Inclusion Body Myositis Associated with Hepatitis C Virus Infection

TSURUTA, Yuko
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University

YAMADA, Takeshi
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University

YOSHIMURA, Takeo
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University

SATAKE, Marie
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University

他

https://doi.org/10.15017/7234034

出版情報：福岡醫學雜誌. 92 (11), pp.370-376, 2001-11-25. 福岡医学会
バージョン：
権利関係：

Fukuoka Acta Med. 92(11) : 370−376, 2001

原　　著

Inclusion Body Myositis Associated with Hepatitis C Virus Infection

Yuko TSURUTA, Takeshi YAMADA, Takeo YOSHIMURA, Marie SATAKE, Katsuya OGATA, Tatsunori YAMAMOTO, Hirokazu FURUYA and Jun-ichi KIRA

Y Tsuruta, T Yamada, T Yoshimura, M Satake, K Ogata, H Furuya, J Kira
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
T Yamamoto
Department of Neurology, University of Occupational and Environmental Health, Kitakyushu, Japan

Abstract Inclusion body myositis (IBM) is a chronic progressive inflammatory myopathy in elders. Three patients with chronic hepatitis C developed IBM. Their muscle biopsies were examined by polymerase chain reaction (PCR) for two patients and immunohistochemistry for three patients. The hepatitis C virus (HCV)-RNA genome was detected in one of the two patients. The degenerated and atrophic muscle fibers were immunoreactive for an anti-HCV antibody in all three patients. Immunoreactivity for an anti-HCV antibody was not apparent in the muscle specimen from an IBM patient without HCV infection. Our findings suggest that HCV infection is related to the pathogenesis in some cases of IBM.

Key words : inclusion body myositis, hepatitis C virus, polymerase chain reaction, immunohistochemistry

1. Introduction

Inclusion body myositis (IBM) is a slowly progressive muscle disorder of the elderly that is characterized histologically by lymphocytic inflammation and autophagic vacuoles called rimmed vacuoles. Ultrastructural studies show abnormal tubulofilamentous materials in the cytoplasm and/or the nuclei of muscle fibers. An abnormal accumulation of various proteins such as amyloid-β and phosphorylated tau is observed in muscle fibers. The accumulation of these proteins is characteristic in Alzheimer disease brains as well[3)7)]. The etiologies of IBM are still unknown. An immune-mediated process, a degenerative disorder, and a possible viral etiology have been suggested but none has been proven[5)8)]. In addition, previous studies suggested a relationship between hepatitis C virus (HCV) infection and inflammatory myopathy[1)2)4)10)13)].

To determine whether HCV infection may be

Correspondence to : Dr Y Tsuruta
Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

involved in the pathogenesis of IBM, we examined muscle biopsies from three patients with IBM who had chronic hepatitis C.

2. Patients and methods

1.1. Patients

Three male patients with chronic hepatitis C (age range, 61 to 72 years) developed muscle weakness following liver dysfunction. The age at onset of muscle weakness ranged from 58 to 64 years (Table). The duration of hepatitis (range, 6 to 17 years) was longer than that of muscle weakness. Two of them were treated with interferon (IFN) (cases 1 and 3) because their liver biopsies showed chronic hepatitis. At that time, a patient with case 1 complained with no muscle disorder, however, muscle weakness preceded the treatment in case 3. Mild muscle weakness was also present in finger flexor, wrist flexor and quadriceps in all patients. Dysphagia was present in cases 1 and 2 who had the illness longer. Serum creatine kinase was elevated in cases 1 and 3. Elevation of serum aspartate aminotransferase and alanine aminotransferase was also seen in all the cases. Electromyogram showed a myogenic pattern in cases 1 and 2 and a myogenic pattern combined with a mild neurogenic pattern in case 3. All muscle biopsies were performed on the biceps brachii in the absence of IFN (case 2) or after cessation of IFN therapy (four years after in case 1 and three months after in case 3).

2.2. Virologic, histological and immunohistochemical studies

Serum HCV subtypes were determined by reverse transcription and polymerase chain reaction (RT-PCR) followed by restriction endonuclease cleavage[15]. The presence of plus-strand or minus-strand HCV-RNA in the biopsied fresh frozen muscle was determined with RT-PCR according to the method of Sherker et al[14].

