THE EFFECT OF INTERFERON-ALPHA A ON TWO CASES OF JAPANESE ENCEPHALITIS IN THAILAND

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INTRODUCTION

Japanese encephalitis (JE) caused by a flavivirus is a mosquito-borne disease characterized clinically by fever, headache, stiff neck, prostration, somnolence, progressing to delirium, convulsions, coma and death, It is considered to be endemic in many countries in Southeast Asia including Thailand where an epidemic form occurs in the rainy season, i.e. during July-August. The disease is prevalent in children aged below 15 years, but it also affects adults. The morbidity of the disease is rather high especially during outbreaks with a mortality rate of 20% to 30%. Many animals including swine, horses and also cattle are reservoirs (Grossman *et al.*, 1973; Gunakasem *et al.*, 1981; Igarashi *et al.*, 1983).

In Thailand, Japanese encephalitis has been recognized as a rural disease, transmitted mainly by *Culex tritaeniorhynchus*, which breeds in rice paddy fields and other rural water-beds. Recently it has been reported that the incidence of the disease is increasing especially in many provinces in north and northeast Thailand, posing serious public health problems. Moreover, in the last few years more JE cases have been admitted to hospitals in Bangkok due to JE transmission occurring in suburban Bangkok where rice paddies and swine rearing houses and small farms were located. More than 70% of recovered cases were left with permanent neurological deficits including mental retardation and deterioration, personality changes, semi-paralysis or paresis, patho-psychological syndrome (Poungvarin *et al.*, 1981; Sangkawibha *et al.*, 1982; Viriyavejakul *et al.*, 1984).

To date there has been no specific drug for the treatment of JE. Patients only receive supportive care and symptomatic treatment. Recently we demonstrated that Recombinant Leukocyte A Interferon (r IFN-alpha A) with strong antiviral properties could inhibit the replication of four local strains of JE virus *in vitro* with the minimal effective doses ranging from 30 I.U./ml to 1,500 I.U./ml (Harinasuta *et al.*, 1984). Based on these findings, a clinical trial to evaluate the efficacy and toxicity of r IFN alpha A in JE patients admitted to the Children's Hospital in Bangkok was initiated. This paper reports on the treatment of 2 JE cases with r IFN alpha A.

MATERIALS AND METHODS

The Patients: Patients admitted to the Children's Hospital with fever and central nervous system involvement with coma were considered for study. The diagnosis of Japanese encephalitis was made clinically and later confirmed serologically by the CSF-JE-IgM and IgG antibody capture technique (Burke *et al.*, 1982) performed at AFRIMS. Patients in whom the diagnosis of JE could not be confirmed were excluded.

The drug: r IFN-alpha A was produced by F. Hoffmann-La Roche and Co.Ltd., Basle, Switzerland. The drug was available as lyophilized powder in vials of either 3×10^6 I.U. or 18×10^6 I.U. The powder was recon-

stituted just prior to use with 1-6 ml sterile water as diluent. Excess drug not immediately used was discarded.

Side-effects: Patients were monitored for clinical side-effects including fever, chills, malaise, nausea and vomiting, as well as laboratory adverse effects such as leukopenia, thrombocytopenia and elevated liver transaminases.

RESULTS

Patients with Japanese encephalitis admitted into the Children's Hospital in Bangkok were studied; two were treated with IFN-alpha A while another 2 cases received only symptomatic treatment. Clinical and laboratory features and results of treatment are given below.

Interferon doses: It was recommended that the maximum effective dose for IFN α in the treatment of encephalitis was 9-10 $\times$ 10^6 I.U. intramuscularly once daily in a patient weighing about 20-30 Kg. To avoid adverse effects of IFN and to develop tachyphylaxis the drug should be started with a small dose of 3 $\times$ 10^6 I.U. and be stepped up to the maximum dose of 9 $\times$ 10^6 I.U. within 5-7 days, a total of 14 day injections. Intrathecal inoculation should be added in order to increase the efficacy of IFN. In a small child the dose of IFN should be half of the mentioned doses. The following doses of r IFN-alpha A were given to our 2 JE cases.

Case No. 1 Wt. 35 Kg.

Day	Intramuscular once daily *Each day*
1, 2	3 $\times$ 10^6 I.U.
3, 4, 5	6 $\times$ 10^6 I.U.
6, 7	9 $\times$ 10^6 I.U.
8 - 14	9 $\times$ 10^6 I.U.

