Clinical Outcomes of Japanese Encephalitis after Combination Treatment of Immunoglobulin, Ribavirin, and Interferon-α2b

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INTRODUCTION

Japanese encephalitis (JE) is one of the most devastating viral encephalitis caused by Japanese encephalitis virus (JEV), which is a single-stranded, positive-sense RNA virus that is mainly spread by mosquito (Culex tritaeniorhynchus) bites.1 Currently there is no specific antiviral treatment for JE, and JEV causes 35,000–50,000 cases of JE and 10,000 deaths per year.2 Approximately 30% of JE patients die, and half of the patients who survive suffer from severe neurological sequelae that cause functional disability.3 Several treatment regimens have been suggested, including steroids, immunoglobulin, antiviral drugs, and interferon, but none of them have been demonstrated to be sufficiently effective to cure the disease.3,4 There is an urgent need for an effective treatment for JE, especially in endemic areas including South Korea, where the incidence of JE has increased since 2010 and

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was highest during 2015, especially among those older than 40 years who were not candidates for a nationwide vaccination program. Considerable economic and social resources of the Korean public health system are used to deal with the disease burden of JE despite the presence of a well-established vaccination program.5

In cases of West Nile virus, which is a member of the Flaviviridae family and Flavivirus genus, and similar to JEV, several antiviral agents including purine analogs (e.g., ribavirin) and interferon have been found to exhibit some efficacy in the laboratory setting,6 with immunoglobulin showing efficacy in animal studies.7 Among other microorganisms that are also very challenging to treat, combination treatment of ribavirin and interferon had been the standard treatment of choice for hepatitis C virus (HCV) infection until 2016, and there are several reports of combination treatment of ribavirin and interferon being associated with improved survival and clinical outcomes in MERS-CoV.8,9

Considering the urgent need to manage the fatal infectious disease JE in the absence of standard guidelines for treatment, we aimed to determine the outcomes of applying combination treatment of immunoglobulin, ribavirin, and interferon-α2b to patients with JE. Here we describe the clinical presentations, laboratory findings, clinical outcomes, and adverse events after combination therapy in patients with JE.

**METHODS**

**Patient enrollment**

Data were collected and reviewed from a prospective cohort of encephalitis patients who were admitted to Seoul National University Hospital between August 1, 2010 and October 31, 2019. The patients had all undergone diagnostic studies, including routine neurological examinations, brain magnetic resonance imaging (MRI) (or computed tomography if MRI was not available), workups for infectious and metabolic etiologies, and lumbar punctures. We identified patients in the cohort data set as being diagnosed with JE if laboratory tests confirmed the presence of JEV-specific immunoglobulin M antibody in serum or cerebrospinal fluid (CSF) samples. The test for the antibody was conducted by the Korea Disease Control and Prevention Agency (KDCA). The patients diagnosed with JE were considered candidates for combination treatment of immunoglobulin, ribavirin, and interferon-α2b, and their clinical presentations, laboratory findings, clinical outcomes, and adverse events were analyzed retrospectively for this study. The study protocol was approved by the Seoul National University Hospital Institutional Review Board and followed the principles of the Declaration of Helsinki (IRB number: 1910-132-1072).

**Treatment regimens**

The use of combination treatment of intravenous immunoglobulin (IVIG), oral ribavirin, and subcutaneous interferon-α2b was initiated as soon as the KDCA report suggested laboratory confirmation of the diagnosis of JE and if the neurological symptoms and signs including altered mental state, confusion, and brainstem signs such as diplopia could not be explained by an etiology other than JEV infection. Another infectious etiology (another virus, bacterium, or fungus) and vascular, inflammatory, and tumorous etiologies also needed to be ruled out to diagnose with JE.

