Eur J Pediatr (1995) 154:295–298

ORIGINAL PAPER

R. S. Weening
G. J. Leitz
R. A. Seger

Recombinant human interferon-gamma in patients with chronic granulomatous disease – European follow up study

Received: 22 June 1994
Accepted: 31 October 1994

R. S. Weening (✉)
Clinical and Laboratory Immunology Unit,
Emma Children's Hospital,
Department of Paediatrics,
Academic Medical Center,
Meibergdreef 9, NL-1105 AZ Amsterdam,
The Netherlands
Tel.: 31–20–5663050
Fax: 31–20–6917735

R. S. Weening
Central Laboratory of the Netherlands
Red Cross Blood Transfusion Service
and Laboratory
for Experimental and Clinical Immunology,
University of Amsterdam,
The Netherlands

G. J. Leitz
Boehringer Ingelheim, Ingelheim,
Germany

R. A. Seger
Division of Immunology/Haematology,
University Children's Hospital,
CH-8032 Zurich, Switzerland

Abstract This was an uncontrolled, open-label follow up study of a previous 12-month, randomized, double-blind, placebo-controlled trial performed to assess the long-term efficacy and safety of Recombinant Human Interferon Gamma (rIFN-γ) in patients with chronic granulomatous disease (CGD). In two centres, 28 patients (24 male, 4 female) with a mean age of 16 years (range 3–37) entered the open-label phase. The patients were treated for a mean of 880 days (range 97–1375 days). Visits were scheduled every 180 days and patients completed one to six visits. rIFN-γ was administered subcutaneously three times weekly at a dose of 0.05 mg per m^2. During the open-label phase of the study 12 patients experienced a serious infection requiring hospitalization within 880 days. The median infection-free time was 993 days. No obvious increase of infections over time was seen. Phagocyte superoxide anion production and phagocyte staphylococcal killing were not influenced by therapy. Seven patients were withdrawn from the study, one because of an adverse reaction, three on their own wish and the other three because they changed to another trial. No patient died during the study.

Conclusion Treatment of patients with CGD with intracellular active antibiotics and additional interferon gamma as infection prophylaxis is safe and justified.

Key words Chronic granulomatous disease · Interferon-gamma · Infections

Abbreviations *CGD* chronic granulomatous disease · *IFN-γ* human interferon-gamma · *rIFN-γ* recombinant human interferon-gamma

Introduction

Chronic granulomatous disease (CGD) is a heterogenous group of inherited disorders of immune function characterized by recurrent pyogenic infections that usually present early in life and may lead to death in childhood [4]. Phagocytes from CGD patients ingest but fail to kill micro-organisms due to the inability to generate superoxide, hydrogen peroxide, and other toxic oxygen metabolites.

The use of prophylactic and aggressive, broad spectrum, intravenous antibiotics has resulted in improved patient survival. In spite of improved therapy, CGD patients still suffer from recurrent infections and granulomatous lesions.

A number of pilot studies with recombinant human interferon-gamma (rIFN-γ), prepared by Genentech, Inc. (South San Francisco, USA), suggested that rIFN-γ might correct the specific respiratory burst defect in certain forms of CGD [1, 2, 5]. Therefore, a randomized, double-blind, placebo-controlled clinical trial was conducted in 13 centres in the U.S.A. and Europe to determine the efficacy and safety of rIFN-γ in patients with CGD. After 12 months of treatment the double-blind part of the trial was

completed and patients were offered open-label rIFN-γ therapy. Double-blind data have been reported elsewhere and showed a beneficial effect of rIFN-γ when compared with placebo [6]. The objectives of the present study were: (1) to determine whether continued rIFN-γ therapy is able to maintain the relative low incidence of serious infections in patients with CGD and (2) to evaluate the safety of long-term rIFN-γ therapy in these patients. This report summarizes data from the open-label phase for the European centres (Zurich and Amsterdam). The open-label phase remains ongoing.