Total IFN = 105 $\times$ 10^6I.U. no intrathecal administration.

Case No. 2 Wt. 9 Kg.

Day	Intramuscular *once daily*	Intrathecal *each day*
1, 2	1.5 $\times$ 10^6 I.U.	0.15 $\times$ 10^6 I.U.
3	omitted (leukopenia)	–
4, 5	2.85 $\times$ 10^6 I.U.	0.15 $\times$ 10^6 I.U.
6	4.5 $\times$ 10^6 I.U.	0.15 $\times$ 10^6 I.U.
7	omitted (leukopenia)	–
8, 9, 10, 11	4.5 $\times$ 10^6 I.U.	–
12, 13, 14	3.0 $\times$ 10^6 I.U.	–
Sub-total	40.2	0.75

Total IFN = 40.95 $\times$ 10^6 I.U.

Case 1

Patient J, a 16-year-old female resident of suburban Bangkok was admitted into the Children's Hospital on 23rd August 1984 with the complaints of fever and severe headache for 4 days. She became unconscious for 18 hours before admission with periodic clonic contractions and spastic convulsions. A clinical diagnosis of Japanese encephalitis was made which was confirmed later by serological tests (Table 1).

Table 1

Serological tests conducted at the Armed Forces Institute of Medical Sciences (AFRIMS) Laboratories.

Specimen	Date	JE-IgM	JE-IgG
Serum	23rd Aug.	23	29
	4th Sept.*	431	671
CSF	23rd Aug.	72	100
	4th Sept.*	490	> 889

*Day 13 of IFN treament.

r IFN alpha A 3×10^6 I.U. was immediately injected intramuscularly, followed by daily intramuscular injections of IFN for a total of 14 days. Complete blood and platelet counts were performed daily during IFN treatment. The clinical and laboratory changes observed were as follows:-

Clinically, the patient remained febrile throughout the treatment period. The persistent fever could have been due to r IFN-alpha A, as fever is a well-known side-effect of interferon administration. The temperature became normal in the 2nd week on termination of IFN. Clonic contractions and spastic convulsions subsided on the 3rd day and improvement of general symptoms was observed from the 5th day on. Mental symptoms slowly improved. Rehabilitation with physiotherapy and speech therapy was started during convalescence.

Laboratory results showed that the white blood count dropped sharply from 12,400/c.mm before therapy to 5,700/c.mm, 5,150/c.mm and 1,500/c.mm on the 2nd-4th day of treatment. Thereafter it remained between 3,000 to 4,650/c.mm throughout the period of IFN administration. The low white cell count was due predominantly to neutropenia. Platelets were adequate and there was no significant change in the hematocrit (31-35%). Liver transaminases rose slightly during IFN administration SGOT/SGPT=80-84/70-73 units but returned to normal after the end of treatment. An EEG showed cerebral dysfunction of the right temporoparietal region which improved on the 12th day after IFN therapy. No other toxic side effects were noted.

Case 2

Patient T, a 3-year-old boy from a suburb of Bangkok, was admitted to the Children's Hospital on 7th October 1984 with the symptoms of fever, headache and lethargy for 3 days. He developed spastic and clonic convulsions (4 times in 2 days) before admission. On admission he was semi-conscious with neck rigidity, and periodic spastic convulsions. The clinical diagnosis was acute bacterial meningitis. However, the CSF was found to be clear, colourless, with 6 mononuclear cells/c.mm. CSF culture showed no growth on two occasions. CSF serology performed on 10th October 1984 was positive for acute JE infection (JE-IgM = 521 units). r IFN-alpha A administered intramuscularly and intrathecally was started on that day, and continued for a total of 14 days.

Clinically fever decreased after 2 days of IFN administration. However, the patient developed pneumonia in both upper lobes with atelectasis of the right upper lobe. This lung complication responded to antibiotic therapy. The mental status of the patient improved from the 6th day of IFN onward and improvement of general symptoms was noted during the 2nd week of treatment. Low grade fever persisted for a few days after IFN was stopped.

