Two neuropsychiatric cases seropositive for bornavirus improved by ribavirin

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Two neuropsychiatric cases seropositive for bornavirus improved by ribavirin

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Summary:

While we had previously detected anti-bornavirus antibodies via radioligand assay in psychiatric patients, we had not examined the viral pathogenicity in these individuals. Herein, we present two psychiatric patients who were seropositive for bornavirus and whose treatment-resistant symptoms improved by oral administration of ribavirin, a broad-spectrum antiviral agent. Cerebrospinal fluid analysis indicated that the ribavirin affected the central nervous system of these patients. Ribavirin ameliorated intermittent involuntary head shaking, which is reminiscent of a symptom observed in bornavirus-infected animals. Using radioligand assays to examine the serial sera of these patients, we found a relationship between the titers of anti-bornavirus antibodies and the change in the patients’ symptoms. Our findings suggest there is a relationship between bornavirus and human symptoms and that ribavirin may be useful in suppressing chronic bornavirus infection in some neuropsychiatric patients. However, the possibility remains that some other known or unknown virus other than bornavirus, which is sensitive to ribavirin, may have caused the symptoms. Additional evidence that directly indicates the causative relationship between bornavirus and human symptoms is needed before establishing the pathogenesis and treatment for human bornavirus infection.
Introduction

Bornavirus—a negative stranded non-segmented RNA virus—was originally studied as a pathogen for Borna disease, an epidemic meningoencephalitis in horses in southwest Germany. Thereafter, it has been proven to cause central nervous system diseases in a variety of vertebrate species worldwide (1,2). In 1985, bornavirus specific antibodies were found in psychiatric patients, especially those with affective psychoses (3,4). This lead to intense investigation of the relationship between bornavirus and human psychiatric diseases.

Interestingly, several new bornavirus strains have been discovered in birds since 2008 (5,6). In 2015, a new strain was detected in the brains of three squirrel breeders who died of acute encephalitis and in their squirrels (7). Although this recent evidence indicates that some bornaviruses can be pathogenic to humans, the pathogenicity of the original bornavirus strains among humans has not yet been elucidated. This is due to lack of standard diagnostic methods for detecting bornavirus infection in humans.

We have previously detected bornavirus antibodies in humans using a highly sensitive and specific radioligand assay (8). Specifically, we detected either anti-bornavirus IgG, IgM, or IgA in approximately 20% of both Japanese psychiatric and control subjects (9). The positive rates were 3.5% in both groups if only samples with high titers were selected. Thus, we concluded that the Japanese population consisting of healthy and psychiatric individuals is exposed to bornavirus or a related
virus. Although most of the infections were speculated to be asymptomatic, bornavirus could have a pathogenic role in other vulnerable humans, especially those with compromised immunity.

We have also seen patients with anti-bornavirus antibodies having unexplainable and treatment-resistant symptoms such as head shaking, acute onset amnesia, severe fatigue, and somatic pain. Ribavirin—a broad spectrum anti-viral agent—has been reported to suppress bornavirus infections both in in vitro (10) and in vivo (11,12) conditions. We therefore previously performed a clinical trial of oral ribavirin administration in nine patients, and reported significant improvement in two of the patients (13,14). In the present study, we further examined the serial sera of two patients with anti-bornavirus antibodies using the radioligand assay.

Materials and Methods:

The antibody examination and ribavirin treatment were both approved by the research ethics committee of Osaka General Medical Center and the studies were performed according to the Declaration of Helsinki. All the patients examined for anti-bornavirus antibodies were those visiting the Department of Psychiatry, Osaka General Medical Center. The IgG, IgM and IgA titers against two bornavirus antigens, nucleoprotein and phosphoprotein, were assayed by radioligand assay as previously described (8,9). Blood samples were stored at 4°C after sampling, and the sera were divided into several tubes
and stored at -80°C on the day of sampling. In the examination of the serial samples, sera which had not been melted and refrozen were used.

Serial samples were simultaneously measured in the same 96-well plates. Each sample was tested on 2 plates in quadruplicate wells. The intra-assay coefficient of variation of 4 results for each sample in 1 plate was 6.2%, and the inter-assay coefficient of variation of 2 results for each sample in 2 plates was 7.8%. The cut-off point for anti-bornavirus-phosphoprotein IgG was the index value of 3.33 (mean + 4 SD for negative samples).