Patients and methods

Study design

All patients who had completed the 12-month double-blind phase of the study were offered treatment with open-label rIFN-γ. In the two centres reported here 28 patients (14 in each centre) were enrolled in the open-label phase (1 August 1989). During the double-blind study 16 patients had received placebo and 12 patients rIFN-γ. Of these 28 patients, 2 discontinued therapy for 1060 and 328 days respectively before restarting with open-label.

The 28 patients were treated between 97 and 1375 days with open-label rIFN-γ. Mean duration of treatment was 880 days. Only few missed injections were reported.

The protocol was approved by the medical ethical committees of the medical centres and conducted according to the declaration of Helsinki.

Each patient received rIFN-γ three times weekly (Monday, Wednesday and Friday). For patients whose body surface was larger than 0.5 m^2 the dose was 0.05 mg/m^2 and for patients whose body surface was less than 0.5 m^2 the dose was 0.0015 mg/kg body weight. rIFN-γ was administered by a subcutaneous injection, to be alternated between the right and left deltoid and the anterior thigh. It was supplied in vials of 0.5 ml sterile liquid with a nominal content of 0.1 mg rIFN-γ. No specific guidelines for concomitant therapy during the open-label study were given. Nineteen of the 28 patients stayed under prophylactic antibiotic treatment throughout the study, while 11 patients used prophylaxis against fungal infections.

The number of visits to the medical centres ranged from one to six visits per patient.

Criteria for effectiveness

Primary endpoint for the assessment of efficacy was the time to serious infection. Serious infection was defined as an infection requiring hospitalization for treatment with parenteral antibiotics.

Secondary endpoints were:

1. The effect of treatment on the severity of an infectious episode as measured by the duration of hospitalization and parenteral antibiotic therapy.

2. Laboratory functional assays, phagocyte superoxide anion production and phagocyte staphylococcal killing.

Effectiveness

Serious infections were recorded by the investigator at each visit with diagnosis, date of diagnosis, required medication and/or surgical procedures and days of hospitalization. Phagocyte superoxide anion production was determined at each visit by spectrophotometric assays [6]. Phagocyte staphylococcal killing after 1 h and 2 h was measured at each visit. A control measurement was always performed [6].

Safety

All adverse events were to be recorded in the case report form with date of onset, severity, relationship to therapy, effect on therapy, required treatment, and clinical outcome.

Clinical and laboratory tests

At each visit a clinical examination was performed at which weight, height, and vital signs were recorded and any abnormalities were documented. A clinical laboratory analysis including haematology, serum chemistry, urine analysis, serology, thyroid function, and erythrocyte sedimentation rate was completed at each visit. Developmental status was judged at each visit for pubic hair, breasts, and testicular size according to the Tanner scale. Also at each visit hormonal levels were measured, and blood samples for determination of anti-IFN-γ antibodies were taken. Visits were scheduled every 180 days while receiving rIFN-γ. At each visit all variables described above were recorded. During data management visits were numbered sequentially starting with visit 1.

Statistical analysis

Data were entered into the database twice by two different individuals. Discrepancies were clarified and corrected in the database. The final report was reviewed for clarity and correctness by a second biometrician not involved in the project. The time from the start of open-label therapy until diagnosis of the first serious infection was analysed by plotting the Kaplan-Meier estimate. Data measured at each visit were analysed by visit number, i.e. no time windows for the visits were defined. For each visit the number and percentage of patients with a serious infection was analysed. Because the number of patients attending a visit differed considerably between visits, the subpopulation of all patients completing visit 4 (approximately after 3 years) was considered and the number and percentage of patients in this subpopulation with a serious infection was tabulated by visit. This type of analysis was also performed for other variables measured in the time course and was intended to eliminate a possible bias due to populations varying over time.

Vital signs, erythrocyte sedimentation rate, and laboratory parameters were also summarized in the time course. Adverse events were tabulated by severity, relationship to therapy, required treatment and clinical outcome. When summarized by severity, an adverse event that was reported more than once was summarized with the highest reported severity.