Patients with JE received intravenous human immunoglobulin G for 5 consecutive days at a dosage of 400 mg/kg/day. Ribavirin (Viramid, Ilsung Pharmaceuticals, Seoul, Korea) was administered orally at a dosage of 400 mg every 12 h for 14 consecutive days. Interferon-α2b (Intron A, Merck & Co, Kenilworth, NJ, USA) was given by subcutaneous injection at a dosage of 3 million units per day for 2 weeks. Some of the patients with JE did not receive all components of the combination therapy due to medical contraindication, economic issues, or personal rejection. All patients received appropriate supportive management as required, including intravenous hydration, nutritional support, oxygenation, mechanical ventilation, or antibiotics, and intensive care.

**Outcomes**

The responses to combination treatment of IVIG, oral ribavirin, and subcutaneous interferon-α2b were categorized into three subgroups: complete, partial, and no recovery. Complete recovery was defined as complete recovery of the patient to their previous functional status. Partial recovery was defined as any improvement in functional status as measured by the modified Rankin Scale (mRS) score at discharge compared with the initial mRS score or as any improvements in the level of consciousness measured by the Glasgow Coma Scale (GCS), symptoms, and signs confirmed by neurological examinations performed by two neurologists independently during the hospital stay. No recovery was defined as no improvement, aggravation of the functional status as measured by the mRS, or death.

**Adverse events**

Adverse events related to IVIG, oral ribavirin, or subcutaneous interferon-α2b were identified and assessed based on the Common Terminology Criteria for Adverse Events (version 5.0).10
RESULTS

Eleven patients diagnosed with JE were included in the study (Table 1). The brain MRI findings of the patients corresponded with those of JE (Figs. 1A, B, C, G, H, and I), and the diagnosis of JE in those patients was confirmed by the antibody test conducted by the KDCA. The median age of the patients was 61 years (range 47–87 years), and five patients were male. Eight patients received combination treatment of IVIG, oral ribavirin, and subcutaneous interferon-α2b according to the common regimen described in the Methods; these patients also received supportive management. Three patients received supportive management only due to their guardians’ decision or medical contraindication to the combination therapy.

The clinical features, laboratory findings, and functional outcomes are listed in Table 1. The most common initial symptom was confusion, while fever, headache, and altered mentality were also observed as initial manifestations. The initial median mRS score was 5 (range 1–5), and the median mRS score at discharge was 4 (range 1–5). Follow-up data at discharge from the hospital were obtained for all patients, and the median hospital stay was 27 days (range 13–181 days).

Four of the eight patients (50%) treated with the combination therapy showed a partial response, while one patient (12.5%) whose initial symptoms and signs included drowsiness, headache, and fever showed complete recovery. These five patients had mRS scores ranging from 0 to 5 at discharge, and the patient with complete recovery had no neurological sequelae at discharge. Among those who showed partial recovery, Patients 2, 4, and 7 showed improved mRS scores, and the level of consciousness of Patient 6 improved during hospitalization, from a drowsy state to a drowsy state in Patient 4 and in awareness in Patient 7, and Patient 2 showed not only an improved mRS score but also improvements in CSF results and follow-up MRI findings. Additionally, radiological improvement was achieved in two patients (Patient 2 and 5) in serial follow-up MRI examinations compared with their initial MRI examinations (Figs. 1D, E, F, J, K, and L). Even though the other three patients (37.5%) showed no recovery, there were no deaths during the hospital stay. During combination treatment of immunoglobulin, ribavirin, and interferon-α2b, two patients (Patient 4 and 8) experienced hemolytic anemia of grade 3 that was related to ribavirin and a febrile reaction to IVIG, respectively.

Among the three patients who received supportive management only, one patient (33.3%) showed partial recovery at discharge, with the mRS score improving from 5 to 3, while the other two patients (66.7%) showed no recovery at discharge. Among those who showed partial recovery, two patients (33.3%) showed partial recovery at discharge. Among those who showed partial recovery, two patients (33.3%) showed partial recovery at discharge. Among those who showed no recovery, there were no deaths during the hospital stay from comatose (GCS score=E1VtM1) to drowsy state in Patient 4 and in awareness in Patient 7, and Patient 2 showed not only an improved mRS score but also improvements in CSF results and follow-up MRI findings. Additionally, radiological improvement was achieved in two patients (Patient 2 and 5) in serial follow-up MRI examinations compared with their initial MRI examinations (Figs. 1D, E, F, J, K, and L). Even though the other three patients (37.5%) showed no recovery, there were no deaths during the hospital stay. During combination treatment of immunoglobulin, ribavirin, and interferon-α2b, two patients (Patient 4 and 8) experienced hemolytic anemia of grade 3 that was related to ribavirin and a febrile reaction to IVIG, respectively.

