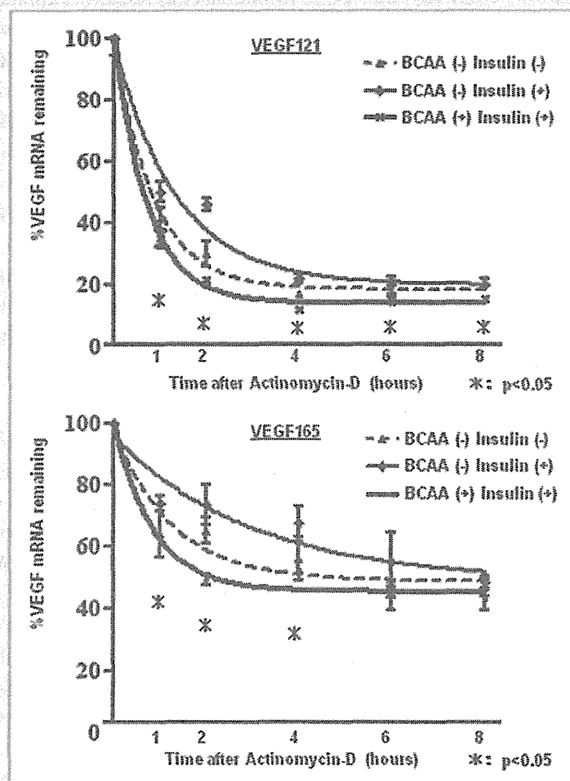

Fig. 4. VEGF121 and VEGF165 mRNA stability after actinomycin-D treatment with or without BCAA and insulin. HepG2 cells were incubated in BCAA-free medium for 20 h. The medium was then changed to fresh BCAA-free medium, or to medium with insulin (200 nM) with or without BCAA (0.8 mM), and actinomycin-D (5 μg/ml) was added to all cultures. mRNA was prepared 0, 1, 2, 4, 6, and 8 h after actinomycin-D treatment and the levels of VEGF121 and VEGF165 mRNA remaining at each time point were analyzed using quantitative PCR. Data represent mRNA expression over control value (VEGF 121 and VEGF 165 mRNA at 0 h), which was arbitrarily set to 100%, and represent the mean ± SD for four separate experiments. Degradation curves were constructed based on a model of exponential decay. (*$P < 0.05$ for VEGF121 and EGF165 mRNA level of BCAA(+)Insulin(+) group compared with BCAA(−)Insulin(+) group at each time).

mRNA, respectively. These data suggested that BCAA decreased VEGF mRNA stability at high-insulin concentrations.

A DEFICIT OF ONE COMPONENT OF BCAA ENHANCED THE EXPRESSION OF VEGF mRNA AT HIGH-INSULIN CONCENTRATIONS

BCAA consist of the three amino acids, Valine, Leucine, and Isoleucine. We examined which component, or combination of BCAA components, suppresses insulin-induced expression of VEGF mRNAs. HepG2 cells were exposed to insulin (200 nM) in BCAA-free medium containing Valine, Leucine, or Isoleucine at 0.8 or 2.4 mM concentrations. The concentration of 2.4 mM was used to adjust the Fischer ratio, which represents the ratio of branched-chain amino acids to aromatic amino acids. The cells were alternatively exposed to insulin (200 nM) and BCAA containing all three components, each at a concentration of 0.8 mM. Following incubation for 4 h, the mRNA expression of VEGF121 and VEGF165 was analyzed by qPCR. The results of this experiment are shown in Figure 5A. BCAA-free medium supplemented with individual amino acids at 0.8 mM concentration, or even at 2.4 mM concentration, could not suppress the expression of VEGF mRNAs at a high-insulin concentration. In contrast, the combination of the three components of BCAA, each at 0.8 mM concentration, strongly suppressed insulin-induced mRNA expression of both VEGF isoforms. We next analyzed the effect of combining two of the components of BCAA on insulin-induced upregulation of VEGF mRNAs. The cells were therefore exposed to insulin (200 nM) in BCAA-free medium supplemented with Valine and Leucine, Valine and Isoleucine, or Leucine and Isoleucine, each at a concentration of 0.8 or 1.2 mM. The cells were also exposed to insulin (200 nM) in media containing the three components of BCAA, each at a concentration of 0.8 mM. As seen for the single supplementation, double supplementation using any combination of the three components of BCAA, with each component at either 0.8 or 1.2 mM, did not suppress insulin-induced expression of VEGF mRNAs (Fig. 5B). These data indicate that all three components of BCAA are needed to accelerate the degradation of VEGF mRNAs at a high-insulin concentration, and thus, a deficiency in any of the components of BCAA stabilizes VEGF mRNA induced by a high-insulin concentration.

DISCUSSION

In this study, we demonstrated that treatment of HepG2 cells with BCAA suppresses insulin-induced VEGF secretion particularly at high-insulin concentrations. Individually BCAA and insulin similarly activate the mTOR pathway and increase the expression of HIF-1α. However, BCAA inhibits insulin-induced expression of VEGF mRNA by decreasing the stability of insulin-induced VEGF mRNAs.

We found that all three of the components of BCAA are required for this inhibitory effect. Neither individual components, nor any combination of two components, are inhibitory. In our experiments with complete BCAA, the concentration of Valine, Leucine, and Isoleucine was set at 0.8 mM each, to yield a Fischer ratio of 3.0. It has been reported that the mean value of the Fischer ratio is 3.5 in healthy controls, 2.7–3.0 in chronic hepatitis, 1.5 in compensated cirrhosis, and 1.1 in decompensated cirrhosis [Kano et al., 1991]. Based on these data we consider that the concentration of BCAA used in our experiments is appropriate for analysis of HCC cell metabolism. Indeed, the suppressive effect of BCAA on insulin-induced VEGF secretion was strengthened as the BCAA concentration was increased to give a Fischer ratio of 3.0 (Fig. 1D).

Several studies [Poon et al., 2001; Kaseb et al., 2009; Schoenleber et al., 2009] have reported a close association between the level of VEGF in tissue or serum and the development and progression of HCC, in which angiogenesis plays an important role. Park et al. [2000] have reported that the expression of VEGF gradually increases with the stepwise development of HCC. Therefore, BCAA administration has the potential to modulate the early stage of hepatocarcinogenesis, rather than the advanced stage of HCC, through the suppression of VEGF secretion.

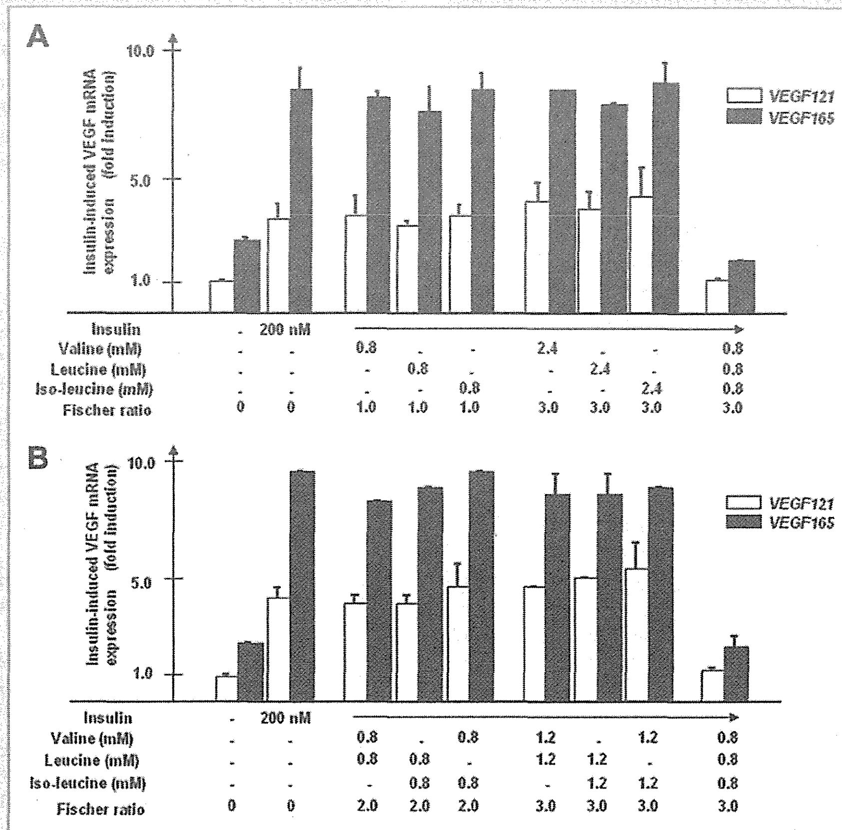

Fig. 5. The mRNA expression of VEGF 121 and VEGF 165 after insulin treatment with or without Valine, Leucine, and/or Isoleucine. A. HepG2 cells were exposed to BCAA-free medium with or without insulin (200 nM) in the presence or absence of individual amino acids, Valine, Leucine, or Isoleucine each at a concentration of 0.8 or 2.4 mM. The cells were alternatively exposed to insulin (200 nM) with BCAA (containing 0.8 mM each of all three amino acids). After 4 h incubation the mRNA expression of VEGF121 and VEGF165 was analyzed using quantitative PCR. B. HepG2 cells were exposed to BCAA free medium with or without insulin (200 nM) in the presence or absence of two amino acids, either Valine and Leucine, Valine and Isoleucine, or Leucine and Isoleucine, each at a concentration of 0.8 or 1.2 mM. The cells were alternatively exposed to insulin (200 nM) with BCAA (containing 0.8 mM each of all three amino acids). After 4 h incubation the mRNA expression of VEGF121 and VEGF165 was analyzed using quantitative PCR. The Fischer ratio, (ratio of branched-chain amino acids to aromatic amino acids) is shown below. Data represent fold induction of VEGF mRNA expression over control value (VEGF121 mRNA in BCAA free medium without insulin), which was arbitrarily set to one, and represent the mean ± SD for four separate experiments.

In our study, BCAA suppressed insulin-induced expression of all isoforms of VEGF mRNAs in a similar manner, including that of VEGF165 mRNA, whose expression has been reported to be linked to poor prognosis of HCC patients [Jeng et al., 2004b]. These results indicate that the process of alternative splicing of the VEGF gene is unaffected by BCAA.

