Intravenous Mycophenolate Mofetil with Low-Dose Oral Tacrolimus and Steroid Induction for Live Donor Liver Transplantation

Ashok Jain,[1] Ravi Mohanka,[1] Mark Orloff,[1] Peter Abt,[1] Randeep Kashyap,[1] Mark Kelley,[2] Kari Burlee,[1] Adel Bozorgzadeh[1]

Objectives: **Mycophenolate mofetil (MMF) is used in liver transplantation (LTx) to reduce rejection, nephrotoxicity, neurotoxicity, and the need for steroids. Lower trough concentrations and bioavailability have been reported with oral MMF in first week after LTx. These parameters improve after the first month postoperatively. Previously published studies have used oral formulations of MMF. In this study, we sought to examine survival, rejection, and nephrotoxicity rates using IV MMF in live donor liver transplantation (LDLT).**

Patients and Methods: **Twenty-eight patients (mean age, 50.1 years; 15 men, 13 women) were examined between January 2000 and January 2004 with a mean follow-up of 17 months for survival, rejection, and renal function.**

Results: **Four patients died at 2, 5, 8, and 18 months after LDLT from sepsis (n = 3) and recurrent hepatocellular carcinoma (n = 1). There were no retransplants; hence, patient and graft survival rates were the same (82.4%). Three patients (10.7%) experienced acute cellular rejection requiring treatment. The mean serum creatinine level prior to LDLT was 0.9 ± 0.4 mg/dL, which remained stable throughout the study. One patient required hemodialysis during the perioperative period for 8 days.**

Conclusions: **In the current study, we demonstrate a new strategy of IV MMF administration with low-dose tacrolimus that provides for lower rates of acute rejection, better preservation of renal function, and one that is better tolerated compared with historical treatments after LTx.**

Key words: *Immunosuppression, live donor liver transplantation, mycophenolate mofetil, nephrotoxicity, tacrolimus*

Owing to the shortage of available deceased donor organs, adult-to-adult live donor liver transplantation (LDLT) has become an accepted modality for treatment of patients with end-stage liver diseases.

During the last 25 years, calcineurin inhibitors (tacrolimus, cyclosporine) have primarily been the immunosuppressive agents used in solid organ transplantation [1]. The main adverse effects of these drugs are nephrotoxicity and neurotoxicity. Usually, these drugs are used with an initial bolus of steroid, after which, patients are subsequently weaned off in most cases. Various protocols have been developed to further reduce the rate of rejection, minimize nephrotoxicity, and allow withdrawal of steroids [2-6].

Mycophenolate mofetil (MMF) has a different mechanism of action than calcineurin inhibitors and does not have any proven nephrotoxicity [7, 8]. An oral formulation of MMF has been used for kidney transplantation (KTx) and liver transplantation (LTx). A prospective randomized multicenter trial for KTx in the United States and Europe and other multicenter trials in Europe, Canada, and Australia have compared oral MMF, cyclosporine, and steroid against cyclosporine, steroid, and azathioprine or placebo. These trials have shown significantly lower rates of rejection with MMF; however, improvement of patient and graft survival rates after kidney transplantation was not shown [8]. Similar combination therapies with oral MMF and either tacrolimus or cyclosporine have met with limited success in LTx. One of the main reasons may be that nearly 30% of

[1]*Department of Surgery, Division of Transplantation,* [2]*Department of Pharmacy, University of Rochester Medical Center, Box SURG, Rochester, NY, 14642, USA*

Acknowledgements: *We acknowledge the help of Dr. Manoj Purohit in finalizing this manuscript.*

Address reprint requests to: *Ashok Jain, MD, University of Rochester Medical Center, Department of Surgery, Division of Transplantation, Box SURG, 601 Elmwood Avenue, Rochester, NY 14642 USA*

Phone*: 00 585 275 29 24* ***Fax:*** *00 585 506 00 54* ***E-mail:*** *ashok_jain@urmc.rochester.edu*

Data were partly presented at the International Symposium on "Living Donor Organ Transplantation," in Essen, Germany, September 2-3, 2005, and the 11th meeting of the International Liver Transplantation Society in Los Angeles, Calif, USA, July 20-23, 2005.

Experimental and Clinical Transplantation (2005) 2: 361-365

liver transplant recipients have preexisting anemia, leucopenia, and/or thrombocytopenia and therefore are less likely to tolerate MMF. Also, gastrointestinal disturbances and infection rates are higher after LTx compared with after KTx. At the same time, the severity and rate of rejections are lower in LTx compared with KTx patients.

