Table 3. Predictive factors associated with sustained virological response (SVR): Multivariate analysis.

		Odds Ratio	95% confidence intervals	P-value
HCV genotype	Non-1	1	-	-
	1	0.182	0.054–0.614	0.006
Serum HCV RNA pre-LT	<500 kIU/mL	1	-	-
	500 kIU/mL≤	0.310	0.130–0.742	0.009

HCV, hepatitis C virus; LT, liver transplantation.
doi:10.1371/journal.pone.0058380.t003

91 of leucine or methionine with VR and SVR were analyzed in 40 patients, whose pre-treatment sera were stored (Table 4). As a result, substitutions of both aa 70 and aa 91 were not significantly associated with VR and SVR.

Predictors of Withdrawal from Therapy

Predictive factors for withdrawal from the treatment protocol were evaluated by comparing 26 patients who withdrew from the treatment protocol and the patients who completed the treatment including patients with SVR, patients who relapsed, and NR. None of the variables analyzed had a significant effect on withdrawal (Data not shown).

Discussion

In this study, we identified 2 independent predictors of SVR in patients with recurrent hepatitis C after LDLT by multivariate analysis: A non-1 HCV genotype and pretransplant serum HCV-RNA levels lower than 500 kIU/mL. The same factors were identified as predictors for VR, which purely indicates response to interferon therapy, by excluding the influences of the premature termination of the therapy and virological relapse after termination of the treatment. In addition, an ABO-incompatible LDLT was identified as an independent variable predicting VR.

In non-transplant settings, pretreatment predictors of response to interferon therapy have been analyzed in many studies, and the viral genotype and pretreatment viral load have been almost invariably shown to be 2 major predictors of SVR [41,42,43,44]. SVR rates were higher in patients infected with a non-1 HCV genotype and in those with a low pretreatment viral load. These 2 factors have been also identified in several reports [16,17,18,19] as factors predicting SVR in patients with recurrent hepatitis C after DDLT. In the present study, a non-1 HCV genotype was again identified as an independent predictive factor for both VR and SVR in patients with recurrent hepatitis C after LDLT by multivariate analysis. A pretreatment viral load <5000 kIU/mL was also a significant predictive factor by univariate analysis, but it was not an independently associated variable by multivariate analysis. On the other hand, pretransplant viral load was identified as an independent variable predictive of both VR and SVR by multivariate analysis.

While reports of factors that can control viral load exist, the mechanism by which serum HCV-RNA levels are regulated has not yet been completely clarified. A correlation between mutations in the ISDR sequence in the NS5A region of the HCV genome and serum HCV RNA levels has been reported. We did not analyze this viral factor in the current study; however, it is possible that the HCV genome sequence determines both pretransplant viremia and response to interferon therapy. The host polymorphism in IL28B, which was identified as a strong predictor of virological response to interferon therapy in patients with hepatitis C, was recently reported to be associated with baseline viral load [26,45]. The allele associated with a better treatment response is associated with a higher baseline viral load. This finding does not correspond with our results showing that a low HCV load predicts a better response to treatment. We speculate that the balance between host immunity and HCV replication regulates the serum HCV load, and that this balance also determines VR. As pretreatment viral load in post-transplant patients is influenced by immunosuppressive agents, the original host-virus balance

Table 4. Association of amino acid substitutions in the core region with virorogical response (VR) and sustained VR (SVR) in 40 patients infected with HCV genotype 1b: Univariate analysis.

		VR	non-VR	*P*	SVR	non-SVR	*P*
		n = 22	n = 13		n = 14	n = 24	
Core aa 70	Arg	9 (75%)	3 (25%)	0.289	7 (50%)	7 (50%)	0.204
	Gln/His	13 (57%)	10 (43%)		7 (29%)	17 (71%)	
Core aa 91	Leu	14 (64%)	8 (36%)	0.902	9 (38%)	15 (63%)	0.912
	Met	8 (62%)	5 (38%)		5 (36%)	9 (64%)	
Core aa 70 and 91	70 Arg and 91 Leu	6 (67%)	3 (33%)	0.784	5 (50%)	5 (50%)	0.320
	Others	16 (62%)	10 (38%)		9 (32%)	19 (68%)	
Core aa 70 and 91	70 Gln/His and 91 Met	5 (50%)	5 (50%)	0.324	3 (30%)	7 (70%)	0.603
	Others	17 (68%)	8 (32%)		11 (39%)	17 (61%)	

NOTE. Data are shown in number. P-values are calculated by Wald test for logistic regression analysis.
Arg, Arginine; Gln, glutamine; His, histidine; Leu, leucine; Met, methionine.
doi:10.1371/journal.pone.0058380.t004

would be reflected better by serum HCV levels before transplantation than by those after transplantation. It is unclear whether this result is specific to LDLT or holds true for both DDLT and LDLT. The significance of pretransplant viral load in DDLT as a predictor for virological response to post-transplant interferon therapy has not been analyzed in most previous studies [10]. Further analysis in patients who receive DDLT could help clarify the underlying mechanism.

Liver transplantation across the ABO blood-type barrier (ABO-incompatible) is generally contraindicated because of the possibility of graft loss caused by antibody-mediated rejection and is performed under exceptional circumstances as a rescue option in an emergent situation. However, ABO-incompatible LDLT has been performed in Japan to overcome organ shortage problems. Recently, rituximab prophylaxis and local infusion of prostaglandin E1 and steroids were established as therapeutic measures for recipients who underwent ABO-incompatible LDLT, and these treatments improved outcomes [46]. Interestingly, in this study, we found that an ABO-mismatched donor is associated with VR to interferon therapy. The reason for this interesting finding is unclear, but it is possible that either subclinical antibody-mediated rejection or drugs such as rituximab and prostaglandin E1 used in ABO-incompatible recipients may contribute to the higher VR to interferon therapy. There is hope that future studies to clarify the basic mechanism underlying this result will lead to a novel strategy to improve the efficacy of interferon therapy in patients with hepatitis C.

Amino acid substitutions of core region of HCV were not associated with treatment response in our analysis. We do not know the reason for the difference of impact of substitution of core aa 70 and aa 91 on virological response to interferon therapy from a previous report, in which SVR rate were significantly higher in transplant recipients with aa 70 of arginine and aa 91 of leucine of core region of HCV [33]. As sample size of both the previous study and our present study are small, and our present study did not assess the other HCV RNA mutations, including ISDR [32] and interferon/ribavirin resistance-determining region [47] in NS5A, and IL28B polymorphism in recipients and donors, further analysis should be required in larger cohorts.

Another aim of this study was to identify predictive variables for adverse events during interferon therapy, but none of the studied factors proved to be statistically significant predictors of withdrawal from the treatment protocol. As patients withdrew from the treatment for diverse reasons, it would be difficult to predict each adverse event before the initiation of interferon therapy. Therefore, careful follow-up during the treatment procedure is important for early detection of adverse events and to prevent progression to severe complications.

In this study, the final outcomes of the treatment including standard interferon plus ribavirin and peginterferon plus ribavirin were analyzed. Difference of the efficacy between standard interferon and peginterferon might affect the results of our present study. We predicted that patients who had virological response to standard interferon would also show the same response to peginterferon, because it is reported that the efficacy of peginterferon plus ribavirin is higher than that of standard interferon plus ribavirin [44,48]. Accordingly, the patients who achieved SVR by standard interferon were included in the present study. On the other hand, all nonresponders and all patients who relapsed by standard interferon plus ribavirin were retreated with peginterferon plus ribavirin, and we analyzed the final outcomes of the peginterferon plus ribavirin therapy. Therefore, we conclude that the difference of treatment regimen has little influence on our results.

In conclusion, SVR to antiviral therapy in patients with recurrent hepatitis C after LDLT is predictable before transplant by serum HCV-RNA level and HCV genotype. In addition, patients who undergo ABO-incompatible LDLT appear to have a better VR to interferon therapy after liver transplantation. Mechanisms underlying these interesting results are unknown at present, but these findings are likely to be useful for improved clinical assessment of patients with hepatitis C after liver transplantation, and could lead to development of new strategies for better outcomes in LDLT recipients with the HCV genotype 1 and/or a higher pretransplant viral load.

Author Contributions

Conceived and designed the experiments: YU HM. Performed the experiments: YU TK YO KO AY KH YF AMH HH HM. Analyzed the data: YU ST. Contributed reagents/materials/analysis tools: YU TK YO KO AY KH YF AMH HH HM. Wrote the paper: YU HM SU TC.

References

1. Berenguer M, Prieto M, San Juan F, Rayon JM, Martinez F, et al. (2002) Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. Hepatology 36: 202–210.
2. Feray C, Caccamo L, Alexander GJ, Ducot B, Gugenheim J, et al. (1999) European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. European Concerted Action on Viral Hepatitis (EUROHEP) Group. Gastroenterology 117: 619–625.
3. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR (2002) The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 122: 889–896.
4. Gane E (2003) The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. Liver Transpl 9: S28–34.
5. Prieto M, Berenguer M, Rayon JM, Cordoba J, Arguello L, et al. (1999) High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 29: 250–256.
6. Sanchez-Fueyo A, Restrepo JC, Quinto L, Bruguera M, Grande L, et al. (2002) Impact of the recurrence of hepatitis C virus infection after liver transplantation on the long-term viability of the graft. Transplantation 73: 56–63.
7. Velidedeoglu E, Mange KC, Frank A, Abt P, Desai NM, et al. (2004) Factors differentially correlated with the outcome of liver transplantation in hcv+ and HCV- recipients. Transplantation 77: 1834–1842.
8. Gordon FD, Kwo P, Vargas HE (2009) Treatment of hepatitis C in liver transplant recipients. Liver Transpl 15: 126–135.
9. Terrault NA (2008) Hepatitis C therapy before and after liver transplantation. Liver Transpl 14 Suppl 2: S58–66.
10. Berenguer M (2008) Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. J Hepatol 49: 274–287.
11. Berardi S, Lodato F, Gramenzi A, D'Errico A, Lenzi M, et al. (2007) High incidence of allograft dysfunction in liver transplanted patients treated with pegylated-interferon alpha-2b and ribavirin for hepatitis C recurrence: possible de novo autoimmune hepatitis? Gut 56: 237–242.
12. Fernandez I, Ulloa E, Colina F, Abradelo M, Jimenez C, et al. (2009) Incidence, risk factors, and outcome of chronic rejection during antiviral therapy for posttransplant recurrent hepatitis C. Liver Transpl 15: 948–955.
13. Stanca CM, Fiel MI, Kontorinis N, Agarwal K, Emre S, et al. (2007) Chronic ductopenic rejection in patients with recurrent hepatitis C virus treated with pegylated interferon alfa-2a and ribavirin. Transplantation 84: 180–186.
14. Berenguer M, Palau A, Fernandez A, Benlloch S, Aguilera V, et al. (2006) Efficacy, predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. Liver Transpl 12: 1067–1076.
15. Carrion JA, Navasa M, Garcia-Retortillo M, Garcia-Pagan JC, Crespo G, et al. (2007) Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. Gastroenterology 132: 1746–1756.
16. Neumann U, Puhl G, Bahra M, Berg T, Langrehr JM, et al. (2006) Treatment of patients with recurrent hepatitis C after liver transplantation with peginterferon alfa-2B plus ribavirin. Transplantation 82: 43–47.
17. Oton E, Barcena R, Moreno-Planas JM, Cuervas-Mons V, Moreno-Zamora A, et al. (2006) Hepatitis C recurrence after liver transplantation: Viral and

