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Failure of interferon alfa and tribavirin in rabies encephalitis

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Abstract

***Objective*—To test the effect of interferon alfa and tribavirin (ribavirin) in patients with rabies encephalitis.**

***Design*—An open trial of chemotherapy and intensive care in patients with early rabies.**

***Setting*—The intensive care unit of a Bangkok hospital.**

***Patients*—Four conscious men with clinical rabies encephalitis.**

***Interventions*—Rapid virological diagnosis of rabies. Treatment with intravenous and intraventricular injections of high doses of lymphoblastoid interferon alfa in three patients and tribavirin in one patient. Intensive care was given throughout.**

***Main outcome measures*—Rabies infection confirmed by antigen detection and virus isolation. Rabies neutralising antibody and specific IgM sought in serum and cerebrospinal fluid. Interferon concentrations monitored before and during treatment in three patients.**

***Results*—Interferon alfa treatment produced high concentrations in serum and cerebrospinal fluid. All four patients died after 5½ to 12½ days of treatment with no evidence of virostatic or clinically beneficial effects from either treatment.**

***Conclusion*—Interferon alfa treatment is not effective in rabies encephalitis. The use of tribavirin warrants further study, possibly combined with new therapeutic methods.**

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Introduction

No records exist of patients surviving furious rabies encephalitis. The three reports of recovery from rabies followed predominantly paralytic illnesses.[1-4] The diagnosis rested on high concentrations of antibody to the rabies virus in serum and cerebrospinal fluid, but no rabies virus or antigen was detected. Further attempts at intensive care have failed to save patients with rabies.[5-12] Clearly, additional therapeutic approaches are necessary.

Interferon prevents replication of the rabies virus in vitro,[13-15] and a single dose of an interferon inducer given 24 hours after inoculation of the virus protects rabbits against rabies.[16] Treatment with interferon from human leucocytes affords protection of infected monkeys even if given 11 days after rabies inoculation. Furthermore, interferon treatment has been most effective when given by combined intramuscular and intralumbar routes in monkeys.[17] Although endogenous interferon has been detected in some humans with rabies, the concentrations were very low, even in brain tissue sampled at necropsy.[18] Treatment with exogenous interferon has been ineffective if used late in the illness[18,19] so we treated patients with early rabies encephalitis with high doses of interferon alfa.

Tribavirin, which selectively blocks the synthesis of viral guanosine nucleotides, is effective against rabies virus, in vitro.[20,21] Protection of mice from rabies by tribavirin treatment has been attempted. A short course of daily injections was given either intramuscularly early or intracerebrally late in the incubation period.[22] Mortality was not affected, but such brief treatment does not exclude benefit from continuous high concentrations of tribavirin in serum and cerebrospinal fluid. Animal experiments with tribavirin to treat other intracranial viral infections indicate that it does not cross the blood-brain barrier rapidly.[23,24] This has been confirmed by pharmacokinetic experiments in monkeys (M Ussery, personal communication), though it has been found in the cerebrospinal fluid of patients with AIDS after several weeks of oral treatment.[25] High doses of intravenous tribavirin have proved effective in Lassa fever in humans.[26] Tribavirin has not been used intrathecally, but toxicity studies in monkeys showed that five daily injections of 2 mg/kg by the lumbar route was safe (G Ward, unpublished findings). We therefore also investigated the use of tribavirin in rabies encephalitis.

We carried out an open trial of combined intravenous and intrathecal interferon or tribavirin in four fully conscious, cooperative patients with early rabies encephalitis.

Patients and methods

All patients were admitted to Bamrasnaradura Hospital, Bangkok, and subsequently transferred to the intensive care isolation unit of the Hospital for Tropical Diseases, Bangkok, when the diagnosis of rabies was strongly suspected on the basis of the history and clinical signs. Patients were transferred only with their own fully informed consent and that of their accompanying family. The study was approved by the ethical committees of the Faculty of Tropical Medicine, Mahidol University, and the Thai Ministry of Public Health.

