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Research report

Detection of Borna disease virus p24 RNA in peripheral blood cells from Brazilian mood and psychotic disorder patients

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Abstract

Background: Borna disease virus (BDV) is a virus that naturally infects a broad range of warm-blooded animals. BDV is an enveloped virus, non-segmented, negative-stranded RNA genome and has an organization characteristic of a member of Bornaviridae in the order of Mononegavirale. In the present work we investigated the presence of BDV p24 RNA in peripheral blood cells from 30 psychiatric patients (19 with mood disorder and 11 with psychotic disorder) and 30 healthy volunteers as the control group.
Methods: All subjects were interviewed by structured diagnostic criteria categorized according to the DSM-IV, Axis I (SCID-V). The presence of BDV p24 RNA was investigated by nested reverse transcriptase PCR (RT-PCR) using specific primers to p24 from BDV. The specificity of the detection was analyzed by the sequencing of PCR products.
Results: The mean duration of illness in mood and psychotic patients with p24 RNA of BDV was 25 (± 12.3) years and the median age was 43.77 (± 15.2) years. There were no significant differences in gender and age among patients and control group, neither duration of illness among patients with mood and psychotic disorders in the presence or absence of p24 RNA of BDV. We found a frequency of 33.33% (10/30) of BDV-RNA on patient's group and 13.33% (4/30) on control group. The given sequences revealed identity with GenBank database sequence for BDV.
Conclusion: The detection of a higher level of BDV-RNA in the peripheral blood cells of patients than on control group should help our understanding of the pathogenesis in the disease.

Keywords: BDV; p24 RNA; Mood and psychotic disorders

1. Introduction

Borna disease virus (BDV) is a neurotropic, enveloped, nonsegmented, negative single-stranded RNA virus and causes changes in brain function resulting in disturbed movement and behavior and may be associated with human psychiatric disorders (Amsterdam et

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al., 1985; Chen et al., 1999; Nakamura et al., 2000; Fukuda et al., 2001; Horning and Lipkin, 2001; Bode et al., 2001; Taieb et al., 2001).

The first report of spontaneous canine BDV in Japan (Okamoto et al., 2002) and epidemiological investigations have revealed that BDV may also infect humans (Bode et al., 1993; Waltrip et al., 1995).

The predilection of the virus for the limbic–hypothalamic region and production in some animals of a syndrome somewhat resembling human affective disorders suggested the possibility that BDV may be involved in some human affective disorders (Amsterdam et al., 1985).

BDV is highly neurotropic in natural and experimental hosts. It replicates in neurons and astrocytes without inducing cytopathic effects (Schneider et al., 2005). This virus infects the central nervous system (CNS) of many animal species and may cause behavioral disturbances reminiscent of autism, schizophrenia and mood disorders (Pletnikov et al., 2002; Tomonaga, 2004).

Bode et al. (1993) reported that the proportion of BDV antibody carriers was higher than 30% among patients with major depression. In addition, they also detected a high rate of viral RNA in peripheral blood mononuclear cells (PBMC) derived from psychiatric patients at a high rate by reverse transcriptase-polymerase chain reaction (RT-PCR) (Bode et al., 1995).

Molecular analysis has indicated that BDV genome consists of at least six open reading frames (ORFs). The ORFs encode nucleoprotein (p40), phosphoprotein (p24), transcriptional activator, matrix protein (gp18), envelope protein (p56), and a predicted RNA-dependent RNA polymerase (p180) in 5′ to 3′order (Schneider et al., 1997).

The second open reading frame (ORFII) codes for a 24 kDa protein (also known as p24), representing the putative phosphoprotein. In addition to the p24 protein, the second ORF also produces a 16 kDa protein by translation from the second in-frame AUG codon. This 16 kDa protein has been detected in BDV infected cultured cells and in brains of experimentally infected animals (Ikuta et al., 2002). Among these proteins, BDV-p40 and p24 are found as abundant proteins in BDV-infected brain cells of experimentally and naturally infected animals (Stitz et al., 2002).

Over the past few years, many reports of patients presenting psychiatric symptoms during viral infection were described. Mood disorder patients were reported to have BDV serum antibodies compared to samples from control group without psychotic and mood disorders (Taieb et al., 2001).

Most epidemiological studies employed RT-PCR or RT PCR-nested methodology to trace viral RNA in biological specimens. Thus, in the work we report the major findings regarding a RT-nested-PCR study of Borna disease virus p24-RNA in mood and psychotic disorder in Brazilian patients and healthy control individuals.

2. Methods

2.1. Subjects

The patients were adult outpatients of the Psychiatric Ambulatory of Londrina State University, Paraná, Brazil during the period from 2001 to 2003. Ethics Committee of Londrina State University approved the present study and the subjects signed a written informed consent to be included on the present study.