It is a well-known fact that a major transcriptional activator of the VEGF gene is HIF1α [Semenza, 2002]. In this study, HIF1α expression was enhanced by combined treatment with both BCAA and insulin. It is likely that BCAA, which was reported to activate mTOR pathways [Ijichi et al., 2003], contribute to upregulate HIF1α expression with additive effect. Nevertheless, secretory VEGF expression was suppressed through the decrease of insulin-induced VEGF mRNA stability by BCAA. This result raises the possibility that the regulation of VEGF mRNA at post-transcriptional level plays an important role in secretory VEGF expression. Regulation of VEGF expression by modulation of its mRNA stability has also been reported to occur during induction of VEGF by hypoxia [von Marschall et al., 2001; Yoo et al., 2006].

Contrary to our expectations, all three components of BCAA are a prerequisite for the decay of VEGF mRNAs. Neither individual components, nor any combination of two components had this inhibitory effect. Interestingly, BCAA suppression was stronger at high-insulin concentrations. This phenomenon is in agreement with the results of a recent large-scale study, which reported that the administration of BCAA reduced the development of HCC in obese cirrhotic patients who had been diagnosed with diabetes mellitus [Muto et al., 2006]. This phenomenon may simply reflect an improvement of a BCAA deficiency rather than a direct effect of added BCAA. In other words, if there is a deficit in even one component of BCAA, VEGF mRNA is not degraded. It is likely that a BCAA deficit is a critical condition for HCC cell survival and that, even in the presence of a growth factor (such as insulin), HCC cells will give priority to the synthesis of proteins that are essential for survival.

In conclusion, we have demonstrated that a deficit of BCAA prevents the degradation of insulin-induced VEGF mRNA. This effect is repressed by supplementation with BCAA. These results

suggest that administration of BCAA to cirrhotic patients exhibiting both hyperinsulinemia and a decreased Fischer ratio has the potential to decrease HCC development or progression through suppression of VEGF expression.

REFERENCES

Ahmed SI, Thomas AL, Steward WP. 2004. Vascular endothelial growth factor (VEGF) inhibition by small molecules. J Chemother 16(Suppl 4):59–63.

Biswas S, Gupta MK, Chattopadhyay D, Mukhopadhyay CK. 2007. Insulin-induced activation of hypoxia-inducible factor-1 requires generation of reactive oxygen species by NADPH oxidase. Am J Physiol Heart Circ Physiol 292:H758–H766.

Bosch FX, Ribes J, Diaz M, Cleries R. 2004. Primary liver cancer: Worldwide incidence and trends. Gastroenterology 127:S5–S16.

El-Serag HB, Mason AC. 2000. Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 160:3227–3230.

Folkman J, Watson K, Ingber D, Hanahan D. 1989. Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339:58–61.

Fukuda R, Hirota K, Fan F, Jung YD, Ellis LM, Semenza GL. 2002. Insulin-like growth factor 1 induces hypoxia-inducible factor 1-mediated vascular endothelial growth factor expression, which is dependent on MAP kinase and phosphatidylinositol 3-kinase signaling in colon cancer cells. J Biol Chem 277:38205–38211.

Hirota K, Semenza GL. 2005. Regulation of hypoxia-inducible factor 1 by prolyl and asparaginyl hydroxylases. Biochem Biophys Res Commun 338: 610–616.

Ijichi C, Matsumura T, Tsuji T, Eto Y. 2003. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. Biochem Biophys Res Commun 303:59–64.

Jeng KS, Sheen IS, Wang YC, Gu SL, Chu CM, Shih SC, Wang PC, Chang WH, Wang HY. 2004. Is the vascular endothelial growth factor messenger RNA expression in resectable hepatocellular carcinoma of prognostic value after resection? World J Gastroenterol 10:676–681.

Jeng KS, Sheen IS, Wang YC, Gu SL, Chu CM, Shih SC, Wang PC, Chang WH, Wang HY. 2004. Prognostic significance of preoperative circulating vascular endothelial growth factor messenger RNA expression in resectable hepatocellular carcinoma: A prospective study. World J Gastroenterol 10: 643–648.

Jiang BH, Jiang G, Zheng JZ, Lu Z, Hunter T, Vogt PK. 2001. Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth Differ 12:363–369.

Kano T, Nagaki M, Takahashi T, Ohnishi H, Saitoh K, Kimura K, Muto Y. 1991. Plasma free amino acid pattern in chronic hepatitis as a sensitive and prognostic index. Gastroenterol Jpn 26:344–349.

Kaseb AO, Hanbali A, Cotant M, Hassan MM, Wollner I, Philip PA. 2009. Vascular endothelial growth factor in the management of hepatocellular carcinoma: A review of literature. Cancer 115:4895–4906.

Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. 1983. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 221:1283–1285.

Komura T, Mizukoshi E, Kita Y, Sakurai M, Takata Y, Arai K, Yamashita T, Ohta T, Shimizu K, Nakamoto Y, Honda M, Takamura T, Kaneko S. 2007. Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. Am J Gastroenterol 102:1939–1946.

Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. 2001. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: Novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol 21:3995–4004.

Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. 1999. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 107:450–455.

Miuma S, Ichikawa T, Taura N, Shibata H, Takeshita S, Akiyama M, Motoyoshi Y, Ozawa E, Fujimoto M, Kawashimo H, Miyaaki H, Eguchi K, Nakao K. 2009. The level of fasting serum insulin, but not adiponectin, is associated with the prognosis of early stage hepatocellular carcinoma. Oncol Rep 22:1415–1424.

Murata K, Moriyama M. 2007. Isoleucine, an essential amino acid, prevents liver metastases of colon cancer by antiangiogenesis. Cancer Res 67:3263–3268.

Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H. 2005. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 3:705–713.

Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H, Ohashi Y. 2006. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. Hepatol Res 35:204–214.

Neufeld G, Tessler S, Gitay-Goren H, Cohen T, Levi BZ. 1994. Vascular endothelial growth factor and its receptors. Prog Growth Factor Res 5: 89–97.

Ng IO, Poon RT, Lee JM, Fan ST, Ng M, Tso WK. 2001. Microvessel density, vascular endothelial growth factor and its receptors Flt-1 and Flk-1/KDR in hepatocellular carcinoma. Am J Clin Pathol 116:838–845.

Park JE, Keller GA, Ferrara N. 1993. The vascular endothelial growth factor (VEGF) isoforms: Differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. Mol Biol Cell 4:1317–1326.

Park YN, Kim YB, Yang KM, Park C. 2000. Increased expression of vascular endothelial growth factor and angiogenesis in the early stage of multistep hepatocarcinogenesis. Arch Pathol Lab Med 124:1061–1065.

Poon RT, Ng IO, Lau C, Zhu LX, Yu WC, Lo CM, Fan ST, Wong J. 2001. Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: A prospective study. Ann Surg 233:227–235.

Schoenleber SJ, Kurtz DM, Talwalkar JA, Roberts LR, Gores GJ. 2009. Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: Systematic review and meta-analysis. Br J Cancer 100:1385–1392.

Semenza GL. 2002. HIF-1 and tumor progression: Pathophysiology and therapeutics. Trends Mol Med 8:S62–S67.

Sharp FR, Bernaudin M. 2004. HIF1 and oxygen sensing in the brain. Nat Rev Neurosci 5:437–448.

Shweiki D, Itin A, Soffer D, Keshet E. 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359:843–845.

Stiehl DP, Jelkmann W, Wenger RH, Hellwig-Burgel T. 2002. Normoxic induction of the hypoxia-inducible factor 1alpha by insulin and interleukin-1beta involves the phosphatidylinositol 3-kinase pathway. FEBS Lett 512: 157–162.

Takahashi H, Shibuya M. 2005. The vascular endothelial growth factor (VEGF)/VEGF receptor system and its role under physiological and pathological conditions. Clin Sci (Lond) 109:227–241.

Taura N, Ichikawa T, Hamasaki K, Nakao K, Nishimura D, Goto T, Fukuta M, Kawashimo H, Fujimoto M, Kusumoto K, Motoyoshi Y, Shibata H, Abiru N, Yamasaki H, Eguchi K. 2006. Association between liver fibrosis and insulin sensitivity in chronic hepatitis C patients. Am J Gastroenterol 101:2752–2759.

Underiner TL, Ruggeri B, Gingrich DE. 2004. Development of vascular endothelial growth factor receptor (VEGFR) kinase inhibitors as

anti-angiogenic agents in cancer therapy. Curr Med Chem 11:731–745.

von Marschall Z, Cramer T, Hocker M, Finkenzeller G, Wiedenmann B, Rosewicz S. 2001. Dual mechanism of vascular endothelial growth factor upregulation by hypoxia in human hepatocellular carcinoma. Gut 48:87–96.

Wang GL, Jiang BH, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 92:5510–5514.

Weidner N, Semple JP, Welch WR, Folkman J. 1991. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 324:1–8.

Yoo PS, Mulkeen AL, Cha CH. 2006. Post-transcriptional regulation of vascular endothelial growth factor: Implications for tumor angiogenesis. World J Gastroenterol 12:4937–4942.

Yoshiji H, Noguchi R, Kaji K, Ikenaka Y, Shirai Y, Namisaki T, Kitade M, Tsujimoto T, Kawaratani H, Fukui H. 2010. Attenuation of insulin-resistance-based hepatocarcinogenesis and angiogenesis by combined treatment with branched-chain amino acids and angiotensin-converting enzyme inhibitor in obese diabetic rats. J Gastroenterol 45:443–450.

Yu MC, Yuan JM. 2004. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 127:S72–S78.

TRANSPLANT
INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Human T-cell leukemia virus type 1 infection worsens prognosis of hepatitis C virus-related living donor liver transplantation

Tatsuki Ichikawa,[1] Naota Taura,[1] Hisamitsu Miyaaki,[1] Toshihisa Matsuzaki,[1] Masashi Ohtani,[1] Susumu Eguchi,[2] Mitsuhisa Takatsuki,[2] Akihisa Soyama,[2] Masaaki Hidaka,[2] Sadayuki Okudaira,[2] Tetsuya Usui,[4] Sayaka Mori,[4] Shimeru Kamihira,[3,4] Takashi Kanematsu,[2] and Kazuhiko Nakao[1]

1 Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
2 Department of Transplantation and Digestive Surgery, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
3 Department of Laboratory Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
4 Central Diagnostic laboratory of Nagasaki University Hospital, Nagasaki, Japan

Keywords
donor age, HCV, HTLV-1, liver transplantation.

