The effect of interferon alpha administration on acute attacks of familial Mediterranean fever: A double-blind, placebo-controlled trial

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This study was financially supported by Schering-Plough Tıbbi Ürünler Tic. AS, Turkey.

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Received on July 2, 2003; accepted in revised form on February 9, 2004.

Clin Exp Rheumatol 2004; 22 (Suppl. 34): S37-S40.

Key words: Familial Mediterranean fever, colchicine, interferon alpha, acute phase reactants, C-reactive protein, serum amyloid Aprotein.

ABSTRACT

Background. *About a quarter of familial Mediterranean fever (FMF) patients are partially or totally resistant to colchicine. A previous observation reported that acute attacks may be shortened by administration of interferon alpha (IFN).*

Objective. *We designed a double-blind, placebo-controlled trial to test our initial observations of a beneficial response with IFN in FMF attacks.*

Methods. *We treated 34 acute abdominal attacks with IFN 5 million IU or placebo sc in the early phase of the attack. Leucocytes, thrombocytes, the erythrocyte sedimentation rate, fibrinogen, C-reactive protein (CRP), serum amyloid A protein (SAA), haptoglobin, transferrin, IL-1β and TNF-α were measured at hours 0, 6, 12, 24 and 48.*

Results. *The median time to recovery in those treated with IFN and placebo was not significantly different, while the leucocytosis and high levels of fibrinogen were significantly more prolonged in placebo-treated patients. CRP and SAA were extremely elevated and peaked at 24h, remaining less marked in the IFN-treated patients but the difference was not statistically significant. Observations regarding the other parameters were unremarkable.*

Conclusions. *Although there were some clues indicating a depressed inflammatory response with IFN, we could not demonstrate a definitive effect of this agent in this double-blind trial. The drug may suppress the acute inflammation of FMF only if administered at the earliest phase. CRP and SAA may be more sensitive indicators of an attack than ESR or fibrinogen.*

Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterised by recurrent febrile attacks of polyserositis resolving spontaneously in 24-72 hours. Although colchicine has been effective in preventing both the attacks and complication of AAamyloidosis, about a quarter of patients still experience occasional attacks and 5-10% of them are resistant to the drug (1). We had observed beneficial response in a colchicine-resistant FMF patient who was treated with interferon alpha (IFN) for chronic hepatitis B disease (2). Our further observations in a pilot study on 21 attacks had shown that IFN might ameliorate acute FMF attacks (3). We have now commenced a double blind and placebo-controlled trial.

Patients and methods

Thirty-four acute abdominal attacks in 22 adult FMF patients on optimal regular colchicine therapy were investigated. All of the patients fulfilled the Tel-Hashomer diagnostic criteria for "definite FMF" (4). There were 16 male and 6 female patients with a mean age of 35.68 (median: 32).

Initially, 24 of the patients with acute attacks were admitted to hospital and treated in a double-blind fashion for 48 hours; another 10 attacks were treated as out-patients and single-blinded. The study was approved by the ethical committee of our institute and informed consent was obtained from each patient.

Either placebo (13 attacks) or 5 million IU of IFN (11 attacks) were administered subcutaneously to hospitalised patients. Each patient was interviewed regarding the commencement and intensity of pain and examined with particular emphasis as to signs of peritonitis once an hour during the first 12 hours, then every 3 hours until they were discharged. A visual analog scale (VAS; 0-

Table I. The medians (IQR in parentheses) of serial determinations of acute phase reactants during the 48 hours of hospitalisation of 24 patients.

		0 hr	6 hr	12 hr	24 hr	48 hr
WBC	IFN	12.4 (11.9-15.0)	9.3 (8.0-11.0)	8.3 (6.8-9.9)	6.7 (5.6-7.6)	5.9 (4.5-7.7)
($x10^9$/L)	Placebo	12.2 (9.2-13.5)	12.1 (9.6-14.6)	10.1 (8.3-13.5)	8.0 (7.1-9.0)	5.9 (5.5-7.7)
CRP	IFN	24.5 (7.4-89.5)	68.9 (36.5-116.5)	88.6 (19.7-133.3)	125.2 (44.3-154.8)	75.9 (31.1-95.9)
(mg/L)	Placebo	26.6 (4.4-59.1)	66.5 (29.2-116)	116 (79.4-141.7)	194.3 (118.9-219.5)	84.2 (46.6-108.9)
SAA	IFN	53.8 (21.7-481.5)	212 (86.6-424.3)	359.5 (84.7-497.3)	664 (59.5-789)	468 (181.3-799)
(mg/L)	Placebo	142 (14.4-213)	273 (152-584)	495 (287-827)	1090 (690.5-1275)	826 (376-1110)
ESR	IFN	14 (5-25)	15 (7-25)	13 (8.5-31.3)	19 (11-39)	30.5 (14.5-43)
(mm/h)	Placebo	21 (7.5-31)	12.5 (4.3-25.3)	17 (5-36)	22 (2.8-52.8)	34.5 (8.5-41.5)
Fibrinogen	IFN	3.2 (3.0-4.3)	3.7 (3.1-5.1)	4.1 (2.8-4.7)	4.2 (3.9-4.8)	4.0 (3.1-4.8)
(g/L)	Placebo	2.3 (1.3-4.5)	3.1 (2.5-4.0)	3.1 (2.4-5.0)	4.5 (3.6-5.9)	4.3 (3.6-6.2)

