Tohoku J. Exp. Med., 2006, **208**, 225-233

Usage of Deoxyspergualin on Steroid-Resistant Acute Rejection in Living Donor Liver Transplantation

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Kawagishi, N., Satoh, K., Enomoto, Y., Akamatsu, Y., Sekiguchi, S. and Satomi, S. *Usage of Deoxyspergualin on Steroid-Resistant Acute Rejection in Living Donor Liver Transplantation*. Tohoku J. Exp. Med., 2006, **208** (3), 225-233 —— Deoxyspergualin (DSG) is an immunosuppressive agent used to treat steroid-resistant acute rejection after kidney transplantation. But in the case of acute rejection after liver transplantation, DSG was reported effective in just a few cases. From July 1991 to November 2005, 96 patients underwent living donor liver transplantation (LDLTx) in our institution. Of them, 9 patients, including 4 ABO incompatible recipients, are presented. Rejection symptoms that did not respond to steroid pulse therapy (methylprednisolone, 10-20 mg/kg/day for 3 days) and were treated with DSG (3 or 5 mg/kg/day) for 4 to 14 days together with a maintenance dose of the steroid. Among them, five responded to treatment with DSG, two did not respond and the other two patients were not evaluated. Six of the nine patients are symptom free at present. Complications such as leukopenia and thrombocytopenia were successfully treated with granulocyte-colony stimulating factor or by platelet transfusion. No recipient died as a direct consequence of the complications induced by DSG. DSG proved effective and safe for some of the LDLTx recipients with steroid-resistant acute rejection but it was not effective for the treatment of accelerated humoral rejection in ABO incompatible recipients. ———— liver transplantation; living donor; deoxyspergualin (DSG); rejection; ABO incompatibility

Deoxyspergualin (DSG) was isolated in the 1980's from the *Bacillus laterosporus* found in Japanese fields (Takeuchi et al. 1981). It was initially expected to be an antitumor antibiotic, but it also had special immunosuppressive effects. As for the mechanisms of the immunosuppressive reaction, it was reported that this was not induced by its inhibitory effect on the production of monokines by macrophages or of interleukin-2 by T lymphocytes, but by its cytostatic effect, that is, the inhibition of the cell cycle at the G0/G1 phase (Nemoto et al. 1987). It also inhibited the maturation of T lymphocytes and the activation, differentiation and maturation of B cells, thereby inhibiting antibody production (Fujii et al. 1990). The immunosuppressive effect of DSG increased with the duration of treatment and DSG was not absorbed from the intestine (Nemoto et al. 1987). Therefore, DSG was recognized as an immunosuppressive agent that could be administered at

Received November 22, 2005; revision accepted for publication December 27, 2005.
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high doses for a short period. The results of phase I and phase II clinical trials showed that DSG was useful in several situations: (i) prophylaxis and treatment of acute rejection, (ii) prevention of antibody response to biologic agents used for immunosuppression, (iii) suppression of the humoral response at the time of transplantation in sensitized patients, and (iv) inhibition of macrophage-mediated damage of cellular transplantation (e.g., islets) (Wahoff et al. 1996). In a randomized study of DSG vs anti-CD3 monoclonal antibody (OKT3) for the treatment of steroid-resistant acute rejection in renal transplantation, no significant difference in efficacy was found between the two groups (Ohkubo et al. 1993). However, the side effects profile of DSG was significantly better than that of OKT3 (Ohkubo et al. 1993).

In Japan, DSG has been licensed as an immunosuppressive agent for steroid-resistant acute rejection in patients subjected to kidney transplantation since 1994. Thereafter, it was revealed that DSG had novel immunosuppressive effects including the reversion of an established rejection and suppression of the antibody response seen in subsets of sensitized patients (Okazaki et al. 1989). But the efficacy of DSG in patients undergoing liver transplantation is still unknown. Some reports indicated that DSG was effective against acute rejection in sensitized patients as well as in kidney recipients (Kato et al. 1997). In our institution, we have treated 9 LDLTx recipients with DSG for acute cellular and humoral rejection, including 4 patients with ABO incompatibility. In this study, we describe the effects of DSG on steroid-resistant acute rejection in living donor liver transplant recipients.

