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Effects of antifibrotic agents on TGF-β1, CTGF and IFN-γ expression in patients with idiopathic pulmonary fibrosis

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Summary
Idiopathic pulmonary fibrosis (IPF) is a deadly disease, largely unresponsive to treatment with corticosteroids and immunosuppressives. The aim of this randomized, prospective, open-label study was to characterize the molecular effects of IFN-γ-1b and colchicine, on biomarkers expression associated with fibrosis (TGF-β, CTGF) and immunomodulatory/antimicrobial activity (IFN-γ), in the lungs of patients with IPF.
Fourteen (14) patients with an established diagnosis of IPF received either 200 μg of IFN-γ-1b subcutaneously three times per week, or 1 mg of oral colchicine per day, for 24 months. Using RT-PCR assay, we evaluated the transcription levels of transforming growth factor β1 (TGF-β1), connective-tissue growth factor (CTGF), and interferon-γ (IFN-γ) genes in lung tissue before and after treatment with IFN-γ-1b or colchicine.
Marked mRNA expression of TGF-β1 and CTGF, but complete lack of interferon-γ was detected in fibrotic lung tissue at entry. After treatment, both groups exhibited increased expression of IFN-γ gene at 6 months that was sustained at 24 months. The expression of CTGF and TGF-β1 remained almost stable before and after treatment, in the IFN-γ-1b group, while TGF-β1 was statistically decreased after therapy, in the colchicine group ($p = 0.0002$). Significant difference in DLCO (% pred), was found between the two treatment groups in favor of IFN-γ-1b group ($p = 0.04$). In addition, the IFN-γ-1b group showed stability in arterial PO_2 while the colchicine group significantly deteriorated ($p = 0.02$).

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In conclusion, we report the effect of antifibrotic agents (IFN-γ-1b and colchicine) in TGF-β, CTGF, and endogenous IFN-γ gene expression, in human fibrosis. However, extended studies are needed to verify the pathophysiological consequences of these findings.

Introduction

Idiopathic pulmonary fibrosis (IPF) belongs to the group of idiopathic interstitial pneumonias (IIPs), leading to the deposition of extracellular matrix and end-stage alveolar fibrosis.[1–4] The median survival of patients with IPF is 3–5 years after the onset of symptoms.[5,6] Currently, two types of therapeutic agents (anti-inflammatory and antifibrotic) have been tested in the treatment of IPF.[7,8] However, the disease is largely unresponsive to treatment and no regimen has been proven effective to improve survival or disease progression.[1,2]

Molecular and cellular studies have identified several growth factors involved in lung fibrosis and the production and deposition of extracellular matrix proteins.[9] Connective tissue growth factor (CTGF) is a cysteine-rich peptide that promotes proliferation, collagen synthesis, and chemotaxis by mesenchymal cells. CTGF is over expressed in a variety of fibrotic disorders, presumably secondary to the activation and production of transforming growth factor-β.[10] The regulation of CTGF appears to be controlled primarily at the level of transcription, and a brief exposure of fibroblasts to transforming growth factor-beta (TGF-β) is sufficient to induce a prolonged high level of CTGF expression. TGF-β plays an important role in normal pulmonary morphogenesis and function. TGF-β is involved in wound healing following lung injury, being necessary for fibroblast proliferation, stimulation of granulation, tissue formation, and collagen deposition. It plays a key role in mediating fibrotic tissue remodeling by increasing the production and decreasing the degradation of connective tissue via several mechanisms.[10,11]

Exogenous interferon gamma (IFN-γ-1b), a cytokine highly homologous with natural IFN-γ is an attractive therapeutic candidate in IPF, because it regulates both macrophage and fibroblast functions. Therefore IFN-γ may influence the course of IPF through its antimicrobial, antifibrotic, antiproliferative and immunomodulatory properties.[7,12] The therapeutic effectiveness of IFN-γ-1b in IPF has been first reported by Ziesche et al.,[13] in a limited study. However, Raghu et al.[12] in a larger clinical trial failed to show a beneficial effect on progression-free survival, pulmonary function or the quality of life after one year of treatment.[12]

Colchicine is a well tolerated antifibrotic drug taken orally, with multiple effects, including the arrest of cell division, the inhibition of granulocytes migration, the release of several proteins from cells, and the blocking of the in vitro release of fibronectin from alveolar macrophages.[14–16] Previous studies suggested that its effects in IPF are similar to that of prednisone with fewer side effects, while the median survival remained unchanged.[14,15]

The aim of this prospective, comparative trial was to characterize molecular effects of subcutaneous IFN-γ-1b agent, versus conventional colchicine, after 6, 12, 24 months of therapy, on the transcription levels of TGF-β1, CTGF and IFN-γ biological markers, in 14 patients with IPF. In this study we report the effect of antifibrotic agents (IFN-γ-1b and colchicine) in TGF-β, CTGF, and endogenous IFN-γ gene expression, in human lung fibrosis.

