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Kinetics of B, T, NK lymphocytes and isoagglutinin titers in ABO incompatible living donor liver transplantation using rituximab and basiliximab ☆

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ABSTRACT

Background: The kinetics of isoagglutinin titers and lymphocyte subpopulations including B, T, and natural killer (NK) cells after ABO incompatible (ABO-I) living donor liver transplantation (LDLT) have not been evaluated.
Methods: From January 2012 to July 2013, consecutive ABO-I LDLT patients were enrolled at the National Cancer Center. Our desensitizing protocol included rituximab, plasma exchanges, basiliximab, and intravenous immune globulin without splenectomy.
Results: Twenty patients (14 males, 6 females) underwent ABO-I LDLT due to HCC (n = 15) or liver cirrhosis (n = 5). There was no hyperacute and antibody-mediated rejection. The isoagglutinin titers were effectively lowered less than 1:16 before operation. CD 19+ B cells were rapidly eliminated after rituximab and suppressed during 6 months postoperatively. CD3+ and CD4+ T cells were elevated higher than CD8+ T cells. CD4/CD8 ratio was increased during first 1 month postoperatively and decreased thereafter. CD16+CD56+ NK cells were lowered and restored after 4 months of LDLT. Among 15 patients with HCC, 5 patients (33.3%) experienced early tumor recurrence (1/8 within Milan and 4/7 beyond Milan).
Conclusions: Our protocol showed effective results in preventing antibody-mediated rejection and suppressing B lymphocytes. Application to advanced hepatocellular carcinoma should be considered due to decreased natural immunity after ABO-I LDLT.

1. Introduction

Living donor liver transplantation (LDLT) has been established as a treatment option for end-stage liver disease and hepatocellular carcinoma (HCC) due to the shortage of deceased organs [1]. In cases where ABO compatible donors are not available, ABO incompatible (ABO-I) LDLT has been performed exclusively in urgent and pediatric cases due to hyperacute or antibody-mediated rejection [2–4]. However, the outcomes of ABO-I LDLT have been improved by various strategies to overcome antibody-mediate rejection including rituximab, graft local infusion, splenectomy, plasma exchange, and aggressive immunosuppressive agents [5–7].

Various combinations of new protocols were shown using rituximab, plasma exchange, basiliximab, intravenous immune globulin (IVIG), splenectomy or graft local infusion therapy [6,8–11]. The purpose of these desensitization protocols is to reduce B cell family and preformed anti-blood type isoagglutinin titer before transplantation. As the understanding of humoral rejection has improved and new monoclonal antibodies have been developed, ABO-I LDLT has been widely performed and good results have been reported. Recently, surgical procedures, such as splenectomy and graft local infusion therapy, have not been performed due to procedure-related complications [6,8]. Medical desensitizing protocols using rituximab, plasma exchange, basiliximab or IVIG, control B cells, T cells, and anti-blood type isoagglutinin titers to prevent antibody-mediate rejection (AMR) between the ABO blood group antigen of transplant liver and the antibody of recipient blood. Especially, kinetics of B cells and isoagglutinin titers after the administration of rituximab and plasma exchange in ABO-I LDLT have been reported [12]. After a single dose of rituximab, CD19+ B cells in blood were depleted in 2 or 3 weeks and isoagglutinin titers could be successfully reduced by several sessions of plasma exchange. However, kinetics of CD3+, CD4+, CD8+ T cell and CD16+CD56+ natural killer (NK) cells in ABO-I LDLT were not elucidated, although

Abbreviations: ABO-I, ABO incompatible; LDLT, living donor liver transplantation; LT, liver transplantation; IVIG, intravenous immune globulin; PT, prothrombin time; AST, aspartate transaminase; ALT, alanine transaminase; CMV, cytomegalovirus; NK, natural killer

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these cells have important roles in postoperative immune defense mechanisms against bacteria, virus, or tumor cells.