The Pittsburgh prospective randomized study comparing tacrolimus and steroid against tacrolimus, steroid, and MMF found a withdrawal rate of 58.9%. Although the rate of acute rejection in the first 3 months was lower with MMF, it was not significantly different during the second year. However, a reduced nephrotoxicity rate and a reduced dialysis need were clear in the postoperative period [9]. Similar withdrawal rates of MMF have been reported by Klupp and colleagues [10] and others [11-13]. When trough concentrations of MPA (mycophenolic acid, an active compound of MMF) were measured over time after oral MMF, they were found to increase with time after LTx [14]. In another pharmacokinetic study, a significantly progressive increase in bioavailability of MMF at 1 week, 2 weeks, and 4 weeks post-LTx was demonstrated [15]. So far, all published studies on MMF have been performed with the oral formulation. Based on this observation, a new strategy was developed at our institution to administer MMF intravenously (IV) instead of orally for the first 3-5 days after LTx with simultaneous use of low-dose oral tacrolimus. Initial observations from ongoing pharmacokinetic studies at our institution with IV and PO MMF, have shown that in LDLT patients, the mean area under the curve (AUC_{0-12}) with IV MMF was more than twice that of oral MMF (26.0 mg/L/hour vs 12.4 mg/L/hour). Also, the mean peak values of IV MPA were significantly higher compared with oral formulation (10.2 mg/L vs 3.0) [16].

Therefore, we sought to examine the role of IV MMF, low-dose oral tacrolimus, and steroid induction in adult-to-adult LDLT patients and to evaluate patient and graft survival, rate of rejection, and renal function.

Materials and Methods

Between January 2000 to January 2004, 115 patients underwent LDLT at our center. Of these, 28 patients (24.3%; 15 men, 13 women; mean age, 50.1 ± 12.4 years) received IV MMF. Patient data were retrospectively reviewed after receiving institutional review board approval. All patients were started on IV MMF (1 g b.i.d. for 3 to 5 days), oral tacrolimus (0.05-0.1 mg/kg/day in 2 divided doses), and steroid bolus. Subsequent adjustments in tacrolimus dosages were made to achieve trough concentrations of 8-10 ng/mL by the third or fourth postoperative day. Oral MMF was started once patients were able to tolerate an oral liquid diet. All patients received 1 g methylprednisolone intraoperatively followed by tapering doses of 600 mg over the next 5 days. After 5 days, 20 mg/day was given and gradually reduced to 5 mg/day by 3 to 6 months. All patients were followed until August 2004 with a mean follow-up of 17 ± 9.6 months. Demographics and diagnoses of the study patients are shown in Table 1. The causes of death, retransplantation, rate of rejection, renal function, and baseline immunosuppression were reviewed at various time points during the study.

Table 1. Demographics and diagnosis

Donor		
	Mean age	37.1 ± 9.3
Recipient		
	Mean age	50.1 ± 12.4
	Sex (male; female)	16; 13
	Mean MELD score	14.8 ± 6.1
Diagnosis		
Hepatitis C		5
Hepatitis C with hepatocellular carcinoma		1
Hepatitis B		1
Laënnec's cirrhosis		6
Primary biliary cirrhosis		5
Primary sclerosing cholangitis		3
Autoimmune		2
Cryptogenic		2
Hemochromatosis		2
Glycogen storage disorder		1

MELD: Model for end-stage liver disease

Statistical analyses

Results are expressed as means ± standard deviation. Patient and graft survival rates and freedom from rejection were estimated using the Kaplan-Meier formula.

Results

Patient and graft survival

Four patients died during follow-up at 2, 5, 8, and 18 months from LTx due to sepsis (n = 3) and recurrent hepatocellular carcinoma (n = 1). None of the patients underwent retransplantation; hence, patient and graft survival rates both were 82.4% at 18 months (Figure 1).

Rate of acute rejection

During follow-up, 17 liver biopsies were performed in 12 patients (42.8 %) as clinically indicated. Routine protocol liver biopsies were not performed. All biopsies were reported by the same pathologist.