Materials and methods

Study subjects

The study was approved by Medical Research Ethics Committee of the Hospital and patients gave their written informed consent. The enrolled patients were a subgroup of a larger randomized controlled study including 50 IPF patients.[17]

Fourteen (14) newly diagnosed, untreated, symptomatic IPF patients with final histopathological diagnosis of IPF/UIP

Table 1 Demographics and lung function tests of the study population at entry.

	Group 1 IFN-γ-1b ($n = 7$)	Group 2 Colchicine ($n = 7$)	*p-value*
Median (range) age (yr)	66 (54–85)	69 (42–82)	
Sex (Male/female)	6/1	6/1	
FVC (%pred)	80.7±11.6	76.3±19.8	0.5
FEV_1 (%pred)	90.5±14.7	88.1±15	0.7
TLC% (%pred)	73.1±14.1	68.4±11.1	0.4
DLCO, (%pred)	71.2±19.8	57.2±18.7	0.1
PaO_2 (mmHg)*	80.0±5.8	71.2±13.4	0.1
$PaCO_2$ (mmHg)*	40.5±3.6	40.1±1.3	0.8

Values are expressed as mean±SD, and age as median (range).
*Breathing Room Air.

according to ERS/ATS Consensus Classification of the Idiopathic Interstitial Pneumonias criteria (2002), including surgical lung biopsy, were included in this study.[2] Eligible patients were 40–75 years of age, had had clinical symptoms of IPF for at least three months, and had a forced vital capacity (FVC) $\geqslant$55% and $\leqslant$90% predicted value, a carbon monoxide diffusing capacity (DLCO) that was at least 35 percent of the predicted value, and a PaO_2 of more than 55 mmHg while they were breathing air at rest (Table 1). Criteria for exclusion were active infection within one week before enrollment, unstable cardiovascular or neurological disease, uncontrolled diabetes, pregnancy, lactation, or any active malignancy.

Study design

The study started with a run-in period of 2 months. During the run-in period, all eligible patients received 50 mg of oral prednisolone per day for 4 weeks, with subsequent tapering of the dose, over 1 month period, to 10 mg per day. Then patients were randomly assigned in 1:1 ratio to receive either 200 μg of interferon gamma-1b, subcutaneously three times per week, plus 10 mg of oral prednisolone daily, or 1 mg of oral colchicine per day, plus 10 mg of oral prednisolone daily. The duration of the treatment was 24-months. All new symptoms other than dyspnea were recorded as adverse events. All patients were followed for the duration of the study regardless of whether they continued IFN-γ-1b or colchicine. PFTs included spirometry, measurement of FVC, TLC, DLCO, and arterial blood gases at baseline and at 6, 12 and 16–24 months were measured (Table 2).

Biological material and RNA extraction

Two groups of patients were evaluated. The first consisted of seven (7) patients treated with IFN-γ-1b, and the second of seven (7) patients treated with colchicine. For the assessment of transforming growth factor β1 (TGF-β1), CTGF and IFN-γ transcription levels, open lung biopsies before, and transbronchial-biopsies specimens after treatment were obtained at 6 and 18–24 months interval. Samples were frozen in liquid nitrogen and stored at $-80\,^{\circ}C$. Total RNA was prepared separately from all patients at all three different time points (0, 6 and 18–24 months). Total RNA was extracted by using the RNeasy mini Kit (Qiagen Extraction Kits, QIAGEN Inc., Valencia, CA, USA). The cDNA sequence of the human TGF-β1, CTGF, IFN-γ and the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) genes (GenBank Accession nos. NM_000660, NM_001901, J00219 and NM_002046) were used to design primers for PCR amplification of the corresponding cDNA fragments (Table 3). G3PDH gene served as internal control. To this end, cDNA was synthesized by using the QIAGEN OneStepRT-PCR Kit (QIAGEN Inc., Valencia, CA, USA) from 2 μg of total RNA, using the reverse transcriptase mix provided with the kit, as described by the manufacturer. The mixture was incubated at 50 °C for 30 min, reverse transcriptase was heat-inactivated and, subsequently, the HotStartTaq DNA Polymerase (QIAGEN Inc., Valencia, CA, USA) was activated by applying an initial heating step at 95 °C for 15 min. Therefore, cDNA was amplified by the polymerase chain reaction (PCR) (40 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and finally 72 °C for 10 min). Aliquots of PCR products were resolved by agarose gel electrophoresis. The image was captured using a Gel Doc digital imaging system (BioRad Gel Doc 2000 System, OH, USA) and analyzed with the BioRad software (BioRad Inc., OH, USA). Relative quantities of mRNAs among different patients were calculated from three replicate RT-PCR experiments. Positive results were based on the presence of DNA bands of the expected size (Table 3).