The subjects were 30 patients with psychiatric disorder from both genders, ranging in age from 18 to 68 years and 30 volunteers control subjects also from both genders ranging in age from 18 to 55 years. They were required to be in good health conditions, defined as the absence of chronic diseases, which affect the immune system as human immunodeficiency virus infection (HIV), hepatitis B and hepatitis C infections. Also, for inclusion in the study the subjects were required not to take immunosuppressive drugs, not to be abusers of alcohol or other dependence substances.

The 30 patients were interviewed by structured diagnostic criteria categorized according to the criteria of the fourth edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders or DSM-IV, Axis I, (SCID-IV) translated into Portuguese by Del Ben et al. (2001). The patients were 19 with mood disorders (major depressive disorder) and 11 psychotic disorders (schizophrenic and schizoaffective). The patients with major depressive disorder were recurrent, without psychotic, catatonic, melancholic, atypical or postpartum onset.

The 30 volunteers were recruited from the community, they were free of any serious medical illnesses, and had never taken psychotropic drugs or presented current or past psychiatric disorders as determined by their reported history during the clinical interview and also by structured diagnostic criteria categorized according to the DSM-IV, Axis I.

2.2. Molecular diagnosis of BDV

2.2.1. Nucleic acid preparation and reverse transcriptase

Leukocytes were prepared from peripheral blood samples after using eritrocytes lysis buffer (0.32 M sucrose; 10mM Tris–HCl pH-7.5; 5 mM $MgCl_2$; 1% Triton X-100). Total cellular RNA was extracted from peripheral white blood cells with Trizol (Trizol LS, Invitrogen, USA) according to the manufacturer's instructions. The RNA was resuspended in 20 μl of sterile water treated with diethylpyrocarbonate (DEPC, Invitrogen). cDNA was generated from 6 μl of total RNA, using specific outer antisense primer and a first strand cDNA synthesis kit (Perkin Elmer GeneAmp RNA PCR kit, Perkin Elmer, USA).

2.2.2. Nested—polymerase chain reaction (PCR)

Four BVD specific primers were used for the amplification of BDV cDNA. The BDV primers were designed based on BDV genome sequence (GenBank—Accession: NC 001607) and were targeted to amplify the p24 protein from BDV genome (BDV1 outer sense 5′ TGACCCAACCAGTAGACCA 3′; BDV2 outer antisense 5′ GTCCCATTCATCCGTTGTC 3′; BDV3 inner sense 5′ TCAGACCCAGACCAGCGAA 3′; BDV4 inner antisense 5′ AGCTGGGGATAAATGCGCG 3′). Reaction conditions for both PCR rounds were the same (20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP and 1.25 unit of Taq polymerase) and consisted of an initial denaturizing step of 94 °C for 5 min followed by 40 cycles of 94 °C, 60 °C and 72 °C for 1 min each and a final extension of 72 °C for 10 min on thermocycling (PCR sprint ThermoHybaid from Biosystems of Brazil). Conditions for the second PCR was the same except for the annealing temperature that was 57 °C. PCR products of 354 base pairs were detected by electrophoresis on a 10% acrylamide gel visualized by silver staining and on a 2% agarose gel visualized by UV fluorescence after staining with ethidium bromide.

2.2.3. Cloning and sequencing

The specificity of the p24-RNA detection was confirmed by fragment cloning and sequencing (MegaBace™-Pharmacia). The PCR products were linked into pGEM-T Easy Vector plasmid (Promega, Madison, USA), transformed into *Escherichia coli* DH5α (Inoue et al., 1990) and sequenced using DYEnamic™ ET dye terminator cycle sequencing kit (Amersham Pharmacia Biotech, USA) in a MegaBace™ sequencer (Amersham Pharmacia Biotech, USA). The resulting sequences were analyzed by comparison with GenBank database.

2.3. Statistical analysis

Data were analyzed by Student's test by Micronal Origin 4.1 Program with the level of significance set at $p < 0.05$.

3. Results

There were no group differences in age, gender and ethnic group between patients and controls (Table 1).

A total of 30 psychiatric disorder patients were examined for BDV RNA in their blood peripheral cells. When the nested RT-PCR technique was applied for the detection of BDV related RNA, clear positive signals were detected in 33.33% (10/30) of the samples.

The mean duration of illness in mood and psychotic patients with p24 RNA of BDV was 25 (± 12.3) years and the median age was 43.77 (± 15.2) years.

From the 30 healthy individuals involved in this study, the p24 BDV-RNA could be detected in only 4, representing 13.33% of the group.

Peripheral blood cells RNA were screened for the presence of BDV p24 sequence, by nested RT-PCR as described in Section 2. Specificity of the amplification products was demonstrated by DNA sequencing. To confirm the presence of BDV RNA, RT-nested PCR was repeated for all the positive samples, and in some cases RNA from patients blood was obtained for the second time and also tested.

