diabetes mellitus occurring as a complication of chronic hepatic diseases associated with HCV infection was higher than that of other chronic hepatic diseases, and that anti-HCV antibody-positive patients aged 40 years or more had a 3.77-fold higher risk of becoming diabetic than anti-HCV antibody-negative patients.[68] In addition, it has been demonstrated that complication by diabetes mellitus is both a risk factor for hepatocellular carcinoma[69] and a prognostic factor in cirrhosis patients.[70] These reports suggest a correlation between HCV infection and type 2 diabetes. Increased insulin resistance and insulin secretory deficiency are considered to be highly involved in the pathogenesis of type 2 diabetes.[71] Petit et al.[72] reported that insulin resistance increases even in chronic hepatitis C patients with slight hepatic impairment and that the index of impairment (HOMA-IR) correlates with the severity of the liver tissue disorder. Tumor necrosis factor (TNF)-α, which closely correlates with hepatic inflammation and fibrillation in chronic hepatitis C,[73] is considered to enhance glucose uptake in peripheral tissue and to promote gluconeogenesis in the liver, leading to the induction of insulin resistance.[74] Shintani et al.[75] confirmed that in transgenic mice with the 1b HCV core genotype, tyrosyl phosphorylation of the insulin receptor substrate 1 in the insulin signal transduction pathway is disrupted and that this disruption causes gluconeogenesis inhibition by insulin in the liver, leading to the induction of marked insulin resistance. These transgenic mice exhibited a high anti-TNF-α antibody level, and insulin resistance was improved by the administration of an anti-TNF-α antibody. These results indicate a close relationship between HCV infection and the pathogenesis of diabetes mellitus. The relationship between HCV infection and hepatocyte fat modification has also attracted attention.[76] Moriya et al.[77] suggested the possible direct involvement of HCV core protein in hepatocyte fat modification, because they observed hepatocyte fat deposits in transgenic mice expressing the HCV core gene. In summary, the above-described findings strongly indicate that hepatitis C has the characteristics of a metabolic disease, and nutritional management is also considered important in the treatment of chronic hepatitis C.

Malignant lymphoma

HCV reproduces in lymphocytes, and studies of a short-term HCV culture system using lymphocytes have been reported.[78,79] Infected lymphocytes may undergo malignant transformation, leading to the development of malignant lymphoma. HCV infection is considered to be associated with the development of malignant lymphoma, particularly in association with the pathogenesis of non-Hodgkin B-cell lymphoma, and many reports suggest a relationship between HCV infection and malignant lymphoma.[80–88] It has been assumed that some cryoglobulinemia patients develop non-Hodgkin B-cell lymphoma in association with *myc* gene mutation.[89] The anti-HCV antibody positivity rates in patients with non-Hodgkin B-cell lymphoma range from 0% to 33%.[80–88] These differences in HCV antibody positivity rates are considered to relate to regional differences in the HCV infection rate. The HCV antibody prevalence tends to be higher in Japan and Italy but lower in Britain and Canada. Studies indicating a relationship between HCV infection and malignant lymphoma have been reported by Ferri et al.[90] and De Vita et al.[91] Ferri et al.[90] reported that 14 of 500 patients with chronic hepatitis C were complicated with non-Hodgkin B-cell lymphoma, and they detected HCV RNA in peripheral blood lymphocytes in all of these patients. De Vita et al.[91] detected positive-strand and negative-strand HCV RNAs in the parotid glands of patients with parotid non-Hodgkin B-cell lymphoma associated with HCV infection, and confirmed the presence of HCV in the parotid gland by in situ hybridization.[91] As shown by these findings, many patients with HCV-associated non-Hodgkin B-cell lymphoma show involvement of extranodal sites such as the liver and salivary glands.[92]

Treatment of HCV-associated malignant lymphoma is similar to that of HCV-associated non-Hodgkin B-cell lymphoma; however, recently, IFN monotherapy or IFN and ribavirin combination therapy have been reported to be effective.[93–96] Vallisa et al.[96] reported that administration of both pegylated IFN and ribavirin to 13 patients with HCV-associated non-Hodgkin B-cell lymphoma achieved a complete response in seven of these patients. It is interesting that IFN-based antiviral therapy has been demonstrated to be useful for malignant lymphoma associated with HCV-associated non-Hodgkin B-cell lymphoma in addition to conventional chemotherapy.

Autoimmune thyroid disease

The relationship between HCV infection and thyroid disease has been analyzed in many studies,[97,100] and a causal relationship between HCV infection and autoimmune thyroid disease has been particularly suggested.[98–100] Antonelli et al.[98] assessed the incidence of thyroid dysfunction in 630 chronic hepatitis C patients without cirrhosis or hepatocellular carcinoma who had not been treated with IFN by recruiting 389 patients from an iodine-deficient area, 268 patients from an iodine-sufficient area, and 86 patients with chronic hepatitis B aged 40 years or older as study subjects. The chronic hepatitis C patients exhibited a higher thyroid-

stimulating hormone level and lower free thyroxine and triiodothyronine levels than the controls. In addition, the chronic hepatitis C patients exhibited hypothyroidism and tended to have antithyroglobulin antibodies and anti-thyroid peroxidase antibodies. These findings suggest a relationship between HCV infection and thyroid disorder.[98] A possible relationship between HCV infection and thyroid cancer has also attracted attention recently.[99] The mechanism underlying the pathogenesis of thyroid disease associated with HCV infection has not yet been elucidated, but a relationship with liver/kidney microsomal antibody type 1 has been suggested.[99] Many patients with thyroid disorder caused by HCV infection are asymptomatic, requiring no special treatment. Thyroid disorder is also known to be an adverse reaction to IFN-α therapy for chronic hepatitis C.[99,101–104] Thyroid hypofunction caused by the administration of IFN-α is usually transient, and the patient recovers spontaneously after the end of the therapy. Hence, discontinuation of IFN-α is not required in many cases.[103]

Idiopathic interstitial pneumonitis

Recently, viral infection has been suggested to be a cause of idiopathic interstitial pneumonitis.[104] With regard to the relationship between HCV infection and idiopathic interstitial pneumonitis, Ueda et al.[106] reported in 1992 that the anti-HCV antibody positivity rate in 66 patients with idiopathic interstitial pneumonitis determined by enzyme-linked immunosorbent assay was 28.8%, which was significantly higher than that in 9464 normal subjects serving as controls.[106] It has not yet been clarified how HCV infection is associated with the pathogenesis of idiopathic interstitial pneumonitis. Kubo et al.[107] suggested that activated T lymphocytes and eosinophils are related to the pathogenesis of idiopathic interstitial pneumonitis associated with HCV infection, because they observed increased activated T-lymphocyte and eosinophil counts in the bronchoalveolar fluid of 13 chronic hepatitis C patients, despite their having the same total cell counts as normal subjects. On the other hand, studies disagree regarding the relationship between HCV infection and idiopathic interstitial pneumonitis,[108] and in-depth studies of this issue are expected. Idiopathic interstitial pneumonitis is also reported to be an adverse reaction to IFN therapy in chronic hepatitis C patients.[109] Such patients often have a high pretreatment KL-6 level, and the potential of their developing idiopathic interstitial pneumonitis is suggested. Recovery from IFN therapy-induced idiopathic interstitial pneumonitis is achieved by the discontinuation of the therapy,[109] but steroid administration is required in some cases.

Rheumatoid arthritis

HCV-associated rheumatoid arthritis complicated by cryoglobulinemia or Sjögren's syndrome has been reported.[10,39] For further information, please refer to the cited references.

Mooren's ulcer

Mooren's ulcer is a progressive ulcer associated with congestion and pain around the cornea.[110] HCV infection has been suggested to contribute to the development of this disease.[111–113] The effectiveness of IFN therapy for HCV-associated Mooren's ulcer has been reported,[110,111] but the exacerbation of ocular pain following the discontinuation of IFN therapy has also been observed; hence, caution is required.[111] Systemic corticoid administration has also been reported to be effective.[112] However, other investigators reported a negative correlation between HCV infection and Mooren's ulcer.[114–116] It is hoped that further detailed studies will clarify this issue.

Conclusion

It is necessary to consider possible complications associated with extrahepatic diseases in the treatment of HCV-infected patients.

References

1. Grey HM, Kohler PF. Cryoimmunoglobulins. Semin Hematol 1973;10:87–112.
2. Zignego AL, Giannelli F, Marrocchi ME, Mazzocca A, Ferri C, Giannini C, et al. T(14:18) translocation in chronic hepatitis C virus infection. Hepatology 2000;31:474–79.
3. Kitay-Cohen Y, Amiel A, Hilzenrat N, Buskila D, Ashur Y, Fejgin M, et al. Bcl-2 rearrangement in patients with chronic hepatitis C associated with essential mixed cryoglobulinemia type II. Blood 2000;96:2910–12.
4. Zuckerman E, Zuckerman T, Sahar D, Streichman S, Attias D, Sabo E, et al. bcl-2 and immunoglobulin gene rearrangement in patients with hepatitis C infection. Br J Haematol 2001;112:364–9.
5. Zignego AL, Ferri C, Giannelli F, Giannini C, Caini P, Monti M, et al. Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas. Ann Intern Med 2002;137:571–80.
6. Sasso EH, Martinez M, Yarfitz SL, Ghillani P, Musset L, Piette JC, et al. Frequent joining of Bcl-2 to a JH6 gene in hepatitis C virus-associated t(14:18). J Immunol 2004;173:3549–56.
7. Curry MP, Golden-Mason L, Doherty DG, Deignan T, Norris S, Duffy M, et al. Expansion of innate CD5pos B cells expressing high levels of CD81 in hepatitis C virus infected liver. J Hepatol 2003;38:642–50.
8. Brouet JC, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. Am J Med 1974;57:775–88.