Patients and Methods

Characteristics of the patients

From July 1991 to November 2005, 96 LRLTx were performed in pediatric and adult patients with end-stage liver disease in the Division of Advanced Surgical Science and Technology, Graduate School of Medicine, Tohoku University. This program was approved by the ethics committees of Tohoku University Hospital and the patients gave their informed consent regarding LDLTx and the use of DSG for steroid-resistant acute rejection. Donors were selected among parents, brothers or sisters and spouses on the basis of liver function tests, serological markers of hepatitis, ABO blood group, graft/recipient size matching, lymphocyte cross-matching and human leukocyte antigen (HLA) typing. Among them steroid-resistant acute rejection occurred in 9 recipients. The primary diseases of the recipients were biliary atresia in 7, primary sclerosing cholangitis (PSC) in 1, and homozygous familial hypercholesterolemia (FH) in 1. Four recipients were ABO incompatible and 3 out of these 4 recipients were subjected to plasmapheresis (PP) and/or double filtrating plasmapheresis (DFPP) because of the high antibody titers they had before transplantation. After transplantation, apheresis was performed to eliminate antibodies in 3 recipients, PP in 2 and PP with continuous hemodiafiltration (CHDF) in 1 (Table 1). In ABO incompatible recipients, an IgM titer of more than 16 was the indication for apheresis. One ABO incompatible recipient was not subjected to apheresis because the antibody titer against donor blood type was low. These 9 recipients consisted of 4 males and 5 females.

Primary immunosuppressive therapy

The immunosuppressive regimen consisted of cyclosporine (CsA), taclorimus (TAC), azathiopurine (AZ) and methylprednisolone (MP). Seven of the 9 recipients were administered TAC initially while the other 2 were administered CsA. TAC was administered orally 12 hrs before transplantation at a dose of 0.075 mg/kg, and from day 1 after transplantation at a dose of 0.1-0.2 mg/kg/day via a nasogastric tube. On the day of transplantation CsA was administered orally or via a nasogastric tube at a dose of 8 mg/kg/day. The doses of TAC and CsA were adjusted according to plasma trough levels. MP was administered intravenously at a dose of 20 mg/kg/day at the time of the operation and then tapered to 1 mg/kg/day during one week. In the ABO incompatible recipients AZ was administered orally or intravenously from Day 3 before transplantation at a dose of 2 mg/kg/day. In one recipient TAC was switched to CsA because of difficulty in maintaining a suitable trough level.

Rescue therapy for rejection

When symptoms of rejection did not improve with steroid pulse therapy (10-20 mg/kg/day for 3 days), DSG (3 or 5 mg/kg/day) was administered daily for 4 to 14 days together with a maintenance dose of TAC and the

steroid. When the recipient had leukopenia (WBC < 3,000/mm^3) or thrombocytopenia (Plt. < 50,000/mm^3), granulocyte colony stimulating factor (G-CSF) (1-2 μg/kg) was administered or platelets were transfused. PP or CHDF was performed if humoral rejection occurred in the ABO incompatible recipients.

