Table 1. ESPEN and A.S.P.E.N. Guidelines for Cirrhotic Patients.

ESPEN guidelines[32]	35–40 kcal/kg/d Enteral nutrition is recommended. Supplemental nutrition, especially BCAA-enriched formula, is preferred.
A.S.P.E.N. guidelines[119]	
Without encephalopathy	25–35 kcal/kg/d
With acute encephalopathy	35 kcal/kg/d
Stable and malnourished	30–40 kcal/kg/d

A.S.P.E.N., American Society for Parenteral and Enteral Nutrition; BCAA, branched-chain amino acid; ESPEN, European Society for Clinical Nutrition and Metabolism.

nutrition support, in addition to oral diet, can reduce the complications after major hepatectomy in cirrhotic patients. PN is indicated for malnourished cirrhotic patients who cannot be nourished sufficiently by either the oral or enteral routes.[58] It has been reported that enteral nutrition (EN) and PN were equally effective for maintaining nutrition status after liver transplantation and decreasing complications and cost.[59] Plauth et al[60] suggested that parenteral feeding might be superior to enteral feeding in patients with portosystemic shunting because enteral feeding might worsen hyperammonemia.

Enteral Nutrition

The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines (see Table 1) advocate that patients with liver cirrhosis should receive 35–40 kcal/kg/d with 1.2–1.5 g/kg protein/d.[32] In general, oral nutrition supplements are recommended for patients without any contraindications such as ileus.[32] If patients cannot maintain adequate oral intake, tube feeding is recommended even when esophageal varices are present.[32] It was recently reported that protein restriction in patients with liver failure has no impact on the encephalopathy and even worsens nutrition status.[61] Cordoba et al[4] reported that diets with a normal protein content can be safely administered to cirrhotic patients with episodic hepatic encephalopathy. Many reports have described the effects of oral nutrition supplementation in patients with alcoholic cirrhosis.[62-66] For example, Le Cornu et al[67] reported that regular dietary counseling is as effective for increasing energy intake as providing a nutrition supplement. Other reports also have exhibited the superiority of the safety and efficacy in terms of postoperative complication rates for postoperative early EN compared with PN.[68-70] The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) guidelines (see Table 1) also support the concept that EN is favored to preserve gut integrity and immune markers and to simplify glycemic management.[71] In adult living donor liver transplantation, Kaido et al[72] reported that EN is a promising strategy to improve postoperative mortality and morbidity rates.

Branched-Chain Amino Acids

Oral supplementation with essential amino acids can improve hepatic encephalopathy and the quality of life in not only cirrhotic patients but also institutionalized elderly patients.[73-75] Supplemental BCAA is commonly applied in patients with liver cirrhosis,[76,77] especially compensated cirrhosis[78,79] or hepatocellular carcinoma.[80-85] The ESPEN guidelines recommend the use of BCAA-enriched supplements in patients with hepatic encephalopathy.[32] It was proposed that depletion of BCAAs, as seen in many patients with advanced liver disease, promotes the development of hepatic encephalopathy by enhancing the passage of AAAs across the blood-brain barrier, resulting in the synthesis of false neurotransmitters.[6] In addition, the administration of solutions enriched with BCAAs has been shown to improve cerebral perfusion in cirrhotic patients.[86]

In prospective studies, it was reported that long-term oral supplementation with a BCAA mixture improved the serum albumin level, as well as the cellular energy metabolism and quality of life in cirrhotic patients.[77,87] The timing of the supplementation with BCAA is also important. It was reported that nocturnal BCAA administration as a late evening snack (LES) improved the serum albumin level in cirrhotic patients who showed no improvement in their serum albumin levels with daytime BCAA administration.[53,88,89] Recently, many reports have shown that BCAA supplementation can improve not only energy metabolism and BTR but also glucose tolerance.[90-95]

There have been reports that perioperative administration of BCAA to patients undergoing hepatic resection quickly improves liver function during the early postoperative period.[81,96,97] Ishikawa et al[98] reported that short-term oral nutrition support with BCAA was associated with higher serum erythropoietin levels in patients with nonhepatitis liver disease who underwent curative hepatic resection. They suggested that it had benefits in protecting liver cells from ischemic injury and preventing intraoperative hemorrhage.[98]

In liver transplantation patients, perioperative supplementation with BCAA-enriched nutrients can improve the nutrition and metabolic disorders associated with end-stage liver disease.[99] Early interventional oral BCAAs might prolong the liver transplant waiting period by preserving the hepatic reserve in patients with cirrhosis.[100] Recently, the effectiveness of amino acid (ie, glycine, taurine, N-acetylcysteine, arginine, and methionine) supplementation in protecting against ischemia/reperfusion injury (IRI) has attracted attention.[101]

Figure 1 shows a schematic representation of the activities of BCAA. BCAA, especially leucine, activates the mTOR signaling pathways and inhibits protein degradation in vitro, thus resulting in the promotion of protein synthesis.[102,103] Furthermore, in a cirrhotic rat model, leucine activated glycogen synthase via mTOR signaling and improved glucose metabolism.[104] Several reports have suggested that BCAA

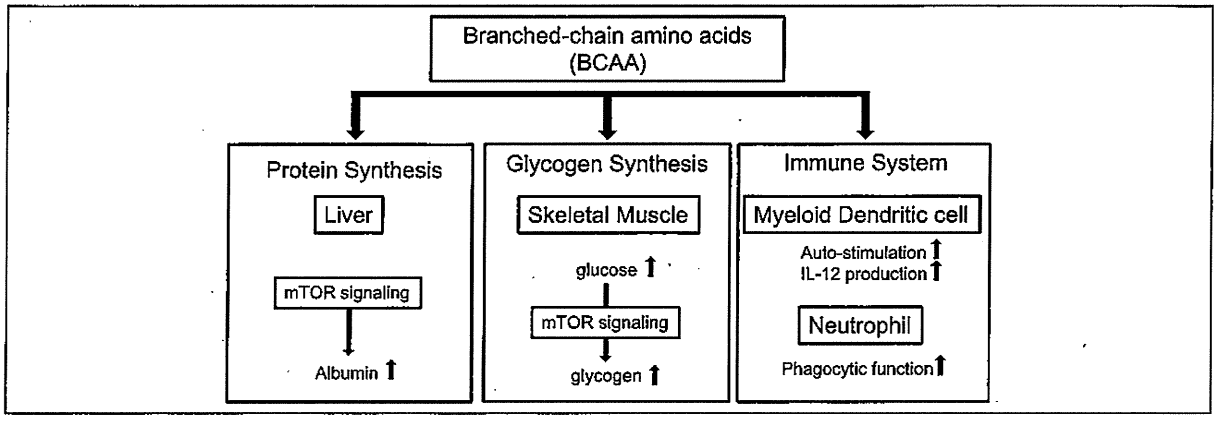

Figure 1. A schematic representation of the activities of branched-chain amino acids (BCAAs). BCAAs promote protein synthesis by activating mTOR signaling in the liver. In the skeletal muscle, BCAAs promote glycogen synthesis via the activation of mTOR signaling. In addition, BCAAs regulate immune system function by activating the myeloid dendritic cell function and improving the phagocytic function of neutrophils.

supplementation can restore or regulate the immune function in patients with advanced cirrhosis.[105-109] Calder[14] reported that the essentiality of BCAA for the function of immune cells relates to protein synthesis. Kakazu et al[107,108] reported that an elevation of the BCAA level improved the function of myeloid dendritic cells and that this was beneficial to immune function. Nakamura et al[105,106] reported that the phagocytic functions of neutrophils and the natural killer activity of lymphocytes obtained from patients with liver cirrhosis were restored by oral supplementation with BCAAs.

Synbiotics

Selective bowel decontamination increases the incidence of cholangitis and bacterial infections.[110] Synbiotics, a combination of pro- and prebiotics, increase the intestinal content of lactic acid–type bacteria in hepatic encephalopathy patients with end-stage liver failure.[48] Sugawara et al[111] reported that preoperative oral administration of synbiotics can enhance immune responses, attenuate systemic postoperative inflammatory responses, and improve the intestinal microbial environment.