100 mm) was used to measure the intensity of pain. Each patient was given 500 mg paracetamol q6h and extra doses were allowed if necessary and noted. Resolution of the attack was defined as the disappearance of abdominal rigidity and attainment of a level of pain which would not interfere with the patient's sleep or daily activities, including going to work or school (VAS ≤ 50). Before being discharged, each patient was requested to give their opinion on the agent administered and asked whether he/she would wish to use the drug again in the future.

A list of side effects which could be attributed to IFN was used at every interview for evaluation and they were scored as none, mild (requiring no extra medical intervention), moderate (requiring extra medical intervention) or severe (requiring hospitalisation).

Blood samples were obtained at hours 0, 6, 12, 24 and 48. Analysis of the white blood cells (WBC), thrombocytes, erythrocyte sedimentation rate (ESR), and fibrinogen determinations were performed immediately at the hospital laboratory. Serum samples for C-reactive protein (CRP), serum amyloid A protein (SAA), haptoglobin, transferrin, interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α) were separated, stored at –70ºC and measured at the end of the study. All serum samples were labelled in a blinded fashion.

High sensitivity immunoassays for CRP and SAA were performed as reported previously (5,6). Transferrin and haptoglobin levels in the serum were measured by rate nephelometry (Array Protein System, Beckman, Ireland). TNF-α and IL-1β levels in the serum were determined by a photometric enzyme-linked immunosorbent assay method (Boehringer Mannheim, Germany).

Since the initial interview and the process of admitting the patient to the hospital inevitably delayed the commencement of drug administration, we later treated another 10 attacks as out-patients in a single-blinded fashion, the IFN dosage remaining the same. Six patients were treated with IFN and 4 received placebo. Each patient made a telephone call at the earliest signs of an acute attack and a member of our team went to the patient and administered the agent along with 500 mg tablet of paracetamol, the patient being blinded. Both the physician and the patient had separate record sheets for documenting follow-up data including possible side effects and response to treatment. The patient remained in close contact with his/her physician and was followed by telephone every 3-4 hours until the resolution of the attack and was re-examined at the end of this period. Again, each patient was requested to estimate the agent administered and asked whether he/she would be willing to re-use the drug in the future. Blood analyses were not performed in this group.

Statistical analysis

Statistical analyses were performed using SAS software, version 6.21 (SAS Institute, Inc., Cary, North Carolina). Between-group comparisons were analysed by the Mann-Whitney U Wilcoxon rank-sum W test. A P value less than or equal to 0.05 was considered statistically significant. P values are quoted exactly. Data are presented as the median and the interquartile range (IQR) from the 25th to 75th percentile in parentheses, unless otherwise noted.

Results

Patients admitted to hospital were treated about 12 hours after the commencement of an attack. The median time to achieving a VAS score of 50 was 7.0h (range: 1-18h) for IFN and 6.0h (range: 1-48h) for the placebo group (p = 0.649). The median time to recovery in patients who were treated with IFN or placebo at home less than 3 hours after the commencement of an attack was 6.5h (range: 2-24h) and 16.5h (range: 2-30h), respectively (p = 0.352).