The dose of ribavirin was decided according to the standard hepatitis C treatment regimen in Japan (i.e. 800 mg per day). If body weight was above 70 kg, 1,000 mg per day was later allowed. The blood cell count was performed frequently to monitor the erythrolytic anemia. The serum concentration of ribavirin was examined by a commercial laboratory.

Results:

Case 1:

A 43-year-old man experienced an acute schizophrenic episode with auditory hallucinations, excitement, and delusion of observation. After 6 weeks of treatment with an oral daily dose of 1.5–3.0 mg haloperidol, his psychotic symptoms eased. Although the acute schizophrenic symptoms recurred one year later, the patient was successfully
re-treated with haloperidol.

After the second psychotic episode, the patient began experiencing intermittent involuntary head shaking with no other psychiatric symptoms once or twice within the year. Head shaking occurred intermittently for a few days and was always accompanied by stressful events, such as business trips. He did not experience any abnormal movements in the absence of stressful events, even while continuously taking small amounts of antipsychotics.

When he was 51 years old, intermittent involuntary head shaking lasting for a minute to several hours began to occur almost every day, even in the absence of stressful events. The shaking included various movements, such as circumnmutating, nodding, and head rotation, sometimes with a frequency around 3 Hz. A serological examination found the presence of anti-bornavirus phosphoprotein IgG.

One and a half years after frequent head shaking, the patient consented to treatment with oral ribavirin. Two weeks after the initiation of ribavirin (800 mg per day), the frequency of his head shaking decreased (Fig. 1A). After 6 weeks of treatment, his white blood cell count increased to 21,600/mm$^3$, and ribavirin administration was stopped. The patient’s head shaking was almost completely suppressed until a year later when these symptoms returned. At that time, ribavirin was administered at a reduced dose of 600 mg per day for 7 weeks. Despite this, his symptoms persisted. The dose was increased to 800 mg per day, which improved head shaking. This time, the increase in
his white blood cell count was mild and transient with mild to moderate anemia. After 8 months of taking 800 mg ribavirin per day, we increased his dose to 1000 mg per day and continued for 9 weeks, with no further improvement observed. Soon after cessation of ribavirin administration, the patient’s head shaking gradually increased and plateaued after 6 months. During this time, the head shaking began to occur during night-time hours. Other drugs including amantadine did not decrease or increase these symptoms (Fig. 1B). Magnetic resonance imaging revealed no abnormalities.

Case 2:

A 38-year-old woman, stressed from her work for a month, suffered from sudden onset amnesia while napping at home after working a night shift. Upon awakening, she became confused and asked her family “what day is it?” and “what am I doing here?” every five minutes. She was admitted to a neighboring hospital. Magnetic resonance imaging, single photon emission computed tomography scan, cerebrospinal fluid examination and blood examinations showed no obvious abnormalities. Low-grade fever continued and amnestic symptoms slightly improved when she was discharged three weeks later.

Arriving at our hospital one month after discharge, she was depressed, severely fatigued, and spent all day on her bed. For more than six months, she recalled a stressful event which occurred ten years before her admittance, and repeatedly discussed it with strong, guilty feelings every time she visited our hospital. Sometimes she could not
recall what she had done the day before. Low-grade fever in the evening persisted and she often had a severe headache. These symptoms did not improve markedly for more than one and a half years (Fig. 2A, B). Amantadine, imipramine, and lithium carbonate were not effective. Antibody against bornavirus phosphoprotein was found in her serum.

One year and 9 months after the onset of the disease, ribavirin treatment (800 mg per day) was prescribed to her. After two and a half weeks, she had an episode where she scolded her daughter for misbehaving. The daughter was surprised at her rapid response, because her mother had been subdued for nearly two years. After 12 weeks’ treatment, depressive mood, sub-fever and general fatigue considerably improved. After 17 weeks, 200 mg per day of amantadine was added. Following this, she could do daily jobs of housekeeping without taking bed rest, and she could recall where she had parked the car after shopping. A slight and transient itching was the only adverse effect of ribavirin in this patient.

Ribavirin administration was stopped after 28 weeks. General fatigue reappeared transiently for several weeks as she recovered. Thereafter, she experienced recurrence of infrequent, general mild fatigue. This was accompanied by sore throat and disappeared within days or weeks. Several years following the cessation of ribavirin treatment, amnesia and depressive mood did not worsen.