histologic response to full-dose PEG-interferon and ribavirin. Am J Transplant 6: 2348–2355.
18. Picciotto FP, Tritto G, Lanza AG, Addario L, De Luca M, et al. (2007) Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. J Hepatol 46: 459–465.
19. Rodriguez-Luna H, Khatib A, Sharma P, De Petris G, Williams JW, et al. (2004) Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. Transplantation 77: 190–194.
20. Sharma P, Marrero JA, Fontana RJ, Greenson JK, Conjeevaram H, et al. (2007) Sustained virologic response to therapy of recurrent hepatitis C after liver transplantation is related to early virologic response and dose adherence. Liver Transpl 13: 1100–1108.
21. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, et al. (2011) Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 364: 1207–1217.
22. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, et al. (2011) Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 364: 2405–2416.
23. Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, et al. (2011) Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 364: 1195–1206.
24. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, et al. (2011) Telaprevir for retreatment of HCV infection. N Engl J Med 364: 2417–2428.
25. Garg V, van Heeswijk R, Lee JE, Alves K, Nadkarni P, et al. (2011) Effect of telaprevir on the pharmacokinetics of cyclosporine and tacrolimus. Hepatology 54: 20–27.
26. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, et al. (2009) Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461: 399–401.
27. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, et al. (2009) IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41: 1100–1104.
28. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, et al. (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41: 1105–1109.
29. Charlton MR, Thompson A, Veldt BJ, Watt K, Tillmann H, et al. (2011) Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. Hepatology 53: 317–324.
30. Fukuhara T, Taketomi A, Motomura T, Okano S, Ninomiya A, et al. (2010) Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. Gastroenterology 139: 1577–1585, 1585 e1571–1573.
31. Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, et al. (2005) Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48: 372–380.
32. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, et al. (1996) Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334: 77–81.
33. Fukuhara T, Taketomi A, Okano S, Ikegami T, Soejima Y, et al. (2010) Mutations in hepatitis C virus genotype 1b and the sensitivity of interferon-ribavirin therapy after liver transplantation. J Hepatol 52: 672–680.
34. Ueda Y, Takada Y, Haga H, Nabeshima M, Marusawa H, et al. (2008) Limited benefit of biochemical response to combination therapy for patients with recurrent hepatitis C after living-donor liver transplantation. Transplantation 85: 855–862.
35. Ueda Y, Takada Y, Marusawa H, Egawa H, Uemoto S, et al. (2010) Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. Transplantation 90: 661–665.
36. Ueda Y, Marusawa H, Kaido T, Ogura Y, Oike F, et al. (2010) Effect of maintenance therapy with low-dose peginterferon for recurrent hepatitis C after living donor liver transplantation. J Viral Hepat.
37. Bedossa P, Poynard T (1996) An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24: 289–293.
38. Poynard T, Bedossa P, Opolon P (1997) Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 349: 825–832.
39. Raut V, Mori A, Kaido T, Ogura Y, Taku I, et al. (2012) Splenectomy does not offer immunological benefits in ABO-incompatible liver transplantation with a preoperative rituximab. Transplantation 93: 99–105.
40. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, et al. (1997) New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 35: 201–207.
41. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, et al. (2002) Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347: 975–982.
42. Ghany MG, Strader DB, Thomas DL, Seeff LB (2009) Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 49: 1335–1374.
43. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, et al. (2004) Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 140: 346–355.
44. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, et al. (2001) Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 358: 958–965.
45. Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, et al. (2010) Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139: 120–129 e118.
46. Egawa H, Teramukai S, Haga H, Tanabe M, Fukushima M, et al. (2008) Present status of ABO-incompatible living donor liver transplantation in Japan. Hepatology 47: 143–152.
47. El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, et al. (2008) Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 48: 38–47.
48. Triantos C, Samonakis D, Stigliano R, Thalheimer U, Patch D, et al. (2005) Liver transplantation and hepatitis C virus: systematic review of antiviral therapy. Transplantation 79: 261–268.

Hepatology Research

Hepatology Research 2013; 43: 67–71 doi: 10.1111/j.1872-034X.2012.01020.x

Original Article

Efficacy and safety of prophylaxis with entecavir and hepatitis B immunoglobulin in preventing hepatitis B recurrence after living-donor liver transplantation

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Aim: Hepatitis B recurrence after liver transplantation can be reduced to less than 10% by combination therapy with lamivudine (LAM) and hepatitis B immunoglobulin (HBIG). The aim of this study was to evaluate the efficacy and safety of prophylaxis with entecavir (ETV), which has higher efficacy and lower resistance rates than LAM, combined with HBIG in preventing hepatitis B recurrence after living-donor liver transplantation (LDLT).

Methods: Twenty-six patients who received ETV plus HBIG (ETV group) after LDLT for hepatitis B virus (HBV)-related end-stage liver disease were analyzed by comparing with 63 control patients who had received LAM plus HBIG (LAM group).

Results: The survival rates of the patients treated with ETV plus HBIG was 73% after both 1 and 3 years, and there was no statistical difference between the patients in the ETV group and LAM group. No HBV recurrence was detected during the median follow-up period of 25.1 months in the ETV group, whereas the HBV recurrence rate was 4% at 3 years and 6% at 5 years in the LAM group. No patients had adverse effects related to ETV administration.

Conclusion: ETV combined with HBIG provides effective and safe prophylaxis in preventing hepatitis B recurrence after LDLT.

Key words: entecavir, hepatitis B, liver transplantation, living donor

INTRODUCTION

THE RECURRENCE OF hepatitis B virus (HBV) infection after liver transplantation for HBV-related diseases resulted in poor outcomes before the development of effective prophylaxis with lamivudine (LAM) and hepatitis B immunoglobulin (HBIG). Without the prophylaxis, the majority of patients developed recurrent infections due to HBV in the early phases after liver transplantation, and the recurrence resulted in rapidly progressive liver injury, early graft loss and reduced survival.[1–3] The development of prophylaxis dramatically reduced the post-transplant recurrence of hepatitis B and markedly improved prognosis. The most widely used prophylaxis so far has been a combination therapy of LAM and i.v. HBIG.

In the non-transplant setting, the long-term use of LAM resulted in high rates of emergence of resistance to the drug, with rates ranging 14–32% after 1 year and 60–70% after 5 years of treatment. In most cases, the resistance was the result of selection of LAM-resistant mutations in the YMDD motif of the DNA polymerase domain of HBV.[4] Moreover, the emergence of HBV strains with mutations that allow escape from hepatitis B surface antibody (anti-HBs) recognition has been reported in patients vaccinated for HBV,[5,6] in patients with chronic hepatitis B[7,8] and in liver transplant recipients after HBIG administration.[9–11] Therefore, the emergence of LAM resistance and HBIG resistance might

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Received 13 February 2012; revision 6 March 2012; accepted 28 March 2012.

increase the risk of recurrence during long-term administration of LAM and HBIG, although the rate of HBV recurrence in liver transplant recipients who received prophylaxis with LAM and HBIG for more than 10 years has not been reported to date. At present, several nucleoside analogs are available for the treatment of chronic hepatitis B[4]. Among them, there is entecavir (ETV), a carbocyclic analogue of 2′-deoxyguanosine, which has been shown to have higher efficacy than LAM in patients with chronic hepatitis B. In addition, ETV has a higher genetic barrier to resistance than LAM. The resistance to ETV requires at least three mutations including rtM204V/I, which causes LAM-resistance, rtL180M, and a mutation at one of the following codons: rtT184, rtS202 or rtM250.[4] Therefore, ETV is now used as a first-line therapy in the treatment of chronic hepatitis B worldwide. Data available in the published work suggest that, in transplant recipients, ETV plus HBIG represents a better prophylaxis protocol than LAM plus HBIG for long-term prevention of HBV recurrence after liver transplantation. However, the efficacy and safety of this treatment is largely unknown.

The aim of this study was to evaluate the efficacy and safety of prophylaxis with ETV and HBIG in preventing hepatitis B recurrence after living-donor liver transplantation (LDLT).

METHODS

Patients

WE RETROSPECTIVELY ANALYZED the medical records of 97 patients who underwent LDLT for HBV-related end-stage liver diseases from September 2002 to December 2010. Of these, eight patients were excluded from our study because they had breakthrough hepatitis due to HBV with LAM-resistant mutations and were prescribed LAM plus adefovir before liver transplantation. Accordingly, 89 patients were enrolled in this study.