Correspondence
Tatsuki Ichikawa, Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel.: +81-95-819-7481; fax: +81-95-849-7482; e-mail: ichikawa@net.nagasaki-u.ac.jp

Conflicts of interest
None.

Received: 7 August 2011
Revision Requested: 13 September 2011
Accepted: 15 January 2012
Published online: 14 February 2012

doi:10.1111/j.1432-2277.2012.01434.x

Summary

Severe and life-threatening donor-transmitted human T-cell leukemia virus type 1 (HTLV-1) infections after solid organ transplantation have been reported. However, in HTLV-1-infected recipients, graft and patient survival were not fully evaluated. A total of 140 patients underwent living donor liver transplantation (LDLT). Of these, 47 of 126 adult recipients showed indications of hepatitis C virus (HCV)-related liver disease. The HTLV-1 prevalence rate was 10 of 140 recipients (7.14%) and three of 140 donors (0.02%). In HCV-related LDLT, graft and patient survival was worsened by HTLV-1 infection in recipients (seven cases). The 1-, 3-, and 5-year survival rates in the HCV/HTLV-1-co-infected group were 67%, 32%, and 15%, respectively, and the corresponding rates in the HCV-mono-infected group were 80%, 67%, and 67%, respectively. Only the 5-year survival rates were statistically significant ($P = 0.04$, log-rank method). HTLV-1 infection in recipients is also an important factor in predicting survival in HTLV-1 endemic areas.

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is highly endemic in the southwestern area of Japan, including Nagasaki as well as in Saharan Africa, South America, the Caribbean islands, and aboriginal Australia [1,2]. However, HTLV-1 infects approximately 15–25 million people worldwide [3] and is associated with adult T-cell leukemia/lymphoma (ATL), HTLV-1-associated myelopathy (HAM), uvetitis, sialadenitis-like Sjögren syndrome (SjS), and a wide variety lymphocyte-mediated disorders [2,4,5]. Severe and life-threatening donor-transmitted HTLV-1 infections after solid organ transplantation have been reported [6–8]. However, in HTLV-1-infected recipients, graft and patient survival has not been fully evaluated. The development of three ATL cases in eight HTLV-1 infected recipients after living donor liver transplantation (LDLT) was reported in Japan [9]. We also reported the development of HAM [10] and sialadenitis-like SjS [11] resulting from HTLV-1 in LDLT recipients. Previous reports state that HTLV-1 infection is associated with a nonresponse to interferon (IFN) monotherapy for chronic hepatitis C (CHC) [12] and flare-up of alanine aminotransferase in hepatitis C virus (HCV)-RNA carriers [13]. HTLV-1/HCV co-infection may affect the course of HCV-associated liver disease and liver cancer [14].

© 2012 The Authors

Additionally, HTLV-1 interferes with intracellular signaling by type 1 IFN and upregulates HCV replication [14–16]. However, the influence of HTLV-1 in recipients on the grafted liver has not been explored. The effect of HTLV-1/HCV co-infections in recipient compared with HCV mono-infections has similarly not been explored.

Between 1988 and 2000, 0.027% of donors reporting to the United Network for Organ Sharing (UNOS) were diagnosed with HTLV-1 infections [6]. However, the prevalence of anti-HTLV-1 antibodies in patients visiting Nagasaki University Hospital between 2000 and 2007 was 13.57% [2], indicating that HTLV-1 carriers are clustered in Nagasaki. To prevent vertical transmission of HTLV-1, the ATL Prevention Program, which is a prefecture-wide breastfeeding intervention study for HTLV-1 carrier mothers, was initiated in Nagasaki in 1987 [17]. As a result, age-specific rates of HTLV-1 among residents in Nagasaki have annually declined (Seropositive rate, 14.5% in 2000; 12.7% in 2007) [2]. The prevalence of anti-HCV antibody increased with age and was higher in populations in the southwestern area of Japan (including Nagasaki) [18]. In endemic areas of HTLV-1 infection, HTLV-1/HCV co-infected patients are frequently observed and increase the probability a person will have a liver transplantation.

The HTLV-1 infection rate is lower in Western countries; however, the influence of HTLV-1 on HCV infection after transplantation has not been examined. It is necessary to evaluate HTLV-1 infection rates in HTLV-1 endemic areas. We examined whether HTLV-1 infection influences patient and graft survival in cases of liver transplantation in endemic areas of HTLV-1 infection in Nagasaki.

Patients and methods

Patients

In total, 126 consecutive adult LDLT patients, 47 of who were HCV-infected, were enrolled in this study. This retrospective cohort study of LDLT recipients included a comparative analysis of HTLV-1-positive and HTLV-1-negative recipients to determine graft and patient survival. In particular, we evaluated whether HTLV-1 infection influenced HCV-related LDLT. Anti-HTLV-1 antibody was detected using an enzyme immunoassay (EIA). In addition, in HTLV-1-positive patients, we used polymerase chain reaction (PCR) analysis to evaluate HTLV-1 proviral DNA in the peripheral blood mononuclear cells. We diagnosed patients with the anti-HTLV-1 antibody and proviral DNA as being HTLV-1 positive. In our hospital, HTLV-1-positive grafts are not used for negative recipients, but are used for positive recipients. Recipient characteristics such as age, gender, body mass index, Child-Pugh score and medical model for end-stage liver disease (MELD) score at the time of transplantation, presence or absence diabetes mellitus (DM), and presence of hepatocellular carcinoma (HCC) were also analyzed. Surgical factors examined included blood type matching, bleeding volume, (ml), and surgery time (min). Donor age was categorized into those less than 50 years old and 50 years old and older. Additional donor characters, such as donor gender, donor BMI, and donor HTLV-1 status were analyzed. HCV factors included genotype (1b or non-1b), titer in 1b, core amino acid mutation in 1b, and IL28B SNP. The HCV-RNA high group (100 000 IU/ml or more in the serum) of patients was analyzed using real-time PCR.

Primary outcomes evaluated included recipient and graft survival. The cause of death was determined using various factors together with biopsy and necropsy. Liver biopsy was performed each year and at exacerbation of liver function.

Methods

The study design, which also included the collection of data from medical records from the associated hospitals mentioned above, was approved by the Ethics Review Board of our hospital.

In this study, 3 *IL28B* SNPs, i.e., rs8099917, rs12979860, and rs12980275, were examined (Nagasaki University Institutional Review Board approval number: 100511184). SNPs were detected using pyrosequence analysis. The sense, antisense, and pyrosequence primers were B-5′-TCCTCCTTTTGTTTTCCTTTCTG-3′, 5′-AAAAAGCCAGCTACCAAACTGT-3′, and 5′-TGGTTCCAATTTGGG-3′ for rs8099917, 5′-GTCGTGCCTGTCGTGTACTGA-3′, 5′-**B**-GGAGCGCGGAGTGCAATT-3′, and 5′-GGAGCTCCCCGAAGG-3′ for rs12979860, and 5′-GCTGTATGATTCCCCCTACATG-3′, 5′-**B**-TACATTGTTCGGCAAGCAATCT-3′, and 5′-AGAAGTCAAATTCCTAGAAA-3′ for rs12980275, respectively. "**B**" in the primer sequences indicates that the primer is biotin-labeled.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC, USA).Graft and patient survival was determined using the Kaplan–Meier method and survival curves were compared using a log-rank test. A cox proportional hazard model was used to determine risk factors for graft and patient survival. Differences between each laboratory data were analyzed using the Mann–Whitney *U*-test and χ^2 test. *P*-values < 0.05 were considered statistically significant.

© 2012 The Authors

Results

We evaluated the impact of HTLV-1 on general graft and patient survival in HCV-infected patients. Of the 140 patients who had undergone LDLT at the Nagasaki University Hospital between 1997 and January 2011, 47 of 126 adult recipients showed indications of HCV-related liver disease. The HTLV-1 prevalence rate was 7.8% (11/140) in the recipients and 2% (3/140) in the donors. Fourteen of the 140 recipients were pediatric recipients. HCV-related LDLT was observed only in adults. All HTLV-1 infected recipients were adult cases. First, we evaluated impact of HTLV-1 for LDLT in adult cases. In HCV-related LDLTs (Fig. 1a), graft and patients survival was worsened by the presence of HTLV-1 infection of recipients. The 1-, 3-, and 5-year survival rates in the HCV/HTLV-1-co-infected group were 67%, 32%, and 15%, respectively, and the corresponding rates in the HCV-mono-infected group were 80%, 67%, and 67%, respectively. Only the 5-year survival rate was found to be statistically significant ($P = 0.04$, log-rank method). However, adult recipients without HCV infection did not develop graft loss and patient death (Fig. 1b). In HCV-related LDLTs, clinical and demographic characteristics in HTLV-1-positive and HTLV-1-negative recipients did not differ between groups, except for donor age (Table 1). We attempted to clarify the factors of graft and patient survival in HCV-infected recipients by univariate analysis. MELD score and donor age at transplantation in the HTLV-1-infected recipients were shown to be significant factors. However, according to multivariate analysis, only donor age was a factor in worsening prognosis ($P < 0.05$; Relative risk 1.048). Three types of IL28B SNPs were not associated with graft and patient survival in HCV infected recipients according to a log rank test and univariate analysis of a Cox proportion hazard test.

Second, we analyzed stratification by donor age. Clinical characteristics, shown in Table 1, in the recipients who tested positive and negative for HTLV-1 did not differ between groups. In HCV-related LDLT recipients from old age donor group (age, 50 years and more; co-infected, 3 cases; HCV mono-infected, 13 cases), graft and patient survival was not worsened by recipient HTLV-1 infection (log-rank test, not significant). However, in the young age donor group (age less than 50 years; co-infected, 4 cases; HCV mono-infected, 34 cases), graft and patient survival was significantly worsened by recipient HTLV-1 infection (log-rank test, $P < 0.05$). However, graft and patient survival in HCV/HTLV-1-co-infected patients did not differ between the old and young donors, and the outcomes of HCV-mono-infected patients differed between the old and young donors according to the log-rank test. On the basis of multivariate analysis using a Cox proportional hazard test, HTLV-1 infection in HCV-infected recipients who received the transplant from younger donors was the only factor contributing to a worsened prognosis ($P = 0.03$; relative risk, 0.207).

