We have previously detected bornavirus antibodies in humans. Diagnostic methods for detecting bornavirus infection in yet been elucidated. This is due to the lack of standard of the original bornavirus strains among humans has not viruses can be pathogenic to humans, the pathogenicity Although this recent evidence indicates that some bornavirus- strain was detected in the brains of 3 squirrel breeders discovered in birds since 2008 (5,6). In 2015, a new virus—was originally studied as a pathogen for Borna disease, an epidemic meningoencephalitis in horses in southwest Germany. Subsequently, 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 an 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 3 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 the 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 vitro (10) and in vivo (11,12). We therefore previously performed a clinical trial of oral ribavirin administration in 9 patients and reported significant improvements in 2 of the patients (13,14). In the present study, we further examined the serial sera of 2 patients with anti-bornavirus antibodies using a radioligand assay.

INTRODUCTION

Bornavirus—a negative-stranded non-segmented RNA virus—was originally studied as a pathogen for Born disease, an epidemic meningoencephalitis in horses in southwest Germany. Subsequently, 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 an 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 3 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 the lack of standard diagnostic methods for detecting bornavirus infection in humans.

SUMMARY: While we previously detected anti-bornavirus antibodies via radioligand assay in psychiatric patients, we did not examine the viral pathogenicity in these individuals. Herein, we present 2 psychiatric patients who were seropositive for bornavirus and whose treatment-resistant symptoms improved after oral administration of ribavirin, a broad-spectrum antiviral agent. Cerebrospinal fluid analysis indicated that 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 infection 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 that is sensitive to ribavirin may have caused the symptoms. Additional evidence that directly indicates the causative relationship between bornavirus infection and human symptoms is needed before establishing the pathogenesis and treatment for human bornavirus infection.

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 2 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. For 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 standard deviations [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. A blood cell count was performed frequently to monitor 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 a daily oral dose of 1.5–3.0 mg haloperidol, his psychotic symptoms eased. Although the acute schizophrenic symptoms recurred 1 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 circumnutation, 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/mm³, 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 1,000 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, having experienced work stress 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 5 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. The low-grade fever continued and amnestic symptoms slightly improved when she was discharged 3 weeks later.

Arriving at our hospital 1 month after discharge, she was depressed, severely fatigued, and spent all day in her bed. For more than 6 months, she recalled a stressful event which occurred 10 years before her admittance and repeatedly discussed it with strong, guilt 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 1 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 2 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 2 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.

![Fig. 2. The symptoms, pharmacotherapy, and the antibody titer 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.](image-url)
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 a sore throat and disappeared within days or weeks. Several years following the cessation of ribavirin treatment, amnesia and depressive mood did not worsen.

**Serial examination of the specific antibodies:**
Results of the specific antibodies against bornavirus-phosphoprotein in serial sera of the 2 cases are shown in Figs. 1C and 2C.

**DISCUSSION**

In both patients, the cause of the treatment-resistant symptoms was 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 erythrolytic 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 infections by these viruses, none of them are thought to have caused the symptoms seen in the 2 cases.

Ribavirin has been reported to suppress bornavirus both in vitro (10) and in vivo (11, 12). 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 to assume that the concentration in the central nervous system will reach the level of partial suppression of bornavirus 2 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. The 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 in the specific antibody titers reflect the viral activity although it is not directly related. The sedation of virus and the disappearance of antigens are followed by a decrease in antibody titers, whereas reactivation of the virus leads to an increase in titers. In our radioligand assay, results were obtained numerically, and if exact volumes of sera and antigens were added to each well of the same plate, changes in titers could be detected. The serial antibody examination of the 2 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 neurobehavioral 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 immunofluorescence 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 = 1,312), 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 further speculated that bornavirus infection might have a 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 the cerebrospinal fluid of 10 patients (19). They successfully treated 1 acute schizophrenic patient and 2 treatment-resistant major depressive patients by cerebrospinal fluid filtration—a method used to treat Guillain-Barré syndrome (20, 21). The specific serum antibodies of the schizophrenic patient increased from 120 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 2 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 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 2 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 entirely clear.

The phenotypes of bornavirus in animals vary 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 disabilities with continuous infection in the central nervous system (23,24). Although the pathogenesis of bornavirus to humans has not yet been confirmed, it is possible that its clinical phenotypes might also 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 bornavirus infection. 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 by mental illness.

Acknowledgments This work was supported by a Grant-in-Aid for Community Health and Medical Care from the Ichou Association for Promotion of Medical Science.

Conflict of interest None to declare.

REFERENCES