Prophylaxis with ETV or LAM combined with HBIG

Lamivudine plus HBIG therapy was given to all recipients with HBV-related end-stage liver diseases from September 2002 to November 2006, as reported previously.[12] From December 2006, we changed the protocol for prophylaxis to ETV plus HBIG. ETV at a dose of 0.5 mg/day or LAM at a dose of 100 mg/day was given before transplantation, usually when the patient was referred to the hospital and scheduled for transplantation. Preoperative ETV or LAM prophylaxis was followed by combination with HBIG after transplantation. The first application of HBIG at a dose of 200 IU/kg body mass was administrated i.v. during the anhepatic phase of LDLT, and repeated every day for the first 5 days post-surgery. HBV serological markers were examined at weekly intervals for the first 2 months after the transplant, then at monthly intervals, and 1000 IU of HBIG was periodically administrated to maintain the serum anti-HBs titers at more than 500 IU/L during the first 6 months and 200 IU/L thereafter throughout the follow-up period.[12]

Immunosuppression

Tacrolimus and low-dose steroid therapy were administrated to induce immunosuppression in most patients.[13] Mycophenolate mofetil was administrated to patients who experienced refractory rejection or required reduction of tacrolimus dose due to adverse events. Patients who received ABO blood-type-incompatible transplants were treated with rituximab, plasma exchange, and hepatic artery or portal vein infusion with prostaglandin E1 and methylprednisolone.[14]

Diagnosis of HBV activation

Activation of HBV was diagnosed when hepatitis B surface antigens (HBsAg) and/or HBV DNA became positive in the serum of the patients. After LDLT, HBsAg, anti-HBs and serum HBV DNA were measured at least at 3 monthly intervals. Serological HBV markers, including HBsAg, anti-HBs, hepatitis B core antibody, hepatitis B e antigen (HBeAg) and antibodies to HBeAg (anti-HBe), were measured by chemiluminescent enzyme immunoassay (Fuji Rebio, Tokyo, Japan). Serum HBV DNA titer was analyzed using a commercial polymerase chain reaction (PCR) assay (Amplicor HBV Monitor; Roche, Branchburg, NJ, USA). LAM-resistant YMDD mutant virus was detected by the PCR enzyme-linked mini-sequence assay.[15]

Statistical analysis

Baseline characteristics are shown in Table 1. For continuous variables, medians and ranges are given, and the significance of the data was analyzed with the Wilcoxon rank sum test. For categorical variables, counts are given, and the data were analyzed with the χ^2-test. Survival rates and the rates of patients who showed HBV activation after LDLT were estimated using the Kaplan–Meier method and compared using log–rank tests. $P < 0.05$ was considered significant.

Table 1 Baseline characteristics of 90 patients

	Entecavir + HBIG (n = 26)	Lamivudine + HBIG (n = 63)	P-value
Age (years)	55 (33–68)	53 (26–64)	0.062†
Men/women	19/7	46/17	0.995‡
Primary disease			0.595‡
Acute liver failure	6 (23%)	9 (14%)	
Liver cirrhosis, HCC$^-$	6 (23%)	20 (32%)	
Liver cirrhosis, HCC$^+$	14 (54%)	34 (54%)	
HBV markers before LDLT			
HBsAg$^+$	24 (92%)	61 (97%)	0.350‡
HBeAg$^+$	6 (23%)	18 (29%)	0.595‡
HBV DNA before LDLT	<2.6 (<2.6–7.6<)	3.7 (<2.6–7.6<)	0.010†
<2.6 log IU/mL	14 (54%)	19 (30%)	0.024‡
Follow-up period (months)	25.1 (0.2–58.6)	70.6 (0.5–109.2)	<0.001†

Qualitative variables are shown in number; and quantitative variables expressed as median (range).
†Wilcoxon rank sum test.
‡χ^2-Test.
HBeAg, hepatitis B e antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LDLT, living-donor liver transplantation.

RESULTS

Patient characteristics

TWENTY-SIX PATIENTS who received ETV plus HBIG (ETV group) after LDLT for HBV-related end-stage liver disease were included in this study. Baseline characteristics of these patients are listed in Table 1 and compared with those of 63 control recipients who received LAM plus HBIG (LAM group) at our institute already present in our database. The two groups of patients did not differ significantly by age, sex, primary diseases or serological markers for HBV before LDLT. Serum HBV DNA levels before LDLT were significantly lower in the ETV group than in the LAM group. Fourteen of 26 patients (54%) showed less than 2.6 log IU/mL of serum HBV DNA in the ETV group. Median follow-up period was 25.1 months (range, 0.2–58.6) in the ETV group, whereas it was 70.6 months (range, 0.5–109.2) in the LAM group.

Efficacy and safety of prophylaxis with ETV plus HBIG

Survival rates of the patients treated with ETV plus HBIG estimated by Kaplan–Meier analysis was 73% at both 1 and 3 years (Fig. 1a). There was no difference between the ETV group and the LAM group, in which survival rates were 81% at 1 year, 78% at 3 years and 73% at

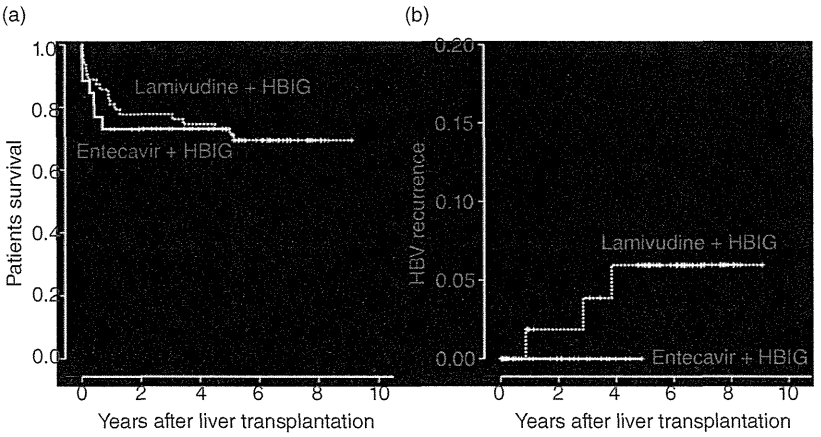

Figure 1 (a) Post-transplantation survival rates and (b) hepatitis B virus (HBV) recurrence after living-donor liver transplantation in HBV positive recipients who received entecavir and hepatitis B immunoglobulin (HBIG) (solid line), or lamivudine and HBIG (dotted line), estimated by Kaplan–Meier method.

5 years. Causes of death in patients in the ETV group were pneumonia ($n = 2$), sepsis ($n = 1$), pulmonary hemorrhage ($n = 1$), cerebral hemorrhage ($n = 1$), graft liver failure ($n = 1$) and multiple organ failure ($n = 1$), none of which were related to ETV. No HBV recurrence was detected in the median follow-up period of 25.1 months in the ETV group, whereas the HBV recurrence rate was 2% at 1 year, 4% at 3 years and 6% at 5 years in the LAM group (Fig. 1b). Three patients in the LAM group had HBV recurrence at 10, 34 and 46 months after LDLT. The emergence of HBV with LAM-resistant mutations in the YMDD motif was confirmed in two of the three patients. HBV mutations of another patient could not be determined because of the low level of serum HBV DNA. As the follow-up period of the ETV group was shorter than that of the LAM group and the HBV recurrence in the LAM group occurred in long-term follow-up after LDLT, the rate of HBV recurrence was not significantly different between the ETV and LAM groups. No patients had adverse events due to ETV administration.

DISCUSSION

IN THIS STUDY, we demonstrated that ETV combined with HBIG provides effective and safe prophylaxis in preventing hepatitis B recurrence after LDLT.

Two studies of patients receiving a combination of ETV and HBIG after liver transplantation have been previously reported.[16,17] One study demonstrated that 30 recipients who received ETV plus HBIG prophylaxis had no recurrence of HBV and no adverse effect relating to ETV.[17] The other study showed that no HBV recurrence was observed in two recipients with HBV-associated cirrhosis receiving ETV, tenofovir and HBIG.[16] Both studies showed the efficacy and safety of prophylaxis with ETV and HBIG in preventing short-term recurrence of HBV after liver transplantation. The current study confirmed their results for longer follow-up periods. Our results showed that prophylaxis with ETV and HBIG has similar efficacy and safety to that with LAM and HBIG, but did not show any further advantage of ETV compared to LAM treatment. Longer follow up might be needed to reveal the difference of HBV recurrence rate. One characteristic of our present report is that all patients in this study underwent LDLT. Our results suggest that prophylaxis with ETV and HBIG in patients after LDLT has similar efficacy and safety to patients after deceased-donor liver transplantation demonstrated in the previous reports.[16,17] More recently, efficacy of ETV monotherapy in preventing recurrence of HBV for liver transplant recipients with chronic hepatitis B was reported.[18] The study demonstrated that most patients showed disappearance of HBsAg and undetectable serum HBV DNA after liver transplantation without HBIG. Although long-term efficacy of ETV monotherapy needs be confirmed, both our data and previous reports suggest that ETV is an effective and safe antiviral agent in the post-transplant setting.

ACKNOWLEDGMENTS

THIS WORK WAS supported by Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (no. 21229009 and 23590972), Health and Labor Sciences Research Grants for Research on Intractable Diseases, and Research on Hepatitis from the Ministry of Health, Labor and Welfare, Japan, and a grant from Bristol-Myers-Squibb.

REFERENCES

1 Davies SE, Portmann BC, O'Grady JG *et al.* Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology* 1991; **13**: 150–7.
2 O'Grady JG, Smith HM, Davies SE *et al.* Hepatitis B virus reinfection after orthotopic liver transplantation. Serological and clinical implications. *J Hepatol* 1992; **14**: 104–11.
3 Todo S, Demetris AJ, Van Thiel D, Teperman L, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. *Hepatology* 1991; **13**: 619–26.
4 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507–39.
5 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325–9.
6 Hsu HY, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; **30**: 1312–7.
7 Kohno H, Inoue T, Tsuda F, Okamoto H, Akahane Y. Mutations in the envelope gene of hepatitis B virus variants co-occurring with antibody to surface antigen in sera from patients with chronic hepatitis B. *J Gen Virol* 1996; **77** (Pt 8): 1825–31.
8 Yamamoto K, Horikita M, Tsuda F *et al.* Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; **68**: 2671–6.

9 Carman WF, Trautwein C, van Deursen FJ *et al.* Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996; **24**: 489–93.
10 Ghany MG, Ayola B, Villamil FG *et al.* Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. *Hepatology* 1998; **27**: 213–22.
11 Ueda Y, Marusawa H, Egawa H *et al.* De novo activation of HBV with escape mutations from hepatitis B surface antibody after living donor liver transplantation. *Antivir Ther* 2011; **16**: 479–87.
12 Ali H, Egawa H, Uryuhara K *et al.* Prevention of hepatitis B virus recurrence after living donor liver transplantation. *Transplant Proc* 2004; **36**: 2764–7.
13 Ueda Y, Takada Y, Haga H *et al.* Limited benefit of biochemical response to combination therapy for patients with recurrent hepatitis C after living-donor liver transplantation. *Transplantation* 2008; **27** (85): 855–62.
14 Raut V, Mori A, Kaido T *et al.* Splenectomy does not offer immunological benefits in ABO-incompatible liver transplantation with a preoperative rituximab. *Transplantation* 2012; **15** (93): 99–105.
15 Kobayashi S, Shimada K, Suzuki H *et al.* Development of a new method for detecting a mutation in the gene encoding hepatitis B virus reverse transcriptase active site (YMDD motif). *Hepatol Res* 2000; **17**: 31–42.
16 Jimenez-Perez M, Saez-Gomez AB, Mongil Poce L, Lozano-Rey JM, de la Cruz-Lombardo J, Rodrigo-Lopez JM. Efficacy and safety of entecavir and/or tenofovir for prophylaxis and treatment of hepatitis B recurrence post-liver transplant. *Transplant Proc* 2010; **42**: 3167–8.
17 Xi ZF, Xia Q, Zhang JJ *et al.* The role of entecavir in preventing hepatitis B recurrence after liver transplantation. *J Dig Dis* 2009; **10**: 321–7.
18 Fung J, Cheung C, Chan SC *et al.* Entecavir monotherapy is effective in suppressing hepatitis B virus after liver transplantation. *Gastroenterology* 2011; **141**: 1212–9.