Finally, we present the profile of seven cases of HCV/HTLV-1 co-infected recipients (Table 2). In the HCV/HTLV-1 co-infected group, chronic rejection (CR) developed in 3 patients, cases 60, 80, and 117, during peg-interferon/ribavirin treatment. CR did not develop in HCV-mono-infected patients. However, the CR rate was not statistically significant between the HCV/HTLV-1-co-infected group and the HCV-mono-infected group. Patients with CR did not have a prior history of acute rejection and used cyclosporine as an immunosuppressant. HCV-RNA disappearance during peg-interferon combination treatment with ribavirin was not observed in 3 CR cases. The period of peg-interferon combination treatment with ribavirin is 47, 23, and 2 months for HTLV-1/HCV co-infected CR patients. The treatment regimen of the

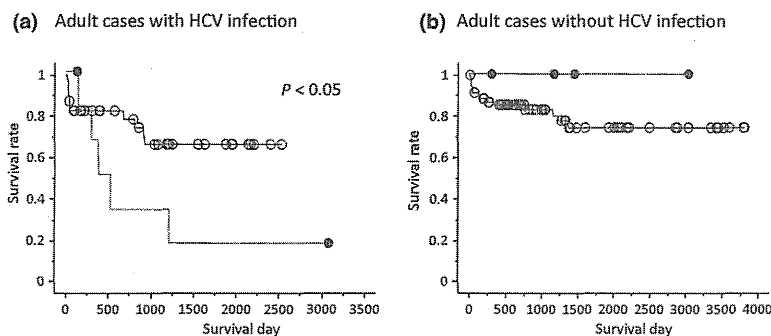

Figure 1 Kaplan–Meier curves for graft and recipient survival in adult transplant cases. A Kaplan–Meier curve revealed adult recipients with HCV infection (a) and without HCV infection (b). Black circle and dot line indicate HTLV-1 positive recipients and white circle and solid line indicate HTLV-1 negative recipients. In adult case with HCV-related LDLT, graft and patient survival of HTLV-1-infected recipients significantly decreased (a, $P < 0.05$).

© 2012 The Authors

Table 1. Clinical characteristics in patients with HCV infection.

	HTLV-1 positive	HTLV-1 negative	*P*-value
Number	7	40	
Age (years)	57.6 (7.16)	58.5 (6.48)	NS
Gender M/F	6/1	20/20	NS
BMI	25.6 (5.17)	25.0 (3.08)	NS
CP-score	10.6 (3.25)	9.85 (2.27)	NS
MELD	11.8 (8.38)	13.6 (8.16)	NS
DM +/-	2/5	20/20	NS
HCC +/-	4/3	27/13	NS
Donor age	48 (14.5)	37.6 (11.8)	0.04
Donor gender M/F	6/1	18/22	NS
Donor BMI	21.9 (2.42)	22.0 (2.62)	NS
Matching +/-	7/0	32/8	NS
Bleeding volume (ml)	5222 (3840)	14 182 (21 230)	NS
Surgery time (min)	902 (171)	934 (215)	NS
HCV GT1/non GT1	6/1	30/10	NS
HCV high titer in GT1	5	28	NS
IL28B SNP Major/Minor	1/2	24/6	NS
SVR rate	1/7	9/40	NS

Data are shown as means (standard deviation) and numbers, with statistical analysis assessed using a Mann–Whitney test for means and χ^2 test for numbers. Statistically significant difference between HTLV-1 positive and negative groups is $P < 0.05$. CP-score, Child–Pugh score; HCV GT, HCV genotype; Matching, Blood type matching; IL28B SNP Major, TT of rs8099917 in recipient and donor; Minor, TG or GG of rs8099917 in recipient and/or donor. BMI, body weight (kg)/height (m)/height (m). SVR, sustained viral response.

Table 2. Clinical characteristics in recipients with HCV and HTLV-1 co-infection.

Case number	20	59	60	80	112	117	132
Age (years)	58	50	68	65	48	54	58
Gender	M	M	M	F	M	M	M
Survival	+	+	–	–	–	–	–
Survival time (day)	3086	1210	528	378	139	301	132
Cause of death	–	HCC	CR	CR	Infect.	CR	–
IFN	+	–	+	+	–	+	–
Viral response	–	–	–	–		–	
IFN period (month)	48		47	23		2	
HCV GT1b	1b	1b	1b	1b	1b	N	1b
BMI	19.3	31.1	23.2	30.5	19.2	30.2	25.9
HCC	–	outside	Milan	Milan	–	Milan	–
MELD	8.1	8.5	4.4	7.3	29.3	15.2	9.9
DM	–	+	+	–	+	+	+
Donor	Sister	Brother	Child	Brother	Brother	Uncle	Child
Donor age	56	45	41	61	46	65	22
Donor HTLV-1	+	–	–	–	–	–	–

Viral response is the disappearance of HCV-RNA in patients under peg-IFN/ribavirin treatment. IFN period is treatment length (month) of peg-IFN/ribavirin treatment. Infect., infection; AIH, autoimmune hepatitis; BA, biliary atresia; LCN, cryptogenic cirrhosis; LCB, hepatitis B virus infected liver cirrhosis. Milan, HCC within Milan criteria, Outside, HCC without Milan criteria.

peg-interferon combination with ribavirin was performed under the rules of our hospital and was the same as was conducted for other HCV-related transplanted patients [19]. Hence, as an immunosuppressive therapy, tacrolimus was used for all HCV-infected patients as an induction therapy combined with steroid tapering; subsequently, tacrolimus treatment was intentionally replaced with cyclosporine treatment to facilitate interferon therapy [20,21] except in case 20. Case 20, which involved an HTLV-1 infected donor, suffered an onset of HAM and sialadenitis under the tacrolimus immunosuppressant regime [11]. Five cases of death occurred in the co-infected group. Causes of death in patients with HTLV-1/HCV co-infection included hepatoma recurrence, infection, and CR. ATL was not observed in this study. Progression of HCV and/or HTLV-1 infection was not always related to death. In particular, all CR cases developed during interferon treatment. Poor survival of HTLV-1/HCV-co-infected patients may have been caused by CR. HCV-RNA levels decreased in the CR cases when the length of peg-IFN/ribavirin treatment was less than 1 year.

Discussion

In this study, we clarified that HTLV-1 infection in HCV-infected recipients is an exacerbation factor involved in survival of both the graft and the patient. Particularly, young donors suffer detrimental effects caused by HTLV-1 infection. Survival of HCV-infected recipients is affected by donor age, MELD score, and HTLV-1 infection. Donor age is the most significant factor in graft and patient survival, and HTLV-1 infection in recipients is the second most important factor in survival in HTLV-1 endemic areas. Donors of advanced age and high MELD scores have been reported as complicating factors [22,23]. We report the impact of HTLV-1 infection on graft and patient survival for the first time.

The presence of HTLV-1 infection as a complicating factor in recipients was revealed after adjusting for age. As HCV/HTLV-1 co-infection occurred in three cases in older donors and four cases in younger donors, it was necessary to determine the role of donor age in HCV/HTLV-1-co-infected recipients. In the HTLV-1 infection-negative group, graft and patient survival was shorter in older donors than in younger donors, but in the HCV/HTLV-1-co-infected group, graft and patient survival did not differ between the old and young donors. Donor age is a complicating factor for graft and patient survival regardless of HTLV-1 infection [23]. The survival rate of the young donor group may initially be high, but survival rate decreases in the presence of HTLV-1 infection. HTLV-I possesses a unique and innate (or acquired) capacity to preserve cellular immunity, such as IL-2 and

© 2012 The Authors

IL-2-receptor induction [24]. HTLV-1 infection may lead to a stronger immune response in recipients when the donor is young than when the donor is old.

The relationship among IFN, HCV, and CR at post-liver transplantation has been previously studied. It is reported that peg-IFN/ribavirin treatment for HCV may trigger rapid CR in patients with therapeutic immunosuppressant trough levels, with or without first inducing acute cellular rejection [25]. Other reports state that the use of cyclosporine, ribavirin discontinuation, a peg-IFN treatment duration of over 1 year, and HCV infection elimination for IFN treatment appear to be associated with CR [26,27]. We suspect that HTLV-1-infected recipients under peg-IFN/ribavirin treatment may be associated with CR for young graft donors and have different immunological mechanisms than HTLV-1 negative recipients.

Recently, the relationship of IL28B SNP and HCV infection has been studied [28]. It has been reported that IL28B SNP is not only related to the effect of IFN treatment, but also to the natural course of HCV infection [29]. We conducted an analysis of IL28B SNP in only 33 pairs of donors and recipients who had obtained agreement in 47 cases of HCV-related liver transplantation. In this study, Il28B SNP was not related to graft and patient survival. However, upon analysis of three types of IL28B SNPs, the survival rate was the same for all three SNPs. Previous reports state that there are no statistical differences in overall graft survival according to recipient and donor IL28B SNPs [30,31].Since it is reported that IL28B SNPs in both recipients and donors is associated with IFN response [30–32], differences in long-term survival between IL28B SNP groups has been examined.

Due to the low prevalence HTLV-1 infection in western countries, the association of liver disease and HTLV-1 infection has not been evaluated. In this study, performed in an HTLV-1 endemic area, we determined that HTLV-1 increases mortality after HCV-related LDLT. Presently, to improve mortality rates, the presence of CR should be determined when HCV/HTLV-1 co-infected transplanted patients are treated using IFN/ribavirin. However, as CR treatment has not been fully evaluated, the mechanism of HTLV-1 infected T cells in HCV-infected graft liver patients under peg-IFN/ribavirin treatment should be determined. The follow-up period of the seven HCV/HTLV-1-co-infected patients was 132–3086 days. As our study population was small and follow-up periods were short, we will extend the follow-up period to validate our results.