Beneficial Effects of Nutrition Support Against Infections Associated With Liver Surgery (Table 2)

Fan et al[57] evaluated the effects of perioperative PN in addition to a typical oral diet, from 7 days before hepatic resection and continued for 7 days after the operation. Perioperative PN in addition to a normal oral diet was associated with a significant reduction in infectious complications (a normal oral diet plus PN: 17% vs control: 37%).[57] This relatively large prospective study has sufficient power to show the benefits of nutrition support for preventing the occurrence of infection after liver surgery. Shirabe et al[69] prospectively compared postoperative early EN and PN. Early enteral feeding had a tendency to reduce the infection rate after major hepatic resection (early EN: 8% vs PN: 31%), without statistical significance due to the small number of patients. Mochizuki et al[70] retrospectively and prospectively compared postoperative EN and PN. Although it was a retrospective study with nonrandomized prospective data, the infection rate of patients in the early EN group, especially high-risk patients, was markedly decreased.[70] Okabayashi et al[112] evaluated the benefits of perioperative supplementation of BCAA-enriched nutrients in patients undergoing hepatic resection. Postoperative surgical site infection tended to be lower in the BCAA supplementation group (5%) than in the control group (15.3%). However, this retrospective study only briefly discussed "infectious" complications, and therefore the impact of BCAA supplementation on preventing postoperative infections still remains insufficiently described, and thus further studies are expected.

In hepatic resection for patients with liver disease, perioperative nutrition support, either enteral or parenteral, can reduce septic complications. Furthermore, postoperative early EN, especially BCAA-enriched nutrition, may prevent postoperative infections.

Beneficial Effects of Nutrition Support Against Infections Associated With Transplantation (Table 3)

Hasse et al[113] evaluated the benefits of receiving EN via nasointestinal feeding tubes before initiating an oral diet. Nutrition support therapy decreased the incidence of viral

Table 2. Beneficial Effects of Perioperative Nutrition Support in Reducing Infections in Patients Undergoing Hepatic Resection.

Authors	Study Design	Patient No.	Nutrition Therapy (Infection Rates)
Fan et al,[57] 1994	Prospective study	124 (64 with PN and 60 with oral diet alone)	Pre- and postoperative PN in addition to oral diet (17%) and oral diet alone (37%)
Shirabe et al,[69] 1997	Prospective study	26 (13 with early EN and 13 with PN)	Postoperative early EN (8%) and PN (31%)
Mochizuki et al,[70] 2000	Retrospective and prospective study	67 (19 with early EN and 48 with PN)	Postoperative early EN (30%) and PN (73.1%)
Okabayashi et al,[112] 2008	Retrospective study	112 (40 with BCAA supplementation and 72 with control)	Pre- and postoperative BCAA-enriched nutrition (5%) and control (15.3%)

BCAA, branched-chain amino acid; EN, enteral nutrition; PN, parenteral nutrition.

Table 3. Beneficial Effects of Perioperative Nutrition Support in Reducing Infections in Patients Undergoing Liver Transplantation.

Authors	Study Design	Patient No.	Nutrition Therapy (Infection Rates)
Hasse et al,[113] 1995	Prospective study	31 (14 with tube feeding and 17 with control)	Postoperative tube feeding (21.4%) and control (47.1%)
Rayes et al,[114] 2002	Prospective study	95 (32 with selective bowel decontamination, 31 with *Lactobacillus*, and 32 with placebo)	Postoperative early EN with *Lactobacillus* (13%) and control (48%)
Rayes et al,[115] 2005	Prospective study	66 (33 with lactic acid bacteria and fiber and 33 with fiber only)	Postoperative early EN with lactic acid bacteria and fiber (3%) and only fiber (48%)
Plank et al,[117] 2005	Retrospective study	32 (15 with perioperative immunonutrition and 17 with control)	Pre- and postoperative immune-enhancing diet (33%) and control (71%)
Kaido et al,[118] 2010	Prospective study	30 (10 with postoperative early immunomodulating nutrition and 20 with conventional enteral diet)	Postoperative early EN with immunomodulating diet (10%) and control (50%)
Shirabe et al,[22] 2011	Retrospective study	236 (129 with BCAA supplementation and 107 with control)	Preoperative BCAA supplementation (6.7%) and control (22.0%)

BCAA, branched-chain amino acid; EN, enteral nutrition.

infection (enteral tube feeding: 0% vs control: 17.7%) and showed a trend to decrease bacterial (enteral tube feeding: 14.3% vs control: 29.4%) and overall (enteral tube feeding: 21.4% vs control: 47.1%) infections.[113] Although this was a small-group study, this proves the benefits of early EN in preventing both viral and bacterial infections. Shirabe et al[22] evaluated the effectiveness of preoperative oral supplementation with BCAA. Preoperative BCAA supplementation reduced the incidence of bacteremia (BCAA supplementation: 6.7% vs control: 22.0%) after living donor liver transplantation. Although it is a retrospective study, it was valuable because it included a large number of patients and the infectious complication rates were quite low in the preoperative BCAA supplementation group. Some *Lactobacillus* species have been shown to initiate immunoglobulin production, restore macrophage function, stimulate apoptosis, and modulate lymphocyte function.[114] In addition, *Lactobacillus* is reported to influence cytokine release, increase mucin production, eliminate toxins, and stimulate mucosal growth.[114] Rayes et al[114,115] reported the benefits of a perioperative supply of synbiotics. The patients who received living *Lactobacillus plantarum 299* plus fiber developed fewer bacterial infections (13%) than did control patients (48%).[114] In addition, the incidence of postoperative bacterial infections was lower (3%) with lactic acid bacteria and fiber than in the 48% of patients who consumed only fiber.[115] Supplementation with ω-3 fatty acids downregulated proinflammatory cytokine production and modulated eicosanoid synthesis.[116] In addition, arginine stimulated the release of growth hormone and insulin, improved nitrogen balance and wound healing, upregulated

immune function, and enhanced nitric oxide (NO) biosynthesis.[116] Plank et al[117] evaluated the effects of a pre- and postoperative enteral immune-enhancing diet. Infectious complications were less common in patients who received immunonutrition (33% in the immunonutrition group and 71% in the control group), although there were no significant differences.[117] Similarly, Kaido et al[118] reported the benefit of postoperative early EN with an immunomodulatory diet. The incidence of posttransplant bacteremia was lower in the immunomodulatory diet group (10%) than in the conventional enteral diet group (50%).[118] This prospective study showed a much lower rate of infectious complications in the immune-enhancing diet group. A randomized controlled study evaluating the effect of preoperative long-term immunonutrition in patients listed for liver transplantation is planned in Europe.[116]

In summary, in patients undergoing liver transplantation, early posttransplant tube feeding is recommended. As early postoperative nutrition, solutions containing pre- and probiotics have some effects to prevent postoperative infections. Preoperative supplementation with BCAA may improve patient malnutrition and reduce the risk of postoperative infections. A pre- and postoperative immune system–enhancing diet increasingly has been demonstrated to have benefits in preventing posttransplant infectious complications.

Conclusions

Malnutrition is common in patients with liver cirrhosis undergoing liver resection or liver transplantation. Oral nutrition support with BCAA, synbiotics, and an immune-enhancing diet can have a beneficial effect on preventing the postoperative infections associated with hepatic resection or liver transplantation.

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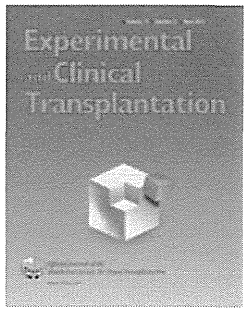
CASE REPORT

Liver Transplant From an ABO-Incompatible and Hepatitis C Antibody-Positive *but an HCV-RNA Negative* Living Donor in a Familial Amyloid Polyneuropathy Patient

Takayuki Takeichi, Katsuhiro Asonuma, Hidekazu Yamamoto, Yuki Ohya, Kenji Okumura, Kwang- Jong Lee, Yukihiro Inomata

Familial amyloid polyneuropathy is a rare, progressively disabling, and ultimately fatal inherited disease. Liver transplant is currently the only available treatment proven to halt the progression of familial amyloid polyneuropathy. We report a 31-year-old woman with familial amyloid polyneuropathy who received a living-donor liver transplant from her husband who was hepatitis C virus antibody-positive but *HCV-RNA* negative and ABO incompatible. Six years after the transplant, both donor and recipient have normal liver biochemistry results; no hepatitis C viral load has been detectable in the recipient. This is the first report of a living ABO-incompatible liver transplant from an anti-hepatitis C virus antibody-positive but an *HCV-RNA* negative donor. This experience suggests that the use of an anti-hepatitis C virus antibody-positive hepatic graft is possible in select circumstances.