LIVER TRANSPLANTATION 19:896–906, 2013

ORIGINAL ARTICLE

Randomized, Multicenter Trial Comparing Tacrolimus Plus Mycophenolate Mofetil to Tacrolimus Plus Steroids in Hepatitis C Virus–Positive Recipients of Living Donor Liver Transplantation

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The purpose of this prospective, randomized, multicenter trial was to evaluate the effects of a steroid-avoiding immunosuppression protocol on hepatitis C virus (HCV)–positive recipients of living donor liver transplantation (LDLT). Seventy-five HCV-positive LDLT recipients were included in this study, and they were randomized to receive tacrolimus (TAC) plus a corticosteroid (ST; n = 35) or TAC plus mycophenolate mofetil (MMF; n = 40). Biopsy-proven acute rejection (BPAR) was treated with steroid pulse therapy in both groups. Protocol biopsy was performed 3, 6, and 12 months after LDLT and annually thereafter. Histological recurrence of HCV (fibrosis stage $\geq$ F1 according to the METAVIR score), BPAR resistant to 2 sets of steroid pulse therapy, hepatocellular carcinoma (HCC) recurrence, retransplantation, and patient death were defined as events, and the primary endpoint was event-free survival. The median follow-up was 55 months. The event-free survival rates at 1, 3, and 5 years were 38.2%, 11.8%, and 5.9%, respectively, for the ST group and 25.0%, 17.5%, and 14.6%, respectively, for the MMF group ($P = 0.45$). The overall 5-year patient survival rates were similar for the ST group (82.7%) and the MMF group (81.0%, $P = 0.28$). Steroid-resistant BPAR occurred in only 1 patient from the MMF group. HCC recurrence occurred for 1 patient from the ST group and 2 patients from the MMF group. HCV recurrence rates with a fibrosis stage $\geq$ F1 1 and 3 years after LDLT were 59.4% and 85.9%, respectively, for the ST group and 74.2% and 81.9%, respectively, for the MMF group ($P = 0.57$). In conclusion, our steroid-avoidance regimen had no apparent impact on LDLT outcomes for HCV-positive recipients. *Liver Transpl 19:896-906, 2013.* © 2013 AASLD.

Received January 31, 2013; accepted May 8, 2013.

Abbreviations: ACR, acute cellular rejection; BPAR, biopsy-proven acute rejection; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; LDLT, living donor liver transplantation; MMF, mycophenolate mofetil; ST, corticosteroid; TAC, tacrolimus.

This study was partly supported by a Health and Labor Sciences Research Grant from the Japanese Ministry of Health, Labor, and Welfare.

The ClinicalTrials.gov registration number is NCT00469131.

The authors of this article have no conflicts of interest to disclose.

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DOI 10.1002/lt.23679
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

Hepatitis C virus (HCV)–related cirrhosis is the most common indication for liver transplantation. However, the recurrence of HCV infections is almost universal, and the rate of fibrosis progression is accelerated so that 20% to 40% of recipients progress to allograft cirrhosis within 5 years.[1-4] The severity of HCV recurrence may be altered by the amount and type of immunosuppression.[5] The treatment of acute cellular rejection (ACR) with repeated bolus injections of a corticosteroid (ST) is generally considered to have negative effects on recurrent HCV.[6,7] However, no convincing data have shown that the use of daily maintenance ST therapy is associated with aggressive recurrent HCV. Although several clinical trials have examined the safety and efficacy of steroid avoidance,[8-14] whether steroid-free regimens would prove less deleterious for recurrent HCV remains controversial.

In Japan, too, HCV-related cirrhosis and hepatocellular carcinoma (HCC) are the most prevalent liver diseases, and living donor liver transplantation (LDLT) has become a treatment option for patients with these diseases.[15] We have already reported the clinical outcomes of LDLT for 91 HCV-positive recipients.[16] In this early experience, the survival rates and rates of progression to severe disease due to HCV recurrence were comparable to those for recipients of deceased donor liver transplantation described in the literature. Our standard immunosuppression protocol has consisted of tacrolimus (TAC) and a low-dose ST, with the administration of steroids gradually tapered and terminated at the end of the third month.[16] Against this background, we conducted a prospective, randomized, multicenter trial to evaluate the effects of a steroid-avoidance regimen on HCV recurrence after LDLT. Because TAC monotherapy was considered weak as an induction immunosuppressive regimen, we adopted mycophenolate mofetil (MMF) as a substitute for steroids in the steroid-avoidance study arm. The aim of this prospective, randomized study was to compare the efficacy and safety of TAC/MMF and conventional TAC/ST regimens in HCV-positive LDLT recipients.

PATIENTS AND METHODS

Study Design

In January 2004, this open-label, randomized, prospective, multicenter trial was started at 6 LDLT centers in Japan (ie, the hospitals of Kyoto University, Kumamoto University, Mie University, Nagoya University, Osaka City University, and Ehime University). This randomized clinical trial was registered at ClinicalTrials.gov (NCT00469131). Patients > 18 years old who were scheduled to undergo LDLT and suffered from HCV-related liver cirrhosis with positive serum results for HCV antibodies were eligible for inclusion in the study. The exclusion criteria were (1) a history of any organ transplantation or retransplantation, (2) ABO blood type incompatibility, (3) positive findings for hepatitis B surface antigen, (4) a small-for-size graft (graft/recipient body weight ratio < 0.8%), (5) renal dysfunction (serum creatinine level > 2 mg/dL), and (6) a platelet count < $3 \times 10^4/mm^3$ or a white blood cell count < $1000/mm^3$. Patients with HCC were not excluded. In the selection criteria for HCC patients, no restrictions were placed on the number or size of tumors, but patients with extrahepatic metastasis or macroscopic venous invasion on preoperative imaging were excluded.[17] HCC was classified according to the tumor-node-metastasis staging criteria.[18]

All subjects provided written, informed consent to participate. Eligible patients were centrally registered and randomized with a Web-based enrollment system at the Translational Research Informatics Center. Randomization was stratified by institutions and HCC stage (stage III or other) and was performed in a 1:1 ratio to an ST group, which received TAC (Prograf, Astellas Pharma, Tokyo, Japan) and an ST, and an MMF group, which received TAC and MMF (CellCept, Hoffmann-La Roche, Basel, Switzerland). The protocol of this study was approved by the medical ethics committee of each participating university.

Histological recurrence of HCV (fibrosis stage ≥ F1 according to the METAVIR score[19]), biopsy-proven acute rejection (BPAR) resistant to 2 sets of steroid pulse therapy, HCC recurrence, retransplantation, and patient death were defined as events, and the primary endpoint of the study was event-free survival. Secondary endpoints included patient survival, HCV viral loads, histological HCV recurrence, BPAR requiring steroid pulse therapy, and chronic rejection.

Immunosuppression Regimens

In both groups, the administration of TAC was started through a nasogastric tube within the first 12 hours after transplantation. The target whole-blood trough level for TAC was 10 to 15 ng/mL during the first 2 weeks, approximately 10 ng/mL thereafter, and 5 to 8 ng/mL from the second month.[16] In the ST group, intravenous methylprednisolone was initiated at 10 mg/kg before graft reperfusion and was then tapered from 1 mg/kg/day on days 1 to 3 to 0.5 mg/kg/day on days 4 to 6 and to 0.3 mg/kg/day on day 7. Subsequently, oral prednisone was continued at 0.3 mg/kg/day until the end of the first month, and this was followed by 0.1 mg/kg/day until the end of the third month. After this time, steroid administration was terminated.[16] In the MMF group (steroid avoidance), a steroid bolus injection immediately before reperfusion was also avoided. MMF was initiated through the nasogastric tube at a starting dose of 10 to 15 mg/kg on day 1, which was gradually increased to a target dose of 30 mg/kg, and this was continued for 6 months.

Assessment and Treatment of HCV Recurrence

The serum HCV RNA load was evaluated with polymerase chain reaction and an Amplicor HCV assay (Cobas Amplicor HCV Monitor, Roche Molecular Systems, Pleasanton, CA). HCV core protein was measured in serum as another index for the viral load with

TABLE 1. Patient, Transplant, and Immunosuppression Profiles

Variables	ST Group (n = 35)	MMF Group (n = 40)
Recipients		
Sex: male/female (n/n)	19/16	22/18
Age (years)*	56 (35-69)	59 (40-69)
Child-Pugh: A/B/C (n/n/n)	1/10/24	3/15/22
Model for End-Stage Liver Disease score*	16 (9-31)	15 (3-28)
HCC: none/stage I or II/stage III [n/n/n (%/%/%)]	15/13/7 (43/37/20)	13/16/11 (32/40/28)
Donors		
Sex: male/female (n/n)	19/16	22/18
Age (years)*	38 (21-59)	42 (22-65)
Graft type: left lobe/right lobe (n/n)	7/28	6/34
Transplants		
Operation time (minutes)*	786 (650-1064)	761 (563-1274)
Blood loss (mL)*	7570 (1150-60,860)	5048 (1150-29,500)
Immunosuppression (days)*†		
TAC	365 (36-365)	365 (11-365)
Steroid	92 (10-93)	—
MMF	—	175 (10-186)
TAC trough levels (ng/mL)*		
Day 28	8.2 (2.3-21.8)	8.3 (0.9-14.2)
Month 3	6.3 (2.0-12.3)	6.7 (2.1-14.6)
Month 6	6.45 (2.4-13.2)	6.1 (1.9-10.4)
Month 12	5.2 (2.4-11.8)	5.0 (1.8-10.4)

NOTE: No significant differences in any variables (except for immunosuppression) were seen between the ST and MMF groups.
*The data are presented as medians and ranges.
†Total number of days of administration during the first year.

a previously reported enzyme immunoassay method.[20] These viral loads were determined before LDLT and 1, 7, 14, and 28 days and 3, 6, and 12 months after LDLT.