Except in one patient with clinically obvious rabies the diagnosis was confirmed before treatment by detection of rabies antigen around the hair follicles from a skin biopsy specimen with an immunofluorescence test.[27]

When the patients arrived blood and cerebrospinal fluid samples were taken from baseline biochemical, haematological, viral, and drug assays. We inserted an Ommaya reservoir into a lateral cerebral ventricle and a tracheostomy tube under general anaesthesia. A temporary pacemaker was introduced under local

anaesthesia. Thereafter the patients were managed with barrier nursing and conventional intensive care techniques, bearing in mind the possible side effects of treatment.

We sought evidence of leakage of protein across the blood-brain barrier by calculating the albumin quotient[28] and used the IgG index[29] to detect intrathecal synthesis of IgG.

Interferon treatment—Interferon alfa derived from the Namalwa human lymphoblastoid cell line (Wellferon batch C1N/4, Wellcome Foundation) was given intravenously at a loading dose of 50 MU/m^2 body surface infused over six hours. This was repeated over the next 18 hours and then given daily as a continuous intravenous infusion. The intravenous solution was stabilised by the addition of human albumin 1·5 mg/ml except in the first patient. The first dose of intrathecal interferon was given by the lumbar route, and thereafter it was given into the cerebral ventricle by the Ommaya reservoir. A loading dose of 2 MU/m^2 was repeated after six hours and then daily. For the third patient the dose was halved after the first week. Samples of serum and cerebrospinal fluid were stored at −70°C for assay of interferon alfa with a Cell-Tec radioimmunoassay kit.

Tribavirin treatment—Tribavirin (Viratek, United States) was given intravenously as a loading dose of 2 g (30 mg/kg) over 20 minutes followed by 1 g six hourly (60 mg/kg/day) for four days and 0·5 g eight hourly (25 mg/kg/day) daily thereafter. Intraventricular tribavirin injections of 100 mg (2 mg/kg) were given daily through the Ommaya reservoir.

Virological examination—A 4 or 6 mm punch skin biopsy specimen, corneal smears, and a brain biopsy specimen (taken through the burr hole at insertion of the Ommaya reservoir or at necropsy) were tested for rabies antigen by a fluorescence antibody test.[30] We tried to isolate virus from saliva, cerebrospinal fluid, and brain tissue by inoculating specimens into suckling mice and sometimes also on to cultures of mouse neuroblastoma cells (donated by G M Baer). Rabies neutralising antibody was measured by the rapid immunofluorescence focus inhibition test[31] and rabies specific IgM by a μ capture radioimmunoassay test by Dr C H Hoke.[32-34]

TABLE I—*Details of exposure to rabies*

Case No	Age (years), sex, occupation	Animal contact	Incubation period
1	54, M, guard	Unprovoked dog bite on right big toe. No vaccine	10 Weeks
2	27, M, labourer	Dog bite on right arm. No vaccine	3 Months
3	14, M, student	Dog bite on left leg. No vaccine	1 Year
4	28, M, farmer	Caught stray dogs. No definite bite. No vaccine	2 Months

TABLE II—*Clinical data on four patients with rabies encephalitis*

Case No	Initial clinical features	Duration of illness before treatment	Treatment	Subsequent clinical events	Time between starting treatment and death
1	Fully conscious. Hyperaesthesia of legs, itching spreading from bitten toe to whole body. Fever, aerophobia, hydrophobia	7 Days	Interferon alfa	Progressive flaccid paralysis and loss of consciousness, areflexia, hypotension, bradycardia, ventricular tachycardia	5½ Days
2	Fully conscious. Widespread hyperaesthesia, itching right hand. Fever, hydrophobia, aerophobia	1½ Days	Interferon alfa	Progressive loss of consciousness, sinus arrest, bradycardia, Cheyne-Stokes respiration, pneumococcal pneumonia and septicaemia, staphylococcal meningitis, hypotension, hypothermia, frequent spasms throughout	10½ Days
3	Fully conscious. Itching at site of bite. Fever, inspiratory spasm, aerophobia	2 Days	Interferon alfa	Cheyne-Stokes respiration. Progressive loss of consciousness, hypothermia, bilateral pneumothorax, pneumonia, frequent spasms, premature ventricular beats, bradycardia, hypotension, haematemesis, brain death	12½ Days
4	Conscious. Leg itching and weakness. Euphoria, dysphagia, mild aerophobia	3 Days	Tribavirin	Progressive areflexia, fasciculation of back muscles, coma, hypothermia, Cheyne-Stokes respiration, pneumonia, hypoxic episode, diabetes insipidus, brain death	9 Days

