

Effect of Interferon- α 2b Therapy on St. Louis Viral Meningoencephalitis: Clinical and Laboratory Results of a Pilot Study

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The safety and potential efficacy of interferon (IFN)- α 2b were determined for 15 patients during an outbreak of meningoencephalitis due to St. Louis encephalitis (SLE) virus. Clinical and laboratory results were compared with those of 17 untreated patients who were admitted to the same hospital during this nonrandomized preliminary trial. Quadriplegia, quadriparesis, or respiratory insufficiency persisted after the first week of hospitalization, for 11 of 17 untreated patients and for only 2 of 15 treated patients. These complications existed after the second week of hospitalization for 5 of the 17 untreated patients and for 1 of the 15 treated patients. Transient neutropenia and/or mild hepatitis occurred in 11 treated patients. Early initiation of IFN- α 2b therapy may reduce the severity and duration of complications due to previously untreatable flavivirus meningoencephalitis. A prospective randomized controlled trial is warranted.

Interferon (IFN)- α 2b is active in vitro against at least 3 members of the *Flaviviridae* family: hepatitis C virus, Japanese encephalitis virus, and West Nile virus [1–3]. All 3 viruses are structurally and genetically related to St. Louis encephalitis (SLE) virus [4].

An outbreak of St. Louis meningoencephalitis due to SLE virus occurred in northeastern Louisiana during the summer of 2001. Thirteen patients were admitted to the same hospital during the first 3 weeks of August. Three became quadriplegic and required ventilatory assistance during the first week of hospitalization.

Because of the taxonomic, serologic, and clinical association between SLE viral disease and West Nile viral disease, as well as the known in vitro activity of IFN- α 2b against West Nile virus, we sought to determine whether early initiation of IFN- α 2b therapy for central nervous system (CNS) disease due to SLE virus would be safe and potentially beneficial, by conducting an open, uncontrolled interventional trial.

Patients, materials, and methods. Patients were included in the present study if they were admitted to the hospital with fever and/or severe headache and cerebrospinal fluid (CSF) pleocytosis. Inclusion in the study initially required documentation of the presence of IgM antibody against SLE virus in serum or CSF. With progression of the outbreak of St. Louis meningoencephalitis, patients with the aforementioned clinical and laboratory findings were included in the study pending the results of serologic testing. If such results were negative, patients were withdrawn from the study. Additional signs noted at, or after, admission to the hospital were nystagmus, diplopia, ophthalmoplegia, respiratory insufficiency, tremor, ataxia, peripheral weakness, confusion, or decreased level of consciousness. Thus, no distinction between meningitis and meningoencephalitis was attempted for the purpose of inclusion of patients in the trial.

Because advanced infection of the CNS with SLE virus produces neuronal degeneration, patients were excluded from the trial if they were seen with clinical, electroencephalographic, or computed tomographic evidence of severe brain damage [5]. For all patients, diagnosis of infection with SLE virus was established by detection of IgM flavivirus antibody in serum or CSF, by use of an IgM indirect fluorescent antibody assay at the Louisiana State Department of Health Laboratories. Assays for the detection of antibody directed against California virus and α virus groups were performed as well. Selected patients were evaluated by the Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC; Fort Collins, CO), by use of the IgM capture ELISA and the plaque reduction neutralization test.

Patients were treated with an initial intravenously administered dose of 3 million U of IFN- α 2b, followed by subcutaneous injection of 3 million U of IFN- α 2b 12 h later and then every

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24 h for 14 days. The dose that was chosen was that which would result in ~30 U of IFN- α 2b/mL of blood, exceeding the lowest in vitro inhibitory concentration of IFN- α 2b against SLE virus [6].

Therapy was temporarily withheld if the absolute neutrophil count decreased to <1000 neutrophils/mm³. IFN- α 2b was provided, as Intron A, by Schering-Plough. Treatment of St. Louis meningoencephalitis with IFN- α 2b was approved by the US Food and Drug Administration, under Investigational New Drug #9984.