Plasmid construction and sequencing

The cDNA sequence of the human interferon-γ (IFN-γ) gene, generated by PCR, was purified by using the QIAquick PCR Purification Kit (QIAGEN GmbH, Germany) and ligated into the pGEM plasmid vector (Promega Corporation, USA). The resultant plasmid was transformed into *Escherichia coli* JM 109 competent cells (Promega Corporation, USA) and the confirmation of the construction was done by restriction analysis (using the *Sal*I and *Nco*I restriction sites of the cloning vector) of several bacterial clones. Plasmid DNA was isolated from these clones using QIAprep Spin Miniprep Kit (QIAGEN GmbH, Germany). Restriction and DNA modification enzymes were provided from MINOTECH (MINOTECH Biotechnology, I.M.B.B.-FO.R.T.H., Heraklion, Crete, Greece) and New England Biolabs Inc. For the amplified IFN-γ fragments both strands were completely sequenced according to the di-deoxy-chain termination method following the manufacturer protocol (Sequenase, USB), using vector specific (T7, SP6) primers and the consensus nucleotide sequence was obtained for two different treated patients, from two sequencing reactions. A LiCor 4200L sequencer (Lincoln, NE, USA) of the laboratory of Microchemistry (IMBB-FORTH, Crete, Greece) was used. Restriction and DNA modification enzymes were provided from MINOTECH

Table 2 Pulmonary function at different time points in IFN-γ-1b and colchicine treated group.

PATIENTS	Interferon-γ-1b group				Colchicine group			
Time-points	Baseline (7 pts)	6 months (7 pts)	12 months (7 pts)	24 months (6 pts)	Baseline (7 pts)	6 months (7 pts)	12 months (7 pts)	24 months (2 pts)
FVC% pred	80.7±11.6	80.5±6.8	83.0±7.7	83.1±8.1	76.3±19.8	69.2±15.2	63.0±14.9	69.0±7.0
TLC% pred	73.1±14.1	69.1±14.1	71.1±10.3	67.5±10.8	68.4±11.1	60.0±5.7	58.5±9.4	53.0±24.0
TL_{CO}% pred	71.2±19.8	79.1±19.1	75.1±14.5	68.6±14.6	57.2±18.7	57.0±10.7	56.0±14.0	59.0±26.8
PO_2 (mmHg)	80.0±5.8	79.7±9.7	80.1±4.7	74.5±10.2	71.2±13.4	67.1±12.6	62.8±11.1	65.5±6.3

(MINOTECH Biotechnology, I.M.B.B.-FO.R.T.H., Heraklion, Crete, Greece) and New England Biolabs.

Quantification of signal intensity

RT-PCR gel images were analyzed with the Scion Image for Windows software (Scion, Fredrick, MD, USA), available free at http://www.scioncorp.com and at http://rsb.info.nih.gov/nih-image/, using standard protocols.[18,19] The resulting measurements were exported to an Excel spreadsheet for quantification. Bar graphs for data representing quantification of signal intensities (arbitrary units) of the transcription levels of G3PDH, TGF-β, CTGF and IFN-γ mRNAs in lung tissue, at different time points (0, 6, 12, 18–24 months) after therapy, in both groups, were employed (Figs. 2 and 4).

Statistical analysis

The statistical analysis was carried out using the SPSS software, version 8.0 (SPSS Inc., Chicago IL, USA). Comparisons between and within groups were made using unpaired *t*-test, paired *t*-test, Mann–Whitney *U* tests or chi-squared statistics as appropriate. Comparisons for PFTs (FVC, TLC, DL_{CO}) and PO_2 in each time point were made by ANCOVA test for repeated measurements. A *p*-value of 0.05 or less was considered statistically significant.