In this study, we treated 20 ABO-I LDLT patients using a novel protocol involving rituximab, plasma exchange, basiliximab, and IVIG without any additional surgical management, such as splenectomy or graft local infusion therapy. Furthermore, we investigated the kinetics of B, T, NK cells and isoagglutinin titers with this protocol.

2. Materials and methods

2.1. Patients

Between January 2012 and July 2013, consecutive patients who underwent ABO-I LDLT at the National Cancer Center were enrolled in liver transplant database. All patients received the right lobe from a liver donor and did not undergo a simultaneous splenectomy and local graft infusion therapy. Same ABO-I LDLT protocol was applied to all patients. Indication for ABO-I LDLT was similar with our policy of ABO compatible LDLT. For HCC patient, on the basis of imaging studies like CT, MRI or PET/CT, patients who met the Milan criteria were selected for transplantation. If patients did not meet the Milan criteria but had neither major vascular invasion nor extrahepatic metastasis and strongly desired LDLT, we performed transplantation. The medical records were retrospectively reviewed including demographics, ABO type, isoagglutinin titers, lymphocyte subpopulations, and postoperative outcome. The study protocol was approved by the Institutional Review Board of our hospital.

2.2. Protocol for ABO-I LDLT

The current ABO-I LDLT protocol started with single dose of rituximab (300 mg/m^2) 2 weeks before LDLT. Several sessions of plasma exchange to decrease preformed anti-donor blood type isoagglutinin antibody titers to <1:16 were followed 1 week before LDLT. We also administered basiliximab for induction therapy (20 mg at operation day and postoperative day 4). A high dose of IVIG (0.8 g/kg) was administered at postoperative day 1 and 4. Immunosuppressive regimen involved high-dose steroid during the operation, followed by tacrolimus and mycophenolate mofetil with a combination of corticosteroid after transplantation. Initial target tacrolimus level ranged from 10 to 12 ng/mL and mycophenolate mofetil was started with a dose of 1.5 g/day. Steroids were tapered to discontinuation by 6 months after LDLT.

The prophylactic regimen consisted of broad spectrum antibiotics (ticarcillin sodium/clavulanate potassium) for 7 days, antifungal agent (fluconazole) for 1 month, and trimethoprim–sulfamethoxazole for 1 year. For cytomegalovirus (CMV) prophylaxis, routine antiviral agents were not given. Instead, CMV antigenemia was checked twice a week until discharge. For the prophylaxis of hepatitis B virus (HBV) recurrence, we used entecavir or tenofovir with high dose of IV hepatitis B immunoglobulin. For recurrent hepatitis C virus (HCV), we treated with pegylated-interferon and ribavirin after the confirmation of biopsy or abnormal liver function test with elevated HCV RNA loads. Catheter for plasma exchange was removed at first outpatient clinic visit. For surveillance of tumor recurrence, we checked alpha-fetoprotein and proteins induced by Vitamin K absence (PIVKA-II) with abdomen and chest computed tomography (CT) every 3 months during first year after LDLT. After diagnosis of tumor recurrence, we treated patients with multidisciplinary approaches such as surgery, chemotherapy, and radiation therapy.

The routine protocol biopsy for detection of rejection was not performed. Instead, if serum level of liver function test was increased 2 or 3-fold higher than the normal limit and isoagglutinin antibody titer was simultaneously elevated over 4 times compared to that at the operative day, we suspected the antibody-mediated rejection and did biopsy. In biopsy proven antibody-mediated rejection, we planned treatment with high dose IVIG (1 g/kg/day), steroid pulse therapy, and plasma exchanges.

2.3. Peripheral blood lymphocyte subpopulations and anti-blood type isoagglutinin titers

The prevalence of peripheral blood lymphocyte subpopulations including CD3+, CD4+, CD8+ T cells, CD19+ B cells, and CD16+ CD56+ NK cells was checked before the administration of rituximab using flow cytometry, and then immediately before LDLT. After LDLT, lymphocyte subpopulations were evaluated twice a week until discharge, weekly until postoperative 1 month, and then monthly thereafter.