Muscle biopsies were quickly frozen in isopentane (−70 °C) for histochemistry and fixed with 2.5% glutaraldehyde for electron microscopy. For immunohistochemistry, frozen muscle sections were fixed with 10% formalin and incubated with a goat anti-HCV polyclonal antibody (Antigenix America Inc., USA). Following washing, the sections were incubated with a biotinylated anti-goat IgG (Vector Laboratories Inc., USA) and then with horseradish peroxidase-conjugated streptavidin (Amersham, UK). The reactive stains were developed with 3, 3'-diaminobenzidine tetrahydrochloride. The anti-HCV antibody was derived from immunization with both capsid and non-structural proteins of HCV. Positive staining with this antibody was confirmed in the liver biopsy from a chronic hepatitis C patient.

3. Results

Circulating HCV-RNA was detected in all the three patients and the subtype was 2a in case 1 and 1b in cases 2 and 3. Quantitative analysis of serum HCV-RNA showed less than 0.50×10^{-3} equivalents/ml in case 1. The viral genome was examined in the fresh frozen muscle in cases 1 and 2. Plus-strand HCV-RNA was detected in case 1 but not in case 2. Minus-strand was not detected in either case (Table).

All muscle biopsy specimens showed marked fiber size variability, increased central nuclei and endomysial fibrosis with intense mononuclear cell infiltration into the endomysium. Mononuclear cells invaded non-necrotic muscle fibers (Fig. 1a and c). Rimmed vacuoles were present in 4.0 % to 8.3% of 500 muscle fibers in each HE section (Table). Numerous necrotic fibers and myophagia were also noted in all the

Table Clinical and pathological characteristics of patients with IBM

Case	1	2	3
Age (Age at onset)	61 (58)	72 (64)	63 (61)
Sex	M	M	M
Duration of hepatitis (years)	6	10	17
CK	704	60	641
AST/ALT	38/118	36/401	119/274
EMG	M	M	M+N
Biopsy	biceps	biceps	biceps
Serum HCV-RNA subtype	2a	1b	1b
Muscle HCV genome	(+) strand	not detected	not examined
Rimmed vacuole (%)*	4.0	8.3	6.0

CK : creatine kinase (normal range 62-287 U/l). AST : aspartate aminotransferase (13-33 U/l). ALT : alanine aminotransferase (6-30 U/l). EMG : electromyogram. M : myogenic pattern. N : neurogenic pattern. HCV : hepatitis C virus
* The number of rimmed vacuoles were counted for 500 muscle fibers with HE staining.

specimens. Small angular fibers and small grouped atrophic fibers were focally present. Ragged red fibers were found in case 1. Vacuolated fibers, especially with rimmed vacuoles, were strongly positive for acid phosphatase. Fine granules were seen in the subsarcolemmal regions or intermyofibrillar portions of abnormal muscle fibers. Type 1 muscle fibers were predominant. Electron microscopic observation demonstrated the accumulation of filamentous materials surrounding myeloid whorls of various sizes (data not shown). These findings indicated inclusion body myositis.

The immunohistochemistry of the anti-HCV antibody revealed positive immunoreactivity in the rim and intermyofibrillar portions in vacuolated fibers in all the three patients (Fig. 1b). The immunoreactivity was also detected in atrophic fibers with diffuse cytoplasmic staining (Fig. 1d). Staining was not observed when only the secondary antibody was used (data not shown). Muscle specimens from an IBM patient without HCV infection was not positive for the anti-HCV antibody (Fig. 1e). A few atrophic fibers from a polymyositis patient with HCV infection presented a faint reaction with the anti-HCV antibody (Fig. 1f).

4. Discussion

All the patients fulfilled the diagnostic criteria for IBM[3)7)]. We demonstrated the positive immunostaining of muscle fibers with an anti-HCV antibody in patients with IBM who had chronic hepatitis C. The presence of HCV genome in the muscle was also confirmed in one patient. Several reports have suggested a relationship between HCV infection and inflammatory myopathy such as polymyositis[1)2)4)10)13)], and one of them described a patient with chronic HCV infection who developed IBM[1)]. However, there was no direct evidence relating the HCV infection to the IBM. Our immunohistochemical and molecular biological studies have demonstrated the association between IBM and HCV infection for the first time.