9. Wong VS, Egner W, Elsey T, Brown D, Alexander GJ. Incidence, character and clinical relevance of mixed cryoglobulinemia in patients with chronic hepatitis C infection. Clin Exp Immunol 1996;104:25–31.
10. Mayo MJ. Extrahepatic manifestation of hepatitis C infection. Am J Med Sci 2002;325:135–48.
11. Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N Engl J Med 1993;328:465–70.
12. Agnello V, Chung RT, Kaplan IM. A roll for hepatitis C virus infection in type II cryoglobulinaemia. N Engl J Med 1992;327: 1490.
13. Okuse C, Yotsuyanagi Y, Okazaki T, Yasuda K, Fujioka T, Tomoe M, et al. Detection, using a novel method, of a high prevalence of cryoglobulinemia in persistent hepatitis C virus infection. Hepatol Res 2003;27:18–22.
14. Lunel F, Musset L, Cacoub P, Frangeul L, Cresta P, Perrin M, et al. Cryoglobulinemia in chronic liver disease: role of hepatitis C virus and liver damage. Gastroenterology 1994;106:1291–300.
15. Misiani R, Bellavita P, Fenili D, Vicari O, Marchesi D, Sironi PL, et al. Interferon alpha-2a therapy in cryoglobulinemia associated with hepatitis C virus. N Engl J Med 1994;330:751–6.
16. Zuckerman E, Keren D, Slobodin G, Rosner I, Rozenbaum M, Toubi E, Sabo E, et al. Treatment of refractory, symptomatic, hepatitis C virus related mixed cryoglobulinemia with ribavirin and interferon-alpha. J Rheumatol 2000;27:2172–8.
17. Cacoub P, Lidove O, Maisonobe T, Duhaut P, Thibault V, Ghillani P, et al. Interferon-alpha and ribavirin treatment in patients with hepatitis C virus-related systemic vasculitis. Arthritis Rheum 2002;46:3317–26.
18. Ubara Y, Hara S, Katori H, Tagami T, Kitamura A, Yokota M, et al. Splenectomy may improve the glomerulopathy of type II mixed cryoglobulinemia. Am J Kidney Dis 2000;35:1186–92.
19. Kasuno K, Ono T, Matsumori A, Nogaki F, Kusano H, Watanabe H, et al. Hepatitis C virus-associated tubulointerstitial injury. Am J Kidney Dis 2003;41:767–75.
20. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, et al. Glomerulonephritis in autopsy cases with hepatitis C virus infection. Intern Med 1998;37:836–40.
21. D'Amico G, Fornasieri A. Cryoglobulinemic glomerulonephritis: a membranoproliferative glomerulonephritis induced by hepatitis C virus. Am J Kidney Dis 1995;25:361–9.
22. Sinico RA, Fornasieri A, D'Amico G. Renal manifestations associated with hepatitis C infection. Ann Med Interne (Paris) 2000;151:41–5.
23. Johnson RJ, Gretch DR, Couser WG, Alpers CE, Willson J, Chung M, et al. Hepatitis C virus-associated glomerulonephritis. Effect of alpha-interferon therapy. Kidney Int 1994;46:1700–4.
24. Lopes E, Lopes LV, Silva AE. Mixed cryoglobulinemia and membranoproliferative glomerulonephritis associated with hepatitis C virus infection. Ann Intern Med 1996;125:781–2.
25. Misiani R, Bellavita P, Baio P, Caldara R, Ferruzzi S, Rossi P, et al. Successful treatment of HCV-associated cryoglobulinaemic glomerulonephritis with a combination of interferon-alpha and ribavirin. Nephrol Dial Transplant 1999;14:1558–60.
26. Sabry AA, Sobh MA, Sheaashaa HA, Kudesia G, Wild G, Fox S, et al. Effect of combination therapy (ribavirin and interferon) in HCV-related glomerulopathy. Nephrol Dial Transplant 2002;17:1924–30.
27. Dammacco F, Sansonno D, Han JH, Shyamala V, Cornacchiulo V, Iacobelli AR, et al. Natural interferon-alpha versus its combination with 6-methyl-prednisolone in the therapy of type II mixed cryoglobulinemia: a long-term, randomized, controlled study. Blood 1994;84:3336–43.
28. Matsumori A. Hepatitis C virus and cardiomyopathy. Herz 2000; 25:249–54.
29. Matsumori A, Yutani C, Ikeda Y, Kawai S, Sasayama S. Hepatitis C virus from the hearts of patients with myocarditis and cardiomyopathy. Lab Invest 2000;80:1137–42.
30. Matsumori A. Role of hepatitis C virus in cardiomyopathies. Ernst Schering Res Found Workshop 2006;55:99–120.
31. Okabe M, Fukuda K, Arakawa K, Kikuchi M. Chronic variant of myocarditis associated with hepatitis C virus infection. Circulation 1997;96:22–4.
32. Maisch B, Ristic AD, Portig I, Pankuweit S. Human viral cardiomyopathy. Front Biosci 2003;8:39–67.
33. Sarkany RP. The management of porphyria cutanea tarda. Clin Exp Dermatol 2001;26:225–32.
34. Fargion S, Piperno A, Cappellini MD, Sampietro M, Fracanzani AL, Romano R, et al. Hepatitis C virus and porphyria cutanea tarda: evidence of a strong association. Hepatology 1992;16: 1322–6.
35. Herrero C, Vicente A, Bruguera M, Ercilla MG, Barrera JM, Vidal J, et al. Is hepatitis C virus infection a trigger of porphyria cutanea tarda? Lancet 1993;341:788–9.
36. Tsukazaki N, Tanaka K, Irifune H, Yoshida H, Watanabe M, Ohgami T, et al. Relationship between porphyria cutanea tarda (PCT) and viral hepatitis. J Dermatol 1994;21:411–4.
37. Hussain I, Hepburn NC, Jones A, O'Rourke K, Hayes PC. The association of hepatitis C viral infection with porphyria cutanea trada in the Lothian region of Scotland. Clin Exp Dermatol 1996;21:283–5.
38. Okano J, Horie Y, Kawasaki H, Kondo M. Interferon treatment of porphyria cutanea tarda associated with chronic hepatitis type C. Hepatogastroenterology 1997;44:525–8.
39. Fox RI. Sjögren's syndrome. Lancet 2005;366:321–21.
40. Nagao Y, Hanada S, Shishido S, Ide T, Kumashiro R, Ueno T, et al. Incidence of Sjögren's syndrome in Japanese patients with HCV infection. J Gastroenterol Hepatol 2003;18:258-66.
41. Koike K, Moriya K, Ishibashi K, Yotsuyanagi H, Shintani Y, Fujie H, et al. Sialadenitis histologically resembling Sjögren syndrome in mice transgenic for hepatitis C virus envelope genes. Proc Natl Acad Sci USA 1997;94:233–6.
42. Takamatsu K, Okayasu I, Koyanagi Y, Yamamoto N. Hepatitis C virus propagates in salivary glands. J Infect Dis 1992;165: 973–4.
43. Arrieta JJ, Rodriguez-Inigo E, Ortiz-Movilla N, Bartolome J, Pardo M, Manzarbeitia F, et al. In situ detection of hepatitis C virus RNA in salivary gland. Am J Pathol 2001;158:259–64.
44. Lunel F, Cacoub P. Treatment of autoimmune and extra-hepatic manifestations of HCV infection. Ann Med Interne (Paris) 2000;151:58–64.
45. Scully C, Beyli M, Ferreiro MC, Ficarra G, Gill Y, Griffiths M, et al. Update on oral lichen planus: etiopathogenesis and management. Crit Rev Oral Biol Med 1998;9:86–122.
46. Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 2002;13:350–65.
47. Rebora A, Robert E, Rongioletti F. Clinical and laboratory presentation of lichen planus patients with chronic liver disease. J Dermatol Sci 1992;4:38–41.
48. Bagan JV, Aguirre JM, del Olmo JA, Milian A, Penarrocha M, Rodrigo JM, et al. Oral lichen planus and chronic liver disease: a clinical and morphometric study of the oral lesions in relation to transaminase elevation. Oral Surg Oral Med Oral Pathol 1994;78:337–42.
49. Bellamn B, Reddy RK, Falanga V. Lichen planus associated with hepatitis C. Lancet 1995;346:1234.
50. Nagao Y, Sata M, Tanikawa K, Itoh K, Kameyama T. Lichen planus and hepatitis C virus in the northern Kyushu region of Japan. Eur J Clin Invest 1995;25:910–4.
51. Ingafou M, Porter SR, Scully C, Teo CG. No evidence of HCV infection or liver disease in British patients with oral lichen planus. Int J Oral Maxillofac Surg 1998;27:65–66.

52. van der Meij EH, van der Waal I. Hepatitis C virus infection and oral lichen planus: a report from the Netherlands. J Oral Pathol Med 2000;29:255–8.
53. Nagao Y, Sata M. Hepatitis C virus and lichen planus. J Gastroenterol Hepatol 2004;19:1101–13.
54. Nagao Y, Kameyama T, Sata M. Hepatitis C virus RNA detection in oral lichen planus tissue. Am J Gastroenterol 1998;93: 850.
55. Nagao Y, Sata M, Noguchi S, Seno'o T, Kinoshita M, Kameyama T, et al. Detection of hepatitis C virus RNA in oral lichen planus and oral cancer tissues. J Oral Pathol Med 2000;29:259–66.
56. Arrieta JJ, Rodriguez-Inigo E, Casqueiro M, Bartolom J, Manzarbeitia F, Herrero M, et al. Detection of hepatitis C virus replication by in situ hybridization in epithelial cells of anti-hepatitis C virus-positive patients with and without oral lichen planus. Hepatology 2000;32:97–103.
57. Pilli M, Penna A, Zerbini A, Vescovi P, Manfredi M, Negro F, et al. Oral lichen planus pathogenesis: a role for the HCV-specific cellular immune response. Hepatology 2002;36:1446–52.
58. Nagao Y, Sata M, Itoh K, Tanikawa K, Kameyama T. Quantitative analysis of HCV RNA and genotype in patients with chronic hepatitis C accompanied by oral lichen planus. Eur J Clin Invest 1996;26:495–8.
59. Lodi G, Carrozzo M, Hallett R, D'Amico E, Piattelli A, Teo CG, et al. HCV genotypes in Italian patients with HCV-related oral lichen planus. J Oral Pathol Med 1997;26:381–4.
60. Nagao Y, Sata M, Suzuki H, Tanigawa K, Itoh K, Kameyama T. Effectiveness of glycyrrhizin for oral lichen planus in patients with chronic HCV infection. J Gastroenterol 1996;31:691–5.
61. Doutre MS, Beylot C, Couzigou P, Long P, Royer P, Beylot J. Lichen planus and virus C hepatitis: disappearance of the lichen under interferon alpha therapy. Dermatology 1992;184:229.
62. Baccia S, Gamberini S, Della Libera M, Strumia R, Ventrurini D. Lichen planus and interferon therapy for hepatitis C. Gastroenterology 1993;105:1921–2.
63. Protzer U, Ochsendorf FR, Leopolder-Ochsendorf A, Holtermuller KH. Exacerbation of lichen planus during interferon alpha-2a therapy for chronic active hepatitis C. Gastroenterology 1993;104:903–5.
64. Nagao Y, Sata M, Suzuki H, Kameyama T, Ueno T. Histological improvement of oral lichen planus in patients with chronic hepatitis C treated with interferon. Gastroenterology 1999;117: 283–4.
65. Nagao Y, Sata M, Tanikawa K, Itoh K, Kameyama T. High prevalence of hepatitis C virus antibody and RNA in patients with oral cancer. J Oral Pathol Med 1995;24:354–60.
66. Nagao Y, Sata M, Itoh K, Chiba I, Komiyama K, Yanoma S, et al. High prevalence of hepatitis C antibody and RNA in patients with head and neck squamous cell carcinoma. Hepatol Res 1997;7:206–12.
67. Allison ME, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol 1994; 21:1135–9.
68. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 2001;133:592–9.
69. Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002;36:1206–13.
70. Bianchi G, Marchesini G, Zoli M, Bugianesi E, Fabbri A, Pisi E. Prognostic significance of diabetes in patients with cirrhosis. Hepatology 1994;20:119–25.
71. Mueckler M. Family of glucose-transporter genes. Implications for glucose homeostasis and diabetes. Diabetes 1990;39:6–11.
72. Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 2001;35:279–83.
73. Zylberberg H, Rimaniol AC, Pol S, Masson A, De Groote D, Berthelot P, et al. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J Hepatol 1999;30:185–91.
74. Pradhan AD, Ridker PM. Do atherosclerosis and type2 diabetes share a common inflammatory basis? Eur Heart J 2002;23: 831–4.
75. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004;126:840–8.
76. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572–7.
77. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol 1997;78:1527–31.
78. Ito T, Yasui K, Mukaigawa J, Katsume A, Kohara M, Mitamura K. Acquisition of susceptibility to hepatitis C virus replication in HepG2 cells by fusion with primary human hepatocytes: establishment of a quantitative assay for hepatitis C virus infectivity in a cell culture system. Hepatology. 2001;34:566–72.
79. Shimizu YK, Iwamoto A, Hijikata M, Purcell RH, Yoshikura H. Evidence for in vitro replication of hepatitis C virus genome in a human T-cell line. Proc Natl Acad Sci USA 1992;89: 5477–81.
80. Ferri C, La Civita L, Caracciolo F, Zignego AL. Non-Hodgkin's lymphoma: possible role of hepatitis C virus. J Am Med Assoc 1994;272:355–6.
81. Ferri C, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G, et al. Hepatitis C virus infection in patients with Hodgkin's lymphoma. Br J Haematol 1994;88:392–4.
82. Bring AM, Watson JP, Burt A, Kestevan P, Wallis J, Proctor SJ, et al. Non-Hodgkin's lymphoma and hepatitis C infection. Leuk Lymphoma 1996;21:127–30.
83. Izumi T, Sasaki R, Miura Y, Okamoto H. Primary hepatosplenic lymphoma: association with hepatitis C infection. Blood 1996;87: 5380–1.
84. Thalen DJ, Raemaekers J, Galama J, Cooreman MP. Absence of hepatitis C virus infection in non-Hodgkin's lymphoma. Br J Haematol 1997;96:880–1.
85. McColl MD, Singer IO, Tait RC, McNeil IR, Cumming RL, Hogg RB. The role of hepatitis C virus in the aetiology of non-Hodgkin's lymphoma—a regional association? Leuk Lymphoma 1997;26:127–30.
86. Kashyap A, Nademanee A, Molina A. Hepatitis C and B-cell lymphoma. Ann Intern Med 1997;128:695.
87. Germanidis G, Haioun C, Pourquier J, Gaulard P, Pawlotsky JM, Dhumeaux D, et al. Hepatitis C virus infection in patients with overt B-cell non-Hodgkin's lymphoma in a French center. Blood 1999;93:1778–9.
88. Collier JD, Zanke B, Moore M, Kessier G, Krajden M, Shepherd F, et al. No association between hepatitis C and B-cell lymphoma. Hepatology 1999;29:1259–61.
89. Perl A, Gorevic PD, Ryan DH, Condemi JJ, Ruszkowski RJ, Abraham GN. Clonal B cell expression in patients with essential mixed cryoglobulinemia. Clin Exp Immunol 1989;76:54–60.
90. Ferri C, La Civita L, Monti M, Longombardo G, Greco F, Pascro G, et al. Can type C hepatitis infection be complicated by malignant lymphoma? Lancet 1995;346:1426–7.
91. De Vita S, Sansonno D, Dolcetti R, Ferraccioli G, Carbone A, Cornacchiulo V, et al. Hepatitis C virus within a malignant lymphoma lesion in the course of type II mixed cryoglobulinemia. Blood 1995;1887–92.
92. De Vita S, Sacco C, Sansonno D, Gloghini A, Dammacco F, Crovatto M, et al. Characterization of overt B-cell lymphomas