Five of the eight patients (62.5%) treated with the combination therapy in our study showed complete or partial recovery; four of these patients showed an improved mRS score at discharge, and the other patient experienced improvement in terms of the neurological evaluation of awareness. The other three patients (37.5%) treated with the combination therapy showed no recovery, as did two of the three patients (66.7%) who received supportive management only. Several studies have suggested high mortality rates of up to 30% for JE, and approximately half of patients with JE did not recover to their functional status prior to the disease. However, there were no patient deaths in our study, which is relatively favorable considering the high mortality rate of JE.

JEV is neurotropic and invades the central nervous system (CNS). Even though the mechanisms by which JEV causes encephalitis are not completely understood, possible models of the immune response to viral infection have been suggested for the damage caused to the CNS and for the disease progression. The best known effects of immunoglobulin are immunomodulation in general via blocking the Fc receptors of macrophages and complement activation, reducing inflammatory cytokines, and suppressing T-cell responses, and it has been applied to several inflammatory autoimmune diseases and infectious conditions. Several factors including antibody replacement, enhanced neutralization, and an augmented opsonocytophobic function have been suggested to contribute to the antimicrobial efficacy of immunoglobulin.

IVIG has also been considered for treating cases of infection with the West Nile virus, which shares the characteristic of being a positive-sense, single-stranded RNA virus with JEV in the Flaviviridae family (Flavivirus genus).
| No. | Sex/age (years) | Initial symptoms | Onset to treatment (days) | MRI lesions | CSF | Recovery | Initial GCS grade | Initial mRS score | mRS score at discharge | Hospital stay (days) | Adverse event |
|-----|----------------|------------------|--------------------------|-------------|-----|----------|-------------------|-------------------|----------------------|-------------------|---------------|
| 1   | M/61           | Confusion        | 44                       | Bilateral thalami and bilateral cerebral peduncles | WBC 60 Ptn 276.7 | No | E4VtM1 | 5 | 5 | 55 |
| 2   | F/73           | Confusion        | 12                       | Bilateral medial temporal lobes and medial thalamus | WBC 630 Ptn 131 | Partial | E4V3M5 | 4 | 3 | 48 |
| 3   | F/50           | Fever, confusion, altered mentality | 10 | Bilateral medial temporal lobes and thalamus | WBC 38 Ptn 43 | No | E4V3M5 | 5 | 5 | 108 |
| 4   | M/59           | Fever, headache, myalgia | 6 | Bilateral BG, thalami, hippocampi, and midbrain | WBC 475 Ptn 143 | Partial | E1VtM4 | 5 | 4 | 24 |
| 5   | M/47           | Confusion        | 32                       | Bilateral medial thalami, left hippocampus, and left SN | WBC 70 Ptn 48.7 | No | E1VtM1 | 5 | 5 | 27 |
| 6   | M/47           | Diplopia, altered mentality | 26 | Bilateral hemisphere, medial temporal lobe, medial thalamus, and tegmentum | WBC 393 Ptn 190.5 | Partial | E1VtM1 | 5 | 5 | 22 |
| 7   | F/68           | Confusion        | 10                       | Normal | WBC 125 Ptn 56 | Partial | E4V4M4 | 5 | 3 | 52 |
| 8   | M/53           | Fever, headache | 7 | Left medial temporal lobe with edema | WBC 413 Ptn 134.4 | Complete | E3V2M5 | 3 | 0 | 13 |
| 9   | F/82           | Fever            | N/A                      | Bilateral cerebral white matter, BG, thalami, pons, and cerebellar peduncles | WBC 25 Ptn 64 | Partial | E1V1M2 | 5 | 3 | 181 |
| 10  | F/87           | Fever, confusion | Bilateral thalamus, medial temporal lobe, and tegmentum | WBC 255 Ptn 134.2 | No | E4V1M4 | 5 | 5 | 18 |
| 11  | F/63           | Confusion, altered mentality | N/A | N/A | No | E1VtM1 | 5 | 5 | 14 |