Laboratory tests showed a marked leukopenia on the 2nd day of treatment which became worse on the 3rd and 7th day (500/c.mm) and the drug had to be withheld on both days. INF was otherwise given according to schedule. White blood cell connts returned to nomal levels in the 2nd week. There were no other toxic side effects noted.

JE cases No. 3 and No. 4 were admitted to Children's Hospital on 13th November 1984 and 1st December 1984 respectively, and who received symptomatic treatment died on the 8th and 9th day of illness respectively.

DISCUSSION

Recent studies of the effect of human interferon on JE virus in Thailand revealed that IFN inhibited the replication of JE virus *in vitro* (Vithanomsat *et al.*, 1984), and had strong antiviral properties *in vitro* against four

local strains of JE virus (Harinasuta *et al.* 1984). These findings encouraged us to perform a clinical trial using Recombinant Leukocyte A Interferon in patients with JE. Results of this preliminary report showed r IFN alpha A to be a promising agent for the treatment of JE.

The reason for performing intrathecal inoculations was that after IFN intramuscular injection the permeability of the blood/brain barrier for IFN during the acute phase of JE is not known. Moreover, it was not certain if the doses of IFN given intramuscularly would have resulted in high enough CSF concentrations of IFN to inhibit replication of JE virus in the brain cells.

The response to IFN of patient No. 1 was satisfactory. Intrathecal inoculations were not given in this case because of awarences of the danger of herniation of brain tissue due to frequent lumbar punctures (the patient had marked brain oedema). Moreover, the patient seemed to show effective response to IFN intramuscular injections and thus intrathecal IFN administration was unnecessary.

Only two minor side-effects were observed in this patient, i e. leukopenia and neutropenia, and slight increase in liver transaminases. Neither side effect necessitated the discontinuation of therapy.

The second patient was admitted on the 3rd day of illness, but the diagnosis of Japanese encephalitis was delayed until the 6th day. r IFN-alpha A was immediately administered both intramuscularly and intrathecally when the patient was at an advanced stage of illness with fever, semiconsciousness progressing to convulsions and coma. Since the weight of this patient was only 9 Kg, it was decided to give half-doses of IFN to him. The clinical responses were slower than those of the 1st case, i.e. recovery from coma was observed on the 6th day of IFN, and improvement of general symptoms was during the 2nd week of the treatment, eventhough the fever was found to decrease to a low level during late 1st week and early 2nd week.

As in patient No. 1 leukopenia and neutropenia were noted in the first week of treatment. The patient also developed acute lung infection on the 3rd day of IFN which responded to antibiotic therapy.

There were no other side effects including general and allergic toxicity, hematological and blood chemistry changes.

The 3rd and 4th JE cases were not treated with IFN, but with usual symptomatic and supportive drugs as before. The patients died on the 5th day of admission, or on the 8th day and the 9th day of the illness respectively.

Thus this study indicated that the Human Recombinant Leukocyte A Interferon is a promising and effective drug in the treatment of Japanese encephalitis in Thailand. At the doses mentioned above the side effects were leukopenia and neutropenia which were only temporary. However, more JE cases are being under further clinical trial in order to obtain definite results on the efficacy of r IFN-alpha A on Japanese encephalitis in Thailand.

SUMMARY

Two Japanese encephalitis cases with serious comatous symptoms were treated with the Human Recombinant Interferon-alpha A. The clinical responses to IFN were found to be satisfactory. The first case showed improvement on the 5th day of IFN treatment and the general condition slowly improved. The second case recovered from the comatous stage on the 6th day of IFN, followed by quick improvement of general symptoms in the 2nd week and complete recovery without any mental sequelae.

Leukopenia and neutropenia occurred during the first week of administration of IFN, but were only temporary. Slight elevation of SGOT and SGPT was observed in the

first case. No other side effects including general toxicity, neurotoxicity or allergy, or any abnormal hematological and blood chemistry changes were observed in these 2 cases.

Two other JE cases (the 3rd and 4th consecutive JE cases) were not treated with IFN, but received the usual regimens of symptomatic and supportive drugs. Both patients died on the 7th-9th day of illness.

This study suggests that the Human Recombinant Leukocyte A Interferon possibly is an effective and promising agent in the treatment of Japanese encephalitis in Thailand. More studies to treat JE cases with this IFN are being performed in order to assess the efficacy, tolerance and safety of rIFN-alpha A on Japanese encephalitis in Thailand.

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