in patients with hepatitis C virus infection. Blood 1997;90:776–82.
93. Hermine O, Lefrere F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis ac virus infection. N Engl J Med 2002;347:89–94.
94. Levine AM, Shimodaira S, Lai MM. Treatment of HCV-related mantle-cell lymphoma with ribavirin and pegylated interferon alpha. N Engl J Med 2003;349:2078–9.
95. Kelaidi C, Rollot F, Park S, Tulliez M, Christoforov B, Calmus Y, et al. Response to antiviral treatment in hepatitis C virus-associated marginal zone lymphomas. Leukemia 2004;18:1711–6.
96. Vallisa D, Bernuzzi P, Arcaini L, Sacchi S, Callea V, Marasca R, et al. Role of anti-hepatitis C virus (HCV) treatment in HCV-related, low-grade, B-cell, non-Hodgkin's lymphoma: a multicenter Italian experience. J Clin Oncol 2005;23:468–73.
97. Montella M, Pezzillo L, Crispo A, Izzo F, Amore A, Marone U, et al. Risk of thyroid cancer and high prevalence of hepatitis C virus. Oncol Rep 2003;10:113–6.
98. Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, et al. Thyroid disorders in chronic hepatitis C. Am J Med 2004;117:10–3.
99. Muratori L, Bogdanos DP, Muratori P, Lenzi M, Granito A, Ma Y, et al. Susceptibility to thyroid disorders in hepatitis C. Clin Gastroenterol Hepatol 2005;3:595–603.
100. Testa A, Castaldi P, Fant V, Fiore GF, Grieco V, DeRosa A, et al. Prevalence of HCV antibodies in autoimmune thyroid disease. Eur Rev Med Pharmacol Sci 2006;10:183–6.
101. Marcellin P, Pouteau M, Benhamou JP. Hepatitis C virus infection, alpha interferon therapy and thyroid dysfunction. J Hepatol 1995;22:364–9.
102. Pittau E, Bogliolo A, Tinti A, Mela Q, Ibba G, Salis G, et al. Development of arthritis and hypothyroidism during alpha-interferon therapy for chronic hepatitis C. Clin Exp Rheumatol 1997;15:415–9.
103. Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid 2003;13:547–51.
104. Bini EJ, Mehandru S. Incidence of thyroid dysfunction during interferon alpha-2b and ribavirin therapy in men with chronic hepatitis C: a prospective cohort study. Arch Intern Med 2004;22:2371–6.
105. Vassallo R. Viral-induced inflammation in interstitial lung disease. Semin Respir Infect 2003;18:55–60.
106. Ueda T, Ohta K, Suzuki N, Yamaguchi M, Hirai K, Horiuchi T, et al. Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus. Am Rev Respir Dis 1992 146;266–8.
107. Kubo K, Yamaguchi S, Fujimoto K, Hanaoka M, Hayasaka M, Honda T, et al. Bronchoalveolar lavage fluid findings in patients with chronic hepatitis C virus infection. Thorax 1996;51:312–4.
108. Irving WL, Day S, Johnston ID. Idiopathic pulmonary fibrosis and hepatitis C virus infection. Am Rev Respir Dis 1993;148:1683–4.
109. Karim A, Ahmed S, Khan A, Steinberg H, Mattana J. Interstitial pneumonitis in a patient treated with alpha-interferon and ribavirin for hepatitis C infection. Am J Med Sci 2001;322:233–5.
110. Wood TO, Kaufman HE. Mooren's ulcer. Am J Ophthalmol 1971;71:417–22.
111. Wilson SE, Lee WM, Murakami C, Weng J, Moninger GA. Mooren-type hepatitis C virus-associated corneal ulceration. Ophthalmology 1994;101:736–45.
112. Moazami G, Auran JD, Florakis GJ, Wilson SE, Srinivasan DB. Interferon treatment of Mooren's ulcers associated with hepatitis C. Am J Ophthalmol 1995;119:365–6.
113. Pluznik D, Butrus SI. Hepatitis C-associated peripheral corneal ulceration: rapid response to intravenous steroids. Cornea 2001;20:888–9.
114. Zegans ME, Srinivasan M, McHugh T, Whitcher JP, Margolis TP, Lietman T, et al. Mooren ulcer in South India: serology and clinical risk factors. Am J Ophthalmol 1999;128:205–10.
115. Zegans ME, Anninger W, Chapman C, Goedon SR. Ocular manifestations of hepatitis C virus infection. Curr Opin Ophthalmol 2002;13:423–7.
116. Jain AK, Sukhija J, Saini JS, Chawla Y, Dhiman RK. Hepatitis C virus-associated keratitis. Eye 2004;18:131–4.

ELSEVIER

Impact of New Methicillin-Resistant *Staphylococcus aureus* Carriage Postoperatively After Living Donor Liver Transplantation

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ABSTRACT

Background. Preoperative carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increased risk of MRSA infection after liver transplantation. It is not known, however, whether new MRSA carriage postoperatively also increases the risk of MRSA infection after liver transplantation.

Methods. We retrospectively reviewed the data from 242 adult patients who underwent living donor liver transplantation (LDLT) including microbiological and medical records from admission to 3 months after LDLT. Uni and multivariate analyses were performed to identify independent risk factors for postoperative MRSA infection among preoperative noncarriers of MRSA.

Results. Postoperative MRSA infection occurred in 18 of 219 preoperative noncarriers of MRSA by median postoperative day 26. Operation time of at least 16 hours and postoperative colonization with MRSA independently predicted postoperative MRSA infection.

Conclusion. Postoperative surveillance cultures should be performed periodically after liver transplantation to identify high-risk candidates for postoperative MRSA infection, even among preoperative noncarriers of MRSA.

STAPHYLOCOCCUS AUREUS is a major cause of bacterial infection after liver transplantation.[1,2] Isolates of *S aureus* causing clinical nosocomial infection can be divided into two groups: methicillin-susceptible *S aureus* and methicillin-resistant *S aureus* (MRSA). MRSA infection frequently complicates the postoperative course after deceased donor liver transplantation (DDLT).[1,3–5] Among several centers, 91% (45 of 49 isolates) of all *S aureus* infections after DDLT were caused by MRSA.[4]

Preoperative MRSA carriage is associated with an increased risk for MRSA infection after DDLT.[1,3–5] In addition, postoperative MRSA colonization is prevalent in DDLT.[6] Positive MRSA cultures on both postoperative and preoperative surveillance is considered important because increased MRSA colonization in a patient during hospitalization increases the risk of MRSA infection.[7] In a prospective study,[7] the relative risk of developing an MRSA infection among patients with MRSA colonization was greater than among patients who were not colonized with *S aureus*. In this particular study, 12 of 394 patients had MRSA colonization during hospitalization, and 4 of the 12 later developed MRSA infection.

It is not known, however, whether new MRSA carriage postoperatively following liver transplantation also increases the risk of MRSA infection. Moreover, MRSA in cases of living donor liver transplantation (LDLT), in which operations are scheduled in a more selective manner, is not well described. The aim of the present study was to assess the details of postoperative MRSA infection among preoperative noncarriers of MRSA and to analyze whether new MRSA carriage postoperatively increased the risk of MRSA infection after LDLT using multivariate analysis.

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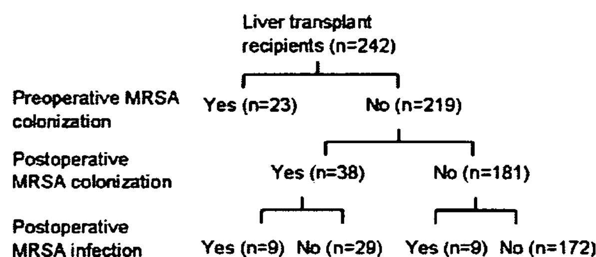

Fig 1. The patient profile of postoperative MRSA colonization and infection. MRSA, methicillin-resistant *S aureus*.

PATIENTS AND METHODS

Patients

We reviewed the 242 patients who underwent LDLT between 1996 and 2004, including 23 colonized with MRSA preoperatively, who were excluded from the study. Of the remaining patients, 119 were men and 100 were women of median age 50 years (range, 19 to 67). The indications included hepatitis C ($n = 62$), followed by primary biliary cirrhosis ($n = 48$) and hepatitis B ($n = 31$). The median Child-Pugh and model for end-stage liver disease (MELD) scores of those patients were 10 (range, 5 to 14) and 13 (range, −3 to 48), respectively. Our donor selection criteria[8] and surgical techniques for recipient and donor operations have been described elsewhere.[9]

Perioperative Management

Antimicrobial prophylaxis consisted of intravenous cefotaxim (1.0 g just before surgery, followed by 1.0 g every 6 hours intraoperatively and thereafter), ampicillin/sulbactam (1.0 g just before surgery, followed by 1.5 g every 12 hours intraoperatively and thereafter), and gentamicin, 60 mg every 12 hours after surgery) for 5 days. Fluconazole (200 mg every 24 hours) was administered intravenously for 7 days after surgery. All patients received the same immunosuppressive regimens using tacrolimus (Prograf, Fujisawa Pharmaceutical Corporation, Tokyo, Japan) and methylprednisolone (Solu-Medrol, Pfizer Inc, New York, NY, USA).[10]

Microbiological Data Collection

All patients were screened preoperatively for *S aureus* after admission for LDLT. Follow-up specimens were collected twice a week during the first month after LDLT and thereafter once a week during the hospital stay. Screened specimens consisted of swabs of the anterior nares, pharynx, sputum, urine, stool, swabs of wound or skin lesions, bile, and abdominal cavity discharge. A catheter or blood sample was also submitted when infection was suspected.

Specimens were plated onto mannitol-salt agar or sheep blood agar. *S aureus* was identified using standard microbiological methods. Methicillin resistance was determined using a disk diffusion test performed on Mueller-Hinton agar after incubation for 24 to 48 hours at 30°C. The strains with an oxacillin minimum inhibitory concentration value of at least 4 μg/mL were defined as MRSA colonization. Patients colonized with *S aureus* at any site and at any time during the hospital stay were considered carriers, and contact precautions were taken in cases with MRSA.

Definition of MRSA Infection

The medical and microbiological records of the patients were reviewed for the occurrence of MRSA infection in the 3 months following LDLT. Only the first MRSA infection was recorded for each patient.

Nosocomial infections were defined according to the reports from the Centers for Disease Control and Prevention in 1988 and in 1992, as described elsewhere.[11] Surgical site infection included superficial incisional, deep incisional, and organ/space infections that occurred within 30 days after surgery. Wound and intra-abdominal cavity infections that occurred more than 1 month after the operation were defined as a gastrointestinal system infection. When an organism isolated from blood culture was compatible with a related nosocomial infection at another site, the bloodstream infection was classified as a secondary bloodstream infection. When MRSA was isolated from culture samples in the presence of nosocomial infection including surgical site infection and other pathogenic organisms were absent, MRSA infection was diagnosed. An MRSA-positive culture sample without the presence of clinical symptoms was diagnosed as MRSA colonization.

Statistical Analysis

Background and clinical data collected for each patient included preoperative, surgical, and postoperative variables. Quantitative variables are presented as medians and ranges. Categorical variables are presented as absolute counts. Univariate analysis was used to identify associations between each of the variables and postoperative MRSA infection. Chi-square test or Fisher exact test was used to compare categorical data.

For multivariate analysis, only variables with a $P < .20$ in the univariate analysis were entered into a logistic regression model by the backward-elimination procedure. The final regression model included covariates associated with a likelihood ratio of $P < .1$. The results of the logistic regression were reported as odds ratios with 95% confidence intervals. A P value of less than .05 was considered statistically significant. All statistical analyses were performed using the JMP5.1 software package (SAS institute Inc, Cary, NC, USA).