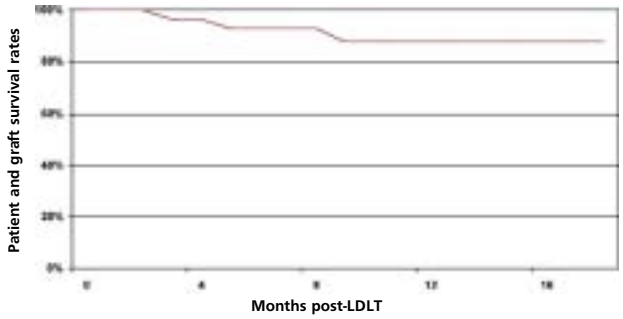

Figure 1. Patient and graft survival curves

Nine liver biopsies (52.9%) showed borderline rejection with a rejection activity index (RAI) of 2 or less. These patients were not treated for acute rejection.

Four biopsies in 3 patients (10.7%) showed acute rejection, the first patient had an RAI of 3-4, (2 weeks after LDLT) that responded to steroid bolus. The second patient had an RAI of 5 on biopsy (5 months after LDLT) and also responded to steroid bolus. The third patient had an RAI of 7 on the first biopsy (9 months after LDLT), which partially responded to steroid bolus, and repeat biopsy showed a decreased RAI of 5. Further response was achieved with steroid bolus. None of the grafts were lost to acute or chronic rejections. Overall freedom from rejection was 84.4% (Figure 2).

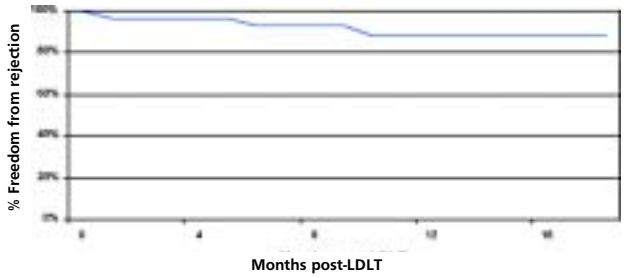

Figure 2. Freedom from rejection

Renal function

Mean serum creatinine prior to LDLT was 0.9 ± 0.4 mg/dL, which remained stable throughout the study period (Figure 3). One patient (3.5%) required hemodialysis for 8 days.

Immunosuppression

Mean tacrolimus dose was 5.8 mg/day at 1 month and remained almost unchanged throughout the first year. The mean trough tacrolimus concentration was 7.7 ± 2.9 ng/dL at 1 month and 7.3 ± 2.8 ng/dL at 12 months (Figure 4). Mean steroid dosage decreased from 10.1 ± 4.3 mg/day at 3 months to 6.0 ± 2.1 mg at 12 months. Of all patients, 58.3% were steroid free by the end of the first year (Table 2).

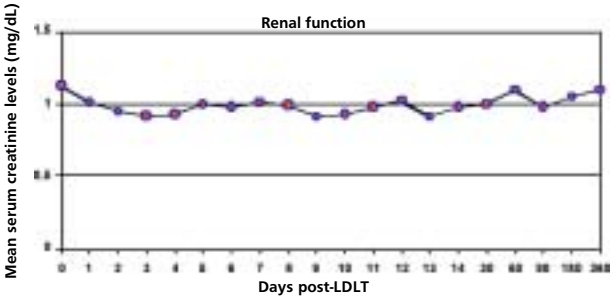

Figure 3. Mean serum creatinine during the study period

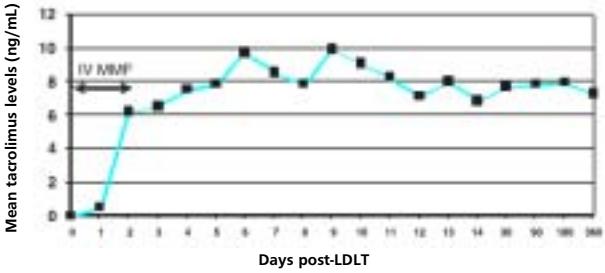

Figure 4. Trough tacrolimus levels

Table 2. Immunosuppression (months posttransplant)

	1	3	6	12
Tacrolimus dose (mg/day)	5.8 ± 4.0	5.1 ± 3.2	6.5 ± 3.8	6.7 ± 4.2
Tacrolimus level (ng/ml)	7.7 ± 2.9	7.9 ± 2.8	8.0 ± 3.0	7.3 ± 2.8
Prednisolone dose (mg/day)*	16.7 ± 4.6	10.1 ± 4.3	6.6 ± 2.8	6.0 ± 2.1
MMF dose (g/day)*	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	1.4 ± 0.5
Patients off MMF (%) #	20.8	29.2	39.1	56.5
Patients off steroids (%)	0	0	8.33	58.3