Diagnosis of rejection

Protocol liver biopsies were taken at the time of transplantation and after surgery percutaneous fine needle biopsy was performed under ultrasonography when rejection was clinically recognized. Biopsy specimens were formalin-fixed and parafin-embedded. Five-μm-thick sections were stained with hematoxylin-eosin and Masson's trichrome for collagen. Individual histological features were scored according to a semiquantitative scoring system, which included the following parameters: degree of portal inflammation, piecemeal necrosis, lobular necrosis, steatosis, fibrosis, portal and central vein endothelitis, acute cholangitis, ductular regeneration and cholestasis. Acute ischemic damage was defined by the presence of confluent central or lobular necrosis associated with a variable degree of swelling and bile staining (cholestasis) and without evidence of inflammatory reaction (International Workshop Party 1995). Acute graft rejection was diagnosed by the concomitance of mixed portal inflammation coupled with bile duct inflammation and/or subendothelial inflammation of portal and terminal hepatic veins, according to international criteria (International Workshop Party 1995). Rejection was classified as mild, moderate, or severe.

Monitoring of anti-ABO alloantibodies

The presence of ABO allo-antibodies was assessed in all ABO incompatible patients 2 weeks before transplantation. Serum samples were analyzed with respect to agglutinin titer against a 2 percent suspension in saline of type A or B red blood cells (RBC) after incubation for 1 hour at room temperature. The titer of IgG antibodies was determined by treating the serum samples with 0.01M dithioethreitol (DTT), followed by incubation for 1 hour at 37°C with type A or B RBC. After repeated washings, polyspecific anti-human globulin serum was added. Changes in ABO antibody titers were expressed as the reciprocal number of the highest serum dilution that caused macroscopic agglutination. After transplantation, anti-A or -B alloantibodies were measured every day for the first 7 days and then twice a week.

Apheresis

PP was performed using a continuous blood flow separator (OP-02^{TM} or OP-05^{TM}, Asahi Medical Co., Tokyo). As a second filter for DFPP, Evaflux^{TM} (Kuraray Co., Tokyo) was used. CHDF was performed using a high-performance membrane (PANFLO^{TM}, Asahi Medical Co.). For a blood access, a double-lumen catheter was placed in the subclavian or femoral vein. Replacement fluids were fresh frozen plasma of AB blood type group or 5% albumin in PP. Nafamostat mesilate (0.15 mg/kg/h) was given as the anticoagulant. PP or DFPP was performed before transplantation on 3 consecutive days in the patients with ABO incompatibility.

Results

Effectiveness for steroid resistant acute rejection

Acute rejection was initially treated with steroid pulse therapy; steroid-resistant acute rejection was occurred once in 9 recipients. They were administered DSG at the dose of 3 or 5 mg/kg/day daily for 4 to 14 days together with the maintenance dose of the steroid (Table 1).

Case 1: The patient was a 5-year-old girl with biliary atresia (BA). She received an ABO incompatible partial liver graft from her mother. Preoperatively PP and DFPP were performed because her anti-A antibody titer was high. On post operative day (POD) 6 liver function tests and histological biopsy indicated acute cellular rejection. She was treated with MP for 3 days. On POD 14 the values of her liver function parameters increased again and the biopsy showed acute cellular rejection. The patient was administered MP followed by DSG at the dose of 3 mg/kg/day for 10 days together with TAC and AZ. PP was also performed to treat her hyperbilirubinemia. After the administration of DSG the value of her liver function parameters decreased and she was discharged on POD 86. During her post operative course no accelerated acute rejection was observed and the anti-A antibody titers decreased after POD 7.

Case 2: The patient was a 1-year-old girl with BA. On POD 6 portal vein thrombosis was detected and thrombectomy was performed under

