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THERAPY OF FOCAL VIRAL ENCEPHALITIS IN CHILDREN WITH ACICLOVIR AND RECOMBINANT β-INTERFERON – RESULTS OF A PLACEBO-CONTROLLED MULTICENTER STUDY

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Abstract: Focal viral encephalitis in childhood is a rare but life-threatening disease. Animal experiments and case reports suggest a positive effect of an additional therapy with interferon-β on the course of the disease. Therefore, we initiated a prospective, double-blind placebo-controlled study to investigate the benefit of a combination therapy of Aciclovir (ACV) and recombinant interferon-ß (rIFN-β) in juvenile focal viral encephalitis.

Initial inclusion criterium was suspicion of focal viral encephalitis. Diagnosis was proven by demonstration of characteristic focal lesions in cerebral imaging or virological evidence of HSV in cerebrospinal fluid. Patients were treated with ACV plus rIFN-β or ACV plus placebo. Neurological outcome was determined 21 days and 3 months after onset of the disease.

Initially 59 patients were enrolled in the study. Encephalitis was proven in 14 patients (7 ACV + rIFN-β, 7 ACV + placebo). The study groups were balanced in terms of important prognostic criteria. 10 patients (5 ACV + rIFN-β, 5 ACV + placebo) were cured or had slight defects, 4 patients (2 ACV + rIFN-β, 2 ACV + placebo) showed moderate to severe defects. There was no significant difference in favour of the additive therapy with rIFN-β.

Key words: encephalitis, Herpes-simplex virus, interferons, aciclovir

INTRODUCTION

Focal viral encephalitis in childhood is a rare but life-threatening disease with an incidence of 2 to 3/1,000,000 per year. It is most commonly caused by herpes simplex virus (HSV), but other viruses as Arboviruses, Epstein Barr virus, measles and enteroviruses may act as etiologic agents as well [13, 16]. The clinical course is characterised by disturbance of conciousness, seizures and paresis of cranial nerves. Aciclovir (ACV) is the standard therapy for HSV encephalitis, but the mortality rate of 28% and a defective healing rate of about 35% are still unsatisfactory [14]. Furthermore, ACV has virtually no effect on most of the other pathogens of viral encephalitis. Interferon-β (IFN-β) is known to have a broad antiviral spectrum [4]. It has been demonstrated in vitro, that IFN-β in combination with ACV has synergistic inhibitory effects on HSV. Data of a retrospective analysis [17] suggested that the combination of ACV and IFN-β may be of benefit in children with focal viral encephalitis. In addition, in experimental HSV 1 encephalitis a significant reduction of mortality in mice with a combination of α-interferon B/D and aciclovir was demonstrated [18].

Case reports suggest a positive effect on the course of the disease, frequency of relapses, and neurological outcome [1, 5, 6, 9, 19]. In addition, in St. Louis encephalitis a remarkable reduction in quadriplegia in patients treated with interferon-alpha2b compared to historical controls was observed [8]. Until now no prospective study has been performed to prove the benefit of this therapy. Therefore, we initiated a prospective, double-blind placebo-controlled study to investigate the benefit of a combination therapy of Aciclovir and IFN-β in juvenile focal viral encephalitis.

PATIENTS AND METHODS

Study design: The study was designed as a randomised double-blind placebo-controlled multicenter trial. The study protocol was approved by the local ethical committees of all hospitals participating in the study. The study drug was provided by Bioferon. Unfortunately, the study had to be terminated prematurely for financial reasons after 1 1/2 year.

Patients: 59 patients with suspicion of encephalitis from 27 hospitals (26 German, 1 Slowenian) were enrolled in the study. 3 patients had to be excluded, one patient with incomplete documentation, 2 patients with invalid inclusion criteria. 2 patients belonged to the IFN-group and 1 to the placebo-group. Therefore the evaluation was based on 28 patients of the interferon group and 28 patients of the control group. Informed consent of all patients was obtained by the parents or legal guardian.

Inclusion criteria: (1) Clinical suspicion of a necrotizing encephalitis with focal neurological signs, (2) Onset of the first clinical symptoms not earlier than 5 days before enrollment, (3) Age of the patients younger than 18 years.

Treatment schedules: Patients were treated with Aciclovir (ACV) and recombinant Interferon-β (rIFN-β) or with ACV and placebo. ACV was administered intravenously at a dose of 3 x 10 mg/kg/d (age <1year or >12 years) or 750 mg/m² (age 1-12 years) for 14 days. This dose was chosen according to the commonly accepted therapy at the time the study was started [7]. For interferon therapy we used recombinant Interferon-ß, a substance which was not licenced at that time. It was administered intravenously in a dose of 0.2 x 10^6 IU/kg for 5 days. This dose was demonstrated to be the maximum well tolerable dose in pilot studies performed for other indications in adults. All patients received additional supportive therapy depending on the severity of their disease.