*Mean values of patients on medication at that time, MMF: Mycophenolate mofetil, # All patients discontinued after switching from IV to PO MMF

It is interesting to note that 20.8% of patients discontinued MMF by the end of 1 month while on oral therapy, and none did so while on IV MMF. The number of patients who discontinued oral MMF progressively increased to 56.5% by 12 months, the rate that is similar to that reported by others [10-13]. The mean MMF dose was 1.4 ± 0.5 m/day in patients who continued MMF.

Discussion

The perioperative course of post-LTx patients is much more complicated than in KTx patients. The majority of complications, including neurotoxicity, renal impairment, rejection, graft loss, and death occur

within the first few weeks of transplantation [1]. The introduction of cyclosporine in the early 1980s almost doubled patient and graft survival rates by reducing the number of grafts lost from immunologic causes [17, 18]. Furthermore, with the development of tacrolimus, immunological loss of liver allografts became extremely rare [19, 20]. However, in the early postoperative period, neurotoxicity and nephrotoxicity remain a major concern [21-25]. The need for a third immunosuppressive agent to reduce the dosage of calcineurin inhibitors and prevent these short- and long-term postoperative complications was of paramount importance, especially as increasing numbers of sicker patients, with greater degrees of pre-LTx renal impairment were undergoing transplantations [26].

With FDA approval of MMF in 1995, various immunosuppressive protocols using MMF and calcineurin inhibitors were developed. Several prospective studies with these protocols reported varying degrees of success with the drug. One of the main drawbacks with MMF was a high withdrawal rate due to preexisting or de novo hematologic and gastrointestinal disorders [9]. Studies from Pittsburgh have reported a lower trough concentration of MPA and lower bioavailability of the drug with oral MMF after LTx in the first few weeks [14, 15]. Pharmacokinetic studies in cirrhotic patients published by the manufacturer revealed 50% lower AUC in these patients than in healthy volunteers. Also, mean MPA AUCs in the early posttransplant period (< 40 days) were approximately 20%-41% lower than they were in the latter posttransplant period, and C_{max} levels were 32%-44% lower during the early period compared with the latter transplant period (3-6 months) [27]. All previous clinical studies on MMF have been published using the oral formulation. MPA drug concentration studies were not conducted in the past. Also, in KTx, dose finding studies have shown that a dose of less than 2 g was less effective [28]. Introduction of intravenous MMF provides an alternative solution and guarantees adequate exposure of the drug. Initial results from ongoing pharmacokinetic studies at our institution comparing IV MMF with oral MMF demonstrate that in live-donor liver transplant patients, the mean area under the curve (AUC_{0-12}) and peak MPA concentrations were significantly higher with IV MMF compared with oral MMF [16].

Interleukin-2 antibodies with calcineurin inhibitors have been successfully tried to preserve renal function [2, 3, 29, 30]. Also, the use of sirolimus with reduced doses of tacrolimus has shown initial promise [4]. Unfortunately, the multicenter randomized trial using sirolimus was halted owing to an increased rate of arterial thrombosis, thrombocytopenia, hyperlipidemia, delayed wound healing, and pneumonitis. At present, there is a black box warning by the FDA for 1 month after LTx, the critical time, when a nonnephrotoxic immunosuppressive agent is needed the most.

This leaves us with few options but to utilize MMF in a more meaningful way. With this background, using IV MMF may be a better therapeutic strategy. This is the first report on IV MMF with low-dose tacrolimus where low tacrolimus levels were maintained in the early post-LTx period and then allowed to rise slowly, to protect renal function. This is the lowest rate of acute rejection in the first 3 months reported so far in any series after LTx, despite sub-therapeutic tacrolimus levels for first 2-3 days after LTx with any immunosuppressive protocol [2, 4, 10, 13, 21, 22, 31-33]. Acute rejection rates varying from 25%- 60% have been reported in the first year after LTx in these studies. Also, a renal dysfunction rate of up to 27% requiring dialysis has been reported in these studies compared with a rate of only 4% for transient renal dysfunction in the present report. Higher creatinine levels at 1 year predict poor long-term patient survival and result in a significantly higher rate of renal failure [34, 35]. With presently available options, we feel that initial use of intravenous MMF, low-dose tacrolimus, and steroid induction provides an effective immunosuppressive strategy to reduce the rate of rejection and preserve renal function.

