A modified protocol with rituximab and intravenous immunoglobulin in emergent ABO-incompatible liver transplantation for acute liver failure

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BACKGROUND: The established procedure for ABO-incompatible liver transplantation (ABO-I LT) was too complicated to be used in case of emergency. We developed a protocol consisting of rituximab and intravenous immunoglobulin (IVIG) for ABO-I LT in patients with acute liver failure (ALF).

METHODS: The data from 101 patients who had undergone liver transplantation (LT) for ALF were retrospectively analyzed. The patients were divided into two groups: ABO-compatible liver transplantation group (ABO-C LT, n=66) and ABO-I LT group (n=35). All the patients in the ABO-I LT group received a single dose of rituximab (375 mg/m²) and IVIG (0.4 g/kg per day) at the beginning of the operation. IVIG was administered for 10 consecutive days after LT. Plasma exchange, splenectomy and graft local infusion were omitted in the protocol. Quadruple immunosuppressive therapy including basiliximab, corticosteroids, tacrolimus and mycophenolatemofetil was used to reinforce immunosuppression.

RESULTS: The 3-year cumulative patient survival rates in the ABO-I LT and ABO-C LT groups were 83.1% and 86.3%, respectively (P>0.05), and the graft survival rates were 80.0% and 86.3%, respectively (P>0.05). Two patients (5.7%) suffered from antibody-mediated rejection in the ABO-I LT group. Other complications such as acute cellular rejection, biliary complication and infection displayed no significant differences between the two groups.

CONCLUSIONS: The simplified treatment consisting of rituximab and IVIG prevented antibody-mediated rejection for LT of blood-type incompatible patients. With this treatment, the patients did not need plasma exchange, splenectomy and graft local infusion. This treatment was safe and efficient for LT of the patients with ALF.

KEY WORDS: ABO-incompatible liver transplantation; rituximab; intravenous immunoglobulin; acute liver failure

Introduction

A cute liver failure (ALF) is a severe clinical syndrome defined by the rapid deterioration of liver function resulting in mental alteration and disturbance of coagulopathy in normal individuals. In most cases, liver transplantation (LT) is recommended for the patients who have received optimal therapy but could not achieve sufficient regeneration of hepatocytes to maintain a life.[1] ALF comprises about 7% of LT cases annually in the United States.[2] However, it is estimated that 24%-43% of patients are unable to receive an available and compatible graft due to the worldwide donation shortage.[3, 4] ABO-incompatible liver transplantation (ABO-I LT) therefore becomes an alternative option for patients with ALF.

ABO-I LT was considered to be a formidable challenge in LT because of the increased risk of infection, antibody-mediated rejection (AMR) and consequent vascular and biliary complications.[5, 6] Early efforts showed that the rates of graft loss and patient death varied from 30% to more than 50%, and the 5-year graft survival rate was less than 20%.[8] The main reason for poor survival was anti-A or anti-B
antibody-mediated rejection which induced a high incidence of hepatic necrosis and intrahepatic biliary complications.\[7\] Fortunately, in the past 20 years, several new methods have been adopted to prevent complications, and the outcome of ABO-I LT has been improved dramatically. Despite practical differences among centers, these methods usually comprise plasma exchange (PE),\[8\] splenectomy,\[9\] graft local infusion (GLI),\[10\] rituximab\[11\] and intravenous immunoglobulin (IVIG).\[12, 13\] At present, the 3-year patient survival rate in patients with ABO-incompatible living donor liver transplantation (ABO-I LDLT) is as high as 70% in Japan, which is not significantly different from that in the ABO-compatible recipients.\[14\] Moreover, Toso et al\[15\] reported that the 1- and 5-year patient and graft survival rates were not significantly different between ABO-I LT and ABO-compatible liver transplantation (ABO-C LT) for ALF. Therefore, adult ABO-I LT has been performed with increasing frequency to treat liver failure.

However, the established immunosuppressive protocols across blood type barrier usually require preparation for several days before transplantation and are not applicable under urgent circumstances. In China, ABO-I LT is always carried out in an emergent condition because the deceased donors are the main source of grafts at present. Furthermore, splenectomy and GLI associated with infection and a high rate of catheter-related complication are now under debate. Faced with this situation, we developed a simple protocol to meet the urgent needs.

The present study described a new treatment for patients with ALF treated by emergent ABO-I LT. This treatment consisted of rituximab and IVIG but no PE, splenectomy or GLI. We compared the results of ABO-I LT with those of ABO-C LT cases.