Key words: Familial amyloid polyneuropathy, Hepatitis C virus, Living-donor liver transplant, ABO-incompatible transplant



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From the Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan
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Corresponding author: Takayuki Takeichi, MD, Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University Hospital
1-1-1 Honjo , Kumamoto 860-8556, Japan
Phone: +81 96 373 5618
E-mail: ttakeichi@fc.kuh.kumamoto-u.ac.jp

Introduction

Familial amyloid polyneuropathy (FAP) is an inherited disorder resulting in systemic deposition of amyloid fibrils containing mutant transthyretin variants.[1] The outcome of this disease is so poor that FAP has long been considered incurable. The first successful liver transplant in a patient with FAP was performed in 1990, and since then, liver transplant has become widely used for patients with FAP as a life-saving treatment.[2, 3] In Japan, there is little deceased-donor liver transplant, but living-donor liver transplant (LDLT) has been done in patients with FAP. The living donor is selected from among the patient's relatives. Because FAP is an inherited disorder, candidates for living donor can be difficult to find among the relatives. This may lead to an increased use of marginal living donors. We report the outcome of an ABO-incompatible (ABO-I) liver transplant from an anti-HCV-positive donor to a recipient with FAP.

Case Report

A 31-year-old woman presented to us with no relevant history of disease during her childhood. Neurologic manifestations had appeared 5 years earlier, and she was diagnosed with FAP 3 years after that. She had a familial history of FAP, and her mother had died of FAP at 43 years of age, while her sister was a gene carrier (although no symptoms had developed). Her father had hepatitis C virus (HCV) cirrhosis. She was indicated for liver transplant, and the transplant had to be done quickly because of her 5-year history of FAP and its late diagnosis and far advanced nature. However, the possibility of deceased-donor liver transplant in Japan is not good. The only possible living-donor candidate was her 26-year-old husband, but he had an HCV infection and had received interferon therapy 5 years earlier. Furthermore, his blood type was A, and the recipient's blood type was O; thus, the blood types were incompatible. The results of his liver function tests were normal: total bilirubin, 0.8 mg/dL; aspartate aminotransferase, 20 IU/L; alanine aminotransferase, 26 IU/L; alkaline phosphatase, 250 U/L; gamma-glutamyl transpeptidase, 41 U/L; albumin, 4.2 g/dL; and prothrombin time, 12.5 seconds (90%). His viral profile was as follows: HBs antigen (-); HBs antibody (-); anti-HCV (+); and HCV-RNA (-). A needle liver biopsy was done, and the histologic findings showed only mild steatosis, no necrosis, no hepatitis, and no fibrosis. Despite the fact that the husband was anti-HCV-positive and ABO-I, we decided to proceed with an LDLT because her disease prognosis was poor and there was little chance of any other liver donor available. Furthermore, the donor was happy to donate his liver to his wife even though there is a risk to both the donor and the recipient with LDLT. Approval was obtained from the Ethics Committee of Kumamoto University Graduate School of Biomedical Sciences after an interview with the donor and the recipient.

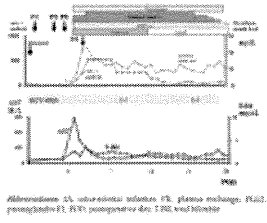

Figure 1. Time Course After Living-Donor Liver Transplant

We performed an LDLT using a left lobe graft without the caudate lobe. The surgical procedure for the donor and the recipient has been described elsewhere.[4] The donor's operative duration was 7 hours 32 minutes. The donor's operative blood loss was 470 mL, and no blood transfusion was performed. The total operative duration for the recipient was 10 hours 28 minutes. The actual graft weight was 470 grams, which was 1.04% of the recipient's body weight. The recipient's operative blood loss was 350 mL; thus, no transfusion was necessary.

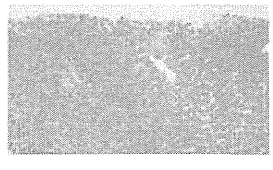

Figure 2. Graft Liver Biopsy 6 Years After Living-Donor Liver Transplant

Because of the ABO-I blood combination, the recipient was treated with an immunosuppression protocol consisting of preoperative rituximab, a plasma exchange, a triple immunosuppressive regimen, intra-arterial infusion therapy, and a splenectomy at surgery (Figure 1). She received 500 mg rituximab intravenously 2 weeks before the LDLT. Her anti-ABO IgM and IgG titers were 512 and 256 one week before the operation. Plasma exchange was performed 3 times within 1 week of the LDLT. Her anti-ABO IgM and IgG titers dropped to 2 and 4 just before the operation. For hepatic artery infusion, an intra-arterial catheter was placed during the operation, and continuous infusion of prostaglandin E1 (0.01 μg/kg/min on days 0 to 14) and methylprednisolone (125 mg/d on days 0 to 7, 50 mg/d on days 8 to 14; then we tapered the dosage and discontinued the drug on day 21). Endoxan (100 mg) was administered from postoperative days (POD) 1 to 14; this was followed by mycophenolate mofetil 500 mg twice a day from POD 15 onward.

Posttransplant immunosuppression consisted of tacrolimus and steroids. The trough level of tacrolimus was maintained between 10 and 15 ng/mL during the first 2 weeks. Because the anti-ABO IgM and IgG titers rose markedly from the day after transplant, we performed a plasma exchange on POD 3. Although the titers did not decrease immediately, the patient's liver function recovered well. The quantity of steroids in the hepatic artery infusion was increased and the titer gradually decreased. The patient had prolific nausea after transplant probably because of the original disease, but her liver function results recovered to normal on POD 21. Her renal functions were normal before and after the transplant. Hepatitis C virus RNA was not detectable by polymerase chain reaction after the transplant. The hepatic artery catheter was removed on POD 31, and she was discharged from hospital with excellent graft condition 50 days after the operation.

At the time of this writing it has been 6 years after the transplant, and the patient has been well, with excellent graft function, unremarkable liver biochemistry, and has been HCV-RNA negative. Figure 2 shows a liver biopsy 6 years after the transplant, with no evidence of cellular rejection or fibrosis. Progression of FAP is controlled and she has an excellent quality of life.

The postoperative course of the donor also was uneventful. Although serum aspartate aminotransferase increased to 225 IU/L on POD 3, it returned to normal by POD 7. The maximum total bilirubin level was 2.5 mg/dL on POD3. He left hospital on POD 17. He returned to work 3 months after the operation. At the time of this writing, after 6 years, his liver function test results are normal, and HCV-RNA is negative.

Discussion

Liver transplant is the only effective treatment for FAP. More than 65 patients in Japan with FAP have undergone a liver transplant, with living donors consisting of parents, siblings, or husbands; there has been 1 deceased donor.[6] In Japan, organs from deceased donors remain scarce, so that living-related liver transplant is more common. Because FAP is an autosomal dominant inherited disease, potential living donors are restricted. In the present case, there were potentially serious problems for the donor and recipient, such as a risk of flare-up of the HCV infection in the donor, and HCV transmission under strong immunosuppression due to ABO-I matching in the recipient.

ABO-I living-related liver transplant increasingly has been performed in Japan to overcome the shortage of donor organs. Initially, the outcome was poor because of antibody-mediated rejection; however, it has dramatically improved with the use of local steroid infusion and rituximab prophylaxis.[7] In the present case, the patient had no antibody-mediated rejection after receiving a living-related liver transplant.

Several single-center studies have shown no significant differences in survival among HCV-positive recipients transplanted with anti-HCV-positive grafts compared with recipients transplanted with anti-HCV-negative donor organs.[8-10] Saab and associates reported that the use of HCV-positive grafts in recipients with HCV infection does not appear to affect patient survival, graft survival, or recurrence of HCV infection when compared with using anti-HCV-negative grafts.[11] There are several reports of HCV flare-up after chemotherapy and bone marrow transplant in patients with anti-HCV-positive/HCV-RNA-positive grafts.[12-14] The persistence of HCV in patients with previously cleared HCV remains controversial. However, we could not find and research reporting on the use of anti-HCV-positive/HCV-RNA-negative allografts in non-HCV recipients.

In kidney transplant, Nicot and associates
have reported the persistence of HCV in immunocompromised transplant patients who were cleared of the virus while on dialysis, but there was no relapse of HCV infection after long-term follow-up despite intensive immunosuppressive therapy.[15] In the current study, although we were concerned about a transmission of HCV virus and de novo HCV hepatitis in the recipient under strong immunosuppression, the patient had a successful posttransplant outcome, with normal liver biochemistry and undetectable HCV in the allograft and serum at 6 years' follow-up.

Conversely, living donor safety is mandatory. In the current case, we also were concerned about an HCV flare-up in the donor after surgery because of the stress of the invasive surgery and liver regeneration, but we could find no reports of an HCV flare-up after hepatectomy. Six years after surgery, the results of the donor's liver function tests are normal and his HCV-RNA remained negative. Although this is a special case of using a marginal donor, an anti-HCV-positive patient with an HCV-RNA negative donor can be taken into consideration for a donor candidate in a special

occasion.

