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BRIEF REPORT

First evidence of the presence of adenovirus type 8 in myocardium of patients with severe idiopathic dilated cardiomyopathy

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Abstract

Previous studies have detected adenovirus and cytomegalovirus (CMV) in cardiac tissue of patients with myocarditis. Therefore, in this study, we investigated the frequency of these viruses, which may be involved in the development of severe dilated cardiomyopathy (DCM). Myocardial tissue from of 23 cardiac transplant candidates with acute idiopathic DCM below the age of 40 years were analyzed by amplification of adenovirus and CMV DNA and subsequent sequencing. Adenovirus was detected in four (17.4%) and CMV in one (4.3%) of the patients. All controls were negative for the presence of both viruses. Our study shows that myocardial infection with adenovirus may play an important role in the pathogenesis of severe DCM and suggests that vaccination against adenovirus might be helpful in decreasing the prevalence of severe idiopathic DCM. This is the first study in which adenovirus type 8 has been detected in the hearts of patients with DCM.

Introduction

Acute dilated cardiomyopathy (DCM) is a severe pathology of the heart that is characterized by a reduction in heart contractibility that finally can lead to congestive heart failure. Affected patients may need heart transplantation in the late stages to prevent sudden cardiac death [4].

Previous work has shown that idiopathic DCM can occur as a late result of viral myocarditis, either due to presence of a virus [7] or due to an autoimmune response triggered by viral infection [9].

Adenovirus and cytomegalovirus (CMV) are two of the most frequently found cardiotropic viruses [5]. Adenoviruses are sometimes localized in the myocardium, which contains adenovirus receptors on the surface of cardiomyocytes [11]. Some studies have reported the occurrence of fatal myocarditis due to CMV infection even in immunocompetent patients [6].

The aim of this study was to investigate whether severe idiopathic DCM might be associated with the presence of adenovirus or CMV in the heart. We evaluated the presence of these viruses in myocardial samples obtained from patients suffering from idiopathic DCM in order to determine their frequency in myocardial tissues of cardiac

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transplant candidates with DCM in comparison with individuals without cardiomyopathy.

Materials and methods

Clinical inclusion criteria: The inclusion criteria for this study were being a cardiac graft candidate and having been diagnosed with congestive heart failure due to acute idiopathic DCM. The patients had no history of any cardiovascular disease. A total of 23 cases (peak age 40 years) were included in the study.

Exclusion criteria: The exclusion criteria were age above 40 years or any other possible causes of cardiac dysfunction such as hypertension, cardiac valve disease, or cardiac ischemia.

Control group: Twenty-six cases of brain death due to a traffic accident were selected as the control group.

Sampling: Six samples (~0.5 g each) were taken from the endomyocardium of the right and left atriums and ventricles and valves of each heart. The samples were snap frozen and kept at -70°C until further analysis.

Extraction of DNA from myocardial samples

PCR assays were done in duplicate. Two myocardial tissue samples were analyzed from each case. Total DNA was extracted using a MagaZorb DNA Mini-Prep Kit (Promega, Madison, Wisconsin, USA). For each assay, positive controls, derived from virus-infected cells, were included.

Nested PCR (polymerase chain reaction)

Viral genomes were detected in myocardial samples using nested PCR. The PCR amplifications were carried out in a final volume of 60 µl. The reaction mix consisted of master mix (Taq PCR buffer, $MgCl_2$, dNTPs (deoxynucleoside triphosphates), Taq polymerase), and 10 pM each primer.

Primers corresponding to the regions encoding the hexon capsid of adenovirus and the membrane protein of CMV were designed using Gene Runner (version 5) (http://www.generunner.net/) and Primer3 (version 0.4.0) software. Primer sequences and thermal profiles for PCR are shown in Tables 1 and 2, respectively in the Supplementary Material.

Type determination by DNA sequencing

Automated sequencing of PCR products using a BigDye DNA Sequencing Kit (Applied Biosystems, Darmstadt, Germany).

Statistical analysis

A comparison between the frequencies of virus detection in the study and control groups was done using Fisher's exact test. A *P*-value less than 0.05 was considered significant. All statistical tests were performed by using SPSS version 18.

Results

PCR analysis

Adenovirus genomes were detected by PCR in four of the 26 myocarditis cases (Fig. 1; Supplementary Material) and were not seen in any of control cases. The frequency of adenoviral detection was significantly higher in patients with severe idiopathic DCM than in the control group (4 subjects [17.4%] vs. 0 subjects [0%], $P < 0.05$). CMV was detected in the myocardial tissue of one patient (4.3%) with concomitant presence of adenovirus (Fig. 2; Supplementary Material).

DNA sequencing

To determine the viral type, PCR products from virus-positive cases were purified and sequenced and compared with published sequences of adenoviruses or CMV. According to BLAST search analysis of the amplicon sequences, all of the adenovirus strains detected had 99% sequence identity to DNA sequences of adenovirus type 8 (accession number AB448769.1). The CMV case was positive for human herpes virus 5 strain JP (accession number GQ221975.1) (100% sequence identity). Comparison of the sequence of the amplified DNA with previously determined sequences of adenovirus and CMV confirmed the specificity of the PCR primer pairs.

Discussion

Accurate identification of the causes of DCM is important for better preventive and therapeutic strategies. It is widely accepted that the presence of viruses in the heart is one of the causes of DCM [8]. Many reports have clearly shown the involvement of adenovirus and CMV in the pathogenesis of myocarditis [1, 5].

Our results suggest that detection of adenovirus or CMV by PCR in cardiac specimens is likely to be indicative of asymptomatic myocarditis due to recent adenoviral or CMV infection associated with DCM and fatal outcome. Hence, caution should be taken when interpreting the results for

establishing the cause of death. Infections with adenovirus and CMV have previously been reported to be a common cause of myocarditis in children [10] and adults [2].

In 2005, an outbreak of acute cardiac decompensation occurred in infants and young children in the city of Havana [10]. Adenovirus type 5 was detected in the myocardium in three (37%) fatal cases, while adenovirus type 8 was detected in our research.

Sequence determination of PCR products indicated the presence of adenovirus type 8, a type of adenovirus commonly seen in patients with keratoconjunctivitis, in all of the samples that were positive for adenovirus.

The role of adenoviruses in the pathogenesis of DCM is further supported by the finding of adenovirus receptors on the heart [3]. Hence, adenoviral-induced cardiomyopathy may need a genetic background [13], and we can also develop the hypothesis that adenoviral infections may have a role in etiology of familial DCM. Toivonen et al. [12] also showed that expression of adenovirus receptor increased in the myocardial layer of hearts with DCM in comparison with normal hearts. Additionally, activation of these receptors can lead to deformation of cytoskeleton in the infected hearts [1].

The data reported herein could have significant therapeutic consequences. The observation of important adverse effects and the occasional ineffectiveness of antiviral drugs against adenoviruses render their use debatable for such infections, and the development and use of a vaccine against adenoviruses might be more useful than antiviral therapy. Further research is required to clarify if vaccines against these infections, especially adenovirus type 8, are effective in reducing the occurrence of DCM.

It should be noted that, in addition to adenovirus and CMV, other viruses such as coxsackievirus, human herpes virus 6 (HHV6), and parvovirus, may also play a role in the pathogenesis of DCM. We did not investigate these viruses, which is one of the limitations in our study.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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