Methods

Patients

A total of 101 adult patients with ALF received LT in emergency from January 2010 to July 2013 at the First Affiliated Hospital, Zhejiang University School of Medicine, China. The patients were divided into two groups: ABO-C LT group (n=66) and ABO-I LT group (n=35). ALF was defined as coagulopathy (prothrombin activity ≤40% or international normalized ratio ≥1.5), jaundice (serum total bilirubin ≥171 μmol/L or a daily increase ≥17.1 μmol/L) and encephalopathy (any degree of altered mental status) within 4 weeks in a patient without pre-existing liver diseases.\[4\] The titers of anti-donor blood-type antibodies immunoglobulin M (IgM) in a perioperative period were monitored. The clinical outcomes including acute cellular rejection (ACR), AMR, biliary complication, infection and survival were compared between the two groups. Diagnosis of AMR was dependent on the clinical course and radiological findings.\[16\] ACR was diagnosed pathologically according to the Banff criteria. The methods of artificial liver support system included PE, plasma perfusion and continuous hemodiafiltration. Blood access was established through a double-lumen catheter via the patient's jugular or femoral vein. PE was performed with a plasma separator Plasmacure PS-06. The total volume of exchanged plasma was about 3300 mL, and the exchange rate was 22-25 mL/min. Continuous hemodiafiltration was performed with Diafilter D-30NR. Plasma perfusion utilizing Adsorba 300C contained 300 g cellulose coated charcoal. LT was approved by the Liver Transplantation Committee of Zhejiang University, and it was performed after informed consent was obtained from the patient. This study was approved by the Ethics Committee of Zhejiang University School of Medicine and in accord with ethical principles of the Declaration of Helsinki.

Immunosuppression and antiviral prophylaxis

Treatment for the blood-type barrier consisted of rituximab and IVIG (Fig. 1). All the patients in the ABO-I LT group received a single dose of rituximab (375 mg/m\(^2\)) and IVIG (0.4 g/kg per day) at the beginning of the operation. IVIG (0.4 g/kg per day) for induction therapy was administered for 10 days after LT. Quadruple regimen was adopted to strengthen the immunosuppression in the two groups. Basiliximab with a dose of 20 mg was used twice a day for day 1 and day 4 after LT. Maintenance immunosuppressive therapy consisted of corticosteroids, tacrolimus and mycophenolate mofetil (MMF). Corticosteroids were withdrawn in one month after transplantation.

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1.** Modified treatment for adult ABO-I LT. PE: plasma exchange; IVIG: intravenous immunoglobulin; MMF: mycophenolate mofetil.
Tacrolimus was administered on the first postoperative day to achieve a trough plasma level of 8-12 ng/mL on the first post-LT month and was titrated down to 5-8 ng/mL for the next month. MMF was started and kept at 500 mg twice daily after LT. To confirm the suppressive effect of rituximab, the peripheral blood CD20+ B cell counts were analyzed by flow cytometry for 3 months post-transplantation.

After LT, patients received the antiviral prophylaxis schedule consisted of 6000 IU of hepatitis B immunoglobulin (HBIG) during the anhepatic phase, followed by 800 IU daily for the next 6 days and weekly for 3 weeks, and then 800 IU monthly thereafter to keep the anti-HBs titer above 100 IU/L, meanwhile entecavir 0.5 mg was administered daily.

Statistical analysis
Continuous variables were presented as mean± SD, and discrete variables as number and percentage. The patient survival rate and graft survival rate were evaluated using the Kaplan-Meier method and difference in the curves was tested by a log-rank test. The software program SAS version 9.1 (SAS Institute Inc., Cary, NC) was used for statistical analysis and P<0.05 was considered statistically significant.

Results
Patients
The mean follow-up period was 18.7±14.1 months (range 1-45). In the ABO-I LT group, the mean age was 46.7±12.1 years (range 22-73); the model of end-stage liver disease (MELD) score was 34.1±4.3 (range 29-43); the primary disease for LT was hepatitis B virus (HBV) associated liver failure (n=32): drug induced liver failure in 2 cases and secondary LT due to the primary non-function (PNF) liver after the first LT in one case. Comparing to the ABO-I LT group, the clinical characteristics of ABO-C LT group not presented any difference, mean age of the patients was 42.6±10.2 years; MELD score was 32.1±11.2; the main cause for LT was HBV associated liver failure. The detailed clinical data of patients are presented in Table 1. The blood type combinations between recipients and donors in the ABO-I LT group are shown in Table 2.

Anti-donor blood-type antibodies titers and CD20 positive cells
The mean of IgM titer was 192 (range 4-1024) before LT. The titers were reduced immediately after LT in all cases (Fig. 2A). The mean titer on day 1 and day 7 after LT was 16 (range 1-32) and 28 (range 4-128). The lower titer levels maintained for several months. The titers of 2 patients who developed AMR were 1:8 and 1:64 before transplant respectively, but displayed rebound elevation during the second week after transplantation (Fig. 2B). The titer of case 2 decreased quickly after salvage therapy with a high dose of IVIG and PE (Fig. 2B). CD20+ B cell counts rapidly decreased to <1% in all cases after transplantation and lasted for several months (Fig. 2C, D).