RESULTS

Postoperative MRSA Colonization and Infection (Fig 1)

Postoperative MRSA infection occurred in 18 patients among the preoperative noncarriers of MRSA: nine patients were new MRSA carriers postoperatively, and nine

Table 1. Postoperative MRSA Infection in 18 Patients

	Colonized with MRSA ($n = 9$)	Noncarriers with MRSA ($n = 9$)	Total ($n = 18$)
Onset of MRSA infection (postoperative day)	16 (7–54)	40 (9–64)	26 (7–64)
Duration between colonization and infection	13 (2–21)	0	1 (0–21)
During hospitalization infection	9	8	17
SSI	6	3	9
Deep incisonal SSI	6	0	6
Organ/space SSI	0	3	3
Gastrointestinal system infection	2*	4	6
Intra-abdominal infection	2	4	6
Pneumonia	0	1	1
Lower respiratory infection	1	0	1
Primary BSI	0	1	1
Laboratory-confirmed BSI	0	1	1

*One patient had secondary surgical site infection. SSI, surgical site infection; BSI, bloodstream infection.

Table 2. Association Between Postoperative MRSA Infection and Preoperative, Surgical, and Postoperative Variables

Variables	MRSA Infection (−) (*n* = 201)	MRSA Infection (+) (*n* = 18)	*P* Value
Preoperative variables			
Age (y) ≥50	51 (19–67)	48 (24–62)	
	111	8	.46
Gender (male/female)	106/95	13/5	.14
Underlying liver disease			
Hepatitis C	55	7	
Primary biliary cirrhosis	46	2	
Hepatitis B	30	1	
Fulminant hepatitis	19	3	
Biliary atresia	10	1	
Autoimmune hepatitis	8	1	
Primary sclerosing cholangitis	8	1	
Metabolic disease	9	0	
Cryptogenic cirrhosis	6	0	
Alcoholic cirrhosis	2	2	
Others	8	0	
Hepatocellular carcinoma	59	5	1.0
Child-Pugh score	10 (5–14)	11 (5–12)	
≥10	105	13	.14
MELD score	12.9 (−3.4–48.2)	14.6 (4.3–29.4)	
≥15	67	6	1.0
Ascites	95	12	.14
Use of diuretics	109	12	.34
Encephalopathy	32	4	.51
Preoperative apheresis	38	4	.76
PT-INR	1.61 (0.89–7.48)	1.60 (1.23–2.35)	
≥1.7	80	6	.80
Serum bilirubin (mg/dL)	4.1 (0.3–38.6)	7.3 (1.2–32.4)	
>3.0	134	13	.80
Serum albumin (g/dL)	2.9 (1.5–4.4)	2.8 (1.8–3.8)	
>2.8	71	9	.31
Serum creatinine (mg/dL)	0.71 (0.2–7.7)	0.62 (0.4–2.4)	
≥1.5	11	2	.29
Steroid pulse therapy	23	2	1.0
Use of antimicrobials	46	8	.08
Beta lactam	37	7	.06
Glycopeptide	2	0	1.0
Fluroquinolone	13	3	.13
Amynoglycoside	5	1	.41
Others	2	0	1.0
History of abdominal surgery	93	8	1.0
Diabetus mellitus	24	2	1.0
MSSA colonization	100	10	.81
Surgical variables			
Operation time (h)	14.9 (10.7–33.2)	16.3 (12.2–19.3)	
≥16	64	11	.02
Blood loss (mL)	5240 (830–53835)	4415 (2590–34800)	
≥5000	106	8	.62
Blood transfusion (mL)	6970 (900–42890)	6385 (4240–26240)	
≥8000	83	6	.62
GV/SLV ratio (%)	46 (25–88)	42 (36–66)	
≥40	160	15	1.0
Duct to duct biliary reconstruction	144	14	.78
Postoperative variables			
ICU stay (d)	5 (3–46)	5 (4–26)	
≥10	18	4	.09
Apheresis	23	6	.02
Reoperation	72	4	.31
Acute rejection	58	5	1.0

Table 2. (continued)

Variables	MRSA Infection (−) (n = 201)	MRSA Infection (+) (n = 18)	P Value
Cytomegalovirus infection	87	5	.22
Fungal infection	6	1	.46
Colonization with MRSA	29	9	.001

PT-INR, the international normalized ratio of prothrombin time; MSSA, methicillin-susuceptible *S aureus*; MRSA, methicillin-resistant *S aureus*; GV, graft volume; SLV, standard liver volume; ICU, intensive care unit.

patients were MRSA noncarriers until the onset of infection. During the study period, 29 patients were asymptomatic carriers of MRSA. Among the nine patients who were colonized with MRSA postoperatively and subsequently developed infection, the MRSA-colonized sites before the onset of infection were sputum in six, stool in six, nares in five, pharynx in five, urine in two, discharge from an abdominal drain in two, and ascites in one patient.

Details of Postoperative MRSA Infection (Table 1)

The median days to onset of MRSA infection in all the patients with infection, in patients colonized with MRSA before infection, and in patients colonized concurrently with infection were postoperative days 26, 16, and 40, respectively. Among patients who were colonized with MRSA before infection, the median duration between the onset of colonization and infection was 13 days. During the study period, median length of hospital stay after LDLT was 50 (range, 6 to 90) days for patients without MRSA infection and 68 (range, 46 to 90) days for those with MRSA infection. MRSA infection occurred during hospitalization in 17 patients and after discharge in one patient.

Surgical site infection was detected in nine patients. One patient with gastrointestinal system infection had a secondary bloodstream infection. We treated MRSA infection with intravenous vancomycin in 12 patients, reoperation and intravenous vancomycin in two, reoperation alone in two, lavage of the intra-abdominal cavity through the surgical drain in one, and debridement of the wound in one. None of the 18 patients with MRSA infection died during the 3 months after LDLT.

Risk Factors for Postoperative MRSA Infection (Tables 2, 3)

Postoperative MRSA infection was significantly associated with operation time (≥16 hours; P = .02), postoperative apheresis (P = .02), and postoperative colonization with MRSA (P = .001, Table 2). In the multivariate analyses (Table 3), 10 risk factors with P values of less than .20 were entered into a logistic regression model using the backward-elimination procedure. In the final model, operation time (≥16 hours; odds ratio, 3.27) and postoperative colonization with MRSA (odds ratio, 7.13) independently predicted postoperative MRSA infection.

Table 3. Multivariate Analysis of Risk Factors for MRSA Infection After LDLT

Variable	Odds Ratio (95% Confidence Interval)	P Value
Preoperative use of beta lactam	3.03 (0.95–9.37)	.06
Operation time (h) ≥16	3.27 (1.15–9.89)	.03
Colonization with MRSA	7.13 (2.43–21.65)	.0004

MRSA, methicillin-resistant *S aureus*.

DISCUSSION

We have shown the impact of postoperative colonization with MRSA on subsequent MRSA infection after LDLT. Among patients with MRSA infection, 9 of 18 (50%) in the present study were colonized with MRSA before the onset of infection. MRSA infection occurred soon after the operation in patients who were new MRSA carriers postoperatively. Of 18 patients with MRSA infection, 10 developed the infection within 1 month after LDLT, among whom seven were colonized with MRSA before the onset of infection. In addition, patients who were colonized with MRSA developed MRSA infection soon after colonization with MRSA. Of nine patients with MRSA colonization and subsequent infection, all developed infections within 3 weeks after colonization with MRSA.

Postoperative surveillance cultures should be performed at multiple sites, including the nares, after LDLT. Although the anterior nares is the most frequent carriage site for *S aureus*,[12] other extranasal sites such as skin, perineum, pharynx, gastrointestinal tract, vagina, and axillae can also harbor the organism.[5,12] Among nine patients who were colonized with MRSA postoperatively and subsequently developed infection, nasal colonization was detected in 5 (56%). If surveillance culture is performed for only the nares as reported in previous studies in DDLT, new postoperative carriers of MRSA at sites other than the nares[1,6] might be overlooked, thereby delaying the administration of appropriate antimicrobials such as vancomycin in patients suspected of MRSA infection.

The results of the present study indicated that postoperative MRSA colonization and prolonged operative time independently increased the risk of postoperative MRSA infection. MRSA infection is well described in previous studies of DDLT.[1,3–5] Most studies have reported that preoperative MRSA carriage increased the risk of MRSA infection, but these studies[2,3,5] were not focused on the impact of new postoperative MRSA carriage on subsequent infection. Of 38 patients, 9 (24%) who were colonized with MRSA subsequently developed MRSA infection in the present study. This rate is comparable to that of the previous reports [around 30%].[7,13] In one recent retrospec-

tive study, 60 of 209 (29%) patients developed subsequent MRSA infection in the 18-month period after the initial MRSA-positive culture.[13] Postoperative surveillance culture should be performed periodically after LDLT to identify new MRSA carriers who are high-risk candidates for subsequent MRSA infection.

Prolonged operative time increased the risk of MRSA infection in the present study. Prolonged surgical duration indicates technically more difficult surgical procedures in which the risk of complication is increased.[14] George et al[15] used multivariate analysis to demonstrate that prolonged surgery duration increased the risk of bacterial infection among in liver transplant recipients. In contrast, Singh et al[1] reported that there was no such association.

Intense antimicrobial use, measured by the administration of preoperative antimicrobials, during the month before LDLT did not correlate with postoperative MRSA infection among preoperative noncarriers of MRSA in the present study. Although there is little doubt that widespread use of antimicrobials provides multidrug-resistant strains of MRSA with a selective survival advantage,[16] the relationship between MRSA and antimicrobials seemed more complex in the current series. Some studies using multivariate analysis have failed to show such an association.[17] In other studies, exposure to specific antimicrobials, such as third-generation cephalosporins, amoxicillin with clavulanic acid, quinolones, and broad-spectrum antibiotics, increased the risk of MRSA infection or colonization.[18] Crowcroft et al[19] found no association between total antimicrobial use and MRSA colonization or infection, suggesting that the problem was inappropriate rather than excessive use of antimicrobials. This discrepancy is probably due to the fact that in the present study, all patients received multiple antimicrobials, resulting in broad coverage as perioperative prophylaxis, per protocol, and it is difficult to detect the effect of a specific antimicrobial.

One limitation to the present study is that we could not differentiate specific MRSA strains. Pulsed-field gel electrophoresis analysis was not accessible. Therefore, we could not analyze the impact of MRSA transmission, such as patient-to-patient transmission by transient carriage on the hands of the medical staff. Similarly, it was not possible to determine whether infection was due to the same strain as that of the colonization or to a newly acquired strain when the infection occurred. Chang et al[4] analyzed isolates from infected sites and those from the anterior nares in seven patients with MRSA infection, reporting detection of the same isolates. Such a detailed analysis might yield further information to elucidate the relationship between new postoperative MRSA carriage and subsequent infection following LDLT.

REFERENCES

1. Singh N, Paterson DL, Chang FY, et al: Methicillin-resistant Staphylococcus aureus: The other emerging resistant gram-positive coccus among livertransplant recipients. Clin Infect Dis 20:322, 2000

2. Bert F, Bellier C, Lassel L, et al: Risk factors for Staphylococcus aureus infection in liver transplant recipients. Liver Transpl 11:1093, 2005

3. Bert F, Galdbart JO, Zarrouk V, et al: Association between nasal carriage of Staphylococcus aureus and infection in liver transplant recipients. Clin Infect Dis 31:1295, 2000

4. Chang FY, Singh N, Gayowski T, et al: Staphylococcus aureus nasal colonization and association with infections in liver transplant recipients. Transplantation 65:1169, 1998

5. Desai D, Desai N, Nightingale P, et al: Carriage of methicillin-resistant Staphylococcus aureus is associated with an increased risk of infection after liver transplantation. Liver Transpl 9:754, 2003

6. Santro-Lopes G, de Gouvea EF, Monterio RC, et al: Colonization with methicillin-resistant Staphylococcus aureus after liver transplantation. Liver Transpl 11:203, 2005

7. Davis KA, Stewart JJ, Crouch HK, et al: Methicillin-resistant Staphylococcus aureus (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Clin Infect Dis 39:776, 2004

8. Kokudo N, Sugawara Y, Imamura H, et al: Tailoring the type of donor hepatectomy for adult living donor liver transplantation. Am J Transplant 5:1694, 2005

9. Sugawara Y, Makuuchi M: Technical advancement in living-related liver transplantation. J Hepatobiliary Pancreat Surg 16:245, 1999

10. Sugawara Y, Makuuchi M, Kaneko J, et al: Correlation between optimal tacrolimus doses and the graft weight in living donor liver transplantation. Clin Transplant 16:102, 2002

11. Horan TC, Gaynes RP, Martone WJ, et al: CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definition of surgical wound infections. Infect Control Hosp Epidemiol 13:606, 1992

12. Williams RE: Healthy carriage of Staphylococcus aureus: its prevalence and importance. Bacteriol Rev 27:56, 1963

13. Huang SS, Platt R: Risk of methicillin-resistant Staphylococcus aureus infection after previous infection or colonization. Clin Infect Dis 36:281, 2003