We cloned all positive PCR samples, from patients group, into pGEM-T Easy and sequenced in a MegaBace™ sequencer, then compared these sequences to GenBank database. All resulting

Table 1
Demographic characteristics of patients and controls

Characteristic	Patients ($n=30$)		Control ($n=30$)
	Mood disorders	Psychotic disorders	
Gender			
Male	04	07	11
Female	15	04	19
Ethnic group			
Asian	01	02	03
Caucasian	18	19	27
Age[a] (S.E.)	37 (± 3.2)	34 (± 6.0)	30.2 (± 2.11)

[a] Median.

sequences revealed more then 98% identity with GenBank Borna disease virus.

4. Discussion

Nowadays, BDV has been observed in a variety of animal species including cats, dogs, cattle, sheep and horses. In addition, the potential role of BDV as a pathogen in human psychiatric diseases has increased the interest in the investigation of this virus and its pathogenenic pathways. Molecular biological analysis revealed important data, such as, type of genomic nucleic acid, genomic organization and BDV specific proteins.

In this study, we investigate the presence of p24 RNA of BDV in mood and psychotic disorders patients and healthy control subjects. We did not verify significant differences in the presence of RNA p24 between males and females. The incidence of p24 RNA of BDV in patients (33.33%) was similar to those (37% and 38.5%) reported by Kishi et al. (1995) and Sauder et al. (1996) respectively.

Normally, products of RT-PCR are detected in agarose gels, but all products of this work were detected in acrylamide gels, which are more sensitive than agarose for this detection.

Despite controversy about potential association with human neuropsychiatry illnesses, BDV affords an intriguing model for the study of these illnesses. Neonatal BDV-infected rats display neurodevelopment, physiologic, and neurobehavioral abnormalities that closely parallel some of the main features associated with several human mental disorders (de la Torre., 2002).

For over two centuries, Borna disease (BD) has been described as a sporadically occurring infectious meningoencephalomyelitis affecting horses and sheep in Central Europe. Over the last decade, the BD epidemiology has been discussed. Firstly, its geographical distribution seems larger than what was previously thought. Secondly, the disease can affect a large number of warm-blooded animal species, including humans. BDV genome has recently been detected in France in the blood and brain of several animal species (horses, bovines, foxes) (Dauphin et al., 2002). Many reports of patients presenting psychiatric symptoms have been made during viral infections. Between 4% and 16.7% of mood disorder patients were reported to have BDV serum antibodies compared to 0% to 4.7% in various control samples without psychiatric disorders (Taieb et al., 2001). In United Kingdom farming populations, some evidences showed that they are exposed to BDV, but no evidence was found that exposure to BDV was associated with poor mental health (Thomas et al., 2005). Brazil is a geographically vast country of continental dimension with several regions where farms with several animal species of bovines and calves are concentrated. We verified that most of our patients live on farms or near them, and all patients positive for p24 RNA BDV, still live or had lived in farms during a period of their lives. Evidence has revealed that BDV uses a unique strategy in its transcription and replication and directly affects cellular functions of infected central nervous systems. BDV research will provide new insights not only into the biology of neurotropic RNA virus but also into neuropsychiatry (Tomonaga et al., 2002).

Hans et al. (2004) showed how a persistent viral infection could selectively interfere with the response of neurons to a neurotrophin without causing any cell death. This illustrates a novel aspect of virus/neuron interactions that may be relevant to the pathogenesis of neurobehavioral diseases.

BDV is now gaining much of the research attention, because the disturbances found in animals resemble those of neuropsychiatric disorders in humans. These observations raise the possibility that BDV infection may be associated with certain human psychiatric disorders. Serological and molecular studies on many samples from human patients with a variety of psychiatric disorders have been performed. Some reported the presence and elevated levels of serum antibodies to BDV (Bode et al., 1992; Terayama et al., 2003). Others reported the presence of BDV-RNA or BDV-antigens in the peripheral blood samples as well as in autopsied brains (Iwata et al., 1998; de la Torre et al., 1996). Taken together these data support the possibility of human infection with BDV. On the contrary, others reported the complete absence of such BDV-markers from their samples, supporting the absence of a link between BDV infection and psychiatric disorders as well as excluding it as a human pathogen (Lieb et al., 1997). Recently, Chalmers et al. (2005) presented a systematic review on BDV and the evidence for human pathogenicity concluding that, although agreed gold standard tests and evidence for test specificity are lacking, there is evidence that humans are exposed to the virus. For the first time in Brazil, our group verified the presence of p24 BDV-RNA in 33.33% of mood and psychotic disorder patients. It has been reported that BDV RNA was detected in the brains of both psychiatric patients and healthy individuals (Haga et al., 1997). We also identified p24 BDV-RNA with a prevalence of 13.33% in healthy donors. Therefore further studies are necessary to clarify the role of BDV infection in the etiology and pathogenesis of mood and psychotic disorders.

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