Results

Twenty-eight patients, 14 from each centre, entered the open-label phase of the study. Seven patients discontinued treatment with rIFN-γ (Table 1). The patient characteristics are given in Table 1. A summary of the demographic data of the patients is given in Table 2. Twelve patients experienced at least one serious infection during the open-label phase within 880 days. Table 3 shows the frequencies of serious infections for each body system.

Table 1 Patient characteristics

	n	%	Study day[a]	
			Mean	Range
Entered open-label	28	100		
Duration of treatment	28	100	880	97–1375
Withdrawn	7	25		
Adverse event	1			
Patient wish	3			
Change to other trial	3			

[a] Study day 1 = start of open-label phase

Table 2 Demographic data

Age [years]	
Mean	16
Range	3–37
Sex [*n* (%) of patients]	
Male	24 (86)
Female	4 (14)
Weight [kg]	
Mean	43.3
Range	16.2–80.0
Body surface area [m^2]	
Mean	1.33
Range	0.65–2.00

Table 3 Serious infections by body system [number (%) of patients]

Body system	All patients *n* = 28
Intestinal tract	6 (21)
Respiratory organ	4 (14)
Nervous and sensory system	2 (7)
Skin	2 (7)
Infections and paradentosis	1 (4)
Unspecified infections	1 (4)
Urinary and genital tract	1 (4)

The time from the start of open-label until the first serious infection was estimated by means of the Kaplan-Meier estimate and is given in Fig. 1. The median of the distribution, which is the time point at which half of the patients had experienced a serious infection, was estimated as 993 days. No trend over time towards an increase or decrease is obvious.

Table 4 summarizes the number of serious infections per year, the number of hospitalizations per year, and the number of days in hospital per year. The number of hospitalizations per year exceeded the number of infections per year, because some patients were hospitalized more than once for one infection. Phagocyte superoxide anion production and phagocyte staphhylococcal killing studies were only performed for neutrophils in the Zurich centre.

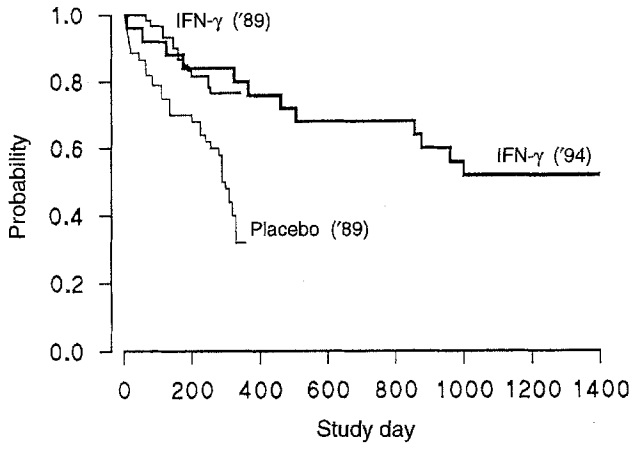

Fig. 1 The Kaplan-Meier estimate of the time to serious infections of the 28 patients in this European open-label study (IFN-γ '94) is superimposed on the data from the blinded study (IFN-γ '89 and placebo '89)

Table 4 Number of serious infections and hospitalization

	All patients *n* = 28
Serious infections per year	
Mean	0.4
Range	0–1.7
Hospitalizations per year	
Mean	0.5
Range	0–6.1
Days of hospitalization per year	
Mean	15.0
Range	0–112.6

Table 5 Drug related adverse events. More than one adverse event per patient possible (number (%) of patients)

Event	All patients (*n* = 28)
Fever	7 (25)
Headache	4 (14)
Digestive system (anorexia, gastritis, colitis, diarrhoea)	4 (14)
Injection-site reaction	2 (7)
Influenza syndrome	1 (4)
Dry mouth	1 (4)
Arthralgia	1 (4)
Asthenia	1 (4)
Anti-nuclear antibodies present	2 (7)

There was no apparent difference between controls and patients (not shown).

Adverse events are summarized in Table 5. Overall, 20 patients experienced at least one adverse event. Severe adverse events were reported by 4 patients (fever) causing

the withdrawal of one patient. No patient died during the study.