14. Arnow PM: Infection following orthotopic liver transplantation. HPB Surg 3:221, 1991

15. George DL, Arnow PM, Fox AS, et al: Bacterial infection as a complication of liver transplantation: epidemiology and risk factors. Rev Infect Dis 13:387, 1991

16. Boyce JM: Understanding and controlling methicillin-resistant Staphylococcus aureus infections. Infect Control Hosp Epidemiol 23:485, 2002

17. Topeli A, Unal S, Akalin HF: Risk factors influencing clinical outcome in Staphylococcus aureus bacteraemia in a Turkish University Hospital. Int J Antimicrob Agents 14:57, 2000

18. Weber SG, Gold HS, Hooper DC, et al: Fluoroquinolones and the risk for methicillin-resistant Staphylococcus aureus in hospitalized patients. Emerg Infect Dis 9:1415, 2003

19. Crowcroft NS, Ronveaux O, Monnet DL, et al: Methicillin-resistant Staphylococcus aureus and antimicrobial use in Belgian hospitals. Infect Control Hosp Epidemiol 20:31, 1999

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Safety and Efficacy of Autologous Progenitor Cell Transplantation for Therapeutic Angiogenesis in Patients With Critical Limb Ischemia

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Background Therapeutic angiogenesis using cell transplantation (TACT) is a treatment strategy for no-option patients with critical limb ischemia (CLI). However, because one-third of treated patients fail to respond, the present study was an exploration of the characteristics of responders and non-responders to this treatment regimen.
Methods and Results Seven CLI patients (3 with Buerger's disease, 4 with arteriosclerosis obliterans undergoing chronic hemodialysis (ASO-HD)) were treated according to the TACT protocol (n=6: bone marrow-mononuclear cells (MNCs); n=1: peripheral blood-MNCs). Subjective symptoms (visual analog scale) and objective findings (extent of ulcer, ankle–brachial pressure index, transcutaneous oxygen pressure, thermography and angiography) were assessed. Numbers of transplanted $CD34^+$, $CD133^+$ and $CD34^+CD133^+$ cells were counted. Changes in circulating $CD34^+$ and $CD133^+$ cell numbers were also examined before and after the treatment. All responders (n=3) had Buerger's disease, and ASO-HD patients did not respond well. Among the responders, the numbers of circulating $CD34^+$ and $CD133^+$ cells persistently increased for 1 month after the treatment, but not in non-responders.
Conclusions The TACT regimen improved CLI in patients with Buerger's disease but not in those with ASO-HD in this small study. In responders, post procedural circulating $CD34^+$ and $CD133^+$ cells persistently increased for 1 month (ClinicalTrials.gov Identifier: NCT00145262, TACT-NAGOYA). (*Circ J* 2007; **71:** 196–201)

Key Words: Angiogenesis; Bone marrow; Limb ischemia; Progenitor cells

In the last decade there has been rapid development of therapeutic angiogenesis, using angiogenic cytokines, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor, and bone marrow (BM)-mononuclear cells (MNCs) in basic and clinical investigations.[1–8]

We previously demonstrated that therapeutic angiogenesis using cell transplantation (TACT) is effective for no-option patients with critical limb ischemia (CLI).[7,9] However, one-third of treated patients did not respond well, presumably because of differences in underlying diseases, age and other clinical background factors. Impaired BM cell function and/or a decreased number of circulating endothelial progenitor cells (EPC) may also cause the unresponsiveness. Indeed, several studies have indicated that the number and/or function of circulating EPC was impaired in patients with coronary risk factors and in those with CLI,[10–13] and thus EPC in non-responders might be impaired, resulting in unresponsiveness to this therapeutic regimen.

Accordingly, we examined patient characteristics, clinical background, and the change in the number of $CD34^+$, $CD133^+$ progenitor cell levels in the studied subjects in terms of responsiveness to the TACT protocol. Furthermore, the number of transplanted MNCs, $CD34^+$, $CD133^+$ progenitor cells and post procedural changes in circulating progenitor cell levels were compared in relation to responsiveness.

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Methods

Patients

Patients were enrolled for TACT if they had severe CLI, including rest pain, non-healing ischemic skin ulcers or both, and were not candidates for conventional surgical or non-surgical revascularization. Patients with poorly controlled diabetes mellitus (DM) (hemoglobin A1c >6.5% or proliferative retinopathy), or with evidence of malignant disorder(s) during the past 5 years were excluded. Hypertension (HT), hyperlipidemia and DM were diagnosed when patients were currently receiving treatment for any of these or had been diagnosed according to the criteria of the Japanese Society of Hypertension (2000), Japanese Atherosclerosis Society (2002) and Japan Diabetes Society (1999), respectively. Written informed consent was given by each patient. The protocol was approved by the Ethics Committee of Nagoya University School of Medicine. In this study,

7 consecutive patients were enrolled from December 2002 to March 2006.

Procedures

In 6 patients, approximately 500 ml of autologous BM was aspirated from the ilium under general anesthesia, and collected into plastic bags containing heparin. In 1 patient, peripheral blood (PB)-MNCs were collected by cell apheresis. We sorted BM- or PB-MNCs using a blood-cell separator (Baxter, Deerfield, IL, USA) to 95% purity and concentrated them into a final volume of 30 ml within 2 h after aspiration. In 6 patients we implanted MNCs approximately 2 h after BM or PB aspiration by direct intramuscular injection into the gastrocnemius of the ischemic legs (4.0×10^6 to 7.0×10^7 cells) and in the remaining patient, we implanted BM-MNCs not only into the gastrocnemius but also into the quadriceps femoris of the ischemic leg, because blood perfusion was significantly reduced at knee level. We implanted approximately 0.75 ml of BM-MNC or PB-MNC suspension into each injection site using a 26-gauge injection needle at 2.5×2.5 cm grid intersect as injection site markers.

Assessment of Limb Ischemia

Safety and efficacy of the treatment, defined as rest pain scale (visual analog scale: VAS), ankle–brachial blood pressure index (ABI) and transcutaneous oxygen pressure (TcO_2) were reconfirmed. We measured ABI and TcO_2 before, 1 day and 1, 2, 4 and 24 weeks after the TACT therapy. We used the criteria of Rutherford et al to assess limb status.[14] To measure ABI, we established Doppler-derived arterial segmental pressures on the ankle and brachium with a standard adult cuff, and indexed ankle systolic blood pressure against brachial systolic blood pressure (normal range >1.0). We judged an increase in ABI of more than 0.1 as an improvement, according to the standard assessment of interventional therapy for peripheral artery disease.[14] We measured TcO_2 using an oxygen monitor (TCM4, Radiometer, Copenhagen, Denmark). After cleansing the measurement site with ethanol, we applied the probe, and heated the skin surface to 43.5°C. When a steady-state temperature was achieved, a value expressed in mmHg was recorded (normal range >60 mmHg). These measurements were performed while patients were supine. TcO_2 was measured repeatedly, and the first measurement was performed when patients were breathing room air, and the second when they were given oxygen (6 L/min) for 5 min. We also assessed subjective symptoms by rest pain scale score [VAS: rated as 11 grades from 0 (pain free) to 10 (maximum pain)] before, 1 day and 1, 2, 4 and 24 weeks after the TACT therapy. Before and 4 weeks after the treatment we performed angiography in which the absolute amount of contrast medium, speed of contrast injection, and the position of the catheter tip were strictly fixed. Angiography and thermography were performed before and 4 weeks after the TACT therapy. Angiographically, we assessed new collateral vessel formation as 0 (no collateral development), +1 (slight), +2 (moderate), or +3 (rich). New collateral vessel formation was assessed by 2 interventional radiologists who were unaware of the treatment regimen.

Progenitor Cell Assay

Fluorescence-activated cell sorting (FACS) analysis revealed that circulating EPC expressed cell surface antigens such as CD34, CD133 and VEGF receptor 2 (VEGFR2).[15]

Table 1 Patient Characteristics

Case no.	Age (years)	Gender	Diagnosis	Ischemic site/ status	ABI	Previous treatment for CLI	Past history
1	37	F	Buerger's disease	Lt. toe (I)/non-healing ulcer	1.09	Medication, sympathectomy	Hyperlipidemia, smoking
2	57	M	ASO	Lt. toe (III)/non-healing ulcer	0.56	Medication	20yo: gout, 43yo: CRF on HD (gout kidney), 45yo: ASO (both legs), 46yo: AMI (stent), smoking
3	58	M	ASO	Rt. foot/rest pain	0.74	Bypass graft, PTA, amputation of rt. finger and lt. foot	33yo: DM, 40yo: CRF on HD (diabetic nephropathy), 50yo: AP (55yo CABG), 51yo: ASO (bil. F-P bypass, 55yo PTA for bil. CIA, 58yo amputation)
4	67	M	ASO	Rt. foot/rest pain	0.29	Medication	52yo: CRF on HD (CGN), 65yo: ASO
5	51	M	Buerger's disease	Rt. toe (I)/rest pain	0.77	Medication, sympathectomy	44yo: DM, 46yo: Buerger's disease (sympathectomy), hyperlipidemia, smoking
6	57	M	ASO	Lt. toe (I, II, V)/non-healing ulcer	0.20	Medication, amputation of rt. lower leg	40yo: CRF on HD (CGN), hypertension, 53yo: AS (AVR), 56yo: ASO (57yo amputation), 57yo: renal artery aneurysm rupture (embolization)
7	47	F	Buerger's disease	Lt. foot/rest pain	0.00	Medication, sympathectomy	Smoking

ABI, ankle-brachial pressure index; CLI, clitical limb ischemia; lt., left; ASO, arteriosclerosis obliterans; yo, year old; CRF, chronic renal failure; HD, hemodialysis; AMI, acute myocardial infarction; rt., right; PTA, percutaneous transluminal angioplasty; DM, diabetes mellitus; AP, angina pectoris; CABG, coronary artery bypass graft; bil., bilateral; F-P bypass, femoro-popliteal bypass graft; CIA, common iliac artery; CGN, chronic glomerulonephritis; AS, aortic stenosis; AVR, aortic valve replacement.

Table 2 Number of Implanted Cell

Case no.	*Implant site*	*Total MNC*	*CD34⁺ cells*	*CD133⁺ cells*	*CD34⁺ CD133⁺ double positive cells*
1	*Lt. gastrocnemius*	*3.7×10⁹*	*3.0×10⁷*	*2.1×10⁷*	*2.1×10⁷*
2	*Lt. gastrocnemius*	*1.2×10⁹*	*1.7×10⁷*	*0.62×10⁷*	*0.62×10⁷*
3	*Rt. gastrocnemius*	*10.0×10⁹*	*0.4×10⁷*	*0.38×10⁷*	*0.35×10⁷*
4	*Rt. gastrocnemius*	*2.8×10⁹*	*3.3×10⁷*	*3.0×10⁷*	*3.0×10⁷*
5	*Rt. gastrocnemius*	*4.36×10⁹*	*7.0×10⁷*	*5.1×10⁷*	*5.1×10⁷*
6	*Lt. gastrocnemius*	*1.6×10⁹*	*2.1×10⁷*	*1.6×10⁷*	*1.6×10⁷*
7	*Lt. gastrocnemius and quadriceps femoris*	*9.0×10⁹*	*1.9×10⁷*	*1.5×10⁷*	*1.5×10⁷*

MNC, mononuclear cells. See Table 1 for other abbreviations.

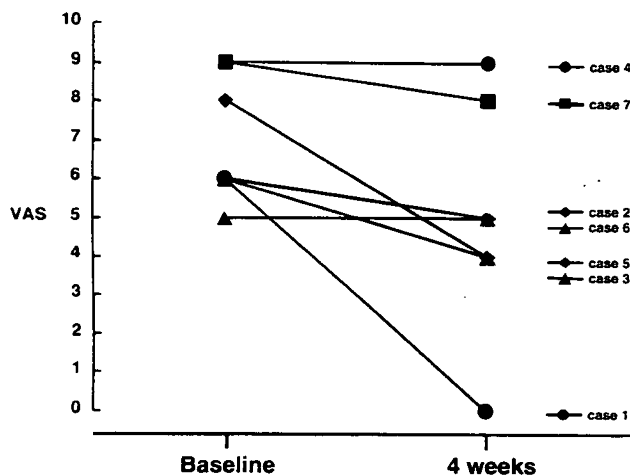

Fig 1. Subjective symptoms as assessed by visual analog scale (VAS) improved in 5 patients (well improved in 2, mildly improved in 3). One patient was completely relieved of rest pain.