Recipient and graft survival
The 3-year cumulative patient and graft survival rates were 83.1% and 80.0% in the ABO-I LT group, and 86.3% and 86.3% in the ABO-C LT group, respectively. (all P>0.05, Fig. 3). Causes of death in ABO-I LT included severe pulmonary infection (n=3), graft-versus-host disease (GVHD, n=1) and cerebral hemorrhage (n=2). In the ABO-C LT group, 5 patients died of severe bacterial or fungal infections, 1 of GVHD, 1 of cerebral
hemorrhage, 1 of intra-abdominal hemorrhage, and 1 of steroid resistant rejection. All the deaths occurred in 2 months after transplantation.

**Fig. 2.** Serial changes of anti-donor blood-type antibody IgM titer and frequency of peripheral blood B cells in all ABO-I LT recipients and 2 AMR patients before and after LT. POD: postoperative day; POM: postoperative month.

**Fig. 3.** Cumulative survival and graft survival curves for ABO-I LT and ABO-C LT groups.

**Table 3.** Complications in the ABO-I LT and ABO-C LT groups (n, %)

<table>
<thead>
<tr>
<th>Complications</th>
<th>ABO-I LT group (n=35)</th>
<th>ABO-C LT group (n=66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial infection</td>
<td>6 (17.1)</td>
<td>13 (19.7)</td>
</tr>
<tr>
<td>Fungal infection</td>
<td>5 (14.3)</td>
<td>7 (10.6)</td>
</tr>
<tr>
<td>AMR</td>
<td>2 (5.7)</td>
<td>0</td>
</tr>
<tr>
<td>ACR</td>
<td>1 (2.9)</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>GVHD</td>
<td>1 (2.9)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Biliary complication</td>
<td>2 (5.7)</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>Intra-abdominal hemorrhage</td>
<td>2 (5.7)</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Cerebral hemorrhage</td>
<td>2 (5.7)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Renal dysfunction</td>
<td>2 (5.7)</td>
<td>4 (6.0)</td>
</tr>
</tbody>
</table>


**Postoperative complications**

The complications after LT in the two groups are shown in Table 3. No significant difference was observed in complications between the two groups. AMR was observed in 2 (5.7%) patients whose blood type was O and received donation from donors with AB blood type. The 2 patients developed AMR at the second week after operation. The clinical manifestations of AMR were intrahepatic biliary complication (case 1, Fig. 4A) and hepatic necrosis (case 2, Fig. 4B). The clinical symptoms of case 1 could not be improved by IVIG and PE, then had to receive ABO-compatible re-transplantation at the sixth month after ABO-I LT. Case 2 recovered after
salvage therapy with a high dose of IVIG (0.8 g/kg per day) combined with PE (Fig. 4C). In the ABO-I LT group, there was one episode of ACR on day 35 after LT, and augmented steroid administration was effective. In the ABO-I LT group, there was one episode of ACR on day 35 after LT, and augmented steroid administration was effective. Infectious complications including bacterial infection (n=6; 17.1%) and fungal infection (n=5; 14.3%) were observed in the ABO-I LT group, but no confirmed viral infection. The incidence of renal dysfunction and infectious complications was similar in the two groups. There were no hepatic artery or portal vein complications in all patients.

Discussion
At present, ABO-C LT was considered as the optimal therapy for ALF in Western countries. However, due to a shortage of donor organ resources, adult ABO-I LT for ALF is increasing. In China, deceased donors are the main source of grafts, ABO-I LT has to be carried out in emergency. However, the established immunosuppressive treatment across the blood type barrier usually requires several days before transplantation and is not applicable in urgent circumstances. For these reasons, modified treatment is warranted for emergent cases such as ALF cases. We therefore developed a simple treatment with rituximab and IVIG in ABO-I LT for ALF.

In our treatment, the omitting of PE was a prominent feature. The general belief is that the high titer of ABO-antibody in the perioperative stage may result in a higher risk of AMR,[13, 16, 17] and the administration of PE to reduce antibody titer before transplantation is essential to the successful ABO-I LT.[18, 19] The titer level less than 1:8 or 1:16 is thought to be relatively safe.[20] However, the role of preoperative ABO-antibody titer in the rejection of ABO-I LT is controversial.[16, 21] Skogsberg et al[22] did not find a significant correlation between titer and AMR. Egawa et al[16] reported that the high preoperative antibody titer had no significant effect on the frequency of AMR. In our study, the average titer level at the operative day was 1:192. Although the patients did not receive any treatment to reduce antibody titer before LT, the rate of AMR was lower (5.7%) than that reported elsewhere.[16] Furthermore, we found that the titer value was decreased immediately on day 1 (average level 16) after LT and maintained at a low level for several months except 2 patients whose serum titers were elevated after LT. The two patients had AMR complications. These findings supported the viewpoint that a preoperative higher antibody titer was not related to the incidence of AMR, and more efforts should be made to prevent the titer elevation after LT. Our data implied that PE administration to reduce the titer before ABO-I LT was not necessary in recipients.