BG: basal ganglia, CSF: cerebrospinal fluid, F: female, GCS: Glasgow Coma Scale, IVIG: intravenous immunoglobulin, M: male, MRI: magnetic resonance imaging, mRS: modified Rankin Scale, Ptn: protein (mg/dL), SN: substantia nigra, WBC: white blood cells (µL)
Fig. 1. Initial and follow-up magnetic resonance imaging (MRI) studies were performed in two patients (Patient 2 and 5) at the initial hospital admission (A, B, C, G, H, and I) and at 2 weeks after symptom onset (D, E, F, J, K, and L), respectively. Relatively symmetric high signal intensities (yellow arrows) in the bilateral medial thalamus and medial temporal lobes (A, B, and C) of Patient 2 and in the bilateral basal ganglia, thalamus, hippocampus, medial temporal lobe, and midbrain (G, H, and I) of Patient 5 were evident in T2-weighted fluid-attenuated inversion recovery (FLAIR) images. Lesions in the initial MRI were decreased on the follow-up FLAIR images (D, E, F, J, K, and L).
Interferon-α2b, which is known to have antiviral activity that is not directly related to interfering with viral replication or the viral life cycle, plays an important role in the innate immune response to virus infection mediated by interferon-stimulated genes (ISGs). Synergistic effects during combination treatment of HCV infection using ribavirin and interferon have been suggested by several studies, in which ribavirin enhances the interferon-α signaling pathway by inducing the expression of ISGs, although the exact mechanism is unknown.

Most of the above-mentioned studies were conducted at the molecular or cellular level, and not in vivo or in clinical settings, and unexpected consequences in the clinical course of the disease remain. Moreover, several side effects of each treatment are known, including hemolytic anemia for ribavirin and bone marrow suppression (especially decreased numbers of granulocytes and thrombocytes), flu-like symptoms (fever, chills, headache, arthralgia, and myalgia), neuropsychiatric side effects, and autoimmune phenomena for interferon. In our study, one case of hemolytic anemia (Patient 4) resulting from ribavirin and one case of febrile reaction to IVIG were observed. Hemolytic anemia of mild to moderate severity was corrected by transfusion with a single pack of 400 mL of red blood cell, and it did not prolong the hospital stay. The febrile reaction subsided after IVIG treatment without further intervention.

This study had several limitations. It had an uncontrolled, observational design with a small number of patients who were not randomly distributed or compared with historical controls adjusted for sex or age. The baseline characteristics of the patients were heterogeneous, and variations in treatment other than the combination therapy were not considered. Other than evaluating functional outcomes and performing neurological examinations, there is no consensus about objective assessment tools for evaluating the disease burden or any changes correlated with the clinical course of JE, such as neither the viral burden nor the follow-up JEV-specific antibody titer. It is considered that the viral load of JEV is not correlated with disease activity, which makes it difficult to assess the effectiveness of treatment.

With only a small spectrum of treatments to choose from for JE, and with no effective antiviral treatment being available, combination treatment of immunoglobulin, ribavirin, and interferon-α2b can be considered due to its relative safety regarding adverse drug reactions. Further prospective controlled studies with larger numbers of patients are warranted to explore the therapeutic potential of the combination treatment of immunoglobulin, ribavirin, and interferon-α2b in JE.

**Author Contributions**

Conceptualization: Soon-Tae Lee, Kon Chu, Sang Kun Lee. Methodology: Soon-Tae Lee, Kon Chu. Investigation: Hyoshin Son, Jun-Sang Sunwoo. Data curation: Hyoshin Son, Jun-Sang Sunwoo. Writing—original draft: Hyoshin Son. Writing—review & editing: all authors.

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**Conflicts of Interest**

The authors have no potential conflicts of interest to disclose.

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