TABLE 1. *Patient demographic characteristics*

No. of Case	Age at Tx/ Gender	P.D.	Blood Type	P.I.	Treatment for Steroid Resistant Acute Rejection	Day on DSG Administration	Outcome
1	5y2m/F	BA	incompatible (A→O)	TAC, AZ, MP	DSG (3 mg/kg/day, 10 days) + PP	19 POD	effective
2	1y5m/F	BA	identical (A→A)	CsA, AZ, MP	DSG (3 mg/kg/day, 4 days)	13 POD	effective
3	11m/M	BA	incompatible (AB→B)	TAC, MP	DSG (3 mg/kg/day, 14 days) + MP	31 POD	effective
4	3y9m/F	BA	identical (A→A)	TAC, MP	DSG (3 mg/kg/day, 7 days 5 mg/kg/day, 3 days)	464 POD	ineffective
5	7y9m/M	BA	identical (O→O)	TAC, MP	DSG (3 mg/kg/day, 4 days)	1452 POD	effective
6	11m/M	BA	identical (B→B)	CsA, AZ, MP	DSG (3 mg/kg/day, 5 days)	14 POD	effective
7	5y11m/M	PSC	identical (B→B)	TAC, MP	DSG (3 mg/kg/day, 9 days)	26 POD	not evaluated
8	10y3m/F	BA	incompatible (A→B)	TAC, AZ, MP	DSG (3 mg/kg/day, 8 days) + splenectomy, PV cannulation, PP, CHDF	4 POD	ineffective
9	2y/F	HFH	incompatible (A→O)	TAC, MMF, MP	DSG (3 mg/kg/day, 10 days) + PP	5 POD	not evaluated

P.D., primary disease; P.I., primary immunosuppression; BA, biliary atresia; PSC, primary sclerosing cholangitis; DSG, deoxyspergualin; TAC, tacrolimus; CsA, cyclosporine A; AZ, azathioprine; MP, methylpredonisolone; PP, plasma pheresis; CHDF, continuous hemodiafiltration; PV, portal vein; HFH, homozygous familial hypercholesterolemia; MMF, mycophenolate mofetil; POD, post operative day.

laparotomy. The biopsy performed at the same time revealed signs of acute rejection. She was administered MP followed by DSG at the dose of 3 mg/kg/day for 4 days but it was discontinued because of severe leukopenia and thrombocytopenia. Her liver function started to improve on the day after discontinuation of DSG.

Case 3: The patient was an 11-month-old boy with BA (Fig. 1). He received an ABO incompatible partial liver graft from her mother. Apheresis was not performed before surgery because his anti-A antibody was low. On POD 18 the results of liver function tests and biopsy indicated acute cellular rejection. He was treated with MP for 3 days. However, the values of liver function parameters increased again and DSG was administered at the dose of 3 mg/kg/day for 14 days together with a 3-day course of MP pulse therapy. After the administration of DSG the concentration of total bilirubin decreased and he was discharged on POD 78. During his POD course no accelerated acute rejection was observed and the anti-A antibody titer remained low.

Case 4: The patient was a 5-year-old girl with BA. She had been discharged without any complication after LDLTx at the age of 3. One year after transplantation she developed post-transplant lymphoproliferative disease and her medication was changed from TAC to CsA. Then she developed acute rejection with severe bile duct damage; she was placed under MP pulse therapy followed by DSG at the dose of 3 mg/kg/day for 7 days and 5 mg/kg/day for 3 days. Her liver function improved but not dramatically; thus, she was administered acyclovir because viral hepatitis was also suspected. After these therapies her laboratory and clinical data gradually improved.

Case 5: The patient was an 11-year-old boy with BA. He had undergone LDLTx at the age of 7. He was administered DSG after the second pulse therapy but the values of his liver function