Conclusion

This is the first study using IV MMF and low-dose tacrolimus reporting effective immunosuppression with low rates of acute rejection as compared with historical data. This is due to the better bioavailability of IV MMF compared with oral MMF. Better MPA availability along with the use of subtherapeutic tacrolimus levels in the initial post-LTx period preserves renal function.

References

1. Jain A, Reyes J, Kashyap R, Dodson SF, Demetris AJ, Ruppert K, et al. Long-term survival after liver transplantation in 4,000 consecu-

tive patients at a single center. Ann Surg 2000; 232: 490-500
2. Emre S, Gondolesi G, Polat K, Ben-Haim M, Artis T, Fishbein TM, et al. Use of daclizumab as initial immunosuppression in liver transplant recipients with impaired renal function. Liver Transpl 2001; 7: 220-225
3. Marino IR, Doria C, Scott VL, Foglieni CS, Lauro A, Piazza T, et al. Efficacy and safety of basiliximab with a tacrolimus-based regimen in liver transplant recipients. Transplantation 2004; 78: 886-891
4. McAlister VC, Peltekian KM, Malatjalian DA, Colohan S, MacDonald S, Bitter-Suermann H, et al. Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. Liver Transpl 2001; 7: 701-708
5. Stegall MD, Wachs ME, Everson G, Steinberg T, Bilir B, Shrestha R, et al. Prednisone withdrawal 14 days after liver transplantation with mycophenolate: a prospective trial of cyclosporine and tacrolimus. Transplantation 1997; 64: 1755-1760
6. Tzakis AG, Tryphonopoulos P, Kato T, Nishida S, Levi DM, Madariaga JR, et al. Preliminary experience with alemtuzumab (Campath-1H) and low-dose tacrolimus immunosuppression in adult liver transplantation. Transplantation 2004; 77: 1209-1214
7. Allison AC, Almquist SJ, Muller CD, Eugui EM. In vitro immunosuppressive effects of mycophenolic acid and an ester pro-drug, RS-61443. Transplant Proc 1991; 23: 10-14
8. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation 1995; 60: 225-232
9. Jain A, Kashyap R, Dodson F, Kramer D, Hamad I, Khan A, et al. A prospective randomized trial of tacrolimus and prednisone versus tacrolimus, prednisone and mycophenolate mofetil in primary adult liver transplantation: a single center report. Transplantation 2001; 72: 1091-1097
10. Klupp J, Glanemann M, Bechstein WO, Platz KP, Langrehr JM, Keck H, et al. Mycophenolate mofetil in combination with tacrolimus versus Neoral after liver transplantation. Transplant Proc 1999; 31: 1113-1114
11. Eckhoff DE, McGuire BM, Frenette LR, Contreras JL, Hudson SL, Bynon JS. Tacrolimus (FK506) and mycophenolate mofetil combination therapy versus tacrolimus in adult liver transplantation. Transplantation 1998; 65: 180-187
12. Fisher RA, Stone JJ, Wolfe LG, Rodgers CM, Anderson ML, Sterling RK, et al. Four-year follow-up of a prospective randomized trial of mycophenolate mofetil with cyclosporine microemulsion or tacrolimus following liver transplantation. Clin Transplant 2004; 18: 463-472
13. Wiesner RH, Shorr JS, Steffen BJ, Chu AH, Gordon RD, Lake JR. Mycophenolate mofetil combination therapy improves long-term outcomes after liver transplantation in patients with and without hepatitis C. Liver Transpl 2005; 11: 750-759.
14. Jain A, Venkataramanan R, Hamad IS, Zuckerman S, Zhang S, Lever J, et al. Pharmacokinetics of mycophenolic acid after mycophenolate mofetil administration in liver transplant patients treated with tacrolimus. J Clin Pharmacol 2001; 41: 268-276.
15. Pisupati J, Jain A, Burckart G, Hamad I, Zuckerman S, Fung J, et al. Intraindividual and interindividual variations in the pharmacokinetics of mycophenolic acid in liver transplant patients. J Clin Pharmacol 2005; 45: 34-41
16. Jain AB, Mohanka R, Kwong T, Mack C, Nelson J, Orloff M, et al. Kinetics of IV mycophenolate mofetil (MMF) and oral MMF in live donor and deceased donor liver transplantation (LTx). Hepatology 2005; 42(suppl 1): 329A