TABLE III—*Results of virological tests on four patients with rabies encephalitis*

Case No	Day of treatment	Rabies virus: Specimen	Rabies virus: Antigen detection*	Rabies virus: Virus isolation	Neutralising antibody: Serum	Neutralising antibody: Cerebrospinal fluid
1	1	Brain	+	+	−	−
		Skin from neck	−			
	2				−	−
	3	Corneal smears	−			
	5	Skin from neck	+		−	−
	6	Brain at necropsy	+	+		
2	1	Skin from neck	+		−	−
		Corneal smear	−			
		Brain	+	+		
	5				−	−
	7	Corneal smear	−			
	8				−	−
	10				−	−
	11	Brain at necropsy	+	+		
3	1	Skin from neck	+		−	−
		Left leg	+			
		Right leg	−†			
		Brain	−	+		
		Corneal smears	−			
	2				−	−
	4	Corneal smears	−		−	−
	5	Saliva		−		
	6	Skin from neck	+		−	−
	8				+	−
	10				+	−
	12	Skin from neck	+		+	+
		Brain at necropsy	+	+		
4	1	Skin from neck	+		−	−
		Corneal smears	−			
		Cerebrospinal fluid		−		
		Saliva		+		
		Brain	+	+		
	10	Skin from leg	+		−	−
		Skin from neck	+			
		Brain at necropsy	+	+		

*Fluorescence antibody test. †No hair follicles in specimen.

Results

Three patients were treated with interferon and one with tribavirin, but all died between five and 12 days after starting treatment. Table I shows the nature of their exposure to rabies infection and table II their clinical features.

Virological examination—Table III shows the results of detection and isolation of rabies virus and serological tests. The skin biopsy method of detecting antigen confirmed the diagnosis during life in every case and gave a positive result on admission in three patients. The corneal smear test yielded negative results in all patients on every occasion. The virus was isolated from the brain biopsy specimens taken from all patients during insertion of the Ommaya reservoir, but no

antigen was detected by the immunofluorescence antibody test in the specimen from the second patient. The needle biopsy brain specimens taken at necropsy contained large amounts of rabies antigen detected by immunofluorescence, and the virus grew readily in suckling mice. The sample from the fourth patient was inoculated on to mouse neuroblastoma cells, and after three days half of the cells were infected, indicating a high virus titre in the brain inoculum. Only the third patient had any detectable rabies antibody (table IV). Neutralising antibody and rabies specific IgM appeared during the second week of illness.

TABLE IV—*Results of serological tests on third patient*

	Serum		Cerebrospinal fluid	
Day of treatment	Neutralising antibody*	IgM†	Neutralising antibody*	IgM†
1	–	–	–	–
2	–	–	–	–
4	–	–	–	–
6	–	–	–	–
8	0·18	–	–	–
10	0·26	2·1	–	–
12	0·75	3·1	0·31	–