**The serial examination of the specific antibodies:**

Results of the specific antibodies against bornavirus-phosphoprotein in serial sera of the two
cases are shown in Fig. 1C and Fig. 2C.

Discussion

In both patients, the cause of the treatment-resistant symptoms were not identified. In Patient 1, the intermittent head shaking observed was different from the involuntary movements typically associated with Parkinsonism, chorea, or neuroleptics. Patient 2 showed sudden onset amnesia, followed by depression, severe fatigue, low-grade fever, and headache. Sudden onset amnesia with gradual but incomplete recovery could be caused by various conditions such as cerebrovascular events, head trauma, and infection. However, physical examinations did not reveal any of these obvious abnormalities.

Ribavirin is a relatively safe antiviral agent and its common side effects are erythrollytic anemia and itching. A more hazardous side effect is teratogenicity, which can occur in both men and women during and 6 months after the treatment. Ribavirin is known to suppress various viruses including hepatitis (B and C), influenza, Lassa fever, herpes simplex, herpes zoster, measles, and respiratory syncytial virus. Although we did not examine for the infections by these viruses, none of them are thought to have caused the symptoms seen in the two cases.

Ribavirin has been reported to suppress bornavirus both in \textit{in vitro} (10) and \textit{in vivo} (11,12) conditions. Partial and complete suppression of bornavirus can be obtained with ribavirin at a concentration of 1 μg/mL and 10 μg/mL, respectively (10). In Patient 2,
the ribavirin concentration in the cerebrospinal fluid after 28 weeks of ribavirin treatment was 1.5 μg/mL, which was above the threshold of partial suppression. Her serum concentration was 2.56 μg/mL. Although we did not examine the cerebrospinal fluid concentration of ribavirin in Patient 1, his serum concentration of ribavirin after 6 months of treatment was 3.37 μg/mL. According to this trend, the concentration of ribavirin in the central nervous system in Patient 1 should have reached a level that could partially suppress bornavirus.

In both cases, symptoms improved 2 weeks after the initiation of ribavirin treatment. Because serum concentration of ribavirin will plateau after 4 weeks of oral administration, it is reasonable that the concentration in the central nervous system will reach the level of partial suppression of bornavirus two weeks after the initiation of the treatment. In Patient 1, after the first 6 weeks’ treatment with ribavirin, the frequency of head shaking reduced and the duration of head shaking shortened from hours to minutes; this was a dramatic improvement. In this patient, suppression lasted for a year. A second round of ribavirin treatment (600 mg per day) did not ameliorate the symptoms, and an increased dose of 800 mg per day was required. This lead to a reduction in head shaking, but the symptom gradually worsened soon after the cessation of the treatment. These observations strongly suggest that ribavirin reduced psychotropic agent-resistant symptoms.

The changes of the specific antibody titres reflect the viral activity though it is not
directly. The sedation of virus and disappearance of antigens are followed by decrease of antibody titres, whereas, reactivation of the virus leads to increase the titers. In our radioligand assay, results are obtained numerically, and if exact volumes of sera and antigens are added to each well of the same plate, changes in titers can be detected. The serial antibody examination of the two patients also supported the relationship between symptoms and viral activities. In Patient 1, the gradual decrease in antibodies after the first treatment trial and the subsequent gradual increase in antibodies coincided with the amelioration and relapse of the patient’s head shaking (Fig. 1C). Consistent high titer levels during the second ribavirin treatment might indicate insufficient suppression of chronic bornavirus activity or reflect repetitive exposure to viral antigens. In Patient 2, the specific antibodies decreased during and after the treatment, which also coincided with her clinical course (Fig. 2C). The gradual decrease in the specific titer levels might be due to the chronic bornavirus activity and continuous exposure to viral antigens after the onset of the symptoms.

