Core Protein of Hepatitis C Virus Induces Cardiomyopathy

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Hepatitis C virus (HCV) has been reported to be associated with cardiomyopathy. However, the mechanism of cardiomyopathy in chronic HCV infection is still unclear. Therefore, we investigated the development of cardiomyopathy in mice transgenic for the HCV-core gene. After the age of 12 months, mice developed cardiomyopathy that appeared as left ventricular dilatation, and systolic and diastolic dysfunction assessed by Doppler echocardiography. Histologically, hypertrophy of cardiomyocytes, cardiac fibrosis, disarray and scarcity of myofibrils, vacuolization and deformity of nuclei, myofibrillar lysis, streaming of Z-bands, and an increased number of bizarre-shaped mitochondria were found in HCV-core transgenic mice. These histological changes are just consistent with cardiomyopathy. In conclusion, the HCV-core protein directly plays an important role in the development of cardiomyopathy.

Hepatitis C virus (HCV) is a major etiologic agent of non-A, non-B acute, and chronic hepatitis. Moreover, HCV has been reported to be associated with several extrahepatic manifestations, such as cryoglobulinemia, glomerulonephritis, B cell non-Hodgkin lymphoma, porphyria cutanea tarda, oral lichen planus, and hyper- or hypothyroidism.1–3 In addition, recent non-Hodgkin lymphoma, porphyria cutanea tarda, oral lichen festations, such as cryoglobulinemia, glomerulonephritis, B cell hyper- or hypothyroidism. 1–3 In addition, recent non-Hodgkin lymphoma, porphyria cutanea tarda, oral lichen festations, such as cryoglobulinemia, glomerulonephritis, B cell hyper- or hypothyroidism. 1–3 In addition, recent non-Hodgkin lymphoma, porphyria cutanea tarda, oral lichen festations, such as cryoglobulinemia, glomerulonephritis, B cell

Materials and Methods

Production of HCV-core gene transgenic mice has been previously described. In the mice, HCV-core expressed in brain, heart, lung, liver, and the other organs, and induced chronic hepatitis.6 The male transgenic mice were mated with wild-type female mice. Mice from the next generation were separated into HCV-core gene negative (WT) and positive (HCV-core) groups by detecting exogenous DNA in tail DNA samples.

Blood pressure (BP) and heart rate (HR) of the conscious mice were measured using the tail-cuff method. Echocardiography, Northem blot hybridization, and electrophoretic mobility shift assay were performed as previously described.7

As shown in Figure 1A, the serial echocardiographic examination revealed that the functional parameters of HCV-core male mice began to change between 6 and 9 months of age. Then, the mice analyzed at 12 months had significantly increased left ventricular end-diastolic and end-systolic dimension (LVDd and LVDs), decreased fractional shortening (FS) and ejection fraction (EF), and increased E wave/A wave velocity ratio (E/A). Figure 1B shows the left ventricular cavity of HCV-core mice is larger than that of WT mice. Myocardial mRNA (Figure 1C) and protein (Figure 1D) expression of HCV-core were observed in only HCV-core mice. Figure 1E shows that myocardial mRNA expression of atrial natriuretic peptide (ANP) and brain natriuretic peptide

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(BNP) was enhanced in HCV-core mice (3.2±1.2 fold and 2.2±0.5 fold versus WT, *P*<0.05, respectively). The mRNA expression of collagen type I and III did not change between the two groups (data not shown). Figure 1F shows that myocardial activation of activator protein-1 (AP-1) did not change at 6 months. However, it is significantly activated in HCV-core mice at 9 and 12 months, compared with WT (1.3±0.2 and 1.5±0.1 fold, *P*<0.01, respectively). The activation of nuclear factor-κB (NF-κB) did not change between the two groups.