In conclusion, we describe the successful transplant of an FAP patient who underwent ABO-I LDLT using a graft from an anti-HCV-positive donor. When the donor is anti-HCV-positive and HCV-RNA-negative with normal liver histology, transplant may be considered in some situations. Long-term follow-up is required for donor and recipient.

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ELSEVIER

A National Survey of Patients With Intestinal Motility Disorders Who Are Potential Candidates for Intestinal Transplantation in Japan

T. Ueno, M. Wada, K. Hoshino, S. Sakamoto, H. Furukawa, and M. Fukuzawa

ABSTRACT

Intestinal motility disorders are a major cause of intestinal failure. Severe cases such as idiopathic pseudo-obstruction represent life-threatening illnessed. Intestinal transplantation is a treatment for severe motility disorders with irreversible intestinal failure. However, the prevalence of severe motility disorders is unknown. We performed a national survey to identify patients with intestinal motility disorders who require an intestinal transplant. The national survey of 302 institutions treating intestinal motility disorders identified 147 patients treated from 2006 to 2011 at 46 institutions. The mean patient age was 12.1 years (range, 0.3–77.5). The mean age of onset was 3.0 years (range, 0.0–68.8). Diagnoses included chronic idiopathic intestinal pseudo-obstruction (n = 96), Hirschsprung disease (n = 29), megacystis microcolon intestinal hypoperistalsis syndrome (n = 18), and other (n = 6). There were 126 survivors and 21 patients who died during the last 5 years. The mortality rate was 14.3%. Eighty-five percent of patients required parenteral nutrition for more than 6 months, which was defined as irreversible intestinal failure. Among surviving patients with irreversible intestinal failure, 8 (9.4 %) developed hepatic failure with jaundice and 27 (31.8%) 2 or more central vein thromboses. In all, at least 35 patients (41%) with irreversible failure due to intestinal motility disorders may be candidates for transplantation. The prevalence of severe intestinal motility disorders was elucidated in Japan. Severe cases should be referred to transplant centers.

INTESTINAL MOTILITY DISORDERS are a major cause of intestinal failure. Severe cases such as idiopathic pseudo-obstruction are life-threatening. Causes of intestinal motility disorders seem to be multifactorial, and only a few have been elucidated. The prognosis is poor for patients with severe illness. The outcome for intestinal failure has improved dramatically due to the development of parenteral nutrition (PN). However PN-related complications, such as central venous catheter infection, thrombosis of venous access points, and PN-associated cholestasis of the liver, are still major problems for patients with intestinal failure. Intestinal transplantation is a treatment for irreversible intestinal failure due to severe disorders of intestinal motility that can significantly improve the prognosis and quality of life for patients. Progress in intestinal transplantation has improved survival. However, the prevalence of severe intestinal motility disorder is unknown. The Therapeutic Guidelines for Intestinal Failure Study Group performed a national survey to identify patients with intestinal motility disorders requiring an intestinal transplant.

METHODS

This national survey was designed as a 5-year retrospective observation study involving 302 institutions that treat intestinal motility disorders. These institutions were members of the Japanese Society of Pediatric Surgeons, the Japanese Society for Small Bowel Transplantation, and the Japanese Study Group for Home Parenteral and Enteral Nutrition. After an initial survey, a questionnaire about each patient was sent to responding institutions from the data center based at Osaka University. Patients with intestinal

From the Pediatric Surgery (T.U., M.F.), Osaka University, Suita, Japan; Pediatric Surgery (M.W.), Tohoku University, Sendai, Japan; Surgery (K.H.), Keio University, Tokyo, Japan; Transplantation Center (S.S.), National Center for Child Health and Development Tokyo, Japan; Gastroentrologic and General Surgery (H.F.), Asahikawa Medical University, Asahikawa, Japan.

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Address reprint requests to Takehisa Ueno, Pediatric Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: ueno@pedsurg.med.osaka-u.ac.jp

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failure treated at each institution from 2006 to 2011 were included. Exclusion criteria were: (1) final diagnosis other than intestinal failure, (2) intestinal failure ultimately resolved, (3) intestinal failure resulting from malignancy, and (4) intestinal failure secondary to diseases in other organs. There were 354 patients reported by 69 institutions. Irreversible intestinal failure was defined as dependence on PN for more than 6 months. Out of these 354 patients, patients with intestinal failure due to motility disorders were identified. The following factors were assessed for possible associations with indications for intestinal transplantation: diagnosis, patient age, age of onset, sex, patient outcome, PN status, liver function tests (LFTs), and central line access. This study was approved by the Osaka University Hospital institutional review board and was supported by Health Science Research Grants from the Ministry of Health, Labor and Welfare of Japan.

RESULTS

There were 147 patients with intestinal motility disorders identified from 46 institutions. The prevalence was approximately one in one million. There were 55 male and 92 female patients. The female-to-male ratio was about 2:1. The mean patient age was 12.1 years (range, 0.3–77.5 years). The mean age of onset was 3.0 years (range, 0.0–68.8 years). Causes of intestinal failure are shown in Fig 1. During the observation period, 126 patients survived and 21 patients died. The mortality rate was 14.3%.

Detailed analysis was added for survivors to determine indications for intestinal transplantation. Of the surviving patients, 91 (62.0%) needed PN at least once a week, and 85 (57.8%) required PN for more than 6 months. Those 85 patients were defined as having irreversible intestinal failure. The following analyses were carried out for patients with irreversible intestinal failure. Catheter-related complications were assessed. The site of central vascular access (internal jugular vein, subclavian vein, and femoral vein) was reported. The number of venous access failures is shown in Fig 2. Twenty-seven patients (31.9%) had 2 or more instances of central vascular access loss.

There were 61 patients (71.8%) who developed abnormal LFTs suggestive of liver injury from PN, including 8 patients (13%) with jaundice. They were considered to have severe liver injury resulting from PN.

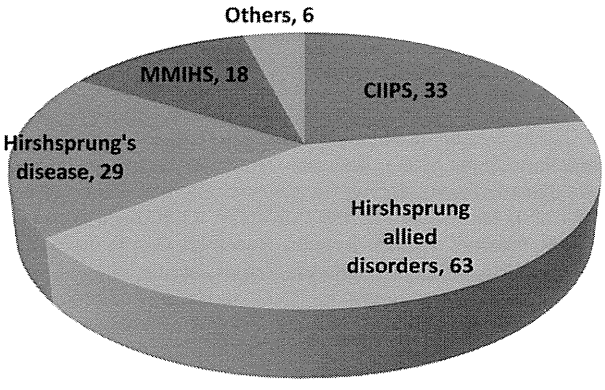

Fig 1. Causes of intestinal failure (n = 147). CIIPS, chronic idiopathic intestinal pseudo-obstruction; MMIHS: megacystis microcolon intestinal hypoperistalsis syndrome.

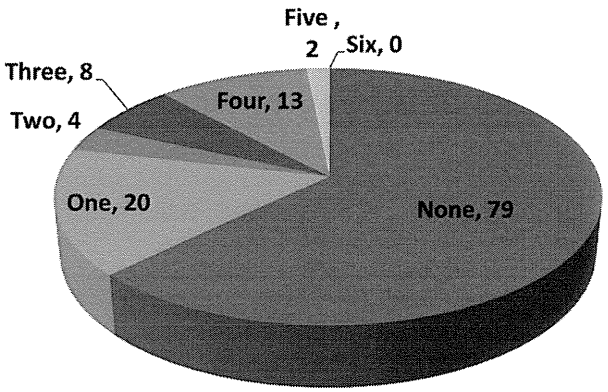

Fig 2. Number of central vascular access losses (n = 126). The number on the left indicates the number of vascular access losses.

Fifty-eight patients required at least 1 hospitalization in the previous year. Nineteen patients (22.4%) required hospitalization for more than 6 months over the previous year. Their quality of life was severely impaired.

A flowchart for identifying possible candidates for intestinal transplantation is shown in Fig 3. Patients dependent on PN for more than 6 months were defined as having irreversible intestinal failure. Those with more than 2 central vascular access losses, and abnormal LFTs with jaundice were considered for candidates for intestinal transplantation. Patients who died from liver failure or infection might be saved by intestinal transplant. They might be candidates for intestinal transplant too. In total, 45 patients were potential candidates for intestinal transplantation. Additionally, the 19 patients who were hospitalized for more than 6 months can be potential candidates given their poor quality of life.

DISCUSSION

Intestinal motility disorders include a wide range of diseases. Chronic intestinal pseudo-obstruction, the most common type of intestinal mobility disorder, is caused by ineffective intestinal contraction. It is characterized by symptoms and signs of intestinal obstruction.[1] Intestinal transplantation can significantly improve the prognosis and quality of life of patients with intestinal motility disorders in Japan.[1] Survival rates in Japan are comparable with rates from the international intestinal transplant registry.[2]

Previously, the prevalence of intestinal motility disorders in Japan was unknown. It was estimated that there were 100 severe cases nationwide. This study supports this figure because surveillance was of a large enough scale to cover the entire nation.