Rituximab as a novel monoclonal anti-CD20 antibody depletes B cells and prevents antibody production. Reports have demonstrated that a single dose of rituximab (375 mg/m²) is sufficient to eliminate B cells for several months and therefore to prevent AMR.[23-25] However, the timing of giving rituximab has not reached a consensus at present.[14, 24, 26] It was suggested that rituximab must be administered for at least 7 days (preferably 3 weeks) before LT to sufficiently terminate humoral reactions.[14] Others[22, 27] administered rituximab immediately after reperfusion or after transplantation to avoid dilution caused by intraoperative bleeding. We administered a single
dose of rituximab at the beginning of LT for the following two reasons. First we did not have enough time to administer rituximab before LT; and second, the administration of rituximab before LT places the recipients at a risk of infection. To validate the suppressive effect of rituximab on B cells, we detected the rate of CD20⁺ cells after LT and found that the count of CD20⁺ cells was significantly decreased from day 1 to 3 months after LT. The results of our study confirmed that the timing of rituximab administration is applicable.

IVIG (0.8 g/kg per day) was considered as an induction therapy after LT or a rescue for treating AMR. It should be emphasized that IVIG is often used in combination with standard alloantibody-depleting therapies such as PE. In the present study, we used IVIG (0.4 g/kg per day) alone for induction therapy and this dosage effectively reduced the alloantibody titers. Furthermore, a lower dose of IVIG remarkably reduced the cost. Thus, the administration of IVIG alone is a novel and effective approach to reduce the antibodies when there is no time for PE.

Although splenectomy and GLI were considered as an integral part of treatment in ABO-I LT in the past, this combination has been under debate since the introduction of rituximab. Furthermore, splenectomy and GLI are associated with high rates of surgical complications and catheter-related complications. In our study, the use of less aggressive procedure minimized complications and morbidity since most of the ALF patients were in very poor health conditions. In our study, AMR was controlled without splenectomy or GLI in most patients.

In this study, acute rejection, infection and biliary complication were the main complications and accounted for graft failure after ABO-I LT. Except in AMR, there was no difference between the two LT groups. Two patients with AMR (5.7%) were diagnosed radiologically and clinically as manifested by infrahepatic biliary complications and hepatic necrosis. The first case did not respond to any treatment with IVIG and PE and eventually received ABO-compatible re-transplantation. The second one was treated effectively with a high dose of IVIG (0.8 g/kg per day). The data of the present study did not support that AMR is directly associated with a higher level of anti-ABO antibody titer preoperatively, but it is likely to happen in patients with rebound elevation of antibody titer postoperatively. Reinforced immunosuppression using basiliximab after LT may be attributed to the lower risk of ACR. In our study, the 3-year patient survival and graft survival rates were 83.1% and 80.0% in the ABO-I LT group respectively, which were similar to those in reported study.

In conclusion, we introduced a modified treatment with rituximab and IVIG but without PE, GLI and splenectomy in adult emergent ABO-I LT for ALF. The results of this study showed that anti ABO-antibody titer level before LT does not significantly affect the outcomes, and that IVIG may play a key role in reducing antibody titer after operation when PE is not available. The results also demonstrated that a single dose of rituximab at the beginning of LT is sufficient to eliminate CD20⁺ cells for several months. This treatment is practical in patients with life-threatening ALF. Large sample size and longer follow-up are needed to validate this treatment.

**Contributors:** ZSS designed the research; ST, LBY and JJJ performed the research and wrote the first draft; WL and LQ analyzed the data; WZY and GL reviewed and revised the paper. All authors contributed to the design and interpretation of the study and to further drafts. ST, LBY and JJJ contributed equally to this article. ZSS is the guarantor.

**Funding:** This work was supported by grants from the National Natural Science Foundation of China (81373160, 81272675 and 81100321), Innovative research group National Natural Science Foundation of China (81121002).

**Ethical approval:** This study was approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine and in accord with ethical principles of the Declaration of Helsinki.

**Competing interest:** No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Protocol of ABO-incompatible liver transplantation


Received February 17, 2014
Accepted after revision April 25, 2014