Authorship

TI, NT, HM, TM, MO, SE, MT, AS, MH, SO, TU, SM: performed study. SK, TK and KN: designed the study. TI: wrote the manuscript.

Funding

None.

References

1. Verdonck K, Gonzalez E, Van DoorenS, Vandamme AM, Vanham G, Gotuzzo E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007; **7**: 266.
2. Koga Y, Iwanaga M, Soda M, *et al.* Trends in HTLV-1 prevalence and incidence of adult T-cell leukemia/lymphoma in Nagasaki, Japan. *J Med Virol* 2010; **82**: 668.
3. Gessain A, Mahieux R. A virus called HTLV-1. Epidemiological aspects. *Presse Med* 2000; **29**: 2233.
4. Goncalves DU, Proietti FA, Ribas JG, *et al.* Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. *Clin Microbiol Rev* 2010; **23**: 577.
5. Terada K, Katamine S, Eguchi K, *et al.* Prevalence of serum and salivary antibodies to HTLV-1 in Sjogren's syndrome. *Lancet* 1994; **344**: 1116.
6. Kusne S. Regarding unexpected severe and life-threatening donor-transmitted viral infections and use of high-risk behavior donors. *Liver Transpl* 2008; **14**: 1564.
7. Toro C, Rodes B, Poveda E, Soriano V. Rapid development of subacute myelopathy in three organ transplant recipients after transmission of human T-cell lymphotropic virus type I from a single donor. *Transplantation* 2003; **75**: 102.
8. Hoshida Y, Li T, Dong Z, *et al.* Lymphoproliferative disorders in renal transplant patients in Japan. *Int J Cancer* 2001; **91**: 869.
9. Kawano N, Shimoda K, Ishikawa F, *et al.* Adult T-cell leukemia development from a human T-cell leukemia virus type I carrier after a living-donor liver transplantation. *transplantation* 2006; **82**: 840.
10. Soyama A, Eguchi S, Takatsuki M, *et al.* Human T-cell leukemia virus type I-associated myelopathy following living-donor liver transplantation. *liver transpl* 2008; **14**: 647.
11. Nakamura H, Ichikawa T, Nakamura T, *et al.* Macrophage-dominant sialadenitis in human T-cell leukemia virus type I-associated myelopathy after living-donor liver transplantation. *Transplant Proc* 2010; **42**: 2797.
12. Kishihara Y, Furusyo N, Kashiwagi K, Mitsutake A, Kashiwagi S, Hayashi J. Human T lymphotropic virus type 1 infection influences hepatitis C virus clearance. *J Infect Dis* 2001; **184**: 1114.
13. Tsuji K, Yamasaki K, Yamanishi M, Kawakami M, Shirahama S. Risk of alanine aminotransferase flare-up among asymptomatic hepatitis C virus RNA carriers: a 10-year follow-up study. *J Gastroenterol Hepatol* 2001; **16**: 536.
14. Boschi-Pinto C, Stuver S, Okayama A, *et al.* A follow-up study of morbidity and mortality associated with hepatitis C virus infection and its interaction with human

© 2012 The Authors

T lymphotropic virus type I in Miyazaki, Japan. *J Infect Dis* 2000; **181**: 35.
15. Zhang J, Yamada O, Kawagishi K, *et al.* Up-regulation of hepatitis C virus replication by human T cell leukemia virus type I-encoded Tax protein. *Virology* 2007; **369**: 198.
16. Feng X, Ratner L. Human T-cell leukemia virus type 1 blunts signaling by interferon alpha. *Virology* 2008; **374**: 210.
17. Hino S, Katamine S, Miyata H, Tsuji Y, Yamabe T, Miyamoto T Primary prevention of HTLV-1 in Japan. *Leukemia* 1997; **11** Suppl 3: 57.
18. Tanaka J, Kumagai J, Katayama K, *et al.* Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995-2000. *Intervirology* 2004; **47**: 32.
19. Ichikawa T, Nakao K, Miyaaki H, *et al.* Hepatitis C virus kinetics during the first phase of pegylated interferon-alpha-2b with ribavirin therapy in patients with living donor liver transplantation. *Hepatol Res* 2009; **39**: 856.
20. Eguchi S, Takatsuki M, Soyama A, *et al.* Intentional conversion from tacrolimus to cyclosporine for HCV-positive patients on preemptive interferon therapy after living donor liver transplantation. *Ann Transplant* 2007; **12**: 11.
21. Hirano K, Ichikawa T, Nakao K, *et al.* Differential effects of calcineurin inhibitors, tacrolimus and cyclosporin a, on interferon-induced antiviral protein in human hepatocyte cells. *Liver Transpl* 2008; **14**: 292.
22. Ishigami M, Honda T, Okumura A, *et al.* Use of the Model for End-Stage Liver Disease (MELD) score to predict 1-year survival of Japanese patients with cirrhosis and to determine who will benefit from living donor liver transplantation. *J Gastroenterol* 2008; **43**: 363.
23. Iwamoto T, Yagi T, Umeda Y, *et al.* The impact of donor age on the outcome of adult living donor liver transplantation. *Transplantation* 2008; **85**: 1240.
24. Casseb J. Possible mechanism for positive interaction of human T cell leukemia type I on liver disease in a hepatitis C virus-infected Japanese cohort. *J Infect Dis* 2000; **182**: 379.
25. Selzner N, Guindi M, Renner EL, Berenguer M. Immune-mediated complications of the graft in interferon-treated Hepatitis C positive Liver transplant recipients. *J Hepatol* 2010; **55**: 207.
26. Stanca CM, Fiel MI, Kontorinis N, Agarwal K, Emre S, Schiano TD. Chronic ductopenic rejection in patients with recurrent hepatitis C virus treated with pegylated interferon alfa-2a and ribavirin. *Transplantation* 2007; **84**: 180.
27. Fernandez I, Ulloa E, Colina F, *et al.* Incidence, risk factors, and outcome of chronic rejection during antiviral therapy for posttransplant recurrent hepatitis C. *Liver Transpl* 2009; **15**: 948.
28. Tanaka Y, Nishida N, Sugiyama M, *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105.
29. Thomas DL, Thio CL, Martin MP, *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798.
30. Charlton MR, Thompson A, Veldt BJ, *et al.* Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. *Hepatology* 2011; **53**: 317.
31. Lange CM, Moradpour D, Doehring A, *et al.* Impact of donor and recipient IL28B rs12979860 genotypes on hepatitis C virus liver graft reinfection. *J Hepatol* 2011; **55**: 322.
32. Fukuhara T, Taketomi A, Motomura T, *et al.* Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology* 2010; **139**: 1577.

© 2012 The Authors

© 2011 John Wiley & Sons A/S

Transplant Infectious Disease, ISSN 1398-2273

Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation?

S. Eguchi, M. Hidaka, A. Soyama, M. Takatsuki, H. Miyaaki, T. Ichikawa, K. Nakao, T. Kanematsu. Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation? Transpl Infect Dis 2012: 14: 156–162. All rights reserved.

Abstract: As treatments for acute cellular rejection (ACR) and recurrent hepatitis caused by hepatitis C virus (HCV) are dramatically different, making a precise diagnosis is considered to be essential in patients after liver transplantation. Therefore, we investigated whether immunohistochemical detection of FOXp3, a marker for regulatory T cells (CD4+ CD25+), could be used to differentiate between recurrent hepatitis C and ACR. From a group of 103 cases of living-donor liver transplantation (LDLT), 48 samples were taken via liver biopsy from 20 patients with HCV infection. An initial diagnosis was made based on hematoxylin and eosin staining, which was scored with the hepatitis activity index (HAI) grading, whereas ARC was scored with the rejection activity index (RAI). The FOXp3 immunohistochemical staining on serial specimens was retrospectively analyzed, scoring from 0 to III. The time after LDLT was a median of 270 (range: 14–2000) days, whereas the median number of biopsies per patient was 3 (range: 1–8). The HAI was significantly different between 0 vs. I, and II vs. III, in terms of the FOXp3 score. On the other hand, a significant difference in the RAI was only found between 0 vs. I. In conclusion, FOXp3 may represent a surrogate marker for recurrent HCV infection after LDLT.

S. Eguchi[1], M. Hidaka[1], A. Soyama[1], M. Takatsuki[1], H. Miyaaki[2], T. Ichikawa[2], K. Nakao[2], T. Kanematsu[1]

[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,
[2]Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Key words: FOXp3; recurrent hepatitis C; acute cellular rejection; living-donor liver transplantation; CD25

Correspondence to:
S. Eguchi, MD,
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki
852-8501, Japan
Tel: 81 95 819 7316
Fax: 81 95 819 7319
E-mail: sueguchi@nagasaki-u.ac.jp

Received 24 May 2011, revised 19 July 2011, accepted for publication 26 August 2011

DOI: 10.1111/j.1399-3062.2011.00690.x
Transpl Infect Dis 2012: **14**: 156–162

Recurrent hepatitis C virus (HCV) infection after liver transplantation (LT) remains a therapeutic challenge, especially in a setting with living donors, where the possibility of retransplantation is limited. To date, the best treatment for chronic HCV infection is interferon (IFN) combined with ribavirin, with a 50% sustained virologic response rate (1). In fact, diagnosis of recurrent hepatitis C is sometimes difficult to make, because the presentation, in the histologic view, has many similarities to rejection (2).

Regulatory T cells (Tregs) are supposed to regulate an over-reactive autoimmune response, and were detected to be CD4+ CD25+. Tregs are engaged in the maintenance of self-tolerance by suppressing the activation and expansion of self-reactive lymphocytes (3–5). Loss of this suppressing function may lead to chronic inflammation and/or autoimmunity (6–12). Currently, the best indicator of Tregs function is thought to be the intracellular expression of forkhead box P3 (FOXp3), which is also crucial for Tregs development (13). One of our co-authors (H.M.) previously reported the usefulness of examining liver-targeted Tregs as indicators of chronic hepatitis B virus and HCV infection (14).

