41)
Statistical value	$\chi^2 = 22.873$	$t = 5.732$	$\chi^2 = 0.198$
P value	< .001	< .05	.656

Abbreviations: SAA, steroid almost-avoidance; SP, standard protocol

Table 3. Comparison of posttransplant complications at the end of follow-up.

Group	Diabetes (cases, %)	Hypertension (cases, %)	Hyperlipidemia (cases, %)	BI (cases, %)	AR (cases, %)
SP group	21.9 (9/41)	21.9 (9/41)	12.2 (5/41)	39.0 (16/41)	7.3 (3/41)
SAA group	4.9 (2/41)	4.9 (2/41)	9.8 (4/41)	14.6 (6/41)	9.8 (4/41)
χ^2 value	5.145	5.145	0.125	6.212	0.156
P value	.023	.023	.724	.013	.693

Abbreviations: AR, acute rejection; BI, bacterial infections; SAA, steroid almost-avoidance; SP, standard protocol

groups, which does not agree with other studies.² Additional long-term follow-up will be required to fully evaluate the effects of this protocol on serum lipid profiles. The infection rate was significantly lower in the 24-h SA group compared to the SP group ($P < .05$), suggesting that steroid elimination within 24 hours may lower the infection rate in transplant recipients.

In summary, the 24-h SA group with basiliximab induction and tacrolimus maintenance is a safe and efficient immunosuppression strategy for ABO compatible liver transplant, which can significantly reduce posttransplant diabetes, hypertension, infections, and other complications owing to long-term use of steroids, without increased acute rejection rate. Considering the limitations of the retrospective design of the current study, a multicenter, prospective, randomized, and controlled study is required to confirm further the benefits of this regimen in liver transplant recipients.

References

1. HE Xiao-shun, Hu An-bin. Development of steroids protocol in liver transplantation, *Chinese J Surg*. 2008;46:1123-1125.
2. Toyoki Y, Hakamada K, Narumi S, et al. Primary immunosuppression regimen of rapid steroid withdrawal after living related liver transplantation: a single-center experience. *Transplant Proc*. 2004;36(8):2279-2281.
3. Innocenti F, Hepp J, Humeres R, et al. Rapid steroid taper and neoral monotherapy in liver transplantation in Chile: a step in the right direction? *Transplant Proc*. 2004;36(6):1675-1676.
4. Hu An-bin, He Xiao-shun, Wu Zhi-peng, et al. Clinical Evaluation of steroids withdrawal at 7 days postoperation in liver transplantation. *Chinese J Surg*. 2008;46:1126-1128.
5. Liu CL, Fan ST, Lo CM, et al. Interleukin-2 receptor antibody (basiliximab) for immunosuppressive induction therapy after liver transplantation: a protocol with early elimination of steroids and reduction of tacrolimus dosage. *Liver Transpl*. 2004;10(6):728-733.
6. Marubashi S, Dono K, Amano K, et al. Steroid-free living-donor liver transplantation in adults. *Transplantation*. 2005;80(5):704-706.
7. Ringe B, Braun F, Schütz E, et al. A novel management strategy of steroid-free immunosuppression after liver transplantation: efficacy and safety of tacrolimus and mycophenolate mofetil. *Transplantation*. 2001;71(4):508-515.
8. Lladó L, Xiol X, Figueras J, et al. Immunosuppression without steroids in liver transplantation is safe and reduces infection and metabolic complications: results from a prospective multicenter randomized study. *J Hepatol*. 2006;44(4):710-716. Erratum in: *J Hepatol*. 2006;45(1):166.