There were over 40 patients who may need intestinal transplantation. However, only 3–4 a year intestinal transplants are performed in Japan, even if 10 times as many patients may be cured by intestinal transplantation.

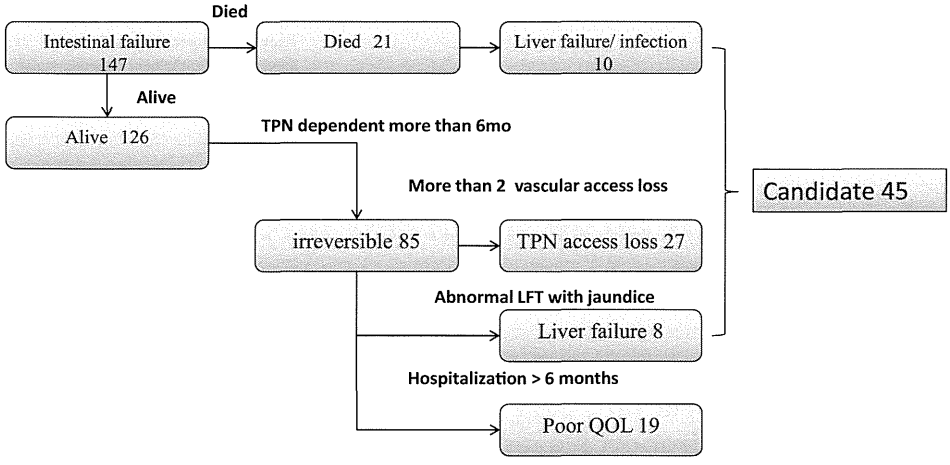

Fig 3. Candidates for intestinal transplantation. TPN, total parental nutrition; QoL, quality of life.

There were 2 major reasons for the relative paucity of intestinal transplants in Japan. One reason is the lack of available organs. For a long time, very few organs from deceased donors were obtainable in Japan. As with other solid organs, most intestinal transplants in Japan are performed with living donors. The shortage of organs has been alleviated due to a new act on organ transplantation that went into effect in 2010. However, the number of intestinal transplant has remained steady.

The financial barrier is the other, more profound reason preventing greater use of intestinal transplantation in Japan. Since the procedure is not covered by health insurance, either the patient or the transplant institution must pay the considerable costs out of pocket.

Some patients develop liver failure with intestinal motility disorders. These patients need simultaneous liver-intestine transplants. A combined liver-intestine transplant has less risk of acute rejection than an isolated intestinal transplant because the liver may have protective effects on the intestine. Current organ allocation guidelines do not allow for simultaneous combined liver-intestine organ retrieval; thus, a simultaneous liver-intestine transplant is impossible from deceased donor sources.

Previously, the laws on organ transplantation banned donors below 15 years of age. Intestinal transplants were not previously possible in infants because of organ size mismatch. Such patients will benefit from intestinal transplants in the future. Moreover, younger patients sometimes develop liver failure.[3] Multiorgan transplantation is a good option for such patients.[4]

It is difficult to determine the optimal timing for intestinal transplants to treat intestinal failure associated with intestinal motility disorders. Severe cases of intestinal motility disorders should be referred to institutions with expertise in transplantation.

In conclusion, the prevalence of severe motility disorders in Japan was elucidated. Patients with irreversible intestinal failure from intestinal motility disorders may be candidates for intestinal transplantation. Severe cases of motility disorder should be referred to transplant centers. Further investigation for patient details is required.

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Immunological detection of large oxidized lipoproteins in hypertriglyceridemic serum

Toshihiro Sakurai[1], Norio Wada[2], Yuji Takahashi[1,2], Ayako Ichikawa[1,3], Akiko Ikuta[1], Hiroaki Furumaki[1], Shu-Ping Hui[1], Shigeki Jin[1], Seiji Takeda[1], Hirotoshi Fuda[1], Masato Fujikawa[2], Chikara Shimizu[3], Hironori Nagasaka[4], Hiroyuki Furukawa[5], Seiichi Kobayashi[1] and Hitoshi Chiba[1]

Abstract

Background: Triglyceride-rich, low-density lipoproteins (TG-rich LDL) have been reported as an oxidized lipoprotein species in patients with severe liver disease. Using TG-rich LDL as an immunogen, we obtained a monoclonal antibody (G11-6) that reacted with TG-rich LDL from patients with liver disease and with metal-oxidized LDL only in the early process of the oxidation reaction. This study determined the G11-6-reactive lipoproteins in hypertriglyceridemic serum.

Methods: Serum samples from healthy volunteers ($n = 12$) and hypertriglyceridemic patients ($n = 9$) were fractionated by gel filtration and subjected to a sandwich enzyme-linked immunosorbent assay (ELISA) using G11-6 and polyclonal anti-apolipoprotein B antibodies.

Results: Small LDL and larger lipoproteins reacted with G11-6. G11-6-reactive small LDL was identified in both the healthy subjects and hypertriglyceridemic patients, whereas G11-6-reactive larger lipoproteins were found only in the hypertriglyceridemic patients.

Conclusions: G11-6 is a useful tool for detecting increased large oxidized lipoproteins in hypertriglyceridemic patients.

Keywords

Lipids, analytes, immunoassay, laboratory methods

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Introduction

The oxidation of lipids and lipoproteins plays a key role in early atherogenesis and is involved in various diseases.[1,2] Among lipoprotein species, the small, dense, low-density lipoproteins (LDL) are more susceptible to oxidation than larger, buoyant LDL.[3] Many studies have reported on the relationship between the concentration of small dense LDL and the development of atherosclerosis.[3–5] However, there are few reports of serum oxidized lipoproteins other than small dense LDL, except for a report on the presence of phosphatidylcholine hydroperoxides in isolated remnant lipoproteins.[6]

We developed a new monoclonal antibody, called G11-6, by immunizing mice with triglyceride (TG)-rich LDL isolated from the serum of a cholestatic patient with severe liver disease.[7] A sandwich enzyme-linked

[1]Faculty of Health Sciences, Hokkaido University, Sapporo, Japan
[2]Departments of Internal Medicine and Clinical Laboratory, Sapporo City General Hospital, Sapporo, Japan
[3]Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan
[4]Department of Pediatrics, Takarazuka City Hospital, Takarazuka, Japan
[5]Department of Organ Transplantation and Regeneration, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Corresponding author:
Hitoshi Chiba, Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.
Email: chibahit@med.hokudai.ac.jp

immunosorbent assay (ELISA) using G11-6 with polyclonal anti-apolipoprotein B (apoB) antibodies detected copper-oxidized LDL only in the early process of the oxidation reaction, while G11-6 reacted with neither native lipoproteins nor copper-oxidized LDL in the late process of the oxidation reaction, supporting the specificity of G11-6 for weakly oxidized lipoproteins.[7] In our previous study, G11-6 reacted with TG-rich LDL in patients with liver disease and with small LDL in normal controls.[7] To further understand weakly oxidized lipoproteins in serum, the present study examined the lipoprotein species in hypertriglyceridemic serum that reacted with G11-6.

Materials and methods

Blood was drawn from 12 healthy volunteers (controls) and nine patients with hypertriglyceridemia after an overnight fast. Hypertriglyceridemia was diagnosed according to the 2007 Guidelines of the Japan Atherosclerosis Society: LDL-cholesterol (LDL-C) $\geq$ 140 mg/dL (3.64 mmol/L), high-density lipoprotein-cholesterol (HDL-C) < 40 mg/dL (1.04 mmol/L), or TG $\geq$ 150 mg/dL (1.65 mmol/L). Serum was separated from the blood by centrifugation at 2000 *g* for 10 min and then stored at 4°C until use.

Serum samples (0.3 mL each) were subjected to gel filtration chromatography on a Superose 6 column (GE Healthcare, Little Chalfont, England) in a liquid chromatography apparatus (Shimadzu, Kyoto, Japan), and 0.5-mL fractions were collected, as described previously.[7] The lipoprotein fractions were stored at 4°C and analysed for lipids and lipoproteins within one day, as described below.

To confirm the elution position of each lipoprotein fraction in this chromatographic system, a serum sample was obtained from a hypertriglyceridemic patient, and then separated into three fractions by ultracentrifugation: chylomicron (CM)-intermediate-density lipoprotein (IDL) ($d < 1.019$ kg/L), LDL ($1.019 < d < 1.063$ kg/L), and HDL plus other serum proteins ($d > 1.063$ kg/L), as reported previously.[7] Each fraction was applied to the same column system, and its elution position was determined by measuring total cholesterol (TC) and TG in the eluates (data not shown).