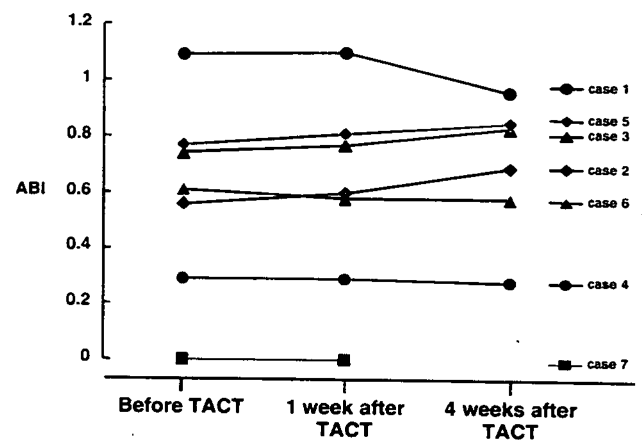

Fig 2. An increase in ankle–brachial pressure index (ABI) of more than 0.1 was not observed in any case. TACT, therapeutic angiogenesis with cell transplantation.

Thus, we counted the numbers of transplanted total MNCs, $CD34^+$, $CD133^+$ and $CD34^+CD133^+$ double positive cells by FACS analysis, and calculated the numbers according to the following equation: the number of transplanted ($CD34^+$, $CD133^+$ and $CD34^+CD133^+$) cells = the number of total MNCs × percentage of ($CD34^+$, $CD133^+$ and $CD34^+CD133^+$) cells measured by FACS analysis.

We also counted those numbers in PB at 1 day, 3 days and 1, 2 and 4 weeks after the treatment according to the following equation: the numbers of ($CD34^+$, $CD133^+$ and $CD34^+CD133^+$) cells = white blood cell count × percentage of ($CD34^+$, $CD133^+$ and $CD34^+CD133^+$) cells measured by FACS analysis.

Statistical Analysis

All statistical analyses were conducted using StatView for Windows version 5.0 (SAS Institute Inc, Cary, NC, USA). All data are expressed as mean ± SEM. Significant differences between responders and non-responders were assessed by unpaired t-test. The value of $p<0.05$ was considered statistically significant. The number of circulating $CD34^+$ and $CD133^+$ cells was each translated into a logarithm, and the changes in the numbers of circulating $CD34^+$ and $CD133^+$ cells were compared between responders and non-responders by repeated measure ANOVA. The value of $p<0.05$ was considered statistically significant.

Results and Discussion

In this preliminary report, we treated 7 patients with CLI with cell transplantation according to the TACT protocol.[7] The clinical profiles of the 7 patients are shown in Table 1: 3 patients had Buerger's disease and the other 4 had arteriosclerosis obliterans undergoing chronic hemodialysis (ASO-HD) (1 diabetic, 3 non-diabetic patients). All patients were treated safely. Among the 4 ASO-HD patients, 2 had a previous history of amputation. The observation period ranged from 5 to 39 months (mean 23.7 months).

Six patients received autologous BM-MNC transplantation (Table 2), and the remaining patient (case 3) received PB-MNCs under intravenous anesthesia because a high risk was expected with general anesthesia. In 1 patient (case 7), we transplanted BM-MNCs not only into the gastrocnemius but also into the quadriceps femoris of the ischemic leg because blood perfusion was significantly reduced even above the knee.

In the early phase until 4 weeks after treatment, subjective symptoms assessed by VAS were improved in the 3 Buerger's disease patients and 2 of the ASO-HD patients (Fig 1). Objective findings did not necessarily parallel the improvement on the VAS (Table 3). Among the 5 patients with VAS improvement, the objective findings were improved in 3 (Fig 2, Table 3). In the present study, we defined responders as those who elicited improvements in both subjective symptoms and objective findings. Thus, 3 of 7 patients were overall responders.

In the acute phase, all 3 responders had Buerger's disease and all 4 non-responders had ASO-HD. In the chronic phase, of the 3 acute phase responders, 1 (case 1) had complete healing of an ischemic ulcer and maintained the improved

Table 3 Outcome at 4 Weeks

Case no.	*Symptoms*	*ABI*	*TcO2*	*Thermography*	*Angiography*
1	*Well improved*	*NC*	*Improved*	*NC*	*Improved*
2	*Mildly improved*	*NC*	*NC*	*NC*	*NC*
3	*Mildly improved*	*NC*	*NC*	*NC*	*NC*
4	*NC*	*NC*	*NC*	*NC*	*NA*
5	*Well improved*	*NC*	*NC*	*Improved*	*NA*
6	*NC*	*NC*	*Improved*	*NC*	*NA*
*7**	*Mildly improved*	*NC*	*Improved*	*Improved*	*NA*

**Two weeks outcome.*
NC, no change; NA, no assessment. See Table 1 for other abbreviations.

status, 1 (case 5) relapsed with rest pain 6 months after cell transplantation, and 1 (case 7) underwent below-knee amputation at postoperative day 16, although above-knee amputation had been thought to be inevitable (Table4) because preoperative angiography had shown almost avascularity at thigh level. Therefore, BM-MNCs were injected into not only the gastrocnemius, but also the quadriceps femoris, and the TcO_2 below knee level had increased 2 weeks after cell transplantation (from 45 to 59mmHg), and ultimately above knee amputation was avoided. For amputees salvage of the distal joints is important for quality of life, so we judged cell transplantation to be effective in this case. Thus, TACT seems to be safe and effective for patients with Buerger's disease.

Among the 4 non-responders (all ASO-HD patients), 3 underwent toes or below-knee amputation and 1 (case 3) died suddenly 5 months after cell transplantation (Table4). Considering the subject had high risk factors for systemic arteriosclerosis (past history including diabetic nephropathy, hemodialysis, coronary artery bypass graft, bypass graft and percutaneous transluminal angioplasty for ASO and previous amputation) and the situation at death, despite the lack of autopsy we consider that the cause of death was a cardiovascular event, such as ventricular tachyarrhythmia, acute myocardial infarction or stroke. This case of sudden death had little relation to the cell transplantation, because the TACT had no effect on therapeutic angiogenesis and seemed not to have any adverse effects on atherosclerosis progression or plaque. Therefore, TACT was less effective for end-stage ASO patients undergoing chronic hemodialysis. In a previous study, some ASO patients responded well to TACT[7] and there are several reasons why the TACT protocol was not so effective in the present patients with ASO-HD. First, their limb status was very poor; for example, there were 2 cases of previous limb amputation. Second, all ASO-HD patients were on relatively long-term hemodialysis (13, 15, 17 and 19 years, respectively). It is well known that patients on chronic hemodialysis have reduced endothelial function,[16] reduced endothelium-derived nitric oxide (EDNO) release,[17] have severe atherosclerotic lesions systemically,[18] and thus have impaired angiogenic capability. We previously showed that EDNO is important for ischemia-induced angiogenesis,[19] and this process might be severely impaired in patients with ASO-HD. Additionally, a recent study showed that number and/or function of circulating EPC is markedly reduced in patients with various coronary risk factors,[10,11] ischemic heart disease[20] and chronic kidney disease (CKD).[12]

Table 4 Long-Term Outcome

Case no.	*Observation period (months)*	*Outcome*
1	*39*	*Complete healing of ulcer*
2	*36*	*Toes amputation (5 weeks & 9 months)*
3	*5*	*Sudden death (5 months)*
4	*25*	*Below-knee amputation (3 months)*
5	*25*	*Pain relapse*
6	*20*	*Below-knee amputation (6 weeks)*
7	*16*	*Below knee amputation (16 days)*

Progenitor Cells in BM and Circulating Progenitor Cells

BM-derived EPCs can be incorporated into neovascularization, a process known as vasculogenesis.[2,21] Therefore, it

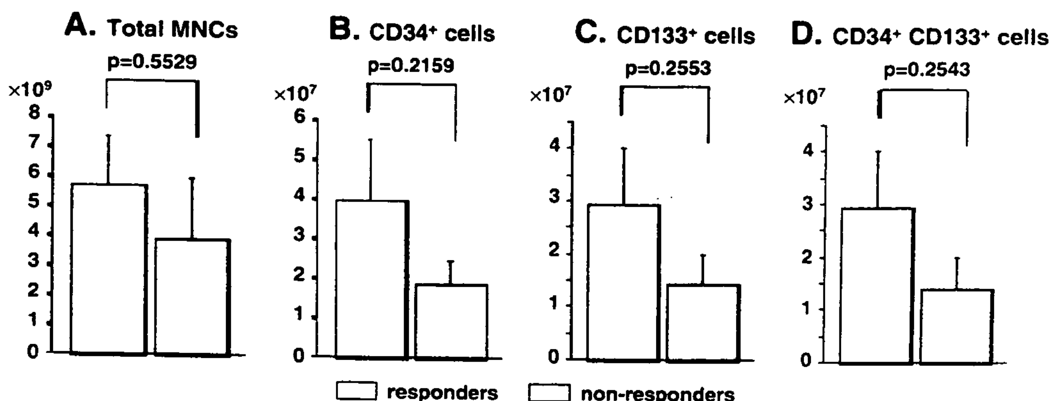

Fig3. Numbers of transplanted total mononuclear cells (MNCs) (A), CD34+ cells (B), CD133+ cells (C) and CD34+CD133+ cells (D) tended to be greater in responders than in non-responders. However, there was no statistically significant difference between the 2 groups.

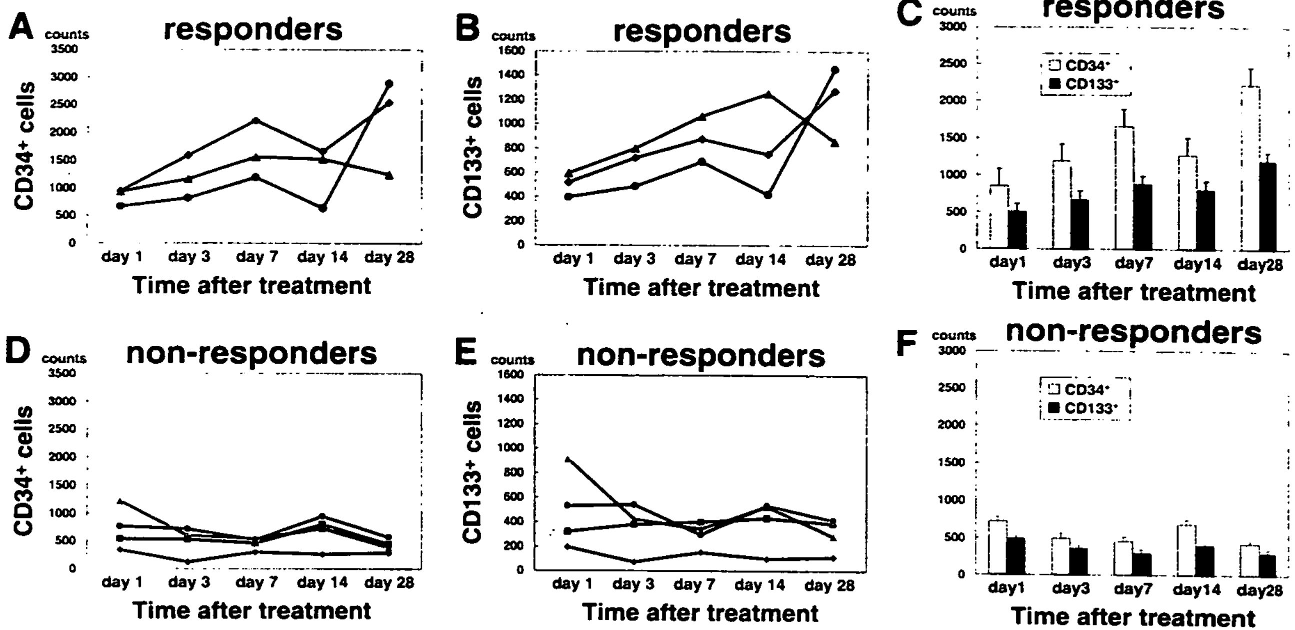

Fig 4. Numbers of circulating CD34+ cells (A) and circulating CD133+ cells (B) increased continuously in responders (C), whereas in non-responders there was no increase in these cells (D–F).