The subtype of circulating HCV-RNA was 2a in case 1, and 1b in cases 2 and 3. Seventy percent of Japanese patients with chronic hepatitis C have type 1b and 15% have type 2a[11)]. Thus, a specific subtype does not appear to be related to the development of IBM. The HCV

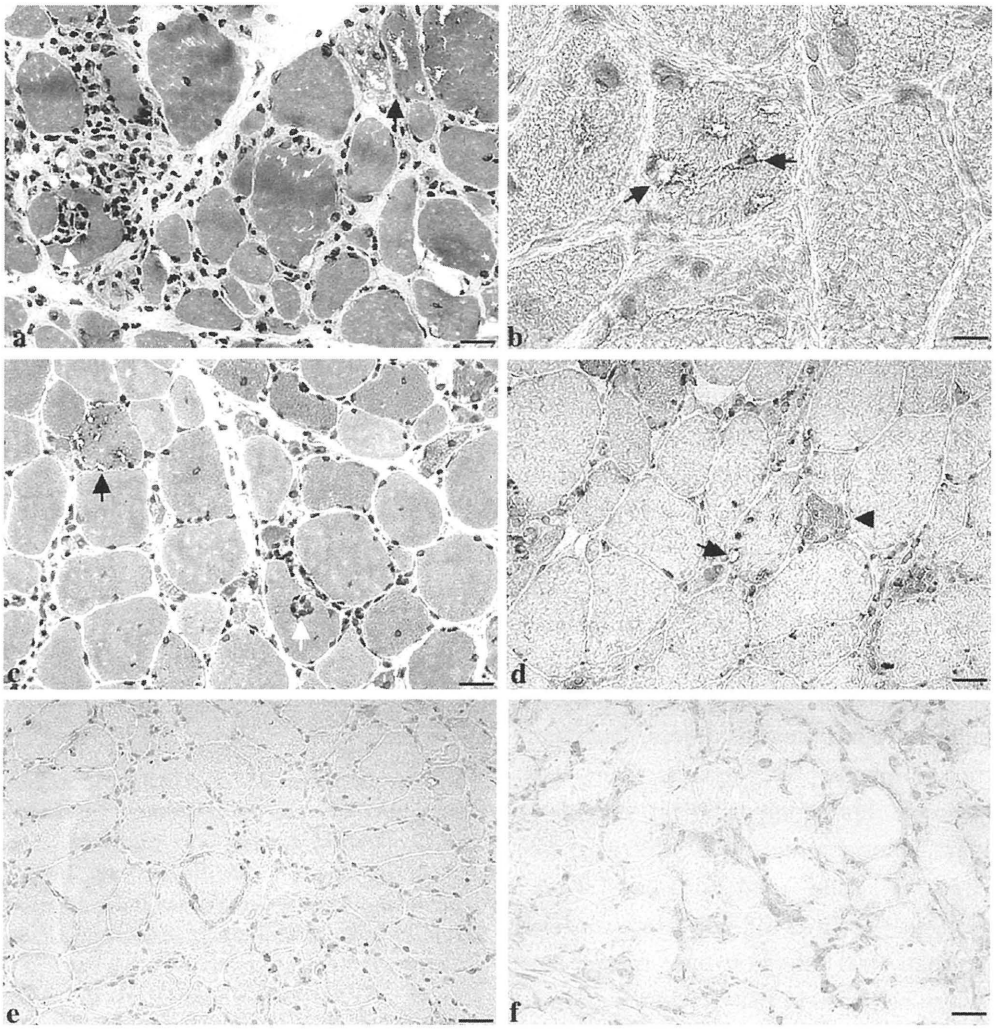

Fig. 1 Degenerative fibers with rimmed vacuoles (arrow) and mononuclear cell invasion (white arrows) are present in severe inflammatory myopathic change in cases 2 and 3 (a and c). Immunoreactivity for an anti-HCV antibody is observed along the rim of vacuoles (arrows in b and d). Some atrophic fibers were diffusely immunostained with the anti-HCV antibody in case 3 (arrowhead in d). The immunoreactivity is not apparent in the vacuolated fibers from an IBM patient without HCV infection (e), while faint reaction is observed in some atrophic fibers from a polymyositis patient infected with HCV (f). a and c, hematoxylin and eosin ; b, d, e, f, immunohistochemistry with an anti-HCV antibody. a and b, case 2 ; c and d, case 3 ; e, IBM without HCV infection ; f, polymyositis with HCV infection. Bar = 20 μm (a, c, d, e, f), bar = 10 μm (b).

genome was detected in the skeletal muscle in case 1. The plus-strand of HCV-RNA was present but not the minus-strand, indicating that HCV is present but does not replicate in the muscle as it does in the liver[6]. Villanova et al.[18] demonstrated with PCR that HCV-RNA

was present in the muscle as well as in the plasma of patients with inflammatory myopathy associated with chronic hepatitis C, while HCV-RNA was not detected in the muscle of patients with chronic hepatitis C without inflammatory myopathy. They speculated on the direct involvement of HCV in inducing muscle fiber damage[18]. Although contamination of blood in the biopsied muscles could not be ruled out completely in our samples as well as those of Villanova et al.[18], our findings also support their notion.

Viral infection and an immune-mediated process have been suggested in the pathogenesis of IBM[5][8]. Cupler et al. have reported three IBM patients infected with human immunodeficiency virus type 1 (HIV-1) or human T cell leukemia virus type 1 (HTLV-1). The HIV-1 or HTLV-1 antigen was present only in macrophages around muscle fibers, but not within muscle fibers. The