Phospholipids (PL), TC, TG, LDL-C, and HDL-C were measured using automated enzymatic methods and commercial kits (Sekisui Medical, Tokyo, Japan). Malondialdehyde (MDA)-modified LDL was detected using a MDA-LDL ELISA kit (Sekisui Medical) with a commercial monoclonal antibody (ML25; Sekisui Medical). The lipoproteins detected by this kit are designated as MDA-LDL here.

G11-6 was used as the solid-phase antibody in combination with polyclonal anti-apolipoprotein B antibodies as the detecting antibody in a sandwich ELISA, which is referred to as the G11-6 ELISA here.[7] Serum samples were diluted 20-fold with 50 mmol/L phosphate buffer (pH 7.4) containing 150 mmol/L sodium chloride and 1 mmol/L ethylenediaminetetraacetic acid before the G11-6 ELISA. Serum G11-6-reactive lipoprotein concentrations were defined as the ratio of the absorbance of each serum sample to that of the control serum obtained from a patient with liver disease. To evaluate the reproducibility of the G11-6 ELISA, serum samples with high or low G11-6-reactive lipoprotein concentrations were measured 10 times to evaluate the within-run variation and four times on four consecutive days to evaluate the between-run variation.

To investigate the possible interaction between G11-6 and lipoprotein(a) [Lp(a)], Lp(a)-deficient serum was prepared as follows. A polyclonal goat anti-Lp(a) antibody (Abcam, Cambridge, MA) was covalently bound to magnetic tosylactivated Dynabeads (Invitrogen), according to the manufacturer's instructions. Magnetic beads bound to non-immune goat IgG (Sigma-Aldrich, St. Louis, MO) were used as a control. The antibody-bound beads were incubated with serum from a hypertriglyceridemic patient overnight at 4°C. After magnetic separation, the resulting Lp(a)-depleted serum was chromatographed on a Superose 6 column, as described above. The eluted fractions were subjected to the G11-6 ELISA and were analysed for Lp(a) content using a latex-enhanced turbidimetric immunoassay [Lp(a)-Latex SEIKEN Kit; Denka Seiken, Tokyo, Japan].

The serum G11-6-reactive lipoprotein concentrations and clinical parameters of the groups, except sex, were compared using the non-parametric Mann–Whitney *U*-test. The influence of sex differences was analysed using Fisher's exact probability test. The statistical analyses were performed using Statcel (OMS, Saitama, Japan). The relationships among the elution positions of G11-6- or ML25-reactive lipoproteins were analysed using the split plot design analysis of variance (ANOVA) on log-transformed concentrations of G11-6-reactive lipoproteins for G11-6 ELISA and MDA-LDL for MDA-LDL ELISA. The statistical analysis was performed using StatFlex (Artech, Osaka, Japan). Values of $P < 0.05$ was considered to indicate statistical significance.

Results

Clinical characteristics

The subjects' clinical data are shown in Table 1. Age, TG, PL, HDL-C, and LDL-C were significantly higher in the hypertriglyceridemic group compared with the control group. The hypertriglyceridemic group was on

Table 1. Clinical parameters and serum lipids in studied groups.

Traits	Healthy volunteer	Hypertriglyceridemia
Age (years), mean ± SD (range)	22.8 ± 1.7 (21–27)	56.6 ± 8.5 (41–68)**
Sex (male/female)	9/3	7/2
Total cholesterol, mmol/L	5.01 ± 0.86	5.96 ± 1.64
Triglycerides, mmol/L	0.85 ± 0.36	3.22 ± 2.25**
Phospholipids, mmol/L	2.77 ± 0.43	3.66 ± 0.65**
HDL-cholesterol, mmol/L	1.69 ± 0.47	1.26 ± 0.52*
LDL-cholesterol, mmol/L	2.93 ± 0.82	4.49 ± 1.00**

*$P < 0.05$ vs. healthy volunteers. **$P < 0.01$ vs. healthy volunteers.
To convert the values for cholesterol, triglycerides and phospholipids from mmol/L to mg/dL, multiply by 38.5, 91.0, and 77.0, respectively.

average older than the control group, while sex and TC did not differ between the groups.

G11-6-reactive lipoprotein concentrations in normal and hypertriglyceridemic serum

The serum G11-6-reactive lipoprotein concentrations did not differ significantly between the healthy subjects and hypertriglyceridemic patients (0.32 ± 0.31 *vs.* 0.36 ± 0.70, mean ± SD; $P = 0.337$). In our assay, the within-assay coefficient of variation was 4.1% for high concentrations (1.72 ± 0.07) and 7.7% for low concentrations (0.52 ± 0.04). The between-assay coefficient of variation was 9.7% for high concentrations (1.61 ± 0.16) and 13.7% for low concentrations (0.35 ± 0.05).

Gel filtration of serum fractions isolated by ultracentrifugation

The CM-IDL fraction that was isolated by ultracentrifugation eluted in fractions 5–16, and TC and TG peaked in fractions 5 and 10 (data not shown). The LDL fraction eluted in fractions 11–16; TC peaked in fractions 13–14, and low TG peaks were observed in fractions 5 and 13. The fraction containing HDL plus other serum proteins eluted in fractions 19–27, and TC and TG peaked in fractions 21–23.

Gel filtration of normal and hypertriglyceridemic serum

Figures 1 and 2 illustrate the gel filtration results for four of the 12 healthy subjects (control) and four of the nine hypertriglyceridemic patients, respectively. They were selected randomly from each group and represented the typical elution profile for each group. Their clinical data are summarized in Table 2. Cases 1–4 had elevated TG and LDL-C.

The elution profiles of the four controls were similar (Figure 1): LDL-C eluted in fractions 11–16 and peaked in fractions 13–14; HDL-C eluted in fractions 19–28 and peaked in fractions 22–23. MDA-LDL eluted in fractions 12–16 and peaked in fractions 13–15 in all four controls. G11-6-reactive lipoproteins eluted in fractions 10–16 and peaked in fractions 14–16 in all four controls.

The four cases also gave similar elution profiles (Figure 2): LDL-C eluted in fractions 11–16 and peaked in fractions 13–14; HDL-C eluted in fractions 19–28 and peaked in fractions 22–23. MDA-LDL eluted in fractions 12–16 and peaked in fractions 13–15 in all four cases. G11-6-reactive lipoproteins eluted in fractions 8–16 and peaked in fractions 10–13 in all four cases.

According to the elution position of the lipoproteins in the gel filtration chromatography, fractions 11–12, 13–14, and 15–16, which we named Fractions I, II, and III, respectively, appeared to correspond to the large TG-rich lipoproteins, large buoyant LDL, and small dense LDL, respectively. Fractions I, II, and III had the G11-6-reactive lipoprotein concentrations of 2.85 ± 1.56 (mean ± SD), 5.80 ± 4.00, and 7.25 ± 4.31, respectively, in the healthy subjects, and 1.84 ± 1.89, 1.30 ± 1.12, and 0.87 ± 0.82, respectively, in the hypertriglyceridemic patients (Figure 3(a)). According to the split plot design ANOVA, significant differences in the G11-6-reactive lipoprotein concentrations were detected between the groups of healthy subjects and hypertriglyceridemic patients ($P = 0.0007$) and among the fractions I to III ($P = 0.0386$). Additionally, there was a significant group × fraction interaction in the G11-6-reactive lipoprotein concentrations ($P < 0.0001$). On the other hand, MDA-LDL concentrations of Fractions I, II, and III were 28.6 ± 26.2 U/L, 111.0 ± 77.1 U/L, and 84.2 ± 4.69 U/L, respectively, in the healthy subjects, and 53.6 ± 42.4 U/L, 268.1 ± 239.6 U/L, and 194.1 ± 184.5 U/L, respectively, in the

