



PCR-based diagnosis of enterovirus and parvovirus B19 in paraffin-embedded heart tissue

A. Baasner*, R. Dettmeyer, M. Graebe, B. Madea

Institute of Legal Medicine, University of Bonn, Stiftsplatz 12, D-53111 Bonn, Germany

Abstract

Although immunohistochemical and molecular biological techniques have improved the diagnosis, the incidence of virus-induced lethal courses of myocarditis is still unclear. Therefore, it is desirable to investigate postmortem myocardial samples in cases of unknown cause of death. While enteroviruses are the most common agents of myocarditis, parvovirus B19 is also known to be highly cardiotropic. The enteroviral genome consists of a single-stranded RNA molecule. Parvovirus B19 is the only known human pathogen virus of the family Parvoviridae and consists of a linear single-stranded DNA molecule. In our investigation, RNA and DNA were specifically isolated and demonstrated from formalin-fixed material. Myocardial samples from 60 autopsy cases with unknown cause of death after autopsy were taken from different regions and investigated with a nested polymerase-chain-reaction (PCR). Enteroviruses could be detected in 14 cases. PCR revealed eight cases with myocardial infection due to parvovirus B19. In the myocardial sample of one case, both enteroviruses and parvovirus B19 were found. Our results emphasize the importance of modern molecular biological methods in cases of sudden death even when histological examination revealed no serious findings in heart muscle tissue.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Enterovirus; Parvovirus B19; PCR; Paraffin-embedded tissue; Myocarditis

1. Introduction

Previous studies of myocarditis in adults have demonstrated that numerous cases of acute myocarditis could not be diagnosed according to the Dallas-criteria by traditional hematoxylin–eosin staining of endomyocardial biopsies. The aim of our study was to analyse enteroviruses and parvovirus B19 from paraffin-embedded heart tissue with a

* Corresponding author. Tel.: +49-228-738356; fax: +49-228-738339.

E-mail address: a.baasner@uni-bonn.de (A. Baasner).

highly sensitive nested PCR. Therefore, we established a reliable method to isolate DNA and RNA from paraffin-fixed material.

2. Materials and methods

Postmortem myocardial samples were obtained from 60 autopsy cases with suspected SIDS. Eight myocardial samples were taken from each heart (right ventricle anterior and posterior, septum interventriculare cranial and caudal, left ventricle anterior wall cranial and caudal, left ventricle posterior wall cranial and caudal).

2.1. RNA/DNA preparation

Paraffin-embedded myocardial samples were dewaxed with xylene and washed with ethanol. The paraffin sections were homogenized using a Micra D-8 homogenizer (Art-moderne Labortechnik, Müllheim-Hügelheim, Germany). Total RNA was extracted with peqGOLD TriFast (peqlab, Biotechnology, Erlangen, Germany) following the manufacturer's protocol. Genomic/viral DNA was extracted with the Gen-ial First-DNA Kit (Gen-ial, Troisdorf, Germany) according to the suppliers protocol.

2.2. PCR protocol

The assumption for the specific virus PCR was the successful amplification of the housekeeping gene cyclophilin [1]. This control PCR amplification verifies the presence of amplifiable nucleic acid extract from each sample.

Since enteroviruses are RNA viruses, reverse transcriptase-PCR (RT-PCR) was used to evaluate these viruses. First-strand cDNA, for use in the detection of enterovirus, was generated from <50 ng of extracted total nucleic acid in the presence of 10 U RNase inhibitor using the Sensiscript Reverse Transcriptase (Qiagen, Hilden, Germany) following the manufacturer's protocol. For the amplification of enteroviruses, a seminested PCR was performed. Primer sequences/First round: EV1b 5'-CAA TTG TCA CCA TAA GCA GCC A-3' and Entero a [2]. Second round: Entero a/Entero b [2].

For parvovirus B19, a nested PCR was performed. The primers were used according to Cassinotti et al. [3].

2.3. Electrophoresis

Each PCR product was analysed on an 8% polyacrylamide gel. The DNA fragments were detected by silver staining.

3. Results and discussion

Enteroviruses could be detected in 14 (23%) out of 60 cases of suspected SIDS, whereas 8 (13%) out of 60 cases were positive for parvovirus B19. In the myocardial

sample of one case, enteroviruses and parvovirus B19 were both found. In all SIDS cases (except one case), the myocardial samples revealed no signs of myocarditis according to the Dallas-criteria using conventional histologic stainings. The PCR-based detection of enteroviruses correlated in seven cases with the detection of enteroviral capsid protein VP 1 (data not shown).

Considering that myocardial infection with enteroviruses causes a myocytopathic effect [4,5] this can be regarded as the cause of death. Fatal myocarditis due to parvovirus B19 is described but these cases seem to be rare [6], only single cases are reported [7]. In pediatric myocarditis cases, enterovirus, cytomegalovirus and adenovirus together accounted for 63% of total cases for whom an etiologic agent could be detected by genome amplification [2].

In the present study, the myocardial tissues had been fixed in neutral buffered formalin (pH 7.4) or in NOTOX (Earth Safe Industries) for a maximum of 48 h. This was crucial for the extraction of intact nucleic acid. From tissues that had been fixed in formaldehyde for months up to years, only highly degraded DNA and RNA could be isolated. In those cases, reproducible PCR-based virus diagnosis was not possible.

The ability to isolate intact viral DNA and RNA from paraffin-embedded tissue permits the analysis of several viruses which cause myocarditis. Our results emphasize the importance of the PCR-based diagnosis in cases of sudden death even when histological examination revealed no serious findings in heart muscle tissue.

References

- [1] J. Ben-Ezra, D.A. Johson, J. Rossi, N. Cook, A. Wu, Effect of fixation on the amplification of nucleic acids from paraffin-embedded material by the polymerase chain reaction, *J. Histochem. Cytochem.* 39 (1991) 351–354.
- [2] A.B. Martin, S. Webber, F.J. Fricjker, R. Jaffe, G. Demmler, D. Kearney, Y.H. Zhang, J. Bodurtha, B. Gelb, J. Ni, T. Bricker, J.A. Towbin, Acute myocarditis: rapid diagnosis by PCR in children, *Circulation* 90 (1994) 330–339.
- [3] P. Cassinotti, M. Weitz, G. Siegl, Human parvovirus B19 infections: routine diagnosis by a new nested polymerase chain reaction assay, *J. Med. Virol.* 40 (1993) 228–234.
- [4] R. Kandolf, M. Sauter, C. Aepinus, J.J. Schnorr, H.C. Selinka, K. Klingel, Mechanisms and consequences of enterovirus persistence in cardiac myocytes and cells of the immune system, *Virus Res.* 62 (1999) 880–884.
- [5] R. Wessely, A. Henke, R. Zell, R. Kandolf, K.U. Knowlton, Low-level expression of a mutant coxsackieviral cDNA induces a myocytopathic effect in culture, *Circulation* 98 (1998) 450–457.
- [6] J.D. Cherry, Parvovirus infections in children and adults, *Adv. Pediatr.* 46 (1999) 245–269.
- [7] K.O. Schowengerdt, J. Ni, S.W. Denfield, R.J. Gajarski, N.E. Bowles, G. Rosenthal, D.L. Kearney, J.K. Price, B.B. Rogers, G.M. Schauer, R.E. Chinnock, J.A. Towbin, Association of parvovirus B19 genome in children with myocarditis and cardiac allograft refection. Diagnosis using the polymerase chain reaction, *Circulation* 96 (1997) 3549–3554.