Evaluation of the diagnosis: Because of theoretical considerations, the greatest benefit of a therapy with IFN-β may be expected if the drug is administered early in the course of the disease. Therefore, the therapy started at once after including a patient in the study. The definite diagnosis of a focal viral encephalitis was evaluated in the following days (1) by the evidence of typical focal lesions in cerebral imaging (MRI or CCT), evaluated blindly by an radiologist* or (2) by the evidence of HSV in the cerebrospinal fluid (CSF) by positive PCR and/or evidence of autochthonous antibody production, also evaluated centrally by an independant laboratory**.

Samples of serum and CSF were taken at least on day 0 and 14.

Virological investigations: We investigated HSV PCR and HSV IgG and IgM in all samples of CSF and additional isoelectric focusing in the sample of day 14. Furthermore, antibody levels of other neurotrop viruses (VZV, EBV, measles, mumps, rubella, coxsackie, enteroviruses A/B, echovirus, influenza A/B, parainfluenza, adenovirus and FSME) and of mycoplasma were determined in all serum samples. If a four fold rise of these levels indicated the possibility of a viral encephalitis, additional specific investigations were done in CSF. HSV encephalitis was considered evident by virological tests if all CSF samples supported this assumption (a) by positive PCR results in more than one sample, (b) by positive PCR results in one sample in combination with a rise of specific antibody levels in CSF or detection of specific oligoclonal bands in isoelectric focusing or (c) a rise of specific antibody levels in CSF and detection of specific oligoclonal bands in isoelectric focusing without evidence of HSV in CSF by PCR. Positive values in only one sample and doubtful values were not regarded as evidence of HSV encephalitis.

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Table 1. Criteria for neurological outcome 3 months after admission to the hospital.

Cure	Complete restoration of health after neurologic disease
Slight defect	Little impairment of motor, psychological or intellectual abilities, further on anticonvulsive therapy
Moderate defect	Functions commensurate with age still possible, but moderate motor or intellectual restrictions, transfer to a special school or medical pedagogic nursery school required or expected
Severe defect	Need for complete nursing care; i.e. tetraspasticity, severe intellectual defect; feasibility to attend school not expected
Death	when attributed to the illness up to 3 months after onset of the disease

Outcome: Neurological outcome was evaluated 3 months after admission to the hospital. A five grade rating scale was used with the categories cure, slight defect, moderate and severe defect and death (Table 1).

Statistical analysis: On the assumption that rIFN-β causes a 50% reduction of mortality and morbidity the estimated number of evaluable patients required per study arm was 36 with an α-error = 0.05 and a power of 0.8. Statistical analysis was done with chi2-test, Wilcoxon test and Fisher´s exact test***.

RESULTS

In 14 out of 56 patients enrolled in the study focal viral encephalitis was confirmed (Table 2). These patients were included in the final analysis. In 12 patients we found typical focal lesions in cerebral imaging. Ten patients showed evidence of HSV in virological investigations. Seven of 14 patients were treated with ACV + rIFN-β and 7 patients with ACV + placebo.

The two study groups were comparable in terms of important prognostic criteria. As shown in the baseline evaluation, there are no significant differences concerning initial glascow coma scale, age, ethnic ori-

Table 2. Final diagnosis in patients primarily enrolled in the study (n = 56).

diagnosis	N
– focal viral encephalitis	14
– other viral encephalitis (FSME, mumps)	2
– clinical suspicion of encephalitis, not proven by cerebral imaging or virological investigations	6
– viral infections with slight encephalitic involvement	6
– other neurological diseases (toxoplasmosis, mycoplasma, tumor, encephalitis disseminata, meningitis, vaccine associated poliomyelitis)	9
– other diseases (febrile convulsions, infection of unknown pathogen)	19

Table 3. Baseline evaluation of patients with evidence of focal viral encephalitis (n = 14).

		therapy		
		ACV + rIFN-β (n = 7)	ACV + placebo (n = 7)	total (n = 14)
Demographic data				
gender	female	4	4	8
	male	3	3	6
age [years]	median	4.6	5.9	
	range	0 - 11	0 - 13	
Neurological history				
encephalitis, meningitis		0	0	0
perinatal asphyxia		1	1	2
developmental disorders		1	1	2
Clinical characteristics				
begin of therapy	< 3 days	3	5	8
(after onset of symptoms)	> 3 days	4	2	6
seizures		5	6	11
glacow-coma-scale < 14	> 7 days	6	5	11
concommittant steroids		4	5	9

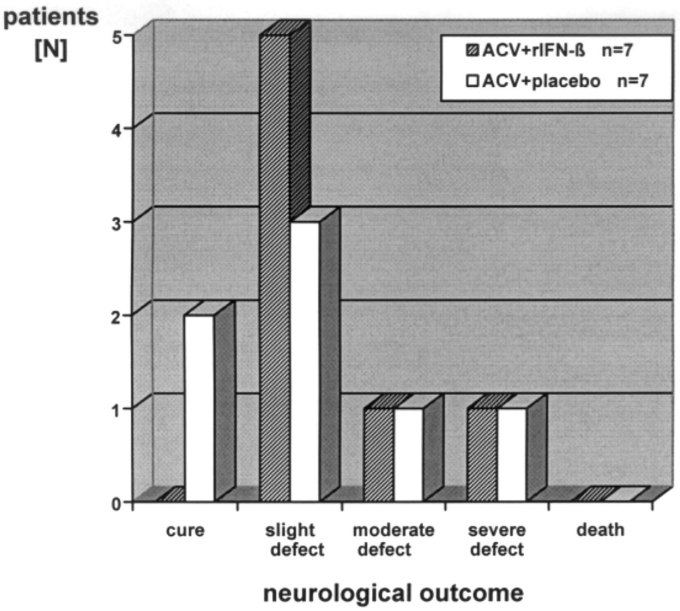

Fig.1. Outcome 3 months after admission to the hospital (N = 14).

gin, neurological history, clinical course and concomittant steroid therapy (Table 3).