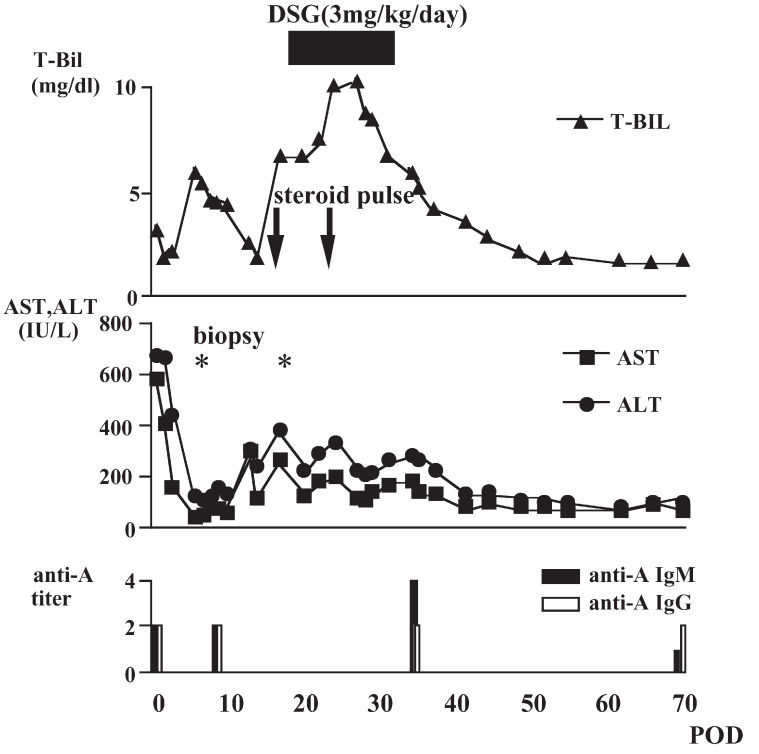

Fig. 1. Rescue therapy by DSG for acute cellular rejection.
Posttransplantation course of case 3. First steroid pulse therapy was ineffective, whereas DSG with MP pulse therapy resulted in improvement of liver function.
DSG, deoxyspergualin; T-Bil, total bilirubin.

parameters were still high after the 4th day on DSG. We speculated this was a case of drug-induced toxicity and withdrew the drug. The biopsy revealed the rejection reaction and drug-induced toxicity had ended. Thereafter his liver function gradually improved.

Case 6: The patient was a 10-month-old boy with BA. On POD 7 the values of liver function parameters and biopsy indicated acute cellular rejection. He was treated with MP for 3 days. However, the values of his liver function parameters increased again and he was administered DSG at the dose of 3 mg/kg/day for 5 days. His liver function improved but his white blood cells count decreased 2 days after the end of the DSG administration and he developed high fever. However, there was no infection and leukopenia improved after treatment with G-CSF.

Case 7: The patient was a 5-year-old boy with PSC. On POD 26 after pulse therapy, he was administered DSG but the value of his liver function parameters were still high after the 5th day on DSG. Examination of a biopsy specimen revealed no signs of rejection but of drug-induced toxicity. DSG was withdrawn on the 9th day of the administration. Thereafter, his liver function gradually improved.

Case 8: The patient was a 10-year-old girl with BA (Fig. 2). She received an ABO incompatible partial liver graft from her mother. Preoperative PP was performed because her anti-A antibody titer was high. On POD 4 the values of liver function parameters and biopsy indicated accelerated acute rejection. She was subjected to PP, splenectomy, MP pulse therapy and DSG. DSG was administered at the dose of 3