17. Iwatsuki S, Starzl TE, Todo S, Gordon RD, Esquivel CO, Tzakis AG, et al. Experience in 1,000 liver transplants under cyclosporine-steroid therapy: a survival report. Transplant Proc 1988; 20 (1 Suppl 1): 498-504
18. Starzl TE, Iwatsuki S, Van Thiel DH, Gartner JC, Zitelli BJ, Malatack JJ, et al. Evolution of liver transplantation. Hepatology 1982; 2: 614-636
19. Jain A, Reyes J, Kashyap R, Rohal S, Abu-Elmagd K, Starzl T, et al. What have we learned about primary liver transplantation under tacrolimus immunosuppression? Long-term follow-up of the first 1000 patients. Ann Surg 1999; 230: 441-448; discussion 448-449
20. Jain A, Demetris AJ, Kashyap R, Blakomer K, Ruppert K, Khan A, et al. Does tacrolimus offer virtual freedom from chronic rejection after primary liver transplantation? Risk and prognostic factors in 1,048 liver transplantations with a mean follow-up of 6 years. Liver Transpl 2001; 7: 623-630
21. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. Lancet 1994; 344: 423-428
22. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. The U.S. Multicenter FK506 Liver Study Group. N Engl J Med 1994; 331: 1110-1115
23. McCauley J, Fung JJ, Brown H, Deballi P, Jain A, Todo S, et al. Renal function after conversion from cyclosporine to FK 506 in liver transplant patients. Transplant Proc 1991; 23: 3148-3149
24. Platz KP, Mueller AR, Blumhardt G, Bachmann S, Bechstein WO, Kahl A, et al. Nephrotoxicity after orthotopic liver transplantation in cyclosporin A and FK 506-treated patients. Transpl Int 1994; 7: S52-S57
25. Porayko MK, Gonwa TA, Klintmalm GB, Wiesner RH. Comparing nephrotoxicity of FK 506 and cyclosporine regimens after liver transplantation: preliminary results from US Multicenter trial. U.S. Multicenter Liver Study Group. Transplant Proc. 1995; 27: 1114-1116.
26. Yoo HY, Thuluvath PJ. Short-term postliver transplant survival after the introduction of MELD scores for organ allocation in the United States. Liver Int 2005; 25: 536-541
27. Roche_Pharmaceuticals. Cellcept Product Information. Available at:http://www.rocheusa.com/products/cellcept/pi.pdf Accessed: 24 October 2005
28. Sollinger HW, Deierhoi MH, Belzer FO, Diethelm AG, Kauffman RS. RS-61443–a phase I clinical trial and pilot rescue study. Transplantation 1992; 53: 428-432
29. Liu CL, Fan ST, Lo CM, Chan SC, Ng IO, Lai CL, 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: 728-733
30. Tector AJ, Fridell JA, Mangus RS, Shah A, Milgrom M, Kwo P, et al. Promising early results with immunosuppression using rabbit anti-thymocyte globulin and steroids with delayed introduction of tacrolimus in adult liver transplant recipients. Liver Transpl 2004; 10: 404-407
31. Busuttil RW, Lake JR. Role of tacrolimus in the evolution of liver transplantation. Transplantation 2004; 77: S44-S51
32. Jain AB, Fung JJ, Todo S, Alessiani M, Takaya S, Abu-Elmagd K, et al. Incidence and treatment of rejection episodes in primary orthotopic liver transplantation under FK 506. Transplant Proc 1991; 23 (1 Pt 2): 928-930
33. Neuhaus P, Langrehr JM, Williams R, Calne RY, Pichlmayr R, McMaster P. Tacrolimus-based immunosuppression after liver transplantation: a randomised study comparing dual versus triple low-dose oral regimens. Transpl Int 1997; 10: 253-261
34. Gonwa TA, Mai ML, Melton LB, Hays SR, Goldstein RM, Levy MF, et al. End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. Transplantation 2001; 72: 1934-1939
35. Gunning TC, Brown MR, Swygert TH, Goldstein R, Husberg BS, Klintmalm GB, et al. Perioperative renal function in patients undergoing orthotopic liver transplantation. A randomized trial of the effects of verapamil. Transplantation 1991; 51: 422-427