Protocol liver biopsy was recommended 3, 6, and 12 months after LDLT and annually thereafter. Event-driven biopsy was performed as clinically indicated. All biopsy samples were evaluated by liver histopathologists at each center. For the diagnosis of HCV recurrence, the necroinflammatory activity (A0-A3) and the fibrosis stage (F0-F4) were assessed with the METAVIR score.[19] HCV recurrence with a necroinflammatory activity classification $\geq$ A2 or a fibrosis stage $\geq$ F1 was considered for antiviral treatment with interferon (IFN) and ribavirin.[21] From 2005 onward, splenectomy was performed during the recipient's transplant operation to increase the platelets and white blood cell counts suppressed by hypersplenism and to enhance the tolerability of IFN therapy.[22]

Assessment and Treatment of Rejection

ACR was confirmed by liver biopsy and assessed according to the Banff criteria.[23] In principle, mild ACR (Banff grade I or rejection activity index score of 1-3) was treated with an increase in the TAC level. Mild to severe ACR (Banff grade II/III or rejection activity index score $\geq$ 4) was treated with steroid pulse therapy (10 mg/kg bolus of methylprednisolone for 3 days) followed by steroid tapering, if necessary, in both groups.

Statistical Methods

The 1-year event-free survival rate for the TAC/steroid arm (the ST group) was estimated to be 20%; this was determined by an examination of historical data (n = 30) from Kyoto University Hospital. The 1-year event-free survival rate for the TAC/MMF arm (the MMF group) was expected to be 40%. Under the assumption of a 1-sided significance level of 5% and a power of 80%, the required sample size was calculated to be 60 patients per arm on the basis of the log-rank test.

Baseline and laboratory test data are summarized as medians and ranges. Categorical variables were compared with the χ^2 test or Fisher's exact test. Continuous variables were compared with the Wilcoxon rank-sum test. Cumulative probability curves of events were calculated with the Kaplan-Meier method. When we evaluated the time to reach fibrosis stage 1 or 2 according to liver biopsy, outcomes were censored at the time of last biopsy or death for patients who did not reach each fibrosis stage. Differences between these curves were compared with the log-rank test. All data were analyzed with an intent-to-treat approach, and $P < 0.05$ was considered statistically significant.

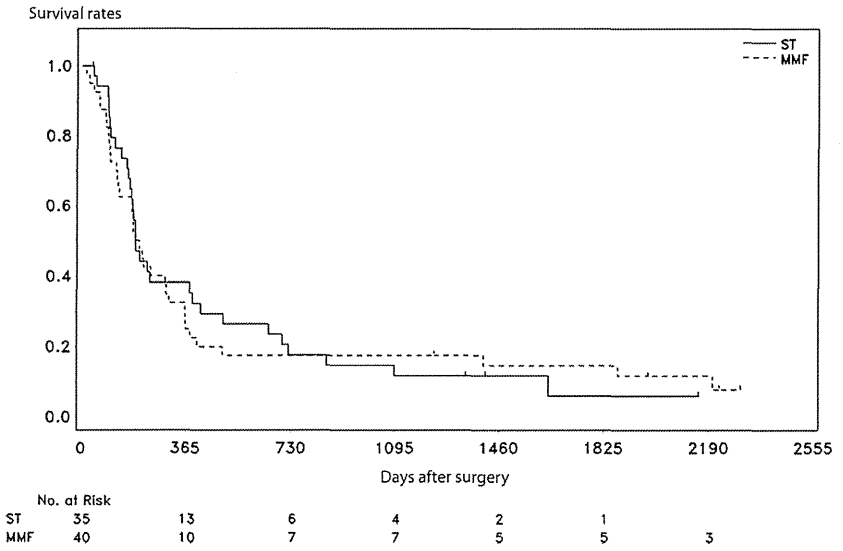

Figure 1. Event-free survival curves. Events were defined as histological HCV recurrence with a fibrosis stage ≥ F1, BPAR resistant to 2 sets of steroid pulse therapy, recurrence of HCC, retransplantation, and patient death. No significant differences in event-free survival were seen between the ST group (TAC + ST) and the MMF group (TAC + MMF; $P = 0.45$, log-rank test).

RESULTS

Study Population

Although the planned sample size was 120 patients, the enrollment period was ended in August 2010 after the enrollment of 79 patients because of shrinkage of the study population. Thirty-seven subjects were randomized to the TAC/ST arm (the ST group), and 42 subjects were randomized to the TAC/MMF arm (MMF group). Two patients in the ST group and 2 in the MMF group were excluded from the study after randomization because of protocol violations, so 35 patients in the ST group and 40 in the MMF group formed the cohort of the present analysis.

Recipient and donor characteristics and operative data are summarized in Table 1. No significant differences in these profiles were evident between the ST and MMF groups. One subject in the ST group withdrew consent to participate in the study on postoperative day 37. Patient outcomes were finally collected at the end of August 2011. The median follow-up was 55 months (range = 1-89 months).

Primary Endpoint

The rates of event-free survival (the primary endpoint) 1, 3, and 5 years after LDLT were 38.2%, 11.8%, and 5.9%, respectively, for the ST group and 25.0%, 17.5%, and 14.6%, respectively, for the MMF group (Fig. 1). No significant differences in event-free survival were evident between the groups (1-sided $P = 0.45$).

Overall Survival

Early mortality (within 4 months after LDLT) occurred for 2 recipients in the ST group and for 3 recipients in the MMF group. The causes of death were graft dysfunction (n = 1) and cerebral bleeding (n = 1) in the ST group and cerebral bleeding (n = 1), bacterial pneumonia (n = 1), and sepsis (n = 1) in the MMF group. In the later period, 3 patients from the ST group died of HCC recurrence (n = 1), malignant lymphoma (n = 1), and graft failure due to a biliary stricture (n = 1), whereas 7 patients from the MMF group died of chronic rejection (n = 1), HCC recurrence (n = 1), HCV recurrence (n = 1), and other causes (n = 4). No recipients in either group underwent retransplantation. The 1-, 3-, and 5-year overall survival rates were 94.1%, 87.6%, and 82.7%, respectively, for the ST group and 92.5%, 84.5%, and 81.0%, respectively, for the MMF group ($P = 0.28$; Fig. 2).

HCV Histological Recurrence and Antiviral Therapy

During follow-up, histological HCV recurrence with a fibrosis stage ≥ F1 was confirmed for 27 patients in the ST group and for 35 patients in the MMF group. Cumulative recurrence rates with a fibrosis stage ≥ F1 (deaths censored) at 1, 2, and 3 years were 59.4%, 78.9%, and 85.9%, respectively, for the ST group and 74.2%, 81.9%, and 81.9%, respectively, for the MMF group ($P = 0.57$; Fig. 3). Recurrence with a fibrosis stage ≥ F2 was confirmed for 9 patients in the ST group and for 13 patients in the MMF group. Cumulative recurrence rates with a fibrosis stage ≥ F2 at 1, 3, and 5 years were 6.3%, 19.0%, and 24.4%, respectively, for the ST group and 16.7%, 28.4%, and 31.6%, respectively, for the MMF group ($P = 0.46$; Fig. 4).

Within 1 year after LDLT, 26 patients in the ST group and 34 patients in the MMF group who had

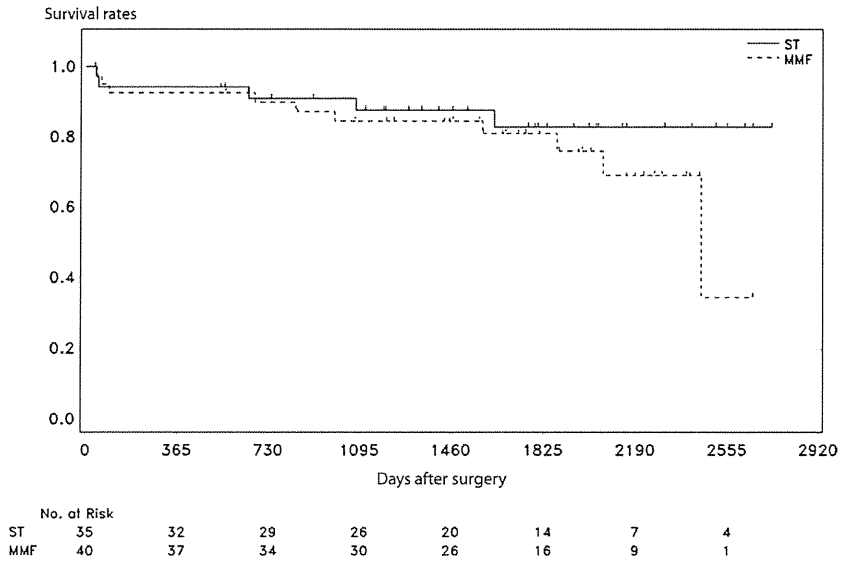

Figure 2. Overall survival curves. No significant differences were apparent between the ST group (TAC + ST) and the MMF group (TAC + MMF; $P = 0.28$, log-rank test).

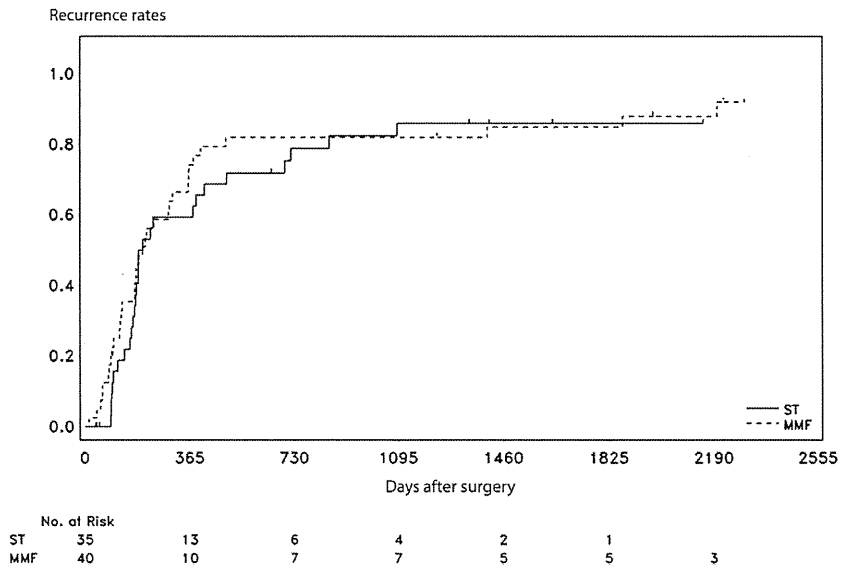

Figure 3. Cumulative recurrence rates with a fibrosis stage ≥ F1. No significant differences were identified in the rates with a fibrosis stage ≥ F1 (deaths censored) between the ST group (TAC + ST) and the MMF group (TAC + MMF; $P = 0.57$, log-rank test).

developed histological recurrence of HCV were considered for antiviral therapy. Among these, 18 patients (69.2%) in the ST group and 21 patients (61.8%) in the MMF group started IFN/ribavirin treatment within 1 year ($P = 0.60$). As a result, a sustained virological response was achieved for 8 of 18 patients in the ST group (44.4%) and for 14 of 21 patients (66.7%) in the MMF group ($P = 0.16$).