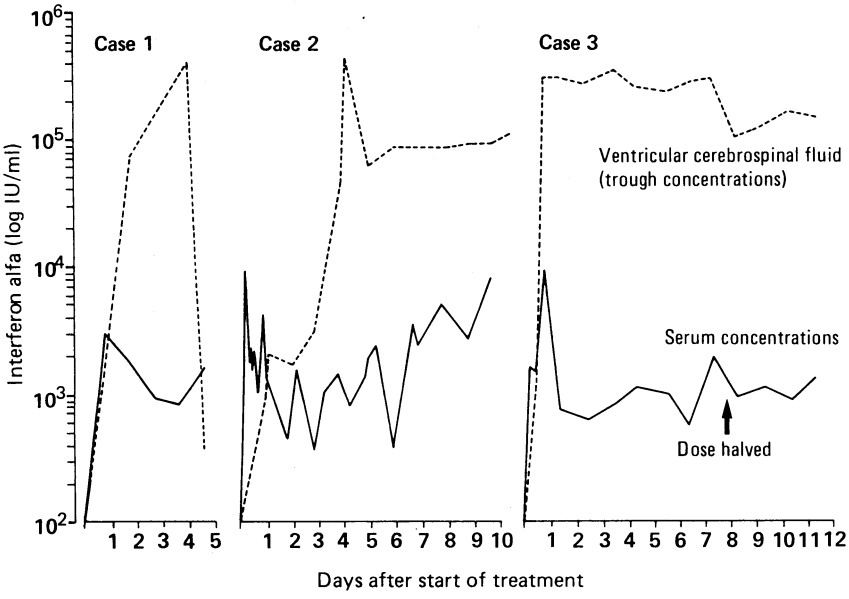
*In IU/ml by rapid immunofluorescence focus inhibition test.
†Rabies specific IgM as ratio of positive to negative in μ capture radioimmunoassay test.

Interferon concentrations—The first and second patients had no detectable interferon (<62 IU/ml) before treatment, but endogenous interferon 80 IU/ml was found in the cerebrospinal fluid of the third patient. The figure shows the high interferon concentrations in serum and ventricular cerebrospinal fluid for each patient. The cerebrospinal fluid samples were obtained before treatment and so gave trough concentrations. Table V shows the ranges of interferon concentrations observed throughout treatment. Accumulation of interferon in the cerebrospinal fluid was seen in the first two patients so the intrathecal dose was halved after one week for the third patient.

TABLE V—*Ranges of interferon alfa concentrations (IU/ml) observed throughout treatment of three patients with rabies encephalitis*

	Serum		Cerebrospinal fluid	
Case No	Maximum	Minimum	Maximum	Minimum*
1	3000	840	400 000	350
2	9200	200	430 000	1150†
3	9600	590	350 000	8550†

*At least 24 hours after starting treatment.
†Lumbar cerebrospinal fluid specimens, all others ventricular.



Interferon concentrations in serum and cerebrospinal fluid in three patients with rabies encephalitis

Analysis of cerebrospinal fluid on admission—The changes in the lumbar cerebrospinal fluid were minor. The first and second patients had 20 000 and 4000 lymphocytes per litre respectively. No abnormalities in total protein and glucose concentrations were detected, and the IgG indices and albumin quotients were normal, indicating no intrathecal production of IgG or leak across the blood-brain barrier.

Histopathological examination of the cerebral cortex biopsy specimens taken on admission showed only minor, non-specific changes in three cases, but the specimen from the third patient showed Negri bodies and some perivascular mononuclear cell infiltration. In needle biopsy samples taken at necropsy, however, Negri bodies were found in all except the third patient, indicating how focal the pathological changes may be. Perivascular infiltration by mononuclear cells and microglial reaction were often seen, and sparse neuronophagia, demyelination, and petechial haemorrhages were occasionally seen.