High initial median values of WBC (x 10^9/L) began to fall from 6h onwards in IFN-treated patients (p = 0.003), while the median WBC of placebo-treated patients declined significantly only at 24h (p = 0.001). The median values of both CRP(mg/L) and SAA(normal < 3 mg/L) significantly rose from 6h onwards (p=0.001), peaking at 24h in placebo-treated patients (p = 0.001 for CRP and p = 0.000 for SAA). This increase was less marked and did not attain statistical significance in the IFN-treated patients (p = 0.141 for CRP and p = 0.684 for SAA at 24 h). However,

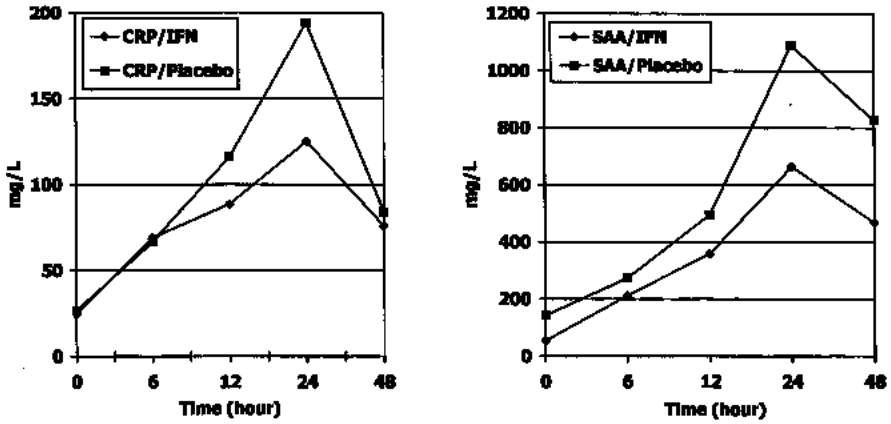

Fig. 1. The sequential levels of CRPand SSAduring the acute attack period of FMF patients.

there was no demonstrable statistical significance in the between-group analyses ($p = 0.067$). Fibrinogen (g/L) was significantly elevated at 24h and 48h only in the placebo-treated group ($p = 0.010$ and 0.024, respectively), ESR (mm/h) elevations were not significant in either group (see Table I for median values and the IQR 25th 75th percentile). The thrombocytes declined slightly, remaining within the normal range and haptoglobin, transferrin, and IL-1β showed a somewhat stepwise rise during the observation period without significant differences between the two groups, while TNF-α values were extremely low or undetectable (data not presented).

Correct recognition of the agent was achieved by only one-third of the hospitalised patients and 8 of the 10 outpatients. The reported and observed side effects were similar and mild to moderate in both groups and most of the symptoms and signs overlapped with the clinical characteristics of FMF.

Discussion

Acute attacks of FMF can be prevented by the daily administration of oral colchicine. However, apart from the occasional patient who is totally resistant, a significant number of patients have unexpected attacks despite full compliance with the drug. Although the exact role and site of action of pyrin encoded by the FMF gene (MEFV) is not yet certain, its N-terminal half has structural similarities with several proteins of the apoptosis pathway (7). A malfunctioning pyrin may cause delay in neutrophil inactivation and the inflammatory process may be prolonged (8). Colchicine inhibits the chemotaxis of neutrophils and, although it is quite efficient prophylactically, the drug cannot stop an ongoing attack probably because the neutrophils, which have already migrated to the peripheral blood from the bone marrow, are "beyond control". Recently it has been shown that IFN gamma or the combination of IFN alpha and colchicine induce MEFV gene expression in neutrophils (9). Therefore, if IFN is administered to the patient at a sufficiently early period during an acute attack, the drug may increase the quantity of the qualitatively defective pyrin and enhance inhibition of the neutrophils. Our previous observations that IFN may be a valuable adjunct treatment for patients resistant to colchicine was later confirmed by another group who administered the drug at regular intervals (10). However, the indefinite administration of twice weekly interferon or weekly supplements of intravenous colchicine, which may be beneficial to some extent (11), does not seem clinically feasible. Our main aim was to use the drug for once at a very early phase and see if it would abort or shorten the attack.

Our study presented a number of technical difficulties, mostly originating from the peculiarities of the disease we were treating. We had observed a beneficial effect of the drug in 18 of 21 attacks in our pilot study (3), but the placebo response was higher than expected in this cohort, which may have been due to the patients'reluctance to be hospitalized and the consequent delay in commencement of treatment.

The median time to recovery was about 7 hours in placebo-treated patients, which is much shorter than that seen in a classical attack of 24-72 hours and may point to a treatment delay. One patient treated with placebo fully recovered in 2 hours, which may represent an incomplete attack or a false alarm. Five of the 6 patients who were treated with IFN at home at the earliest signs responded in 6 hours, while the patient treated within 3 hours of the attack resolved in 24 hours. The first 2 hours therefore seem to be crucial for a beneficial effect.

During the acute attack FMF patients have high fever, severe fatigue, nausea and vomiting, constipation and generalised myalgia. These symptoms are quite similar to the side effect profile of IFN and those receiving IFN would be expected to have an even more severe attack than the placebo-treated patients. This was not the case and patients tolerated the treatment without difficulty.