HCV Viral Load

Changes in HCV RNA and HCV core protein loads are shown in Fig. 5. On day 28, HCV RNA levels were significantly lower for the MMF group (median = 250 KIU/mL, range = 0-17,660 KIU/mL) versus the ST group (median = 1700 KIU/mL, range = 0.02-13,000 KIU/mL, $P < 0.05$; Fig. 5A). HCV core protein levels were also significantly lower for the MMF group

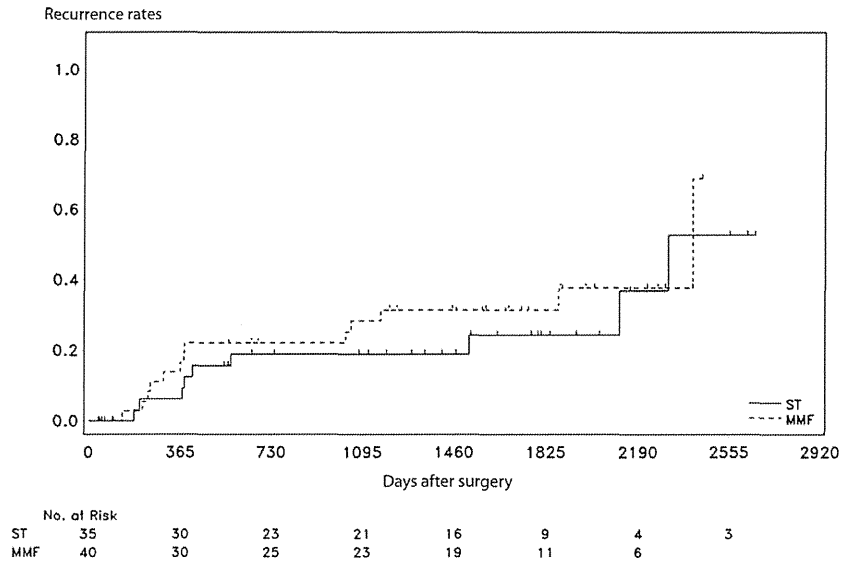

Figure 4. Cumulative recurrence rates with a fibrosis stage ≥ F2. No significant differences were identified in the rates with a fibrosis stage ≥ F2 (deaths censored) between the ST group (TAC + ST) and the MMF group (TAC + MMF; $P = 0.46$, log-rank test).

(median = 719 fmol/L, range = 20-26,200 fmol/L) versus the ST group (median = 7200 fmol/L, range = 20-32,000 fmol/L; $P < 0.01$; Fig. 5B). Thereafter, the viral loads were similar for the groups.

Immunosuppression and Rejection

The median total durations of the administration of steroids (in the ST group) and MMF (in the MMF group) were 92 and 175 days, respectively (Table 1). TAC whole-blood trough levels at 3, 6, and 12 months did not differ significantly between the ST and MMF groups. The addition of MMF or an ST was decided on the basis of the judgment of the investigators for 1 patient in the ST group and for 3 patients in the MMF group, respectively. BPAR requiring treatment with an ST bolus injection occurred in 4 patients from the ST group and in 13 patients from the MMF group ($P = 0.051$). According to the Banff criteria, ACR was mild for 2, moderate for 1, and severe for 1 in the ST group and mild for 3 and moderate for 10 in the MMF group. All episodes were observed within the first 3 months after LDLT in both groups. BPAR resistant to 2 sets of ST bolus treatment occurred in only 1 patient from the MMF group. Chronic rejection was diagnosed in only 1 patient, again from the MMF group.

Seventeen of the 75 patients experienced BPAR treated with ST pulse treatment. The overall survival rate for the 17 patients with BPAR was significantly lower than the rate for the 58 patients without BPAR (68.2% versus 90.9% at 3 years and 51.9% versus 90.9% at 5 years, $P < 0.001$; Fig. 6). However, histological HCV recurrence rates did not differ between patients with BPAR and patients without BPAR. Rates of progression to a fibrosis stage ≥ F1 at 1 year were 78.5% and 64.9%, respectively ($P = 0.66$), and rates of progression to a fibrosis stage ≥ F2 at 3 years were 30.8% and 22.3%, respectively ($P = 0.31$).

Adverse Events

As for infectious complications, bacterial and fungal infections such as sepsis and pneumonia were diagnosed for 1 patient in the ST group and for 4 patients in the MMF group. A cytomegalovirus infection was diagnosed for 2 patients in the ST group and for 1 patient in the MMF group. Clinical test results with respect to metabolic complications are summarized in Table 2. No significant differences in these data were apparent between the groups.

DISCUSSION

Several risk factors have been implicated in the frequency and severity of recurrent HCV after liver transplantation.[6,24] Many studies have found deleterious effects of potent immunosuppression (eg, high numbers of bolus injections of methylprednisolone, the use of anti-lymphocyte preparations, and high total cumulative doses of steroids).[24] On the other hand, it had been not clarified as of 2004 (when the present study was started) whether the use of daily maintenance ST therapy was associated with aggressive recurrent HCV. Although there have been, to the best of our knowledge, 7 prospective, randomized studies reported as full articles since then,[8-14] the issue of steroid-free regimens remains controversial. All 7 studies included a calcineurin inhibitor as part of the immunosuppressive regimen (cyclosporin A in 2 studies and TAC in 5 studies). In 4 studies,[8,9,11,12] the ST was simply removed from the regimen of the control

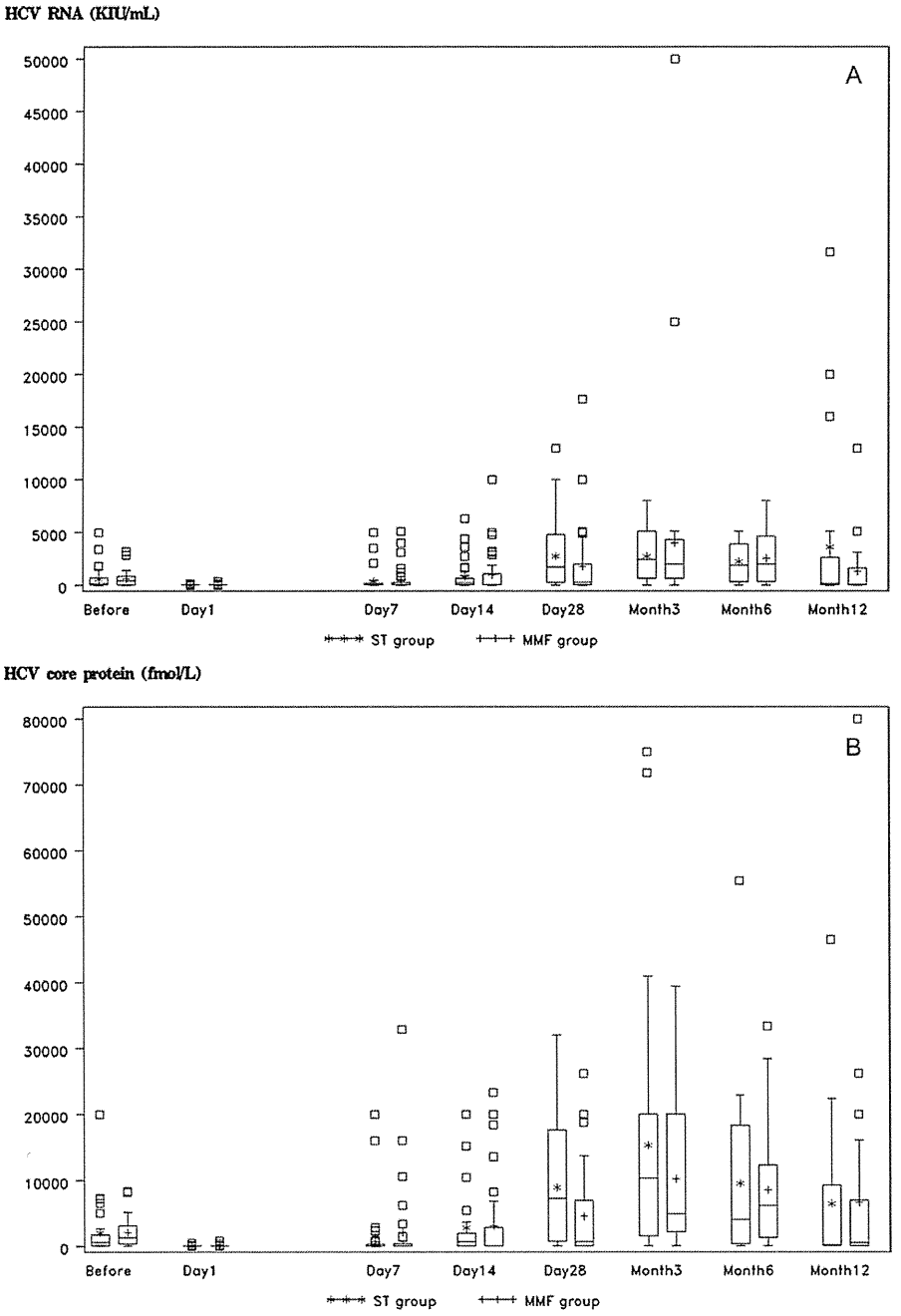

Figure 5. Changes in (A) HCV RNA and (B) HCV core protein levels: box and whisker plots. The bottom and top of each box represent the lower quartile and the upper quartile, whereas the bar in the box shows the median. The plot in the box is the mean. The ends of the whiskers represent the lowest and highest data still within 1.5 interquartile ranges (upper quartile − lower quartile) of the median. Small boxes not included between whiskers represent outliers. (A) HCV RNA levels were significantly lower for the MMF group versus the ST group ($P < 0.05$) on day 28. (B) HCV core protein levels were also significantly lower for the MMF group versus the ST group ($P < 0.01$) on day 28.