Three months after admission to the hospital neurological outcome was evaluated as shown in Figure 1. Two patients of the placebo group were rated as cured, five patients of the verum group and three patients in the placebo group showed slight defects. Two patients of each group had moderate or severe defects. The differences between the two therapy groups were not statistically significant. There was no trend in favour of the verum group.

Adverse events

Adverse events were evaluated in all 56 patients who were initially enrolled in the study. 28 patients were treated with ACV + rIFN-β and 28 with ACV + placebo. The application of rIFN-β was well tolerated. Mean body temperature was slightly higher in the rIFN-β group from day three on. This difference was statistically significant at day four to five (38.2oC versus 37.4oC at day 4).

In five of 28 patients of the verum group (18%) and four of 28 patients of the placebo group (14%) adverse events were reported by the local investigators. In the rIFN-β group fever (two patients), hypotension (one patient), elevation of creatinine (one patient), liver enzymes (one patient) and PTT (one patient), thrombocytopenia (one patient), elevation of sodium (one patient) and reduction of potassium (one patient) and supraventricular extrasystoles (one patient) were

observed. One patient died due to multiorgan failure of unknown origin with the symptoms of coma and status epilepticus. An association of this complication to the therapy with rIFN-β seems to be unlikely because major problems, i.e. signs of liver failure were already present at the time of enrollment in the study.

In the placebo group fever (two patients), elevation of creatinine (one patient), organic psychosyndrome (one patient) and vomiting (one patient) were mentioned. The relation of the adverse events to the therapy was rated as unrelated to probable in both groups by the local investigators.

Discussion

The introduction of ACV has clearly improved the outcome of severe focal encephalitis like herpes simplex encephalitis. However, mortality rate (28%) and defective healing rate (about 35%) are still unsatisfactory [11, 13]. Positive results from a retrospective study evaluating the effect of a combination of ACV and natural β-interferon as well as an animal study prompted us to initiate this prospective randomized double blind trial [17, 18]. Also for interferon-alpha2b a remarkable reduction in quadriplegia in patients with St. Louis encephalitis compared to historical controls was reported [8]. On the other hand Solomon et al. [12] found no beneficial effects of alpha-interferon in children with Japanese encephalitis in a randomised double-blind placebo controlled study. Therefore, we initiated a prospective randomised study to evaluate the benefit of additional ß-interferon in the treatement of focal viral encephalitis.

As suggested by theoretical considerations therapy with interferon has to be introduced in the first days of the disease. Therefore, therapy was started immediately after enrollment of a patient. This procedure seemed to be justified by the severity of the underlying disease. In 14 of 56 patients, who were initially enrolled in the study focal viral encephalitis (i.e. HSV encephalitis) could be verified. These patients were included in the analysis of the efficacy.

Unfortunately the study had to be terminated prematurely for withdrawal of financial support. In spite of 72 patients (36 patients for each arm) only 14 patients (7 patients in each arm) were enrolled. Nevertheless, both groups were well balanced in terms of important prognostic factors. In this population no positive effect in favour of rIFN-β administration was observed.

The result of this study does not support the results of the preceding retrospective study of our group [14]. One explanation might be that the size of the present study was too small to show a positive effect of rIFN-β which then is expected not to be high. Secondly, the retrospective design of the former study may have shown a false positive effect. Methods of comfirmation of the diagnosis of focal encephalitis, start of the therapy with ACV and IFN might be different between the hospitals included.

In addition, in the above mentioned retrospective study natural interferon-ß at a higher dose was used. It is unknown whether a dose of 200 000 U/kg rIFN-β is as effective as the recommended dose of 500 000 U/kg for natural interferon-ß. Also one might speculate if the natural interferon-ß might have a higher efficacy in vivo. For the present study natural interferon-ß was not available and the used dose of rIFN-β was demonstrated to be the maximum tolerable dose in pilot studies performed for other indications in adults [3].

Several case reports showed positive effects of the combination of ACV and ß-interferon in patients with HSV encephalitis [1, 5, 9]. However, the natural course of this disease shows a great variability. rIFN-β was well tolerated. In the verum group only a slight but significant elevation of the body temperature was observed at day four and five after the start of the therapy. No adverse event according to the WHO-classification was rated as severe (grade 3 or more).

In conclusion in this well balanced study we observed no positive effect in favour of a combination regimen of rIFN-β with ACV in the therapy of focal viral encephalitis in children. However, the power of the study was to weak for a definite answer.

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