Bornavirus-infected horses show various neuro-behavioral symptoms including head shaking (1), frequent head nodding, and tonic deviation of the head and neck (15). Similarly, bornavirus-infected cats also experience head shaking (16), whereas experimentally infected cats turn their heads with a twitchy movement (17). The similarity of symptoms suggests that there might be a link between our patient’s head shaking and exposure to bornavirus or a related virus.
Bechter et al. (18) used indirect immuno-fluorescence assays to detect the specific antibodies. They demonstrated that psychiatric and neurological populations less than 50 years old had higher seropositivity than control populations: 6.02% (n=1312), 3.7% (n=653) vs. 2.2% (n=276), respectively. In people older than 50 years, the seropositivities were 5.73%, 5.54%, 4.8%, respectively (18). They then speculated that bornavirus might have causal relation to some of the seropositive patients in younger populations. They examined cerebrospinal fluid in 38 seropositive psychiatric patients younger than 50 and found elevated bornavirus specific antibodies in cerebrospinal fluid in 10 patients (19). They successfully treated one acute schizophrenic patient and two treatment-resistant major depressive patients by cerebrospinal fluid filtration—a method to treat Guillain-Barré syndrome (20,21). The specific serum antibodies of the schizophrenic patient increased from 1:20 up to 1:160 in the first 5 weeks, and the specific antibody was also found in his cerebrospinal fluid. They reported that the negative symptoms and suicidal thoughts, which remained after pharmacotherapy, dramatically improved. The two depressive patients also had physical symptoms suggesting mild encephalitis. One of them showed reduced short-term memory, low performance, headache and depressive mood with guilt feelings; the combination of these symptoms markedly resembles those observed in Case 2 of our study.

On the other hand, Bode et al. (22) detected the specific antibodies, circulating immune-complexes, and antigens with their original enzyme immunoassay in the
human sera. They focused on affective disorders and reported that amantadine was effective for acute major depression and bipolar depression (22). Although amantadine was not apparently effective in the two cases, we continued the administration of this drug after the cessation of ribavirin treatment because other drugs that suppress the bornavirus are not available. Whether amantadine prevented the relapse of the patients’ symptoms is not fully elucidated.

The phenotypes of bornavirus in animals are various according to the species of animals, genetic background, the age of infection, and other factors. For example, experimentally infected adult rats exhibit cell-mediated encephalitis, whereas, experimentally infected newborn rats do not develop encephalitis but show social and learning disability with continuous infection in the central nervous system (23,24). Although the pathogenesis of bornavirus to human has not yet been confirmed, it is possible that its clinical phenotypes might be varied as suggested by Bechter et al. (18).

The major limitation of our study was the lack of direct evidence for the causal relationship between the symptoms and the infection of bornavirus. We have not ruled out the possibility that some other known or unknown ribavirin-sensitive virus might have caused the symptoms. It is therefore necessary to carefully evaluate the pathogenesis of bornaviruses in humans to decipher their disease-related effects, particularly in those affected with mental-illness.
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Conflict of interest

None to declare.
References


Figure Legends

Fig. 1. The symptoms, pharmacotherapy and the antibody titres of Patient 1

A: The frequency of head shaking noted for Patient 1. Days were divided into 4 time periods: wake-up time to noon, noon to 6 PM, 6 PM to bedtime, and during sleep. The frequency of the day was rated from 0 to 4 according to the number of the time period of the day in which the head shaking occurred. Each bar indicates the score of each day. B: Doses of the drugs during the treatment. C: The serial examination of the titers of anti-bornavirus-phosphoprotein antibody.

Fig. 2. The symptoms, pharmacotherapy, and the antibody titres of Patient 2

A: The course of the symptoms for Patient 2. Memory disturbance was evaluated with Wechsler Memory Scale-Revised (WMS-R) and Rivermead Behavioral Memory Test (RBMT). General fatigue was evaluated with Performance Status scored from 0 to 9. B: Doses of the drugs administered during treatment. C: The serial examination of the titers of anti-bornavirus-phosphoprotein antibody.
Fig. 1

A) Frequency score

B) Ribavirin:
- 800mg

Haloperidol:
- 4.5mg
- 3mg
- 2.25mg

Risperidone:
- 1mg
- 2mg

Biperidene:
- 6mg
- 3mg
- 5mg
- 2mg
- 6mg
- 3mg

Amantadine:
- 150mg

C) Index value

- plate 1
- plate 2
- plate 3

(time)
Fig. 2

A  
Amnesia:

Depressive Mood:

General Fatigue:

Performance Status:  7~8  5~6  3~4  1~2

B  
Ribavirin:

Amantadine:  100mg  200mg  800mg

Imipramine:  50mg  100mg  25mg

Lithium:  400mg

C  
Index value

Index value

cutoff point

plate 1  plate 2

(time)