Patients were examined at admission to the hospital and daily thereafter by one of the authors (L.L.T.). A neurologic function score was determined weekly. The neurologic capacities assessed and the possible number of points assigned to each neurologic capacity were as follows: asymptomatic status (1 or 0 points); walking, talking, thinking, and swallowing (2, 1, or 0 points per each capacity assessed); and unassisted respiration (1 or 0 points).

Asymptomatic patients received a neurologic function score of 10. Patients who had quadriplegia and required ventilatory assistance received a score of 0. These neurologic function scores and other pertinent clinical and laboratory data were recorded by 2 other authors (J.J.R. [who recorded clinical data] and T.R. [who recorded laboratory data]). These authors reviewed the hospital and follow-up outpatient records of all patients retrospectively.

The same information was obtained from the records of the first 13 untreated patients who were admitted to the hospital with SLE virus infection before the availability of IFN- α 2b and from 4 patients who declined treatment. These 17 patients routinely had been evaluated by the same physician, by use of the aforementioned neurologic function score, which was recorded within notes on patient progress. Severe neurologic complications and their definitions were as follows: "quadriplegia" was defined as absence of muscle strength in all extremities, "quadriparesis" was defined as muscle strength of 1–3 (of a highest possible strength of 5) in all extremities, and "respiratory insufficiency" was defined as a requirement for ventilatory assistance.

SLE virus strain LA-01-5981 was cultured from *Culex quinquefasciatus* collected in Monroe, Louisiana, on 4 September 2001, and provided by H. M. Savage of the CDC. The in vitro system for the susceptibility assay was identical to that previously reported for West Nile virus, with the exception of substitution of baby hamster kidney cells for Vero cells to achieve adequate replication of the SLE virus [3].

Informed consent was obtained from each treated patient or his/her guardian. Human experimentation guidelines of the US Department of Health and Human Services were followed.

Results. Thirty-two patients were considered to have St. Louis viral meningoencephalitis. All had IgM flavivirus antibody detected by indirect fluorescent assay, and the results of all tests for California and α virus group antibody were negative. Four patients studied by the CDC demonstrated cross-reactions

between SLE viral antigens and West Nile viral antigens. However, plaque reduction neutralization assays yielded higher titers against SLE virus (range, 1:40–1:2560) than against West Nile virus (range, <1:10–1:40). The clinical symptoms and signs of St. Louis viral meningoencephalitis included fever (temperature, $\geq 38.3^\circ\text{C}$) in 24 patients, headache in 25, gastrointestinal symptoms in 17, nuchal rigidity in 11, ataxia in 7, tremor in 7, myalgia in 6, diplopia in 2, and ophthalmoplegia in 1 patient.

All patients had CSF pleocytosis. The range of total white blood cell (WBC) counts in the CSF was 7–688 WBCs/mm³, the percentage of neutrophils noted was 1%–87%, and the percentage of lymphocytes noted was 9%–81%. The range of protein concentrations in the CSF was 31–149 mg/dL, and that of the glucose concentrations was 44–187 mg/dL.

Fifteen patients were treated with IFN- α 2b for 2 weeks, starting on days 1–4 after admission to the hospital (mean time to initiation of treatment with IFN- α 2b, 1.93 days). The full course of therapy, including treatment interruptions resulting from development of granulocytopenia or hepatitis, required 14–24 days (mean, 18.8 days). Of the untreated patients, 13 were admitted before the onset of the IFN- α 2b protocol. Four other untreated patients chose not to be treated. One untreated patient experienced a relapse after discharge from the hospital, and was readmitted and treated.

Nine untreated patients were female, and 8 were male. Ten were white, 5 were black, and 2 were Latino. The mean age of the untreated patients was 49.5 years. Of the treated patients, 7 were female and 8 were male; 13 were white and 2 were black. The mean age of the treated patients was 44.5 years. Important underlying disease was rare in both groups. Hypertension, which was the most frequently occurring abnormality, was noted in 5 untreated patients and in 2 treated patients. Diabetes mellitus was present in 2 patients (1 treated patient and 1 untreated patient). Two untreated patients and 1 treated patient had atrial fibrillation. Migraine headache, hyperthyroidism, and cardiomyopathy were each noted once among 3 separate patients.

One patient who had coma and quadriplegia and who was ventilator dependent for 2 weeks was inadvertently enrolled in the trial after transfer to the study site. Data on the course of her disease were excluded from data analysis.