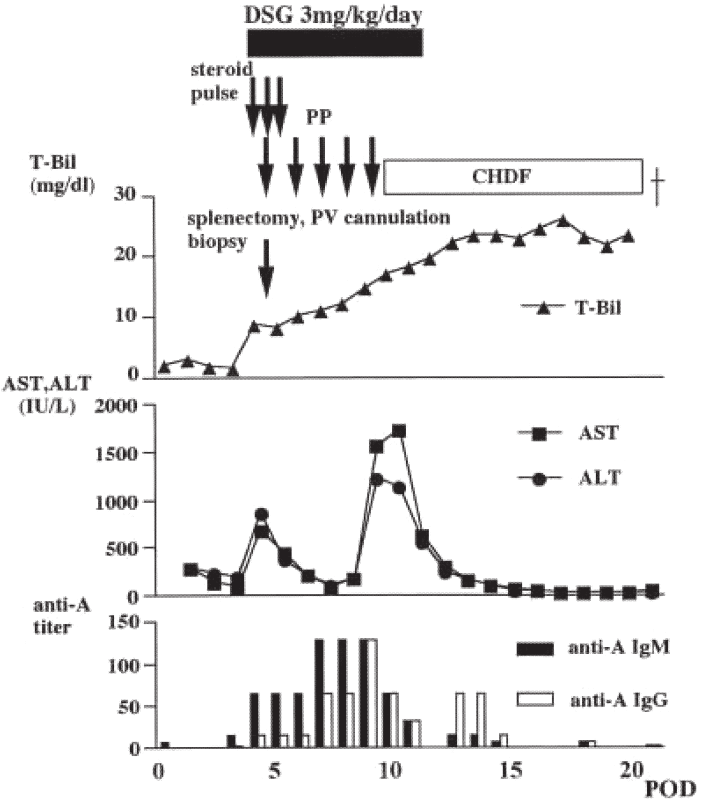

Fig. 2. Accelerated acute rejecion in case with ABO-incompatible.
Posttransplantation course of case 8. On 4 POD her liver function tests and histological biopsy indicated accelerated acute rejection. She was treated by PP, splenectomy, MP pulse therapy and DSG, however she was not rescued.
PP, plasmapheresis; CHDF, continuous hemodiafiltration; DSG, deoxyspergualin;
T-Bil, total bilirubin.

mg/kg/day for 8 days together with PP. The titer of anti-A antibody decreased after the 9th POD but hyperbilirubinemia persisted. Her liver function gradually improved but she died on POD 22 because of cardiac failure.

Case 9: The patient was a 2-year-old girl with homozygous familial hypercholesterolemia. She received an ABO incompatible partial liver graft from her mother. Preoperative PP was performed because her anti-A antibody titer was high. On POD 3 the values of liver function parameters and anti-A antibody titer indicated humoral rejection. She was subjected to PP and DSG. DSG was administered at the dose of 3 mg/kg/day for 10 days together with PP. The titer of anti-A antibody decreased after POD 11. She suffered from biliary stenosis 6 months after LDLTx, but her liver function improved after percutaneous transhepatic biliary drainage.

Adverse events

As for adverse events, three recipients had leukopenia and/or thrombocytopenia and these were rescued by G-CSF or platelet transfusion (Table 2). Three recipients showed DSG-induced hepatotoxicity but their liver function tests improved within one week after withdrawal of the drug. None of the recipients died because of complications directly induced by DSG.

TABLE 2. *Adverse events observed during the anti-rejection therapy*

	n
Leukopenia	4
	(Use of G-CSF 2)
Thrombocytopenia	2
	(Platelet transfusion 1)
Drug induced toxicity	3

G-CSF, granulocyte colony stimulating factor.

Long-term follow-up

As for long-term follow-up, seven recipients are alive and six of them are symptom free at present (Table 3).

TABLE 3. *Long-term follow up*

No. of Case	Long term status	Period after LDLTx
1	symptom free	11y 5m (alive)
2	symptom free	11y 4m (alive)
3	symptom free	10y 5m (alive)
4	symptom free	9y 1m (alive)
5	symptom free	8y 10m (alive)
6	symptom free	8y 8m (alive)
7	recurrence of primary disease	7y5m (dead)
8	dead on 22 POD	-
9	biliary stenosis	1y (alive)

DISCUSSION

Steroid-resistant acute rejection is treated with DSG after living donor liver transplantation in our institution. Progressive graft rejection in a patient with a liver graft is life threatening and when steroids and/or other immunosuppressive agents fail to reverse the process retransplantation has been the only option. The cases described here show that DSG is effective for steroid-resistant acute rejection in living donor liver transplant recipients. DSG has been recognized as an effective immunosuppressive agent in kidney transplantation, particularly in Japan. The drug is mainly used as a prophylactic and/or rescue therapy for acute rejection. Particularly, in the case of rescue therapy for acute rejection after kidney transplantation, it was reported that therapy using DSG combined with MP was one-hundred percent effective while DSG alone was effective in more than 70% of the cases (Kenmochi et al. 1990). Moreover, it was reported that the efficacy of DSG and that of OKT3 against steroid resistant acute rejection were comparable (Ohkubo 1993).