In the setting of LT, it was also reported that needle biopsy could provide a source for determining FOXp3 messenger RNA (mRNA) expression after LT (15). In addition, it was reported that FOXp3 mRNA in peripheral blood is a useful marker for acute cellular rejection (ACR), whereas CD4+ CD25+ numbers in peripheral blood may be a marker to predict recurrence of HCV after LT (16, 17).

However, to the best of our knowledge, studies have not previously examined the use of FOXp3 in

Characteristics of liver transplant patients with HCV

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis HAI	RAI	FOXp3	IFN
1	58/M	1b	1800	5	4	0	+
			2000	5	3	1	+
2	57/F	1b	540	0	0	0	+
			720	3	0	2	+
3	53/F	1b	30	3	3	0	–
			180	6	6	1	–
			540	3	7	0	+
			1020	3	0	2	+
4	52/M	II	20	1	8	1	–
			35	6	4	2	–
5	63/F	1b	150	3	4	1	+
			270	4	0	2	+
			510	5	0	2	+
			630	0	0	1	– (SVR)
6	57/M	1b	14	1	4	0	–
			180	6	2	2	+
			540	6	0	0	+
7	55/F	1b	180	3	6	1	–
			194	7	5	3	–
			208	6	4	2	–
			360	7	3	1	+
			1080	7	3	1	+
8	64/F	1b	21	2	3	2	–
			74	2	3	2	–
			180	6	3	3	+
9	61/F	1b	30	3	0	3	–
			720	4	2	1	–
			780	4	2	1	–
10	62/M	II	60	6	4	3	+
11	67/M	1b	14	7	7	1	–
			400	7	3	2	+
			720	3	5	1	–
12	58/M	1b	90	2	1	0	–
			360	3	6	1	+
			720	2	4	2	+ (SVR)
13	51/F	1b	450	10	5	3	–
			900	10	6	3	–
			1380	10	5	3	+
			1835	10	4	3	+
14	59/M	1b	360	2	0	0	+ (SVR)
15	54/M	1b	390	0	0	0	– (SVR)
16	68/F	1b	150	3	3	1	–
			300	6	3	1	+
17	59/F	1b	180	4	0	0	–
			360	5	0	0	+
			480	2	1	0	+
18	65/F	1b	30	4	4	1	–
			120	9	6	3	+
			135	3	3	2	+

Table 1 continued

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis HAI	RAI	FOXp3	IFN
19	59/M	1b	21	4	3	0	–
			41	1	3	0	–
20	65/M	1b	480	4	0	2	+
			570	3	0	1	+

HCV, hepatitis C virus; Pt no., patient number; LDLT, living-donor liver transplantation; HAI, hepatitis activity index; RAI, rejection activity index; FOXp3, marker for regulatory T cells; IFN, interferon; M, male; F, female; SVR, sustained virologic response.

Table 1

liver infiltrating lymphocytes to differentiate recurrent HCV infection from ACR.

Patients and methods

Patients

Of 103 cases of living-donor LT (LDLT), 29 patients (mean age: 57.8 ± 10.6, male:female ratio: 17:12) were positive for anti-HCV antibodies. Fifty-eight samples were taken via liver biopsy from 20 patients (Table 1). Liver biopsy tissue specimens were taken by a needle puncture for diagnostic purposes. HCV serotype was type I in 18 of those patients, whereas it was type II in 2 patients. In all patients, IFN therapy was eventually attempted. Immunosuppression was based on our protocol using cyclosporine as previously reported (1).

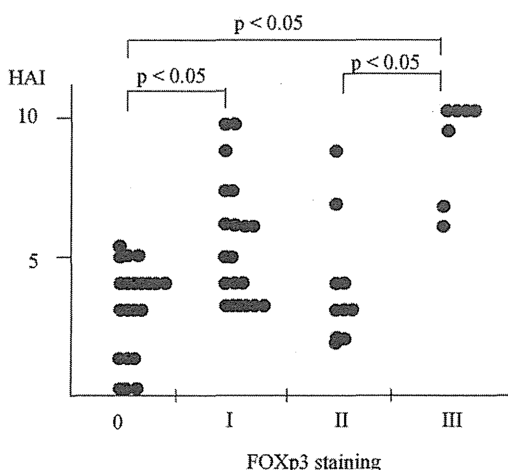

Fig. 1. The relationship between hepatitis activity index (HAI) grading and FOXp3 staining. Significant differences were seen between 0 and I, II and III, and 0 and III with regard to the FOXp3 staining.

Methods

All tissues were fixed in 10% neutral buffered formalin and were then embedded in paraffin, and 4-mm-thick serial sections were cut from each paraffin block. T cells were examined immunohistochemically using an anti-CD4 antibody (Novocastra, Newcastle, UK).

Initial diagnosis was made based on hematoxylin and eosin (H&E) staining, followed by FOXp3 immunohistochemical staining (eBioscience, San Diego, California, USA) on serial specimens. Among aggregated lymphocytes, the number of FOXp3-positive CD4+ lymphocytes was scored as 0 = none, I = 1–9 cells, II = 10–19 cells, and III = >20 cells, as in our previous report (14). The association of FOXp3 with hepatitis activity index (HAI) and/or rejection activity index (RAI) (median 3, range: 0–8) was investigated.

To classify the degree of hepatic inflammation (hepatic activity), we used the HAI score as described by Knodell et al. (18). Based on their criteria, the H&E-stained specimens of the non-cancerous liver tissues were examined and classified into 4 categories. ACR was scored based on the RAI according to the Banff schema (19, 20).

All data are expressed as the median values with ranges. The statistical analysis was performed using the Mann–Whitney *U*-test for continuous values, and the chi-squared test for categorical values. A significant difference was defined as a *P*-value of <0.05. The StatView 5.0 statistical software package (Abacus Concepts, Berkeley, California, USA) was used for all statistical analyses.

Fig. 2. The relationship between the rejection activity index (RAI) and FOXp3 staining. A significant difference was only seen between 0 and I in terms of FOXp3 staining with regard to the RAI.

Results

Table 1 showed characteristics of the patients and a summary of the histologic findings. The median days from the time of liver biopsy was 270 days (range: 14–2000 days) after LT. The median grade, based on the HAI, was 4 (0–10). The median degree of rejection, based on the RAI, was 3 (0–8). The difference in HAI was significant between 0 vs. I, as well as II vs. III, based on the number of FOXp3-positive cells (Fig. 1). On the other hand, a significant difference in the RAI was only seen for 0 vs. I (Fig. 2).

Figure 3 shows a representative liver biopsy specimen of a patient with recurrent HCV infection after LT. The patient was a 58-year-old woman who had undergone LT 4 years earlier. Her aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels were elevated at 107/80. H&E staining revealed an HAI grade of 10 and RAI of 5.

Figure 4 shows another representative liver biopsy specimen. This patient was a 62-year-old woman who underwent LT 4 months before the biopsy. Her AST/ALT levels were elevated at 68/65. H&E staining revealed an HAI grade of 9 and RAI of 6.

On the other hand, Figure 5 shows a representative liver biopsy specimen from a patient with ACR. The patient was 58-year-old woman who had undergone LT 4 years before the biopsy samples were taken. Her AST/ALT levels were elevated to 120/108. H&E staining revealed HAI grade of 3 and RAI of 6.

Discussion

This study examined the distribution and frequency of the appearance of Tregs in the liver after LT. After LT, in patients with hepatitis C, both ACR and recurrent HCV can lead to elevation of transaminases, in

HAI 10, RAI 5, FOXp3 III (Case 13)

Fig. 3. Case 13. Representative findings in a liver with recurrent hepatitis C. Many liver infiltrating lymphocytes were positive for CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

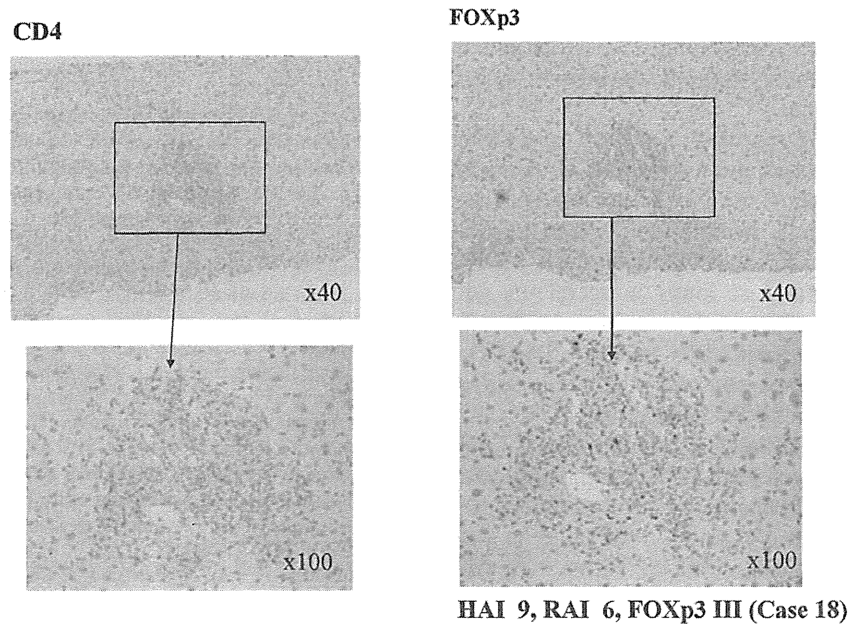

Fig. 4. Case 18. Representative findings in a liver with recurrent hepatitis C. Many liver infiltrating lymphocytes were positive for CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

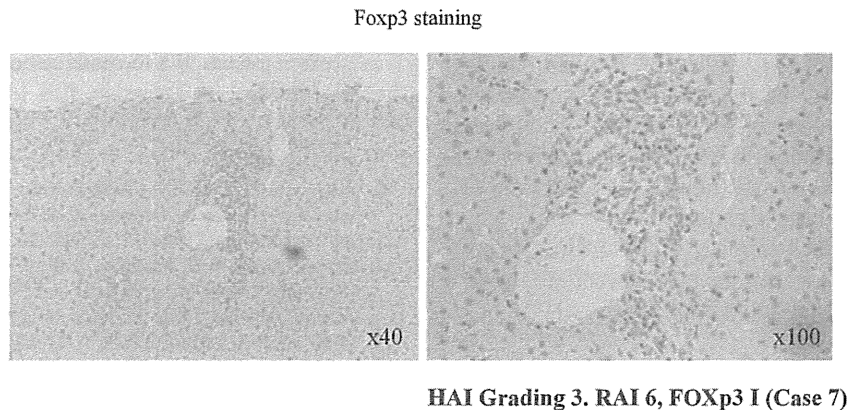

Fig. 5. Case 7. Representative findings in a liver with acute cellular rejection. Few liver infiltrating lymphocytes were positive for both CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

addition to possible vascular abnormalities. Previously, Jain et al. (21) reported the significance of C4d expression in infiltrating lymphocytes, as CD4, CD8, and CD56 were similar in both ACR and recurrent HCV infection. However, accurately differentiating ACR from hepatitis C can sometimes be very difficult.