arm, whereas the other 3 studies replaced the ST with daclizumab.[10,13,14] Although each steroid-free protocol was found to be safe and feasible in all studies, the results were divergent with respect to the effects on HCV recurrence. Lladó et al.[11] reported favorable results for a steroid-free group in terms of short-term histological HCV recurrence. However, Manousou et al.[12] reported contradictory findings. Moreover, the

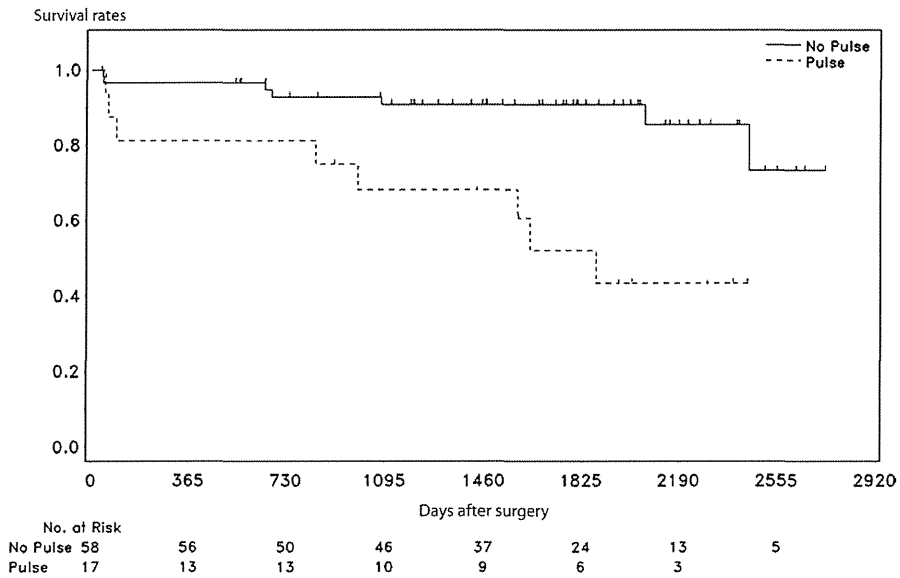

Figure 6. Overall survival rates for patients with or without steroid pulse treatment for BPAR. Seventeen of the 75 patients experienced BPAR treated with steroid pulse treatment. The overall survival rate was significantly lower for the 17 patients with BPAR versus the 58 patients without BPAR ($P < 0.001$, log-rank test).

TABLE 2. Clinical Test Results

	Before	Month 3	Month 6	Month 12
Systolic blood pressure (mm Hg)				
ST group	102 (84-144)	120 (84-154)	120 (96-154)	124 (102-164)
MMF group	113 (84-157)	121 (76-177)	122 (100-172)	120 (94-142)
Diastolic blood pressure (mm Hg)				
ST group	62 (48-86)	73 (52-94)	70 (60-102)	83 (59-90)
MMF group	66 (40-95)	77 (40-98)	72 (59-114)	74 (56-95)
Fasting blood glucose level (mg/dL)				
ST group	101 (69-226)	104 (63-226)	105 (72-224)	105 (74-176)
MMF group	114 (68-288)	117 (77-297)	111 (82-186)	105 (71-200)
Serum creatinine level (mg/dL)				
ST group	0.8 (0.5-1.9)	0.9 (0.2-2.1)	0.9 (0.5-1.2)	0.8 (0.5-1.3)
MMF group	0.8 (0.4-1.4)	0.8 (0.4-3.5)	0.8 (0.4-1.3)	0.9 (0.4-1.7)

NOTE: The data are presented as medians and ranges. No significant differences in any variables were seen between the ST and MMF groups.

remaining 5 studies found no significant differences between steroid-free and control groups.

The present investigation was a randomized comparative study conducted among LDLT recipients to investigate the impact of a steroid-avoidance immunosuppression protocol on HCV recurrence. In the steroid-avoidance arm, steroids were completely removed; this included a methylprednisolone bolus injection immediately before reperfusion. The safety and efficacy of omitting steroids during and after liver transplantation have been demonstrated in clinical studies.[25] To really evaluate the steroid-free effects, the regimen of the study arm should simply remove STs from the regimen of the control arm, as in the previous 4 studies.[8,9,11,12] However, because we thought that TAC monotherapy would prove weak as an induction immunosuppressive regimen, we adopted MMF as a substitute for steroids in the steroid-avoidance arm of the study. Because MMF, an inositol monophosphate dehydrogenase inhibitor like ribavirin, has been proposed to exert antiviral effects,[26] we also expected additional anti-HCV effects from the use of MMF in the study arm. Several studies have evaluated the anti-HCV effects of MMF in clinical liver transplantation settings.[27-30] Although antiviral properties have remained unconfirmed in

most studies,[27-29] Fasola et al.[30] reported a delay in the recurrence of severe hepatitis among recipients treated with MMF.

Consequently, the present study compared immunosuppressive regimens using an ST or MMF in combination with TAC. The primary endpoint was defined as event-free survival. For HCV-positive recipients, optimal immunosuppression is required to both prevent rejection and control the recurrence of HCV. Less potent immunosuppression is considered to reduce the severity of HCV recurrence. On the other hand, the risk of rejection will be increased, and this may lead to intractable rejection requiring repeated steroid boluses and thus adversely affect the risk of HCV recurrence. The risk of HCC recurrence and bacterial or viral infectious complications, which often cause fatal outcomes, are also affected by the strength of immunosuppression.

Unfortunately, the present study failed to demonstrate any favorable results for the MMF group. Several factors should be considered in interpreting the present results. First of all, the final sample size (n = 75) was smaller than planned (n = 120). One reason was the recent trend toward cases using a small-for-size or ABO-incompatible graft, both of which were excluded from the present study. At Kyoto University, from the perspective of donor safety and with the development of strategies for small-for-size syndrome, the algorithm for graft type selection has been modified to primarily consider left lobe donation.[31] Between 2006 and 2008, the selection of a small-for-size graft (graft/recipient body weight ratio < 0.8%) increased to 23.9% of all adult cases.[30] Similarly, with improvements in ABO-incompatible protocols such as preoperative rituximab, ABO-incompatible cases have increased to more than 20%.[32] The post hoc type II error based on the completed sample size of 75 patients and prespecified settings was elevated by 38%. However, the predictive power,[33] representing the probability of obtaining a significant result after completion of the trial (n = 120) with the observed data (n = 75), was only 1% under the assumption of a uniform prior distribution. Therefore, although definitive conclusions cannot be reached with this decreased statistical power, we considered that significant differences regarding the primary endpoint may still not have been obtained even if all 120 subjects had been enrolled.

BPAR resistant to 2 sets of steroid pulse therapy was encountered in only 1 patient in this study, but BPAR treated with steroid pulse therapy tended to be more frequent in the MMF group versus the ST group (P = 0.051). The immunosuppressive power might plausibly have been weaker for the former group. In contrast to a steroid being intravenously injected during the first postoperative week, MMF was administered orally or via a nasogastric tube even immediately after the operation. Because the postoperative recovery of oral intake and gastrointestinal function is retarded for most LDLT recipients, the dosage or absorption of MMF might not have been sufficient to reach optimal blood levels in the immediate postoperative period. The addition of induction with interleukin-2 receptor antibody drugs, as in other studies,[8,10,11,13,14] was considered at the time of the protocol's creation, but the idea was abandoned because these agents were not covered by National Health Insurance in Japan. In any case, it could be argued that the beneficial effects of the steroid-avoidance regimen on HCV recurrence might be offset by the increased use of steroid bolus injections. Kato et al.[10] reported the occurrence of acute rejection during the first year as the only factor associated with increased hepatic fibrosis at 1 year. However, this speculation is not supported by the finding that histological HCV recurrence rates did not differ between patients with BPAR treated with steroid pulse therapy and patients without it.

On the other hand, according to the analysis of the total study population, patients receiving steroid pulse treatment for BPAR showed significantly lower overall survival than those without steroid pulse treatment (Fig. 6). The causes of early mortality for patients receiving steroid pulse treatment were mostly related to infectious complications. Acute rejection is an established risk factor for infections within the first posttransplant year.[34,35] Obviously, an optimal immunosuppression protocol for sufficiently decreasing the risk of acute rejection remains a prerequisite for HCV-positive recipients.

Notably, in the present study, the posttransplant HCV viral load was lower for the MMF group versus the ST group shortly after the operation. On day 28, the HCV RNA and HCV core protein levels were significantly lower for the MMF group. These findings suggest that HCV replication was suppressed in the MMF group, especially in the immediate posttransplant period. However, viral loads subsequently showed similar increases at 3 and 6 months, and they were decreased by 12 months in both groups. These changes were closely influenced by the induction of antiviral therapy. According to our criteria for starting antiviral therapy, 18 patients in the ST group and 21 patients in the MMF group started IFN/ribavirin treatment between 3 and 12 months. Antiviral therapy could also affect histological HCV recurrence and diminish the effects of differences in immunosuppression protocols.

In conclusion, this study failed to show any significant results. It is unknown whether this was due to the limited size of the sample (reducing the power to detect differences) or because our steroid-avoidance regimen had a minimal impact on LDLT outcomes for HCV-positive recipients. Recent meta-analyses[36,37] have favored steroid-free protocols in terms of HCV recurrence, but the included studies have been heterogeneous and complicated to interpret. As previously mentioned, most individual trials have not shown results reaching the level of statistical significance. A larger multicenter trial with a more optimal protocol is required to better define the role of steroid-free regimens for HCV-positive recipients.

ACKNOWLEDGMENT

The authors acknowledge Ms. Naoko Kashiwagi and Ms. Emiko Uno for their support with the data management.