In the field of liver transplantation there have been only a few reports that refer to the efficacy of DSG. Kato et al. (1997) reported 3 cases, including 2 ABO incompatible recipients, who were rescued by DSG after steroid-resistant acute cellular rejection. Groth et al. (1990) reported the reversal of acute rejection with DSG in a patient in whom previous treatment with steroid and

OKT-3 had failed. In our experience, DSG was effective for steroid-resistant acute cellular rejection in 6 out of 8 liver transplantation cases. But in one case with accelerated humoral rejection, DSG was not effective.

The precise mechanism of the immunological effect of DSG is not known but it is believed to inhibit a protein called heat shock protein 70, which is necessary for the translocation of transcription factors such as nuclear factor-kappa B (NF-kB) to the nucleus (Nadler et al. 1992). As a result, DSG inhibits not only the maturation of T lymphocytes but also the activation, differentiation and maturation of B lymphocytes, inhibiting thereby antibody production. Therefore, DSG is useful for sensitized or ABO incompatible recipients, relying on its ability to suppress humoral immunity. In the field of kidney transplantation, Okazaki et al. (1991) reported the efficacy of DSG in recipients after donor specific transfusion. In a study involving 44 ABO incompatible kidney recipients, in which DSG was used together with standard induction therapy, PP, splenectomy and local graft irradiation, the results of transplantation were excellent, i.e., graft survival was 83% at one year and 80% at three years (Takahashi et al. 1993). In liver transplantation, the efficacy of preformed lymphocytotoxic antidonor antibodies on graft survival still remains controversial. Several hypotheses have been proposed as possible explanations for the discrepancies among various studies (Donaldson et al. 1995; Manez et al. 1995). These discrepancies relate, in particular, to the relatively small numbers of patients as well as to methodological differences and sensitivity of the assays in renal transplantation.

As for liver transplantation, the survival rate of ABO incompatible patients has been reported to be significantly worse than that of ABO compatible patients (Farges et al. 1995). The presence of preformed anti A and/or anti B antibodies in the recipient and the wide expression of these antigens on endothelial cells in vessels and parenchymal epithelial cells in the graft are indeed risk factors for a hyper acute rejection (Ernst et al. 1984). Therefore, liver transplantation from ABO mismatched donors has been justified only in emergency cases, especially in children, due to the shortage of appropriate donor grafts. However, in living donor liver transplantation we have to use ABO mismatched grafts even in elective cases. In the case of blood type mismatched grafts, we take a lot of measures in an effort to succeed such as pre- and post- transplant PP, splenectomy and strong immunosuppressive therapy (Kawagishi et al. 2001). In our institution we experienced 11 patients who received an ABO blood type incompatible graft. We used DSG in four cases of acute cellular rejection and in two the therapy proved successful. But one patient with accelerated humoral rejection could not overcome severe rejection despite DSG combined with PP, splenectomy and portal cannulation (Case 8). In recipients of kidney transplants, it was reported that DSG was effective even for accelerated acute rejection (Amemiya et al. 1990).

The adverse effects observed in kidney transplant patients included numbness of the face, lips and limbs, gastrointestinal toxicity, bone marrow suppression and the occurrence of infection (Amemiya et al. 1990). In our experience none of the side effects, such as leukopenia, thrombocytopenia and drug toxicity, persisted for a long period. Infectious complications were prevented with antibiotics and stringent screening for bacterial and viral infection.

In conclusion, DSG proved effective for steroid-resistant acute rejection in some LDLTx recipients without inducing severe adverse effects. But DSG was not effective in patients with accelerated humoral rejection.

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