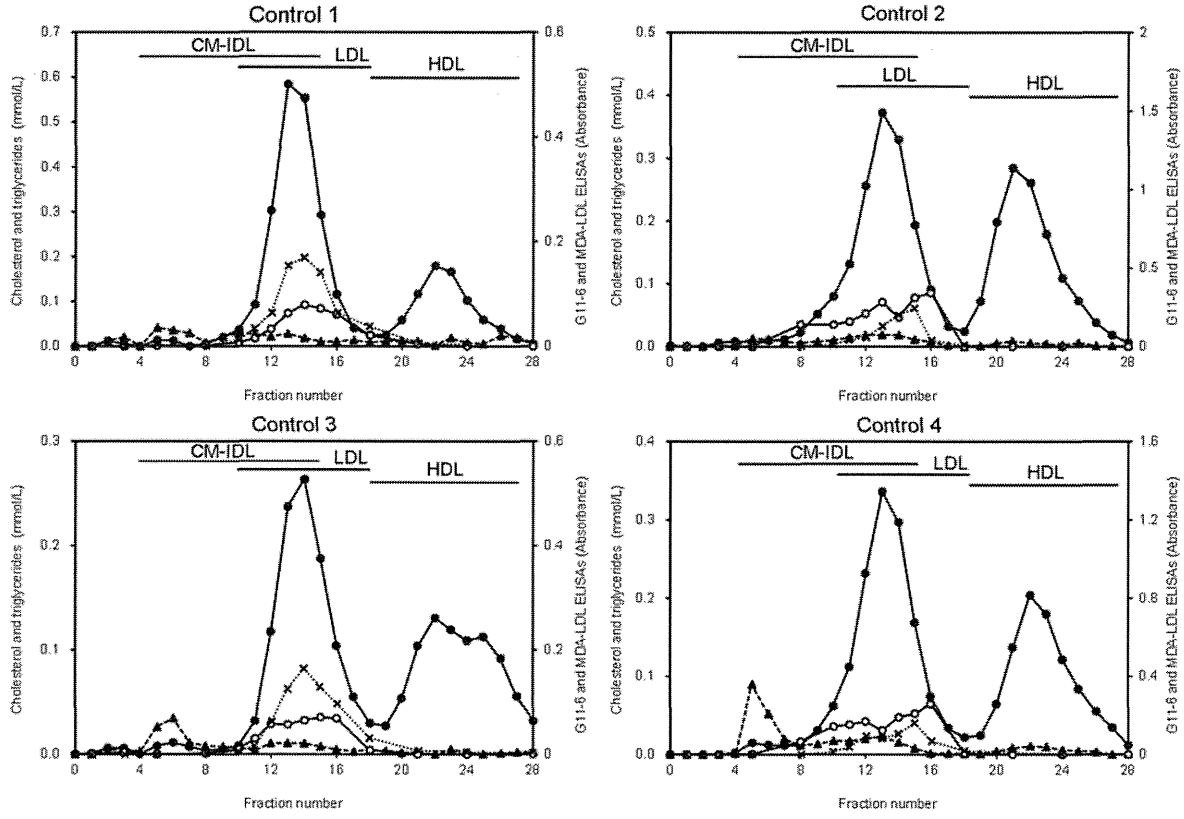

Figure 1. Typical gel filtration chromatography elution profile for serum from healthy subjects. Total cholesterol (•, solid line, left axis), triglycerides (▲, dashed line, left axis), ELISA using G11-6 (○, solid line, right axis), and MDA-LDL (×, dotted line, right axis).

hypertriglyceridemic patients (Figure 3(b)). According to the split plot design ANOVA, significant differences in the MDA-LDL concentrations were detected among the fractions I to III ($P < 0.0001$), whereas no significant difference was found between the groups of healthy subjects and hypertriglyceridemic patients ($P = 0.1506$). Additionally, there was no significant group × fraction interaction in the MDA-LDL concentrations ($P = 0.9580$).

Lp(a) depletion experiment

The absence of Lp(a) in the eluates from the Superose 6 column loaded with Lp(a)-depleted hypertriglyceridemic serum was confirmed by turbidimetric measurement of Lp(a). The eluates had essentially the same elution profiles with and without Lp(a) depletion, indicating that G11-6 did not recognize Lp(a) (data not shown).

Discussion

When free radical-mediated oxidation of unsaturated fatty acids in LDL occurs, a chain reaction leads to the massive formation of PL hydroperoxides.[6,8] The PL hydroperoxides undergo carbon–carbon bond cleavage via alkoxyl radicals in the presence of transition metals, forming short-chain unesterified aldehydes and short acyl-chain PL.[8] The short-chain unesterified aldehydes such as MDA and 4-hydroxy-2-nonenal bind to the positively charged amino groups of apoB.[8,9] The MDA-modified apoB can be detected with monoclonal antibody ML-25 in a MDA-LDL ELISA.[9] By contrast, Itabe and Ueda[10,11] reported that short acyl-chain PL is recognized by DLH3 antibody.

In our previous report, G11-6 reacted with copper-oxidized LDL during the early process of the oxidation reaction.[7] ML-25 and DLH3, however, showed time-courses different from that for G11-6. ML-25 reacted with copper-oxidized LDL in both the early and late processes of oxidation, and DLH3 reacted in the latest process of oxidation.[7] Furthermore, G11-6 did not react with artificially prepared MDA-modified LDL, in contrast to ML-25 and 4E6, another monoclonal antibody against oxidized LDL reported by Holvoet *et al.*[12] These data clearly show the unique immunological property of G11-6 among the reported antibodies to oxidized LDL. In addition, the amino acid

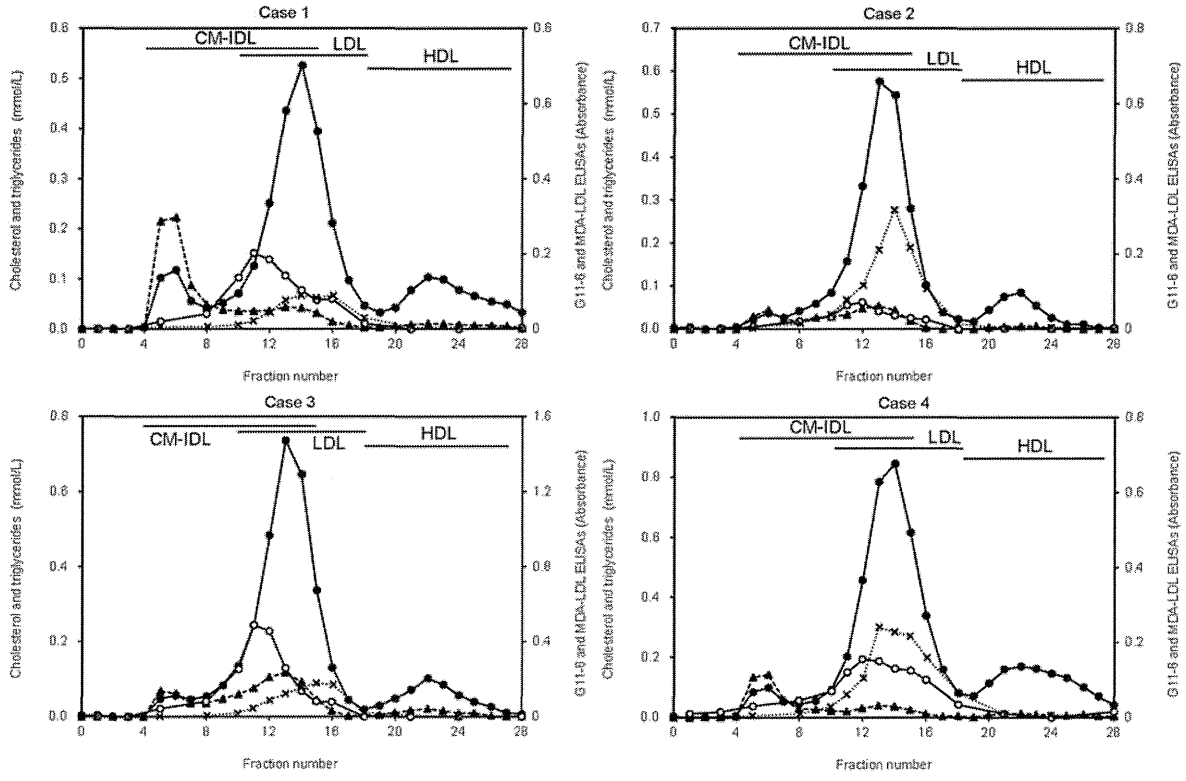

Figure 2. Typical gel filtration chromatography elution profile for serum from hypertriglyceridemic patients. Total cholesterol (•, solid line, left axis), triglycerides (▲, dashed line, left axis), G11-6 ELISA (○, solid line, right axis), and MDA-LDL (×, dotted line, right axis).

Table 2. Clinical characteristics of the controls and the cases.