In most patient total serum globulin decreased, while in 6 patients globulin increased during infections. In 5 patients rheumatoid factors were elevated. In 2 patients a positive antinuclear antibody titre was found (not shown). Although growth is often retarded in CGD patients [3], no effect of IFN-γ treatment on this parameter was observed. Head, ears, eyes, nose and throat, lymph nodes, and skin were the areas where new abnormalities were noted most frequently. They occurred with about equal frequency at each visit. For the other body areas new findings were only sporadic and were reported for 3 patients. Anti-IFN-γ antibodies were only measured in the Zürich centre. No antibodies were detected.

Discussion

In an earlier double-blind, placebo-controlled study, the efficacy of rIFN-γ injected subcutaneously, three times weekly, in reducing the frequency of serious infections in patients with CGD was clearly established. This therapy was effective in all genetic types of CGD, while children younger than 10 years of age benefited most. No antibodies to IFN-γ were detected and the study showed that this drug has only few side-effects.

Despite previous indications [1, 2, 5], that study failed to show improvement in phagocyte function; thus, the mode of action of this clinical benefit remains unknown. The present study was conducted to investigate whether the improvement in clinical condition remained stable during the next 3 years and to monitor the long-term side-effects of IFN-γ. This drug is approved by the FDA and most European countries for this indication. Therefore, the present study had to be an uncontrolled, open-label study. Only patients from the double-blind study were enrolled. Our study shows that the clinical improvement is at least stable during the follow up period. Again, no influence of IFN-γ on superoxide formation and phagocyte staphylococcus killing was observed by the polymorphonuclear cells of the patients in one of the two centres.

Most patients in the blinded as well as in this open-label study used prophylactic antibiotics. The mean age was comparable in both studies. Because the blinded study established in a prospective way that CGD patients on IFN-γ plus prophylactic antibiotics showed less serious infections than on prophylactic antibiotics alone, the patients with serious infections were all motivated for the open-label study. We decided not to include the remaining group of patients on prophylactic antibiotics as comparison, because this group would consist of the less serious infected patients. Fever and headache were the most frequent side-effects. Furthermore, 5 patients with positive rheumatoid factors and 2 with positive antinuclear antibody titres have to be followed to see whether these are transient or precede clinical implications. Therefore, this study is still ongoing. Nevertheless, a remarkable resemblence of this open-label study with the IFN-γ arm of the blinded study was seen, regarding the number of serious infections. The same trend was independently noted in the U.S.A. (J.C. Curnutte, personal communication).

We conclude that treatment of patients with CGD with intracellularly active antibiotics and IFN-γ for infection prophylaxis is justified, and that this treatment is safe, without serious long-term side-effects.

Acknowledgements We acknowledge Dr. W. Sauermann, DATAMAP, for careful statistical analysis of the data.

References

1. Ezekowitz RAB, Orkin SH, Newburger PE (1987) Recombinant interferon gamma augments phagocyte superoxide production and X-chronic granulomatous disease gene expression in X-linked variant chronic granulomatous disease. J Clin Invest 80:1009–1016
2. Ezekowitz RAB, Dinauer MC, Jaffe HS, Orkin SH, Newburger PE (1988) Partial correction of the phagocyte defect in patients with X-linked chronic granulomatous disease by subcutaneous interferon gamma. N Engl J Med 319:146–151
3. Forrest CB, Forehand JR, Axtell RA, Roberts RL, Johnston RB (1988) Clinical features and current management of chronic granulomatous disease. Hematol Oncol Clin N Am 2:253–267
4. Johnston RB Jr, Newman SL (1977) Chronic granulomatous disease. Pediatr Clin North Am 24:365–376
5. Sechler JMG, Malech HL, White CJ, Gallin JI (1988) Recombinant human interferon-γ reconstitutes defective phagocyte function in patients with chronic granulomatous disease of childhood. Proc Nat Acad Sci USA 85:4874–4878
6. The International Chronic Granulomatous Disease Cooperative Study Group (1991) A controlled trial of interferon-γ to prevent infection in chronic granulomatous disease. N Engl J Med 324:509–516