In previous reports regarding LT, the significance of Tregs in the grafted liver has been controversial. One report showed a relationship between ACR and an increase in Tregs. Intrahepatic detection of FOXp3 gene expression after LT can be accomplished using minimally invasive aspiration biopsy (15). With regard to recurrent hepatitis C, FOXp3 mRNA expression was used to differentiate between the two conditions. Based on needle biopsy, they reported that intrahepatic FOXp3 levels are associated with HCV re-infection and a history of acute rejection, and that the level increased within the first year after LT (15).

Generally speaking, Tregs are associated with graft tolerance in organ transplantation. It seems likely that FOXp3 mRNA expression is associated with graft acceptance (22). It was reported that CD4+ FOXp3 cells are present within grafts in a subset of tolerant patients after human LT (23). However, in the present study, no clear relationship was observed between

ACR and Tregs, except to find a statistical difference between 0 and I in FOXp3 staining. This relationship needs further investigation without the interference of HCV infection.

Sakamoto et al. (24) reported increased expression of FOXp3 mRNA immediately after LDLT, probably because of the activation of T cells, including Tregs and other T-cell subsets. In addition, it was reported that expression of FOXp3 mRNA on days 14, 21, and 28 after transplantation were lower in recipients with ACR within 60 days after LDLT. In our study, the median time since transplantation was 270 days. This is different from previous reports, which focused on short-term diagnosis using FOXp3 staining in the liver and peripheral blood. Usually, 6 months after LT, the level of immunosuppression is stabilized. HCV infection could occur during this period, and antiviral therapy is often initiated. In our study, most patients were undergoing or had already received antiviral therapy with IFN and ribavirin. Although we showed a relationship with FOXp3 expression, we were unable to clarify the function of Tregs in recurrent HCV infection after LT. Further investigation will be needed.

After effective IFN therapy, the number of infiltrating lymphocytes seemed to decrease, which made scoring FOXp3 staining difficult. It was unclear whether the character of the infiltrating lymphocytes changed over the course of treatment. In settings other than transplantation, the FOXp3 staining system may be used to differentiate hepatitis C from autoimmune-like disease or other causes of hepatitis.

In conclusion, FOXp3 staining in infiltrating lymphocytes in the liver may represent a surrogate marker for recurrent HCV infection after LDLT.

Acknowledgements:

Authors' contributions: S.E., T.K., and K.N carried out study conception and design. M.H. and A.S. provided acquisition of data. M.T., T.I., and H.M. performed analysis and interpretation of data. M.T. and S.E. were responsible for drafting of the manuscript. T.K., S.E., and K.N. performed critical revision.

References

1. Eguchi S, Takatsuki M, Soyama Y, et al. Intentional conversion from tacrolimus to cyclosporine for HCV-positive patients on preemptive interferon therapy after living donor liver transplantation. Ann Transplant 2007; 12 (4): 11–15.
2. Regev A, Molina E, Moura R, et al. Reliability of histopathologic assessment for the differentiation of recurrent hepatitis C from acute rejection after liver transplantation. Liver Transpl 2004; 10 (10): 1233–1239.
3. Sakaguchi S. Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol 2004; 22: 531–562.
4. Piccirillo CA, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring CD4+ CD25+ regulatory T cells. Trends Immunol 2004; 25 (7): 374–380.
5. Jiang H, Chess L. An integrated view of suppressor T cell subsets in immunoregulation. J Clin Invest 2004; 114 (9): 1198–1200.
6. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 2001; 182: 18–32.
7. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol 2001; 2 (9): 816–822.
8. Hori S, Sakaguchi S. Foxp3: a critical regulator of the development and function of regulatory T cells. Microbes Infect 2004; 6 (8): 745–751.
9. Coutinho A, Hori S, Carvalho T, Caramalho I, Demengeot J. Regulatory T cells: the physiology of autoreactivity in dominant tolerance and 'quality control' of immune responses. Immunol Rev 2001; 182: 89–98.
10. Lan RY, Ansari AA, Lian ZX, Gershwin ME Regulatory T cells: development, function and role in autoimmunity. Autoimmun Rev 2005; 4 (6): 351–363.
11. Furuno K, Yuge T, Kusuhara K, et al. CD25+ CD4+ regulatory T cells in patients with Kawasaki disease. J Pediatr 2004; 145 (3): 385–390.
12. Kukreja A, Cost G, Marker J, et al. Multiple immuno-regulatory defects in type-1 diabetes. J Clin Invest 2002; 109 (1): 131–140.
13. Sakaguchi S. Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 2005; 6 (4): 345–352.
14. Miyaaki H, Zhou H, Ichikawa T, et al. Study of liver-targeted regulatory T cells in hepatitis B and C virus in chronically infected patients. Liver Int 2009; 29 (5): 702–707.
15. Demirkiran A, Baan CC, Kok A, Metselaar HJ, Tilanus HW, van der Laan LJ. Intrahepatic detection of FOXP3 gene expression after liver transplantation using minimally invasive aspiration biopsy. Transplantation 2007; 83 (6): 819–823.
16. Brown K, Wong W. Diagnostic value of regulatory T cells: a new facet of a much studied cell population. Transplantation 2008; 86 (11): 1485–1491.
17. Perrella A, Arenga G, Pisaniello D, et al. Elevated CD4+/CD25+ T-cell frequency and function during hepatitis C virus recurrence after liver transplantation. Transplant Proc 2009; 41 (5): 1761–1766.
18. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1 (5): 431–435.
19. [Anonymous.] Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997; 25 (3): 658–663.
20. Demetris A, Adams D, Bellamy C, et al. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for histopathological staging and

reporting of chronic rejection. An International Panel. Hepatology 2000; 31 (3): 792–799.
21. Jain A, Ryan C, Mohanca R, et al. Characterization of CD4, CD8, CD56 positive lymphocytes and C4d deposits to distinguish acute cellular rejection from recurrent hepatitis C in post-liver transplant. Clin Transplant 2006; 20 (5): 624–633.
22. Fehervari Z, Sakaguchi S. CD4+ tregs and immune control. J Clin Invest 2004; 114 (9): 1209–1217.
23. Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+ CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol 2005; 174 (4): 1783–1786.
24. Sakamoto R, Asonuma K, Zeledon Ramirez ME, Yoshimoto K, Nishimori A, Inomata Y. Forkhead box P3 (FOXP3) mRNA expression immediately after living-donor liver transplant. Exp Clin Transplant 2009; 7 (1): 8–12.

Acta Med. Nagasaki 57: 1−4

Anti-hepatitis C virus activity of geranylgeranylacetone treatment in hepatitis C-infected patients

Tohei YAMAGUCHI, Tatsuki ICHIKAWA, Shigeyuki TAKESHITA, Naota TAURA, Hisamitsu MIYAAKI, Toru MURAOKA, Hidetaka SHIBATA, Takuya HONDA, Keisuke HAMASAKI, Yuji KATO, Fuminao TAKESHIMA, Kazuhiko NAKAO

Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Background. Geranylgeranylacetone (GGA), which is an isoprenoid compound, has been used orally as an antiulcer drug in Japan. GGA induces antiviral gene expression by stimulating the formation of interferon-stimulated gene factor 3 in human hepatoma cells. This study verified the anti-hepatitis C virus (HCV) activity of GGA in chronic hepatitis C-infected patients.
Methods. The present prospective study included 20 consecutive anti-HCV antibody-positive, HCV-genotype 1b, and chronic gastritis patients who visited Nagasaki University Hospital between January 1999 and December 1999. GGA (150 mg per day, which is the dose generally used for chronic gastritis) was taken orally for four weeks. We evaluated HCV-RNA titers and other clinical parameters at pretreatment, posttreatment, and at the endpoint of the study. Pretreatment was the beginning point of GGA treatment. Posttreatment was the termination point of GGA treatment. The endpoint was the point four weeks after the posttreatment point.
Results. All patients completed four weeks of GGA treatment and four weeks of observation. HCV-RNA titers at postpoint were not significantly diminished compared to those at pretreatment. However, HCV-RNA titers were significantly diminished at endtreatment compared to pretreatment. Unfortunately, we did not observe a case with no titer of HCV-RNA. Alanine aminotransferase values and other parameters were not affected by GGA treatment.
Conclusion. GGA has anti-HCV activities in chronic hepatitis C-infected patients. In the future, it will be necessary to examine the clinical effectiveness of the combination of treatment with both GGA and interferon in HCV patients.

ACTA MEDICA NAGASAKIENSIA 57: 1−4, 2012

Keywords: Hepatitis C virus, geranylgeranylacetone, chronic hepatitis C

Introduction

Currently, chronic hepatitis C virus (HCV) infections are the major cause of hepatocellular carcinoma (HCC) worldwide (1). Therefore, an anti-HCV strategy is important for the prevention of carcinogenesis. The treatment of HCV with a combination of pegylated interferon (IFN) and ribavirin is effective in 80% of HCV genotype 2 or 3 cases but is less than 50% effective in genotype 1 cases. New anti-HCV agents designed to inhibit the life cycle of HCV have been developed and are used in combination with IFN-α to ameliorate the salvage rate of HCV infection (2). However, this combination therapy cannot completely eliminate chronic HCV infections. Therefore, long-term management and safety drugs for chronic hepatitis C (CHC) patients are required.