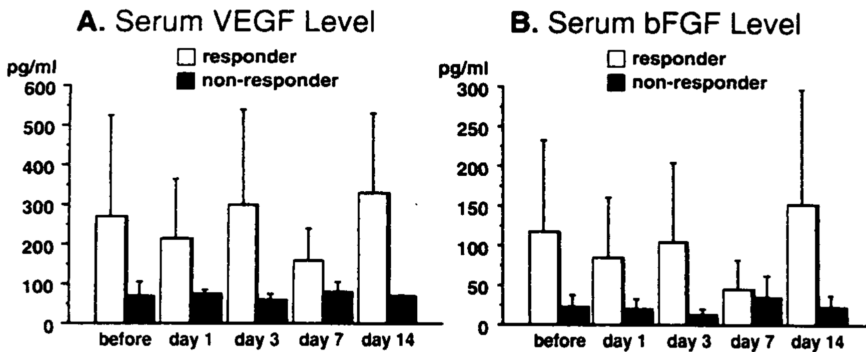

Fig 5. Serum vascular endothelial growth factor (VEGF) levels and serum basic fibroblast growth factor (bFGF) levels at each time point were measured in 5 patients (cases 3–7). The changes in the levels of these cytokines did not differ statistically between the responders and non-responders.

can be expected that the transplantation of a larger number of BM-MNC or progenitor cells would be more efficacious. Although the numbers of transplanted MNCs, CD34+, CD133+ and CD34+CD133+ cells were not significantly different between responders and non-responders in the present study, they tended to be greater in responders than non-responders (Table 2, Fig 3). Among subjects with BM-MNC transplantation, the number of transplanted MNCs tended to be larger in responders than in non-responders (5.687±1.668 vs 1.867±0.481, $p=0.09$). However, the differences in the number of transplanted CD34+, CD133+ and CD34+CD133+ cells between the 2 groups was not so great (3.967±1.550 vs 2.367±0.481, $p=0.38$, 2.900±1.114 vs 1.740±0.691, $p=0.43$, 2.900±1.114 vs 1.740±0.691, $p=0.43$, for CD34+, CD133+ and CD34+CD133+ cells, respectively). Therefore, the clinical efficacy of this procedure might depend on the number of transplanted MNCs rather than on the number of transplanted progenitor cell.

However, the number of circulating CD34+ and CD133+ cells increased continuously after cell transplantation in responders but not in non-responders [$p=0.005$ (CD34+ cells) and $p=0.014$ (CD133+ cells), responders vs non-responders] (Fig 4). Therefore, the postprocedural increase in circulating progenitor cell levels may indicate more powerful mobilization of progenitor cells from the BM in responders than in non-responders. Several mechanisms account for the relation between the increase in EPC level and clinical course. First, transplanted BM-MNCs secrete various angiogenic cytokines, such as VEGF, basic fibroblast growth factor (Fig 5) and angiopoietin-1, at the injection sites.[7,22,23] BM-MNC transplantation might cause further postprocedural mobilization of progenitor cells via secretion of these cytokines. Second, the number of circulating EPC is reduced in patients with risk factors for atherosclerosis, such as HT, DM, smoking, aging, and in patients with CKD.[10–13,24] Transplantation of BM physically helps progenitor cells mobilize in ischemic tissue. In this study, all non-responders had ASO-HD but because the sample size was only 3, the efficacy and feasibility of the present TACT protocol for ASO-HD subjects must be re-evaluated in a larger sample.

Passuer et al reported that patients on long-term hemodialysis showed reduced numbers of circulating CD34+ and CD34+CD133+ cells, impaired migratory activity and EPC adhesion compared with control subjects, although the blood VEGF level was significantly elevated in the dialysis

patients.[25] Taken together, augmentation of EPC mobilization from the BM might be impaired in patients with ASO-HD, probably because of reduced BM function.

Study Limitations

First, because the number of patients in this study is very small, larger scale clinical trials are needed. Second, mechanisms for the unresponsiveness of ASO-HD cases to this protocol need to be further clarified (eg, involvement of uremic toxin, impaired production of erythropoietin, etc). In addition, the mechanisms of the differential time course of the number of circulating EPCs according to responsiveness and the type of underlying disease (eg, Buerger's disease or ASO-HD) should be further investigated. We could not determine whether that the different time course of the progenitor cell levels in the subjects reflects responsiveness or disease condition or both.

Conclusions

TACT was performed safely, and the procedure per se was feasible in patients with CLI. Favorable efficacy occurred for patients with Buerger's disease but not in those with ASO-HD. The responders showed a continuous increase in the number of circulating progenitor cells after TACT and this unique feature may be related to the clinical efficacy of the procedure. The safety and efficacy of autologous progenitor cell transplantation were again reconfirmed in this study.

Acknowledgments

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References

1. Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, et al. Therapeutic angiogenesis: A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. *J Clin Invest* 1994; **93:** 662–670.
2. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275:** 964–967.
3. Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease: Part I: Angiogenic cytokines. *Circulation* 2004; **109:** 2487–2491.
4. Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease: Part II: Cell-based therapies. *Circulation* 2004; **109:** 2692–2697.
5. Isner JM, Baumgartner I, Rauh G, Schainfeld R, Blair R, Manor O, et al. Treatment of thromboangiitis obliterans (Buerger's disease) by intramuscular gene transfer of vascular endothelial growth factor: Preliminary clinical results. *J Vasc Surg* 1998; **28:** 964–973; discussion 973–975.
6. Morishita R. Recent progress in gene therapy for cardiovascular disease. *Circ J* 2002; **66:** 1077–1086.
7. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomized controlled trial. *Lancet* 2002; **360:** 427–435.
8. Saigawa T, Kato K, Ozawa T, Toba K, Makiyama Y, Minagawa S, et al. Clinical application of bone marrow implantation in patients with arteriosclerosis obliterans, and the association between efficacy and the number of implanted bone marrow cells. *Circ J* 2004; **68:** 1189–1193.
9. Yamamoto K, Kondo T, Suzuki S, Izawa H, Kobayashi M, Emi N, et al. Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: Therapeutic effect by stem cell transplantation. *Arterioscler Thromb Vasc Biol* 2004; **24:** e192–e196.
10. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; **348:** 593–600.
11. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001; **89:** E1–E7.
12. Choi JH, Kim KL, Huh W, Kim B, Byun J, Suh W, et al. Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 2004; **24:** 1246–1252.
13. Kondo T, Hayashi M, Takeshita K, Numaguchi Y, Kobayashi K, Iino S, et al. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 2004; **24:** 1442–1447.
14. Rutherford RB, Flanigan DP, Gupta SK, Johnston KW, Karmody A, Whittemore AD, et al. Suggested standards for reports dealing with lower extremity ischemia. *J Vasc Surg* 1986; **4:** 80–94.
15. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000; **95:** 952–958.
16. Joannides R, Bakkali EH, Le Roy F, Rivault O, Godin M, Moore N, et al. Altered flow-dependent vasodilatation of conduit arteries in maintenance haemodialysis. *Nephrol Dial Transplant* 1997; **12:** 2623–2628.
17. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992; **339:** 572–575.
18. O'Hare A, Johansen K. Lower-extremity peripheral arterial disease among patients with end-stage renal disease. *J Am Soc Nephrol* 2001; **12:** 2838–2847.
19. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, et al. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 1998; **101:** 2567–2578.
20. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004; **109:** 1615–1622.
21. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999; **85:** 221–228.
22. Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. *Circulation* 2001; **103:** 897–903.
23. Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001; **104:** 1046–1052.
24. Heiss C, Keymel S, Niesler U, Ziemann J, Kelm M, Kalka C. Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005; **45:** 1441–1448.
25. Herbrig K, Pistrosch F, Oelschlaegel U, Wichmann G, Wagner A, Foerster S, et al. Increased total number but impaired migratory activity and adhesion of endothelial progenitor cells in patients on long-term hemodialysis. *Am J Kidney Dis* 2004; **44:** 840–849.

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Letter to the Editors-in-Chief

Pitavastatin attenuates the upregulation of tissue factor in restraint-stressed mice

To the Editor:

Hypercoagulability and thrombotic tendency are frequently induced by a variety of stressors. Indeed, the presence of psychosocial stressors has been associated with increased risk of acute myocardial infarction [1]. The restraint stress model often has been used to investigate the stress response experimentally in terms of pharmacologic, physiologic, or pathologic phenomena in vivo [2]. Tissue factor (TF) is a key procoagulant gene because it is the primary cellular initiator of the coagulation protease cascade and serves as a specific cofactor for plasma factors VII/VIIa [3]. We previously reported that a restraint (immobilization) stress, a typical physicopsychological stress, to mice induced the TF gene expression in several tissues, including kidneys and adipose tissues [4]. Statins, 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors, have been broadly used for the prevention from cardiovascular diseases primarily with their lowering serum cholesterol levels. Statins also exert pleiotropic and beneficial effects on coagulation system, which are regarded to be independent of cholesterol lowering action. In particular, statins have been shown to reduce the TF expression in lipopolysaccharide-stimulated macrophages and smooth muscle cells in vitro, and in carotid lesions of cholesterol-fed rabbits in vivo [5,6].

We have observed that pitavastatin attenuated the upregulation of TF gene in restraint-stressed mice. Eight-week-old male C57BL/6J mice were administrated with 10 mg/kg/day of pitavastatin or atorvastatin for 3 weeks before the animals received restraint stress. Restraint stress, RNA extraction and RT-PCR assay were performed, as described previously [4]. All procedures were carried out according to the protocol approved by the Animal Care and Use Committee of Nagoya University. Twenty hours of restraint stress to mice caused a substantial induction of TF mRNA in the kidney and adipose tissues [4], which has been regarded to be a major source of TF [7]. Pitavastatin attenuated the induction of TF mRNA by stress in these tissues in about 50% of the control (i.e., statin free) mice, while atorvastatin did not (Fig. 1). As plasma cholesterol levels were not affected by statins in these mice (not shown), pitavastatin could suppress the upregulation of TF gene independently of cholesterol lowering action in restraint-stressed

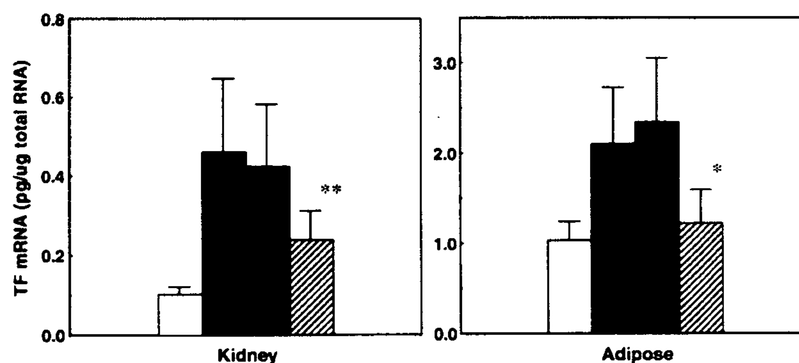

Figure 1 Eight-week-old mice had been administered with pitavastatin (10 mg/kg/day) or atorvastatin (10 mg/kg/day) for 3 weeks ($n=6$, respectively), followed by 20-h restraint stress. As a control group, we prepared non-stressed mice and 20-h-stressed mice without statin therapy ($n=6$, respectively). Kidneys and adipose tissues were harvested and analyzed for TF mRNA by quantitative RT-PCR. White bars: no restraint stress; black bars: only 20-h restraint stress; dark gray bars: pre-treatment with atorvastatin followed by 20-h restraint stress; hatched bars: pre-treatment with pitavastatin followed by 20-h restraint stress. *$p<0.05$; **$p<0.04$.

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mice. The inhibition of HMG-CoA reductase by statins leads to the decreased synthesis of cholesterol and its associated precursors, which are isoprenoid products (e.g., geranylgeranylpyrophosphates) from mevalonate. It has also been reported that statins reduces the TF expression by suppressing the formation of a geranylgeranylated proteins required for the proper synthesis of TF [8]. Thus, pitavastatin could attenuate the TF induction in restraint-stressed mice through the inhibition of geranylgeranylated protein synthesis.

Several differences are observed in pleiotropic effects between statins. Although there have been some reports on the inhibitory effect of atorvastatin on the TF expression in vitro or ex vivo [9,10], its suppressive effect in vivo is still controversial [11,12]. Restraint stress induces inflammatory cytokines (e.g., TNF-α) [4] and oxidative stress markers (e.g., 4-hydroxynonenal and 8-hydroxy-2′-deoxyguanosine) [13], both of which could upregulate TF gene expression in vivo [4,14]. In this context, statins inhibit TNF-α-induced nuclear factor κB (NF-κB) activation [15], which stimulates the TF expression, although they differ in their ability to block NF-κB activation [16]. Pitavastatin could strongly suppress the molecular responses against stress insults, which include the induction of cytokine-induced NF-κB and the production of oxidative stress markers in the ischemic model in vivo in comparison with atorvastatin [17]. Taken together, pitavastatin would attenuate the TF expression induced by stress through the inhibition of TNF-α-induced NF-κB activation (i.e., anti-inflammatory) and its antioxidative effect. The finding in this study suggests that pitavastatin contributes to the prevention from thrombotic cardiovascular diseases associated with physicopsychological stress although additional studies to elucidate its mechanism are required.