muscle fibers expressed major histocompatibility complex (MHC) class 1 antigen and were infiltrated by CD8-positive T cells predominantly. They considered that the virus did not infect muscle fibers but that viral infection may trigger an inflammatory response in IBM[5]. In our pathological studies, severe lymphocytic inflammation was observed in endomysium. This finding seems to suggest that HCV infection may also have triggered an immune-mediate mechanism leading to IBM, as seen in HIV-1 and HTLV-1 carriers.

The mechanisms of liver cell damage by HCV infection remain unknown. HCV does not seem to directly destroy the liver cells because the regions of hepatic necrosis are not usually coincident with the location of HCV antigens, and hepatocytes stained intensely with an anti-HCV antibody do not show any apparent degenerative changes[17]. Some other mechanisms may be responsible. Humoral immune responses[16], cellular immunity[12] or apoptotic processes[9] may be triggered by HCV infection. These proposed mechanisms of liver damage in chronic hepatitis C may play a role in the muscle fiber degeneration in IBM as well.

These three patients demonstrate that IBM can occur in patients with chronic hepatitis C, and we conclude that HCV infection may be a trigger of inflammatory myopathies such as IBM.

Acknowledgments

We would like to thank Ms. K. Hatanaka for excellent technical assistance.

References

1) Alexander JA and Huebner CJ : Hepatitis C and inclusion body myositis, Am J Gastroenterol. 91 : 1845-1847, 1996.

2) Arai H, Tanaka M, Ohta K, Kojo T, Niijima K and Imawari M : Symptomatic myopathy associated with interferon therapy for chronic hepatitis C [letter]. Lancet 345 : 582, 1995.

3) Askanas V and Engel WK : Inclusion-body myositis : newest concepts of pathogenesis and relation to aging and Alzheimer disease. J Neuropathol Exper Neurol. 60 : 1-14, 2001.

4) Ayabe M, Kawamoto M, Ijima H, Hino H and Shoji H : Clinical characteristics and muscle histopathology in polymyositis positive anti-hepatitis with C virus antibody. Rinsho Shinkeigaku - Clinical Neurology 37 : 208-211, 1997 [In Japanese].

5) Cupler EJ, Leon-Monzon M, Miller J, Semino-Mora C, Anderson TL and Dalakas MC : Inclusion body myositis in HIV-1 and HTLV-1 infected patients. Brain 119 : 1887-1893, 1996.