This study provided us with the unique opportunity of following prospectively the serum levels of various acute phase reactants during an ongoing acute attack of FMF. None of these acute phase reactants are specific for FMF and their relative sensitivities have not yet been determined. Some of these parameters revealed an earlier regression of the inflammatory response among the IFN-treated patients and may be regarded as an objective sign of the efficacy of the therapeutic intervention. The most striking elevations were observed with CRP and SAA, rising several hundredfold above normal (see Table I and Fig. 1). SAA is an essential prerequisite for the development of AA amyloidosis and suppressing its levels below 10 mg/Lhas a favourable effect on the survival of patients with established amyloidosis (12). Indeed, two studies have observed that CRP and SAA may be more sensitive than ESR or fibrinogen as indicators of an acute attack and ongoing subclinical inflammation in FMF (13, 14). Furthermore, we could de-

monstrate that the serum levels of such parameters as IL-1β or TNF-α do not rise significantly, if at all, during an acute attack of FMF.

This study could not reach a definite conclusion regarding the efficacy of a single administration of IFN during an FMF attack. The drug may be effective in shortening the acute attack period of FMF if administered at the earliest phase. Further studies on larger groups of patients will determine the most appropriate time and dosage for this treatment.

Acknowledgements

The authors would like to thank Dr. Mesut Akarsu and Dr. Kutlay Naci Tutucu for their help and Hatice Üstün for the statistical analysis.

References

1. LIVNEH A, LANGEVITZ P, ZEMER D *et al*.: The changing face of familial Mediterranean fever. *Semin Arthritis Rheum* 1996; 26: 612-7.
2. TANKURT E, TUNCA M, AKBAYLAR H, GÖNEN Ö: Resolving familial Mediterranean fever attacks with interferon alpha. *Br J Rheumatol* 1996; 35: 1188-9.
3. TUNCA M, TANKURT E, AKBAYLAR AKPINAR H, AKAR S, HIZLI N, GÖNEN Ö: The efficacy of interferon alpha on colchicine-resistant familial Mediterranean fever attacks: a pilot study. *Br J Rheumatol* 1997; 36: 1005-08.
4. PRAS M: Familial Mediterranean fever – from the clinical syndrome to the cloning of the pyrin gene. *Scand J Rheumatol* 1998; 27: 1-6.
5. WILKINS J, GALLIMORE JR, MOORE EG, PEPYS MB: Rapid automated high sensitivity enzyme immunoassay of C-reactive protein. *Clin Chem* 1998; 44: 1358-61.
6. WILKINS J, GALLIMORE JR, TENNET GA *et al*.: Rapid automated enzyme immunoassay of serum amyloid A. *Clin Chem* 1994; 40: 1284-90.
7. RICHARDS N, SCHANER P, DIAZ A, STUCKEY J, SHELDEN E, WADHWA A, GUMUCHIO DL: Interaction between pyrin and the apoptotic spec protein (ASC) modulates ASC-induced apoptosis. *J Biol Chem* 2001; 276: 39320-9.
8. CHAE JJ, KOMAROW HD, CHENG J, WOOD G, RABEN N, LIU PP,KASTNER DL: Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol Cell* 2003; 11: 591-604.
9. CENTOLA M, WOOD G, FRUCHT DM *et al*.: The gene for familial Mediterranean fever, MEFV, is expressed in early leukocyte development and is regulated in response to inflammatory mediators. *Blood* 2000; 95: 3223-31.
10. CALGUNERI M, APRAS S, OZBALKAN Z, OZTURK MA, ERTENLI I, KIRAZ S: The efficacy of the interferon alpha on colchicine-resistant familial Mediterranean fever (FMF). *Clin Exp Rheumatol* 2002; 20 (Suppl. 26): S106 (abstract).
11. LIDAR M, KEDEM R, LANGEVITZ P, PRAS M, LIVNEH A: Intravenous colchicine for treatment of patients with familial Mediterranean fever unresponsive to oral colchicine. *J Rheumatol* 2003; 30: 2620-3.
12. GILLMORE JD, LOVAT LB, PERSEY MR, PEPYS MB, HAWKINS PN: Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet* 2001; 358: 24-29.
13. KORKMAZ C, OZDOGAN H, KASAPCOPUR O, YAZICI H: Acute phase response in familial Mediterranean fever. *Ann Rheum Dis* 2002; 61: 79-81.
14. DUZOVA A, BAKKALOGLU A, BESBAS N *et al*.: Role of A-SAAin monitoring subclinical inflammation and in colchicine dosage in familial Mediterranean fever. *Clin Exp Rheumatol* 2003; 21: 509-14.