Haematological and biochemical changes possibly caused by treatment—The first patient had no haematological or biochemical abnormality suggesting interferon toxicity, though the white cell counts of the second and third patients fell to about 3×10^9/l in five days but returned to about 10×10^9/l before death. The serum potassium concentration of the second patient rose to 9·8 mmol/l before death, which was associated with creatinine concentrations rising to 630 μmol/l, plasma sodium to 156 mmol/l, and blood urea to 22 mmol/l. There was no proteinuria. The serum aspartate aminotransferase activity rose from 53 IU/l on admission to 268 IU/l before death, and the bilirubin concentration was 64 μmol/l (direct) or 98 μmol/l (total). The plasma sodium concentration of the third patient also rose from 145 to 161 mmol/l before death with normal blood urea and potassium concentrations. The serum aspartate aminotransferase activity rose from 28 to 158 IU/l. The only detectable possible side effect of tribavirin treatment in the fourth patient was a fall in haemoglobin concentration from 144 to 107 g/l in eight days.

Discussion

Consistently high concentrations of interferon alfa in the serum and cerebrospinal fluid for several days did not noticeably influence the clinical progression of rabies in three patients. Virus was cultured from brain samples taken at necropsy, and large amounts of rabies antigen were seen by immunofluorescence. There was no evidence that interferon was virostatic in the patients, who started treatment while fully conscious.

A low concentration of endogenous interferon was found in the cerebrospinal fluid of one patient, but experimental evidence suggests that it may have little effect on replication of the rabies virus. An important antiviral action of interferon is stimulation of 2-5A synthetase to activate a nuclease, which disrupts viral RNA. The endogenous interferon induced by experimental infection with rabies virus stimulates a form of 2-5A synthetase that is incapable of activating the RNA disrupting nuclease.[35]

Rabies neutralising antibody usually appears within two weeks after the onset of symptoms,[12] but we found it in only one patient treated with interferon. Merigan *et al* attributed low rabies antibody concentrations to the immunosuppressive effect of interferon,[18] which is associated with high interferon concentrations, but interferon treatment in the presence of antigen can increase antibody production experimentally.[36] Tribavirin is unlikely to have been immunosuppressive.[37]

To our knowledge this is the first report of the use of intrathecal tribavirin in humans. No adverse effects were observed. Intravenous tribavirin was given to a patient with rabies in the United States, but treatment was begun in the third week of illness without apparent benefit.[38] Tribavirin has a virostatic effect in vitro. A concentration of 25 μg/ml inhibits the release of 90% of infectious virions.[20] This concentration (equivalent to 10 μM tribavirin) would have been attained in the plasma within minutes and lasted at least 12 hours after the initial dose.[39] The maintenance of blood concentration is predicted to be well above the inhibitory concentration. Despite nine days' treatment the brain tissue taken at necropsy was full of infectious virus, and so either the drug did not reach the infected cells or it was not virostatic in vivo.

The expected side effects of interferon treatment, lymphopenia and hepatotoxicity, were not serious, but the hyperkalaemia of the second patient was probably fatal. This infrequent complication has been reported by Rohatiner *et al*.[40]

Rapid confirmation of the diagnosis of rabies is essential if other treatment regimens are to be tried in patients with early signs of encephalitis. We found that antigen detection by immunofluorescence of a skin biopsy specimen was as reliable as an immunofluorescence test on a brain smear at necropsy.[34] Blenden *et al* also advocate this technique.[41]

In conclusion, the use of interferon alfa or tribavirin alone does not influence the course of rabies encephalitis. The use of these and other compounds such as tungstoantimoniate derivatives[21] in combination may yet effect a cure. The only treatment that has been effective after the onset of clinical rabies encephalitis is intrathecal vaccination with a live attenuated rabies virus in dogs.[42] A more attenuated virus, which is apathogenic when injected intrathecally into monkeys, is now available[43] and may be used in humans in the future.

At the time of the study MJW worked at Sir William Dunn School of Pathology, University of Oxford. NJW, REP, and DAW are at Nuffield department of clinical medicine, University of Oxford.

The clinical team also included Drs Adisorn Vongsa, Chaisin Viravan, May Ho, Yupaporn Wattanagoon, and George Watt from the Hospital for Tropical Diseases, Bangkok; and Drs Chindra Yongjaiyuth, Somsakdi Chandsri, Somyot Khunjak, and Weera Sinpornchai from Pramonkut Klao Hospital, Bangkok.

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