The mean neurologic function score of 15 untreated patients at admission to the hospital (i.e., at "week 0") was 5.6 (SD, ± 2.1), and that of the treated patients was 6.2 (SD, ± 1.1), of a possible range of 0–10. The mean neurologic function scores of treated and untreated patients, from the time of admission to the hospital to weeks 1, 2, and 3 are shown in figure 1. Only 9 of 17 untreated patients were seen at follow-up visits at the end of weeks 2 and 3.

Quadriparesis or quadriplegia occurred in 8 untreated patients and in 4 treated patients. Seven untreated patients and 3 treated patients had respiratory insufficiency that required

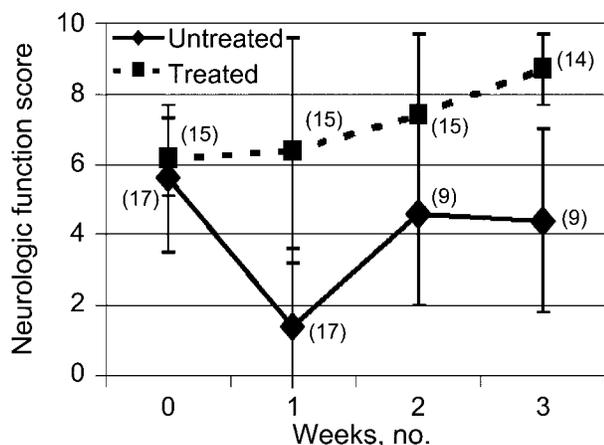


Figure 1. Combined neurologic function scores, over time, for 15 patients with St. Louis meningoencephalitis treated with interferon- α 2b and for 17 untreated patients. Nos. in parentheses denote the no. of patients seen for follow-up at each weekly interval.

ventilatory assistance. Persistence of these complications after the first week of follow-up occurred among 11 untreated patients and 2 treated patients, and persistence after the second week of follow-up occurred among 5 untreated patients and 1 treated patient (table 1).

All patients, whether treated or untreated, survived after the 4-week follow-up period. The mean length of acute-care hospitalization was 15.0 days for 17 untreated patients and 11.3 days for 15 treated patients.

Eleven of 15 treated patients developed a total WBC count of <4000 WBCs/mm³. Absolute neutrophil counts decreased to <1000 neutrophils/mm³ in 3 patients (all 3 had counts of 900–1000 neutrophils/mm³), and the counts recovered to >1000 neutrophils/mm³ after IFN- α 2b therapy was withheld temporarily. No secondary bacterial infections occurred. Elevation of liver enzyme levels was noted in 3 of 32 patients, untreated and treated, at admission to the hospital. Of the individuals who were treated with IFN- α 2b, 5 of 15 developed elevations of liver enzyme levels of 50–200 U/mL during the first week,

and 11 of 15 developed elevations of 50–250 U/mL during the second week. Such elevations of liver enzyme levels occurred in 3 of 8 untreated patients, all of whom had peak levels of <200 U/mL. No episodes of severe hepatitis occurred, and all abnormalities resolved after completion of IFN- α 2b therapy.

In duplicate experiments, IFN- α 2b, in 2-fold increasing concentrations, was added to baby hamster kidney cells 1–2 h after infection with SLE virus. At an IFN- α 2b concentration of 23.4 U/mL, an increase in viability of 13%–29% occurred in such cells, compared with infected control cells. Cell viability increased to 49%–68% after the addition of 750 U of IFN- α 2b/mL. The addition of IFN- α 2b before infection of cells by SLE virus resulted in a greater effect: IFN- α 2b concentrations of 23.4–750 U/mL yielded increased cell viability, from 50%–56% to 64%–94%.

Discussion. The results of the present nonrandomized, unblinded, interventional pilot study of the use of IFN- α 2b therapy for St. Louis viral meningoencephalitis suggest a potential beneficial effect of IFN- α 2b on the early neurologic course of treated patients. However, the absence of randomization in this initial trial introduces the potential for bias and prohibits any definite conclusion regarding the efficacy of IFN- α 2b in the treatment of St. Louis viral meningoencephalitis. We consider the results of the present study to be supportive of subsequent prospective, randomized, double-blinded, placebo-controlled trials of IFN- α 2b therapy for flavivirus meningoencephalitis. These results do not support current, empirical use of IFN- α 2b for the treatment of undiagnosed or suspected flavivirus meningoencephalitis. Such therapy remains experimental pending the results of future controlled trials.