	Healthy volunteer					Hypertriglyceridemia				
Traits	Controls	1	2	3	4	Cases	1	2	3	4
Age, years		22	23	22	24		55	67	61	68
Sex		Male	Female	Male	Male		Male	Female	Female	Male
Total cholesterol, mmol/L		5.40	5.23	3.81	4.30		5.88	7.57	6.80	4.78
Triglycerides, mmol/L		0.83	0.38	0.55	0.73		2.09*	1.96*	1.84*	2.49*
Phospholipids, mmol/L		2.75	2.86	2.24	2.39		3.17	3.52	3.44	3.07
HDL-cholesterol, mmol/L		1.31	2.20	1.54	1.65		1.27	1.09	1.22	1.07
LDL-cholesterol, mmol/L		3.64	2.57	1.90	2.39		3.69*	5.28*	5.05 *	4.58*

*Satisfied the criteria of the dyslipidemia according to the 2007 guidelines of the Japan Atherosclerosis Society.

sequence in the hypervariable region of G11-6 was not found in BLAST search (unpublished data). Given that no detergent was needed for the immunoreaction between G11-6 and oxidized LDL, the epitope of G11-6 must be exposed on the surface of LDL particles, although its identity remains to be elucidated.

The serum G11-6-reactive lipoprotein concentrations measured by G11-6 ELISA did not differ significantly between the healthy subjects and hypertriglyceridemic patients. In comparison, the G11-6-reactive lipoprotein elution profiles in gel filtration chromatography differed significantly between them: G11-6-reactive lipoproteins eluted most abundantly in Fraction III (small LDL) in the healthy subjects and in Fraction I (lipoproteins larger than LDL) in the hypertriglyceridemic patients (Figure 3). Many studies have reported the relevance of small LDL to oxidation. Small dense LDL is more oxidizable *in vitro* than large buoyant LDL[4,5] and is able to enhance foam cell formation by THP-1 macrophages

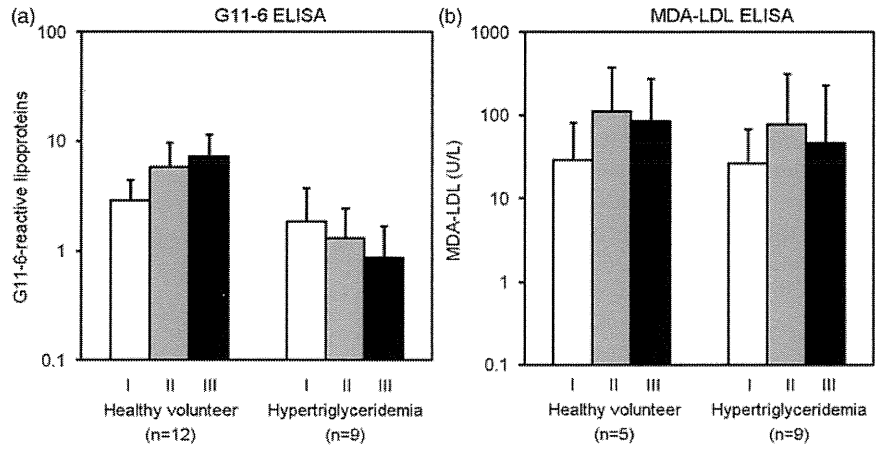

Figure 3. Distributions of G11-6-reactive lipoproteins and MDA-LDL classified by elution position in gel filtration: (a) G11-6 ELISA and (b) MDA-LDL ELISA. Note that log scale is used for the G11-6-reactive lipoprotein and MDA-LDL concentrations. G11-6-reactive lipoprotein concentrations were defined as the ratio of the absorbance of each sample to that of the control serum obtained from a patient with liver disease. According to the elution position of the lipoproteins in the gel filtration chromatography, fractions 11–12, 13–14, and 15–16, which we named Fractions I, II, and III, respectively, appeared to correspond to the large TG-rich lipoproteins, large buoyant LDL, and small dense LDL, respectively. Difference between the groups of healthy subjects and hypertriglyceridemic patients was significant ($P = 0.0007$) for the G11-6-reactive lipoprotein concentrations, but not significant for the MDA-LDL concentrations, according to the split plot design ANOVA. Additionally, group × fraction interaction was significant in the G11-6-reactive lipoprotein concentrations ($P < 0.0001$), but not significant in the MDA-LDL concentrations.

without copper-induced oxidation.[4] Furthermore, ML-25, or the antibody to MDA-LDL, has been reported to react with isolated small dense LDL.[9] LDL isolated from hypertriglyceridemic serum has reduced affinity for the LDL receptor[13] and a prolonged plasma half-life (3.2 days for hypertriglyceridemic LDL *vs.* 2.0 days for normal LDL), which may underlie the higher oxidizability *in vivo* of small LDL.[14]

The elution position of the large G11-6-reactive lipoproteins coincided with that of the CM-IDL fraction. We speculate that the large G11-6-reactive lipoproteins observed in hypertriglyceridemic patients are oxidized remnant lipoproteins. Remnant lipoproteins are reported to have elevated thiobarbituric acid-reactive substance (TBARS) and greater oxidizability *in vitro* than very low-density lipoproteins (VLDL).[15] Since CM elute in the void volume with our column system (data not shown), they are not the G11-6-reactive lipoproteins. The presence of circulating oxidized Lp(a) in normal and hypertensive subjects has been reported.[16] With our gel filtration system, Lp(a) elutes at a position similar to that of remnant lipoproteins. However, G11-6 does not seem to react with Lp(a), according to the result of the Lp(a) depletion experiment.

Fraction I had different triglyceride contents in the healthy subjects and hypertriglyceridemic patients, as shown in Figures 1 and 2. Here, we defined Fraction I as the lipoprotein fraction with a density < 1.019 g/mL and elutes in gel filtration chromatography at the positions that G11-6-reactive lipoproteins are detected. Hence, Fraction I may be composed of IDL, VLDL, and possibly small contaminating amounts of LDL. In hypertriglyceridemic patients, IDL and VLDL should increase in Fraction I. Since these lipoproteins are rich in triglyceride and poor in cholesterol, their increase should result in the elevation of triglyceride in Fraction I.

Isolated remnant lipoproteins are reported to contain detectable amounts of phosphatidylcholine hydroperoxides.[17] In addition, the hypertriglyceridemic VLDL remnants induce cholesteryl ester accumulation in cultured macrophages as efficiently as oxidized LDL.[18] Hypertriglyceridemic remnant lipoproteins also induce the expression of proatherothrombogenic molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor, in endothelial cells via a mechanism that is suppressed by antioxidants.[19] Hypertriglyceridemic remnant lipoproteins have an echinocytogenic effect on red blood cells, which is also suppressible by antioxidants.[16] Consequently, remnant lipoproteins have chemical and biochemical properties corresponding to those of oxidized lipoproteins. Thus the immunological change in remnant lipoproteins may have been caused by oxidation.

The observed increase in oxidized remnant lipoproteins in hypertriglyceridemic serum may be partly explained by delayed metabolism of remnant lipoproteins in hypertriglyceridemic patients. Cortner *et al.*[20] reported the prolonged clearance of CM remnant lipoproteins (CM-free d < 1.006 fraction), with half-times

of 14.1 ± 9.7 and 50.7 ± 20.8 min in controls and hypertriglyceridemic patients, respectively. They speculated that this delay was largely due to the overproduction of VLDL particles in the liver and the consequent competition between VLDL and CM remnants for hepatic uptake via apoE receptor-mediated endocytosis. Although VLDL remnants were not mentioned in the literature, it is likely that their clearance is also delayed in hypertriglyceridemic patients.

Interestingly, ML-25 did not react with the large lipoproteins that were reactive with G11-6 (Figure 2). The oxidative change in this fraction might be too weak to be recognized by ML-25. We speculate that the clearance of TG-rich lipoproteins is more rapid than that of small dense LDL,[14] and therefore, little MDA-modification occurs in TG-rich lipoproteins.

The acquisition of an adequate quantity of standard substance for G11-6 ELISA remains to be solved. Although TG-rich LDL isolated from patients with advanced liver disease can be used as a standard substance in G11-6 ELISA, it is difficult to obtain a large volume of serum from patients. The use of copper-oxidized LDL might solve this problem, as reported by Kotani *et al.*[9] in their MDA-LDL ELISA.

In conclusion, G11-6 has the advantage of detecting large oxidized lipoproteins, probably oxidized remnant lipoproteins, which are increased in hypertriglyceridemic patients. G11-6 might be useful in elucidating the role of large oxidized lipoproteins in cardiovascular disease.

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Declaration of conflicting interests

None.

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Ethical approval

The study was approved by the ethics review board at the Faculty of Health Sciences, Hokkaido University (approval number 08-57).

Guarantor

HC.

Contributorship

TS and HC researched literature and conceived the study. A Ichikawa, AIkuta, H Furumaki, S-PH, SJ, ST and H Fuda were involved in lipoprotein separations and lipid measurements. NW, YT, MF, CS and H Furukawa were involved in providing samples and patient recruitment. SK and TS were involved in the preparation and characterization of monoclonal antibodies. HN was involved in discussion concerning the role for abnormal lipoproteins. TS wrote the first draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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