As shown in Figure 2A and 2B, hypertrophy of cardiomyocytes, disarray of myofibers, scarcity of myofibrils, vacuolization, and pleomorphic nuclear changes were observed in HCV-core mice at 12 months. No infiltration of lymphocytes was observed at 6, 9, and 12 months of age, thereby suggesting that apparent myocarditis were absence. The cardiomyocyte cross-sectional area in HCV-core mice is larger than that of WT (WT, 210±14 μm²; HCV-core, 256±18 μm²; *P*<0.01). As shown in Figure 2C and 2D, cardiac fibrosis was observed in HCV-core mice (WT, 0.9±0.4%; HCV-core, 4.9±0.5%; *P*<0.01). TUNEL-positive nuclei/cardiomyocyte area was not significantly different between the two groups (data not shown). Electron microscopic examination shows that irregular shaped nuclei, myofiber disorganization, myofilament loss, an increased number of bizarre-shaped mitochondria (Figure 2F), Z-band abnormalities (Figure 2G, arrows), aggregation of mitochondria (Figure 2H), and cavity formation of mitochondria (Figure 2I) were observed in HCV-core mice. The expression of HCV-core protein was mainly observed in mitochondria (Figure 2K, arrows).

**Figure 1.** A, Doppler-echocardiographic study. **P**<0.01 vs WT. B, Typical heart morphology in a short-axis view of the ventricles. C, HCV-core mRNA expression in the ventricles of 12-month-old mice. D, Immunofluorescence detection of HCV-core. E, Ventricular ANP and BNP mRNA expression. F, Ventricular AP-1 and NF-κB activities at 6, 9, and 12 months of age.

**Figure 2.** Pathological changes in the heart of 12-month-old mice. Ventricular myocardium stained by hematoxylin and eosin in WT (A) and HCV-core (B) and Sirius red in WT (C) and HCV-core (D). Electron microscopic examination of WT (E) and HCV-core (F, G, H, and I). immunoelectron microscopic examination of WT (J) and HCV-core (K). Scale bars represent 50 μm in A, B, C, and D; 20 μm in C, D, E, and F; 1 μm in G; and 100 nm in H and I.
Discussion
HCV-core protein is a major component of viral nucleocapsids and regulates the growth of hepatocytes by affecting the transcription of cellular protooncogenes such as ras oncogene, c-myc, and c-jun.9,10 Therefore, HCV-core protein in liver cells is strongly related to transformation of the liver cells.

In the present study, to determine the long-term effects of HCV-core expression on the heart, Doppler-echocardiography was performed in mice at every 3 months. Then, male HCV-core mice analyzed at 12 months of age had the dilatation of the LV cavity and systolic dysfunction. The abnormal left ventricular inflow pattern (increased E/A) may suggest diastolic dysfunction. Myocardial mRNA expression of ANP and BNP, which are useful markers of hypertrophic cardiomyopathy, BP, HR, and body weight did not change between the two groups and pleural effusion was not observed in HCV-core mice, thereby suggesting that pressure overload and/or water retention may not be the cause of cardiomyopathy in this model. As one of the molecular mechanisms of the long-term effects of HCV-core expression in heart, myocardial AP-1 was activated in HCV-core mice at 9 months when the functional parameters began to diverge from WT mice. It has been demonstrated that AP-1 is activated in the liver of HCV-core mice, and suggested that this pathway may contribute to hepatocarcinogenesis.10 We have previously reported that activation of AP-1 induced cardiomyocyte hypertrophy,11 and the activation was observed in progressive ventricular remodeling.7 Taken together with these findings, myocardial AP-1 activation by HCV-core may be one of the important pathways for cardiomyopathic changes in this model. In histological examination, cardiomyocytes hypertrophy and cardiac fibrosis were often observed in HCV-core mice. Electron microscopic examination shows myofibrillar and mitochondrial abnormalities in HCV-core mice. These histological changes are just consistent with cardiomyopathy.12 Furthermore, it is demonstrated that mitochondrial abnormalities were partially related to dilated cardiomyopathy.13 Interestingly, the expression of HCV-core was observed in mitochondria of cardiomyocyte as same as that of liver,14 thereby suggesting the possibility of mitochondrial dysfunction by HCV-core directly.

In conclusion, our observations demonstrate that the expression of the HCV-core gene results in progressive morphological and functional changes that ultimately result in the development of cardiomyopathy. Our results indicate that chronic myocarditis is not an absolute prerequisite for the cardiomyopathy, and that HCV itself is directly involved in the development of cardiomyopathy.

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References

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