Geranylgeranylacetone (GGA) is an isoprenoid compound, which includes retinoids. GGA was developed in Japan and has been used orally as an antiulcer drug (3). GGA protects the gastric mucosa from various types of stress without affecting gastric acid secretion (4,5). Moreover, GGA suppresses cell growth and induces differentiation or apoptosis

Address correspondence: Tatsuki Ichikawa, Department of Gastroenterology and Hepatology, Graduate School of Biomedical Sciences, Nagasaki University 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
Phone: +81-95-819-7482, Fax: +81-95-819-7481, E-mail address: ichikawa@net.nagasaki-u.ac.jp, All authors: There are no conflicts of interest

Received September 15, 2011; Accepted January 6, 2012

in several human leukemia cells (6,7). 3,7,11,15-Tetramethyl-2,4,6,-10,14-hexadecapentaenoic acid is another isoprenoid compound that was designated as an acyclic retinoid because it has the ability to interact with nuclear retinoid receptors (8) that cause apoptosis in certain human hepatoma cells (9). GGA acts as a potent inducer of antiviral gene expression, and it induces the expression by stimulating the formation of IFN-stimulated gene factor 3 (ISGF3) in human hepatoma cells (10). GGA induces the expression of antiviral proteins such as 2′5′-oligoadenylate synthetase (2′5′-OAS) and double-stranded RNA-dependent protein kinase (PKR) in hepatoma cell lines. GGA stimulates 2′5′-OAS and PKR gene expression at the transcriptional level through the formation of ISGF-3, which regulates the transcription of both genes. GGA induces the expression of signal transducers and activators of transcription 1, 2 (STAT-1, STAT-2) and p48 proteins, components of ISGF3, together with the phosphorylation of STAT1 (10). However, the anti-HCV activity of GGA has not been observed in vivo and in vitro.

At present, new treatments for CHC patients are necessary, and GGA has an IFN-like action in hepatoma cells (10). Therefore, we attempted to verify the anti-HCV activity of GGA in CHC patients.

Methods

Patients

The present prospective study included 20 consecutive anti-HCV antibody-positive, HCV-genotype 1b, and chronic gastritis patients who visited the Nagasaki University Hospital between January 1999 and December 1999. Patients were enrolled in the study after informed consent was obtained. The patients had not been previously treated with IFN therapy and were diagnosed with CHC on the basis of clinical data. The patients were evaluated with a HCV-RNA polymerase chain reaction (PCR) method (Amplicor method). The HCV-RNA high group (100,000 IU/mL or more in the serum) was identified by quantitative PCR. The criteria for HCC were assessed by abdominal imaging methods and by HCC history. The patients who were not previously diagnosed with diabetes mellitus (DM) were evaluated by the 75-g oral glucose tolerance test (OGTT). All subjects underwent OGTT with 75 g of glucose according to the recommendations of the National Diabetes Data Group of the National Institute of Health. Blood samples were taken at 0, 30, 60, 90, 120, and 180 min after administration in order to measure the plasma glucose (PG) and insulin concentrations. In this study, the DM group consisted of patients with clinically diagnosed DM or ≥110 mg/dL fasting PG and/or 140 mg/dL or high PG at 120 min.

White blood cell counts, red blood cell counts, platelet counts, hemoglobin A1c levels, alanine aminotransferase (ALT) levels, aspartate aminotransferase (AST) levels, and γ-glutamyl transpeptidase (GTP) levels were determined by hematometry and standard laboratory techniques. Clinical characteristics are shown in the Table.g

Table. Clinical characteristics at pre-GGA treatment

Characteristic	mean (SD) or number
Age (years)	56 (16)
Sex (F/M)	10/10
BMI	21.0 (3.02)
Genotype 1b	20
HCV high titer	14
HCV-RNA titer	489 (378)
HCC +/-	0/20
WBC count	6004 (1585)
RBC count	447 (60)
Plt count	18.9 (7.9)
Alb level	4.46 (3.0)
AST level	49.5 (21.2)
ALT level	71 (28)
γ-GTP level	50.5 (32)
DM +/-	0/20
HbA1c level	5.05 (0.8)
FPG level	96 (13)

Data are shown as means (standard deviation) and numbers.
BMI, body mass index; HCV, Hepatitis C virus; HCC, hepatocellular carcinoma; WBC, white blood cells; RBC, red blood cells; Plt, platelets; Alb, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; DM, diabetes mellitus; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose.
Normal values in laboratory tests: ALT (IU/L), 5-40; AST (IU/L), 10-40; γ-GTP (IU/L), <70 in men, <30 in women; Alb (g/dL), 4.0-5.0; WBC (cells/μL), 3500-9000; RBC (× 10^4 cells/μL), 450-580 in men, 380-480 in women; Plt (× 10^4 platelets/μL), 14-33; ferritin (ng/mL), 39.4-340 in men, 3.6-114 in women; FPG (mg/dL), 70-110; HbA1c (%), 4.3-5.8; BMI, body weight (kg)/height^2 (m).

Methods

The dose of 150 mg of GGA per day, which is generally used to treat chronic gastritis in Japan, was taken orally for four weeks, and it was assumed that patients took one dose a day. Pretreatment was the beginning point of GGA treatment. We evaluated HCV-RNA titers and other clinical parameters at pretreatment, posttreatment, and study endpoint. Posttreatment was the termination point of GGA treatment. Endpoint was the point four weeks after the

posttreatment of GGA. During this study, all patients were not treated with Stronger Neo-Minophagen C (Minophagen Pharmaceutical Co., Ltd., Tokyo, Japan) because of its anti-hepatitis effects or with IFN because of its anti-HCV effects.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC). The differences in the values of each laboratory parameter were analyzed with a t-test. P values less than 0.05 were considered statistically significant.

Results

GGA decreased the HCV-RNA titers in patients but did not affect the values of ALT

All patients completed four weeks of GGA treatment and four weeks of observation. Adverse effects were not observed in any patient. The titers of HCV-RNA (Fig. 1A) changed after the patients completed GGA treatment. Compared with HCV-RNA titers at pretreatment, titers at endpoint did not diminish significantly. However, compared to HCV-RNA titers at pretreatment, the titers were significantly diminished at posttreatment. Unfortunately, we did not observe a case with no titer of HCV-RNA. Values of ALT (Fig. 1B) and other parameters were not changed by GGA

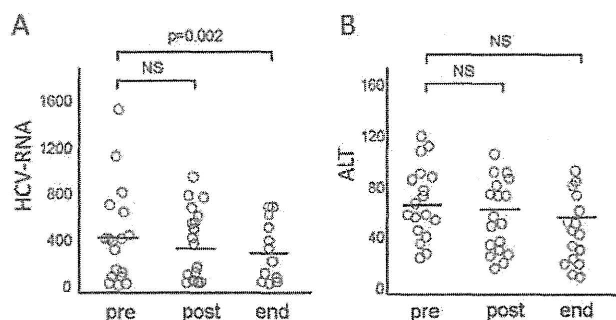

Figure 1. Titers of hepatitis C virus (HCV)-RNA at the endpoint were decreased compared to the levels at pretreatment (A), but alanine aminotransferase (ALT) levels were not changed (B).

Panel A shows serum HCV-RNA titers, and panel B shows serum ALT levels at each of the indicated points. The bar indicates the mean value. Statistical significance was accepted with p-values less than 0.05. "Pre" indicates the point of pre-geranylgeranylacetone (GGA) treatment. "Post" indicates the termination point of GGA treatment. "End" indicates the point four weeks after posttreatment of GGA. Compared to HCV-RNA titers at pretreatment, GGA treatment decreased the titer at pretreatment but not at posttreatment.

treatment. The diminished HCV-RNA titers at the posttreatment point were increased at the endpoint, which was four weeks after the posttreatment point.

In Fig. 2, we present the case of a patient who had the most diminished HCV-RNA titers among the 20 GGA-treated patients (Fig. 2). This case had mild fluctuations of ALT levels before GGA treatment. The HCV-RNA titer was 420 K copies/mL and 380 K copies/mL at 12 weeks before treatment and at the pretreatment point, respectively. After GGA treatment, HCV-RNA titers were decreased to 2 K copies/mL and 4 K copies/mL at the endpoint and at the posttreatment point, respectively. In this case, the ALT values were also diminished in a similar manner as HCV-RNA. After the observation period, +12 weeks, HCV-RNA titers and ALT values were increased compared to those at the pretreatment point.

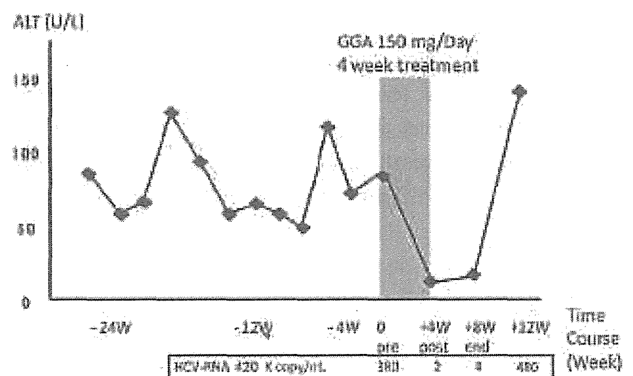

Figure 2. The clinical course of a geranylgeranylacetone (GGA)-treated chronic hepatitis C (CHC) patient.

Here, we present a case of a 53-year-old man who was an outpatient of our hospital for 5 years. He was diagnosed with chronic hepatitis on the basis of clinical data. The patient had not been previously treated with interferon (IFN). The y-axis indicates alanine aminotransferase (ALT) levels, and the x-axis indicates the time course. The duration of the GGA treatment periods is shown in the gray field. The zero point on the x-axis is the GGA treatment-starting day. HCV-RNA titers are 420, 380, 2, and 4 K copies/mL at -12 weeks, 0 weeks (pretreatment), +4 weeks (posttreatment), and +8 weeks (end of follow-up period), respectively.

Discussion

GGA demonstrated anti-HCV activity in this study. The anti-HCV effect that was due to GGA did not result in a disappearance of HCV-RNA titers in CHC patients. An adverse effect was not observed with GGA treatment.

GGA is a non-toxic heat shock protein (HSP) 70 inducer (11). Various GGA activities outside of the stomach are also related to HSP induction (12,13,14). GGA induces