REFERENCES

1. Prieto M, Berenguer M, Rayón JM, Córdoba J, Argüello L, Carrasco D, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 1999;29:250-256.
2. Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. Liver Transpl 2003;9:S28-S34.
3. Velidedeoglu E, Mange KC, Frank A, Abt P, Desai NM, Markmann JW, et al. Factors differentially correlated with the outcome of liver transplantation in HCV+ and HCV− recipients. Transplantation 2004;77:1834-1842.
4. Berenguer M, Prieto M, San Juan F, Rayón JM, Martinez F, Carrasco D, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. Hepatology 2002;36:202-210.
5. Irish WD, Arcona S, Bowers D, Trotter JF. Cyclosporine versus tacrolimus treated liver transplant recipients with chronic hepatitis C: outcomes analysis of the UNOS/OPTN database. Am J Transplant 2011;11:1676-1685.
6. Wiesner RH, Sorrell M, Villamil F; for International Liver Transplantation Society Expert Panel. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. Liver Transpl 2003;9:S1-S9.
7. Watt K, Veldt B, Charlton M. A practical guide to the management of HCV infection following liver transplantation. Am J Transplant 2009;9:1707-1713.
8. Filipponi F, Callea F, Salizzoni M, Grazi GL, Fassati LR, Rossi M, et al. Double-blind comparison of hepatitis C histological recurrence rate in HCV+ liver transplant recipients given basiliximab + steroids or basiliximab + placebo, in addition to cyclosporine and azathioprine. Transplantation 2004;78:1488-1495.
9. Margarit C, Bilbao I, Castells L, Lopez I, Pou L, Allende E, Escartin A. A prospective randomized trial comparing tacrolimus and steroids with tacrolimus monotherapy in liver transplantation: the impact on recurrence of hepatitis C. Transpl Int 2005;18:1336-1345.
10. Kato T, Gaynor JJ, Yoshida H, Montalvano M, Takahashi H, Pyrsopoulos N, et al. Randomized trial of steroid-free induction versus corticosteroid maintenance among orthotopic liver transplant recipients with hepatitis C virus: impact on hepatic fibrosis progression at one year. Transplantation 2007;84:829-835.
11. Lladó L, Fabregat J, Castellote J, Ramos E, Xiol X, Torras J, et al.; for THOSIN Study Group. Impact of immunosuppression without steroids on rejection and hepatitis C virus evolution after liver transplantation: results of a prospective randomized study. Liver Transpl 2008;14:1752-1760.
12. Manousou P, Samonakis D, Cholongitas E, Patch D, O'Beirne J, Dhillon AP, et al. Outcome of recurrent hepatitis C virus after liver transplantation in a randomized trial of tacrolimus monotherapy versus triple therapy. Liver Transpl 2009;15:1783-1791.
13. Klintmalm GB, Davis GL, Teperman L, Netto GJ, Washburn K, Rudich SM, et al. A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. Liver Transpl 2011;17:1394-1403.
14. Neumann U, Samuel D, Trunečka P, Gugenheim J, Gerunda GE, Friman S. A randomized multicenter study comparing a tacrolimus-based protocol with and without steroids in HCV-positive liver allograft recipients. J Transplant 2012;2012:894215.
15. Japanese Liver Transplantation Society. Liver transplantation in Japan—registration by the Japanese Liver Transplantation Society [in Japanese]. Ishoku 2010;45:621-631.
16. Takada Y, Haga H, Ito T, Nabeshima M, Ogawa K, Kasahara M, et al. Clinical outcomes of living donor liver transplantation for hepatitis C virus (HCV)-positive patients. Transplantation 2006;81:350-354.
17. Takada Y, Ueda M, Ito T, Sakamoto S, Haga H, Maetani Y, et al. Living donor liver transplantation as a second-line therapeutic strategy for patients with hepatocellular carcinoma. Liver Transpl 2006;12:912-919.
18. Makuuchi M, Belghiti J, Belli G, Fan ST, Lau JW, Ringe B, et al.; for Working Group of the International Scientific Committee of the International Hepato-Pancreato-Biliary Association. IHPBA concordant classification of primary liver cancer: working group report. J Hepatobiliary Pancreat Surg 2003;10:26-30.
19. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 1996;24:289-293.
20. Tanaka E, Ohue C, Aoyagi K, Yamaguchi K, Yagi S, Kiyosawa K, Alter HJ. Evaluation of a new enzyme immunoassay for hepatitis C virus (HCV) core antigen with clinical sensitivity approximating that of genomic amplification of HCV RNA. Hepatology 2000;32:388-393.
21. Ueda Y, Takada Y, Marusawa H, Egawa H, Uemoto S, Chiba T. Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. Transplantation 2010;90:661-665.
22. Takada Y, Uemoto S. Living donor liver transplantation for hepatitis C. Surg Today; doi:10.1007/s00595-012-0361-z.
23. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25:658-663.
24. Berenguer M. Host and donor risk factors before and after liver transplantation that impact HCV recurrence. Liver Transpl 2003;9:S44-S47.
25. Pirenne J, Aerts R, Koshiba T, Van Gelder F, Roskams T, Schetz M, et al. Steroid-free immunosuppression during and after liver transplantation—a 3-yr follow-up report. Clin Transplant 2003;17:177-182.
26. Neyts J, Meerbach A, McKenna P, De Clercq E. Use of the yellow fever virus vaccine strain 17D for the study of strategies for the treatment of yellow fever virus infections. Antiviral Res 1996;30:125-132.
27. Bahra M, Neumann UP, Harren M, Jacob D, Langrehr JM, Berg T, et al. Significance of mycophenolate mofetil treatment in patients with HCV reinfection after liver transplantation: impact on clinical course and histologic damage. Transplant Proc 2002;34:2934-2935.
28. Jain A, Kashyap R, Demetris AJ, Eghstesad B, Pokharna R, Fung JJ. A prospective randomized trial of mycophenolate mofetil in liver transplant recipients with hepatitis C. Liver Transpl 2002;8:40-46.
29. Germani G, Pleguezuelo M, Villamil F, Vaghjiani S, Tsochatzis E, Andreana L, Burroughs AK. Azathioprine in liver transplantation: a reevaluation of its use and a comparison with mycophenolate mofetil. Am J Transplant 2009;9:1725-1731.
30. Fasola CG, Netto GJ, Christensen LL, Molmenti EP, Sanchez EQ, Levy MF, et al. Delay of hepatitis C recurrence in liver transplant recipients: impact of mycophenolate

mofetil on transplant recipients with severe acute rejection or with renal dysfunction. Transplant Proc 2002;34:1561-1562.

31. Ogura Y, Hori T, El Moghazy WM, Yoshizawa A, Oike F, Mori A, et al. Portal pressure <15 mm Hg is a key for successful adult living donor liver transplantation utilizing smaller grafts than before. Liver Transpl 2010;16:718-728.
32. Raut V, Mori A, Kaido T, Ogura Y, Taku I, Nagai K, et al. Splenectomy does not offer immunological benefits in ABO-incompatible liver transplantation with a preoperative rituximab. Transplantation 2012;93:99-105.
33. Jennison C, Turnbull BW. Stochastic curtailment. In: Jennison C, Turnbull BW, eds. Group Sequential Methods With Applications to Clinical Trials. Boca Raton, FL: Chapman and Hall/CRC; 2000:205-220.
34. San Juan R, Aguado JM, Lumbreras C, Díaz-Pedroche C, López-Medrano F, Lizasoain M, et al.; for RESITRA Network, Spain. Incidence, clinical characteristics and risk factors of late infection in solid organ transplant recipients: data from the RESITRA study group. Am J Transplant 2007;7:964-971.
35. Fishman JA. Infection in solid-organ transplant recipients. N Engl J Med 2007;357:2601-2614.
36. Segev DL, Sozio SM, Shin EJ, Nazarian SM, Nathan H, Thuluvath PJ, et al. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. Liver Transpl 2008;14:512-525.
37. Sgourakis G, Radtke A, Fouzas I, Mylona S, Goumas K, Gockel I, et al. Corticosteroid-free immunosuppression in liver transplantation: a meta-analysis and meta-regression of outcomes. Transpl Int 2009;22:892-905.

Surg Today (2013) 43:709–714
DOI 10.1007/s00595-012-0361-z

REVIEW ARTICLE

Living donor liver transplantation for hepatitis C

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Received: 14 May 2012 / Accepted: 5 July 2012 / Published online: 6 October 2012

Abstract Liver cirrhosis and hepatocellular carcinoma related to chronic hepatitis C virus (HCV) infection are currently the most common indications for liver transplantation. The number of living donor liver transplantation (LDLT) procedures has increased given the shortage of donor organs from deceased donors. However, recurrence of HCV infection is universal and affects graft survival. This mini-review compared the outcomes for HCV-positive recipients after LDLT with those after deceased donor liver transplantation.

Keywords Living donor liver transplantation · Deceased donor · Recurrence of hepatitis C · Anti-viral therapy

Introduction

Chronic hepatitis C is a global epidemic, with an estimated 200 million people infected worldwide. End-stage liver disease secondary to chronic hepatitis C virus (HCV) infection is currently the most common indication for liver transplantation in the United States and Europe [1, 2]. Living donor liver transplantation (LDLT) is an important means of expanding the donor pool, given the shortage of deceased donor organs, making transplantation available to an increasing number of patients. HCV-related cirrhosis and hepatocellular carcinoma (HCC) represent the most prevalent liver diseases in Japan, and LDLT is a viable option for patients with these diseases [3, 4].

However, recurrence of HCV infection is nearly universal and often occurs immediately after transplantation [5]. Indeed, the prevalence of chronic HCV in HCV-positive liver transplant recipients is 70–90 % after 1 year, and the progression of fibrosis is accelerated, which means that 8–44 % of patients progress to allograft cirrhosis within 5–10 years [6–8]. Therefore, graft and patient survival is significantly reduced for HCV-positive recipients in comparison to HCV-negative recipients [9, 10]. In addition, prior reports from some Western transplant centers have raised concerns that HCV recurrence may occur earlier and with greater severity and that graft loss caused by recurrent HCV may be more frequent for LDLT than for deceased donor liver transplantation (DDLT) [11–16]. Various mechanisms for an increase in graft damage in HCV-infected LDLT recipients have been postulated [17, 18]. First, because the partial liver graft undergoes intense regeneration immediately after LDLT, specific cellular changes occurring during this vigorous proliferative response may facilitate entry of HCV into hepatocytes or promote HCV replication. Second, most living donors are primary relatives of the recipient, thus the increased genetic similarity and the higher degree of HLA matching between the donor and the recipient with LDLT, in comparison to DDLT, may enhance the severity of recurrent HCV infection. However, the outcomes may be better among recipients of LDLT in comparison to recipients of DDLT because the donors are typically younger and the ischemia times are shorter with LDLT than with DDLT.

Although more recent studies have reported comparable results between LDLT and DDLT [19–26], it is debatable whether transplant outcomes for HCV-positive patients are

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