Anti-blood type isoagglutinin titers were tested with the immediate spin technique [13]. Isoagglutinin titers were checked before the administration of rituximab, and then daily measured after starting plasma exchanges until discharge. After discharge, isoagglutinin titers were followed up weekly until postoperative 1 month, and every other week or monthly thereafter.

2.4. Statistical analysis

Continuous variables in Table were expressed as the mean and standard deviations. Non-normal distribution quantitative variables in Figure were expressed as the median (centile 25; centile 75). Statistical analysis used SAS® version 9.1.3 for Windows® (SAS Institute, Cary, NC, USA).

3. Results

3.1. Patient characteristics

Twenty patients who underwent ABO-I LDLT with our protocol were enrolled in this study (follow-up time ranging from 4 to 22 months, Table 1). Among them, there were 14 males and 6 females. Fifteen patients underwent ABO-I LDLT due to hepatocellular carcinoma, and 5 patients had only liver cirrhosis. The etiology of liver disease consisted of hepatitis B viral infection (n = 15), hepatitis C viral infection (n = 1), and alcoholic cirrhosis (n = 4). Mean age of recipient and donor was 51.9 ± 8.6 and 36.7 ± 15.0, respectively. MELD score of recipient was 11.7 ± 4.4. Most common ABO type of recipient and donor was O+ and A+, respectively. Mean value of graft-to-recipient weight ratio, operation time, total ischemic time, and postoperative hospital stay was 0.85 ± 0.21, 127.4 ± 34.8 min, and 14.5 ± 5.1 days, respectively. The number of transfusion of packed RBC during operation was 5.3 ± 7.5 units.

3.2. Kinetics of anti-blood type isoagglutinin titers

Preformed anti-blood isoagglutinin titers of recipient showed 1:256 (n = 1), 1:128 (n = 2), 1:64 (n = 3), 1:32 (n = 6), 1:16 (n = 2), 1:8 (n = 4), and 1:4 (n = 2). All patients demonstrated decreased isoagglutinin titer to less than 1:16 with several sessions of plasma exchange before LDLT (4 times of plasma exchange: 2 patients, 3 times: 2 patients, 2 times: 9 patients, 1 time: 5 patients). After ABO-I LDLT, isoagglutinin titers were stabilized less than 1:16 (Fig. 1). Four patients displayed temporary elevation of the isoagglutinin titer over 4 times at first week after LDLT compared to that at operation day. Among them, three patients did not show simultaneous increase of liver function test, so we performed one or two cycles of plasma exchange to prevent further elevation. One patient showed concomitant elevation of liver function test, then we performed biopsy with plasma exchange and steroid pulse therapy to differentiate acute rejection. However, the biopsy did not reveal any kind of rejection. Elevated isoagglutinin titers of above patients were decreased within 1 week and stabilized thereafter.

Interestingly, in 8 O+ recipient patients, anti-donor isoagglutinin titers remained less than 1:16, but non anti-donor isoagglutinin titers restored to original level prior to plasma exchange after LDLT (Fig. 2 shows isoagglutinin titers of 6 patients with follow-up of more than 6 months). Catheter-related complications were not demonstrated in these patients.

3.3. Kinetics of lymphocyte subpopulations

CD19+ B cells in peripheral blood rapidly disappeared 2 weeks after the administration of rituximab (Fig. 3A). The level of CD19+ B cells restored approximately 8 months after LDLT. However, during follow-up, the level of CD19+ B cells was generally suppressed compare to that prior to rituximab. For CD16+CD56+ NK cells, the percentage in peripheral blood was decreased during first 8 weeks after LDLT, then restored thereafter (Fig. 3B). Especially, during first 2 weeks after LDLT, the level of CD16+CD56+ NK cells was steeply decreased.