References

[1] Rosengren A, Hawken S, Ôunpuu S, Sliwa K, Zubaid M, Almahmeed WA, et al. Association of psychosocial risk factors with risk of acute myocardial infarction in 11119 cases and 13648 controls from 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;**364**:953-62.
[2] Glavin GB, Pare WP, Sandbak T, Bakke HK, Murison R. Restraint stress in biomedical research: an update. *Neurosci Biobehav Rev* 1994;**18**:223-49.
[3] Edgington TS, Mackman N, Brand K, Ruf W. The structural biology of expression and function of tissue factor. *Thromb Haemost* 1991;**66**:67-79.
[4] Yamamoto K, Shimokawa T, Yi H, Isobe K, Kojima T, Loskutoff DJ, et al. Aging and obesity augment the stress-induced expression of tissue factor gene in the mouse. *Blood* 2002;**100**:4011-8.
[5] Markle RA, Han J, Summers BD, Yokoyama T, Hajjar KA, Hajjar DP, et al. Pitavastatin alters the expression of thrombotic and fibrinolytic proteins in human vascular cells. *J Cell Biochem* 2003;**90**:23-32.
[6] Baetta R, Camera M, Comparato C, Altana C, Ezekowitz MD, Tremoli E. Fluvastatin reduces tissue factor expression and macrophage accumulation in carotid lesions of cholesterol-fed rabbits in the absence of lipid lowering. *Arterioscler Thromb Vasc Biol* 2002;**22**:692-8.
[7] Samad F, Pandey M, Loskutoff DJ. Regulation of tissue factor gene expression in obesity. *Blood* 2001;**98**:3353-8.
[8] Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol* 1997;**17**:265-72.
[9] Bruni F, Puccetti L, Pasqui AL, Pastorelli M, Bova G, Cercignani M, et al. Different effect induced by treatment with several statins on monocyte tissue factor expression in hypercholesterolemic subjects. *Clin Exp Med* 2003;**3**:45-53.
[10] Krysiak R, Okopien B, Herman Z. Effects of HMG-CoA reductase inhibitors on coagulation and fibrinolysis processes. *Drugs* 2003;**63**:1821-54.
[11] Olivotti L, Ghigliotti G, Spallarossa P, Leslie S, Rossettin P, Barsotti A, et al. High doses of atorvastatin do not affect activity of prothrombinase in patients with acute coronary syndromes. *Blood Coagul Fibrinolysis* 2002;**13**:315-22.
[12] Bartok A, Steiner S, Seidinger D, Jetzl A, Huber K, Minar E, et al. Atorvastatin reduces thrombin generation after percutaneous coronary intervention independent of soluble tissue factor. *Thromb Res* 2005;**115**:469-74.
[13] Zaidi SMKR, Al-Qirim TM, Hoda N, Banu N. Modulation of restraint stress induced oxidative changes in rats by antioxidant vitamins. *J Nutr Biochem* 2003;**14**:633-6.
[14] Gorlach A, Brandes RP, Bassus S, Kronemann N, Kirchmaier CM, Busse R, et al. Oxidative stress and expression of p22phox are involved in the up-regulation of tissue factor in vascular smooth muscle cells in response to activated platelets. *FASEB J* 2000;**14**:1518-28.
[15] Holschermann H, Schuster D, Parviz B, Haberbosch W, Tillmanns H, Muth H. Statins prevent NF-kappaB transactivation independently of the IKK-pathway in human endothelial cells. *Atherosclerosis* 2006;**185**:240-5.
[16] Hilgendorff A, Muth H, Parviz B, Staubitz A, Haberbosch W, Tillmanns H, et al. Statins differ in their ability to block NF-kappaB activation in human blood monocytes. *Int J Clin Pharmacol Ther* 2003;**41**:397-401.
[17] Hayashi T, Hamakawa K, Nagotani S, Jin G, Li F, Deguchi K, et al. HMG CoA reductase inhibitors reduce ischemic brain injury of Wistar rats through decreasing oxidative stress on neurons. *Brain Res* 2005;**1037**:52-8.

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Intravenous Immunoglobulin Therapy for Acquired Coagulation Inhibitors: A Critical Review

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Abstract

Intravenous immunoglobulin (IVIG) therapy has been used for autoimmune diseases and disorders involving autoantibodies, including coagulation inhibitors. In this review, we have evaluated the efficacy and safety of IVIG therapy for acquired coagulation inhibitors, including factor VIII inhibitor, and for acquired von Willebrand syndrome on the basis of 44 reports published between 1965 and 2005. Among 35 patients with factor VIII inhibitor, we estimated the efficacy of IVIG therapy alone (which includes complete remissions and partial responses with a clinical benefit) to be 30% (11 cases), whereas the response to combination therapy with IVIG plus immunosuppressive agents (eg, corticosteroid, cyclophosphamide) seemed to be better (approximately 70%, 33/45 cases) than with IVIG therapy alone. In acquired von Willebrand syndrome, the efficacy of IVIG therapy was estimated to be 30%. The response to IVIG therapy appears to occur rapidly, and coagulation inhibitors seem to be neutralized immediately. Moreover, severe complications or side effects rarely occur during IVIG treatment. IVIG therapy thus may be considered one choice for treating acquired coagulation inhibitors, although its efficacy improves when used in combination with immunosuppressive agents.
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Key words: Intravenous immunoglobulin therapy; Acquired coagulation inhibitors; Autoimmune disease; Factor VIII inhibitor; von Willebrand syndrome

1. Introduction

Intravenous immunoglobulin (IVIG), a highly purified immunoglobulin G (IgG) fraction derived from pooled human plasma, is currently one of the most widely used plasma components in the world [1,2]. It was originally introduced as replacement therapy for patients with primary immunodeficiency disorders. In 1981, Imbach et al reported a serendipitous observation that a high-dose infusion of IVIG (2 g/kg of body weight infused over 5 days) was able to transiently increase the platelet count in children with idiopathic thrombocytopenic purpura (ITP) [3]. With the encouragement of this and other reports on ITP [4], the clinical applications of IVIG have increased markedly over the past 25 years to include many autoimmune diseases. IVIG has been shown to be efficacious in clinical trials for graft-versus-host disease [5], myasthenia gravis [6], Guillain-Barré syndrome [7], Kawasaki disease [8], and chronic inflammatory demyelinating polyneuropathy [9]. It has also been used to treat immune neutropenia and coagulation inhibitors [10-12], but its efficacy and safety have not been firmly established.

Coagulation inhibitors, antibodies against individual clotting factors, interfere with blood coagulation. The most common coagulation inhibitor is factor VIII inhibitor, an antibody against factor VIII that neutralizes the coagulant activity of factor VIII. Factor VIII inhibitor develops in patients with hemophilia A as an alloantibody after replacement therapy or spontaneously as an autoantibody in nonhemophilic patients [13], including postpartum patients and those with autoimmune disease, malignancy, or diabetes [14]. Once developed in such patients, factor VIII inhibitor poses a serious problem for the management of bleeding episodes, because any infused factor VIII will be rapidly neutralized and will not be available to induce hemostasis [15]. Although IVIG therapy has been used as one of the immunotherapies

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for eradicating coagulation inhibitors, such an indication is considered off label [2].

The aim of this review is to examine the efficacy and safety of IVIG therapy in patients with acquired inhibitors against factors VIII, IX, or V, and in patients with acquired von Willebrand disease. Cases with lupus anticoagulant were not included in this review. An electronic search of the Medline/PubMed database from 1965 to 2005 was performed to identify relevant articles. This search yielded 108 citations, 72 of which were considered appropriate and reviewed. The bibliography of each review paper was examined to identify articles that may have been missed by our electronic searches.

2. History

In 1983, Nilsson et al reported an interesting observation [11]. A patient with severe hemophilia B and factor IX inhibitor was treated with extracorporeal protein A–Sepharose adsorption to remove the inhibitor, followed by the administration of factor IX concentrate and cyclophosphamide. This procedure produced a 15-fold increase in factor IX inhibitor on one occasion but did not cause any increase of the inhibitor titer on another occasion, when 5 g of IVIG was also given to the patient to restore the reduced IgG level. The investigators suggested that the administration of IVIG appeared to suppress antibody synthesis in hemophilia B patients with factor IX inhibitor.

Three groups of investigators reported the use of IVIG in the management of factor VIII inhibitors in 1984 [12,16,17]. IVIG therapy combined with vincristine produced a transient disappearance of acquired factor VIII inhibitor along with a slow rise of factor VIII activity in a 13-year-old boy with autoimmune disease [16]. IVIG therapy was ineffective in 2 patients with hemophilia A inhibitor [17]. Sultan et al [12] reported that IVIG therapy (0.4 g/kg body weight per day for 5 days) resulted in the rapid, marked, and prolonged suppression of factor VIII inhibitor in 2 patients with acquired factor VIII antibody (autoantibody) but that it had little or no effect in 2 hemophilic patients with factor VIII antibody (alloantibody). They showed by in vitro experiments that IVIG preparations were able to neutralize the anti–factor VIII activity of the patients' plasma and the IgG fraction of the patients' sera. Many articles were subsequently published on the effect of IVIG on acquired factor VIII inhibitors, as is discussed later.

3. Possible Mechanisms of Action

The rapid rise in the platelet count in ITP following IVIG administration is thought to occur through binding to and blocking Fcγ receptors on macrophages, thereby preventing the removal of antibody-coated platelets by the reticuloendothelial system in the spleen and liver [4]. This mechanism, however, does not appear to explain the effect on coagulation inhibitors.

Several hypotheses on the mechanisms of action of IVIG on factor VIII inhibitor have been put forward. Sultan et al and Kazatchkine and Kaveri postulated that anti-idiotypic antibodies present in IVIG preparations neutralize factor VIII autoantibodies [12,18]. $F(ab')_2$ fragments from IVIG preparations inhibited anti–factor VIII activity in $F(ab')_2$ fragments from the patient's plasma. Anti–factor VIII $F(ab')_2$ fragments were specifically retained on an affinity column of Sepharose-bound $F(ab')_2$ from IVIG, indicating that a direct interaction occurred through the antibody-binding sites of both immunoglobulins [19]. Anti-idiotypes against various autoantibodies were shown to be present in pooled normal human polyspecific immunoglobulin. In addition, IgG prepared from elderly donors and multiparous women was reported to contain a higher frequency of neutralizing antibodies against factor VIII autoantibodies [20]. It is puzzling that such an in vitro antibody-neutralizing effect was not always demonstrated, even though in vivo administration of IVIG produced a marked reduction of the inhibitor titer [21,22].

The fall in inhibitor titer following IVIG therapy without simultaneous immunosuppressive treatment appears to be rapid (within several days) in most cases [12,23,24] but is slow (more than 10 days) in others [22,25]. There must be slow effects of IVIG on autoantibody production. In addition to its direct and immediate action on antibodies, IVIG has been proposed to suppress antibody formation by B-cells, a process mediated through the down-regulation of Fcγ receptors [26]. Furthermore, IVIG may induce T-cell suppressor activity [27]. These observations taken together suggest that IVIG exerts its effect on the inhibitor titer through more than one mode of action.

4. Efficacy

4.1. Factor VIII Inhibitor

We extensively reviewed the international literature published from 1965 to 2005. The typical IVIG dosage used for treating factor VIII inhibitor was 0.4 g/kg per day for 5 consecutive days.

The efficacy criteria (ie, the response to IVIG therapy) were as follows [28]: Complete remission (CR) was defined as the disappearance of the inhibitor, partial response (PR) was defined as a decrease in the inhibitor titer by at least 25% of the baseline value, and failure was defined as other than CR and PR.

In Table 1, we present all of the cases in which the efficacy of IVIG treatment alone was evaluated [12,22-25,28-40]. The response to IVIG therapy alone was failure in 11 cases (31.4%) and PR in 21 cases (60.0%), but with a subsequent clinical benefit in only 8 patients. Finally, 3 patients (8.6%) achieved CR. The efficacy of IVIG therapy alone, which includes CR and PR with a clinical benefit, among these 35 patients was estimated to be 31.4% (11 cases). In most cases of CR or PR, the response to IVIG treatment was rapid, and factor VIII inhibitor seemed to be neutralized immediately.

We summarize the responses to combined therapy with IVIG plus immunosuppressive agents in Table 2 [21,25, 28,32,35,38-52]. The response to IVIG plus steroid and/or cyclophosphamide therapy was better than to IVIG treatment alone. CR was achieved in 19 (73%) of 26 patients who were treated with IVIG plus steroid. In addition, 14 (74%) of 19 patients who received IVIG plus steroid and