6) Fong TL, Shindo M, Feinstone SM, Hoofnagle JH and DiBisceglie AM : Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. J Clin Invest. 88 : 1058-1060, 1991.

7) Griggs RC, Askanas V, DiMauro S, Engel A, Karpati G, Mendell JR and Rowland LP : Inclusion body myositis and myopathies. Ann Neurol. 38 : 705-713, 1995.

8) Hilton DA, Fletcher A and Pringle JH: Absence of Coxsackie viruses in idiopathic inflammatory muscle disease by in situ hybridization. Neuropathol Appl Neurobio. 20: 238-242, 1994.

9) Hiramatsu N, Hayashi N, Katayama K, Mochizuki K, Kawanishi Y, Kasahara A, Fusamoto H and Kamada T: Immunohistochemical detection of Fas antigen in liver tissue of patients with chronic hepatitis C. Hepatology 19: 1354-1359, 1994.

10) Horsmans Y and Geubel AP: Symptomatic myopathy in hepatitis C infection without interferon therapy [letter; comment]. Lancet 345: 1236, 1995.

11) Kato N, Ootsuyama Y, Tanaka T, Nakagawa T, Nakazato T, Muraiso K, Hijikata M and Shimotohno K: Marked sequence diversity in the putative envelope protein of hepatitis C viruses. Virus Res. 22: 107-123, 1992.

12) Koziel MJ, Dudley D, Wong JT, Dienstag J, Houghton M Ralston R and Walker BD: Intrahepatic cytotoxic T lymphocytes specific for hepatitis C virus with chronic hepatitis. J Immunol. 149: 3339-3344, 1992.

13) Monger RM and West SG: Inflammatory myopathy and hepatitis C virus infection. Arthritis & Rheumatism 41: 1323-1324, 1998.

14) Sherker AH, Twu JS, Reyes GR and Robinson WS: Presence of viral replicative intermediates in the liver and serum of patients infected with hepatitis C virus. J Med Virol. 39: 91-96, 1993.

15) Simmonds P, Holmes EC, Cha SW, McOmish F, Irveine B, Beall E, Yap PL, Kolberg J and Urdea MS: Classification of hepatitis C virus into six major genotypes and a series by phylogenetic analysis of the NS-5 region. J Gen Virol. 74: 2391-2399, 1993.

16) Torgano G, Vecchi M, Arosio E, Santambrogio D, Ronchi G, Annoni G, Tomasini M, Contessini E and de Franchis R: Changes of IgG-bearing cell populations in the portal tracts of patients with chronic liver disease of viral etiology: an evaluation by immunoperoxidase method and computerized image analysis. Hepatology 16: 19-23, 1992.

17) Uchida T, Shikata T, Tanaka E and Kiyosawa K: Immunoperoxidase staining of hepatitis C virus in formalin-fixed, paraffin-embedded needle liver biopsies. Virchows Arch. 424: 465-469, 1994.

18) Villanova M, Caudai C, Sabatelli P, Toti P, Malandrini A, Luzi P, Maraldi NM, Valensin PE and Merlini L: Hepatitis C virus infection and myositis: a polymerase chain reaction study. Acta Neuropathol. 99: 271-276, 2000.

(Received for publication March 22, 2001)

(和文抄録)

C型肝炎ウイルス感染症を伴う封入体筋炎

九州大学大学院医学研究院脳神経病研究施設神経内科
鶴田裕子・山田　猛・由村健夫・佐竹真理恵
緒方勝也・古谷博和・吉良潤一
産業医科大学神経内科
山本辰紀

封入体筋炎は中年期に発症する慢性進行性炎症性筋疾患である．封入体筋炎を発症した慢性C型肝炎患者3名の骨格筋生検サンプルを用い免疫組織化学法を施行し，うち2名についてはpolymerase chain reaction法を行い検索した．C型肝炎ウイルス（HCV）-RNAゲノムは2名中1名の骨格筋で認められた．全患者で変性および萎縮筋線維に抗HCV抗体に対する染色性が見られた．HCV感染のない一人の封入体筋炎患者の筋標本では抗HCV抗体の染色性は認められなかった．封入体筋炎の中にはHCV感染が病因に関与している例がある．