St. Louis and West Nile viral meningoencephalitis present with very similar clinical findings and provoke cross-reactive antibody responses. Thus, the diagnostic distinction between these infections may require special studies, such as the plaque reduction neutralization test. The plaque reduction neutralization test confirmed the diagnosis of infection with SLE virus for 4 of the treated patients in the present study, as well as for 34 of 70 case patients from the same geographic area whose cases

Table 1. Proportion of patients treated with interferon (IFN)- α 2b and untreated patients with severe neurologic impairment or respiratory insufficiency at admission to the hospital and after 1–4 weeks of hospitalization.

Clinical complication ^a	Patients with severe neurologic impairment or respiratory insufficiency, by week of hospitalization									
	Treated with IFN- α 2b (n = 15)					Untreated (n = 17)				
	Week 0 ^b	Week 1	Week 2	Week 3	Week 4	Week 0 ^b	Week 1	Week 2	Week 3	Week 4
Quadriplegia	0	0	0	0	0	0	0	2	0	1
Quadriparesis	0	2	1	1	0	0	0	2	2	1
Respiratory insufficiency	1	2	0	0	0	0	4	2	0	1

NOTE. Data are no. of patients with ≥ 1 of each of the 3 complications at each weekly follow-up visit; data are not cumulative.

^a Of the treated patients, 1 had ≥ 1 of the 3 clinical complications after 2 weeks of follow-up, and 2 had ≥ 1 of the 3 clinical complications after 1 week of follow-up. Of the untreated patients, 5 had ≥ 1 of the 3 clinical complications after 2 weeks of follow-up, and 11 had ≥ 1 of the 3 clinical complications after 1 week of follow-up.

^b Time of admission to the hospital.

were reported by the Louisiana Office of Public Health [7]. Entomological studies performed by the CDC demonstrated the prevalence of infection with SLE virus to be 3–5 cases/1000 *C. quinquefasciatus* mosquitoes. These data provide strong supportive evidence for SLE virus as the cause of infection among our treated and untreated patients.

The results of the present study are consistent with findings regarding the therapeutic effect of IFN- α 2b against flavivirus encephalitis in an animal model. A study of the use of IFN- α 2b for the treatment of flavivirus (Modoc virus) encephalitis in SCID mice showed a significantly increased mean survival time for treated mice, compared with untreated mice. Treatment significantly reduced the levels of viral RNA in the serum, brain, and spleen [8].

The present study follows the suggestion made by Merigan [9, p. 2515], in 1982, that the action of IFN- α 2b against “CNS viral infections that are particularly severe and caused by RNA viruses for which we have no other available therapy” be examined. The present study was initiated as an emergency intervention, in an attempt to lessen the neurologic effects associated with an ongoing outbreak of severe SLE viral meningoencephalitis. Thus, a placebo-controlled, randomized, blinded study was not possible. However, the proportion of patients who experienced quadriplegia, quadriparesis, or respiratory insufficiency after the first or second week of hospitalization was greater among untreated patients than among patients who received IFN- α 2b. The mean neurologic function score at admission to the hospital was similar among treated and untreated patients.

The present study has demonstrated that IFN- α 2b is active against SLE virus in vitro; that 2 weeks of treatment with IFN- α 2b for CNS infection due to SLE virus is well tolerated, except for the development of transient neutropenia and/or mild hepatitis; and that the clinical course of treated patients appears to be favorable, compared with that of untreated patients, within

the same outbreak at the same hospital. A definitive conclusion regarding the beneficial effect of IFN- α 2b against St. Louis or other flavivirus meningoencephalitis awaits a placebo-controlled, prospective, randomized, blinded study. On the basis of the results of this pilot study, we conclude that further investigation of early initiation of IFN- α 2b therapy for previously untreatable CNS flavivirus infection is warranted.

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