Results

Fourteen patients were included in the study and underwent randomization (1 to 1) to receive either 200 μg of interferon gamma-1b subcutaneously three times per week, or 1 mg of oral colchicine per day; 7 patients received IFN-γ-1b and 7 colchicine. The majority of patients were male, the median age was 66 (median range 54–85), in the IFN-γ-1b group, and 69 (median range 42–82), in the colchicine group. Baseline demographics characteristics among the two groups are presented in Table 2.

Effects of IFN-γ-1b and colchicine on the transcription of *TGF-β1*, *CTGF* and *IFN-γ* genes

The molecular effects of IFN-γ-1b agent in IPF, the transcription of *TGF-β1*, *CTGF* and *IFN-γ* genes in lung tissue are shown in Fig. 1. Before treatment, all patients exhibited marked mRNA expression of TGF-β1 and CTGF, but not IFN-γ (Fig. 2a). The expression of TGF-β1 remained almost stable after treatment, in the IFN-γ-1b group (Fig. 2b and c). Four out of seven patients showed transcription of IFN-γ gene at 6 (1 patient) and at 18–24 (3 patients) months after treatment (Fig. 2b and c). The expression of CTGF gene remained almost stable (non-noticeable differences) before and after treatment (Fig. 1). The transcription of G3PDH gene (served as internal control) was constitutively expressed before and after treatment (Fig. 1). This observation indicated that IFN-γ-1b agent enhanced the expression of the patients' endogenous gene. Moreover, no significant difference in the intensity of G3PDH was observed between treated and non-treated patients, a finding that indicates that IFN-γ-1b does not affect the stability of the relevant mRNAs.

The effect of colchicine treatment is shown in Fig. 3. After colchicine treatment three out of seven patients presented increased expression of IFN-γ mRNA (Fig. 3). The expression of CTGF gene remained almost stable before and

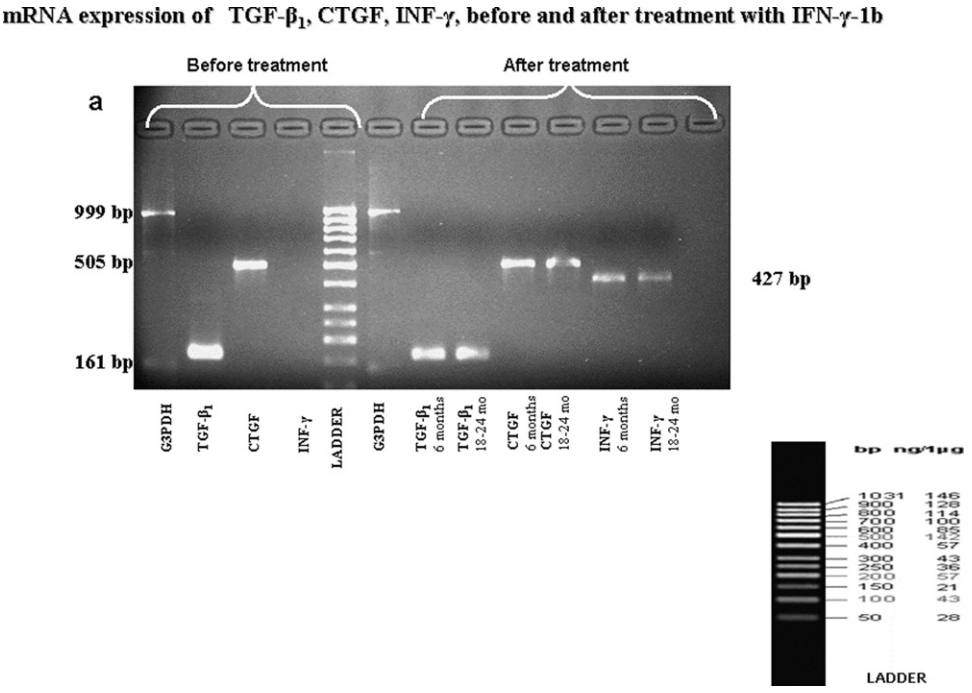

Figure 1 mRNA expression of TGF-β1, CTGF, INF-γ, before and after treatment with *IFN-γ-1b*. The insert in the right corner pictures the DNA ladder used.

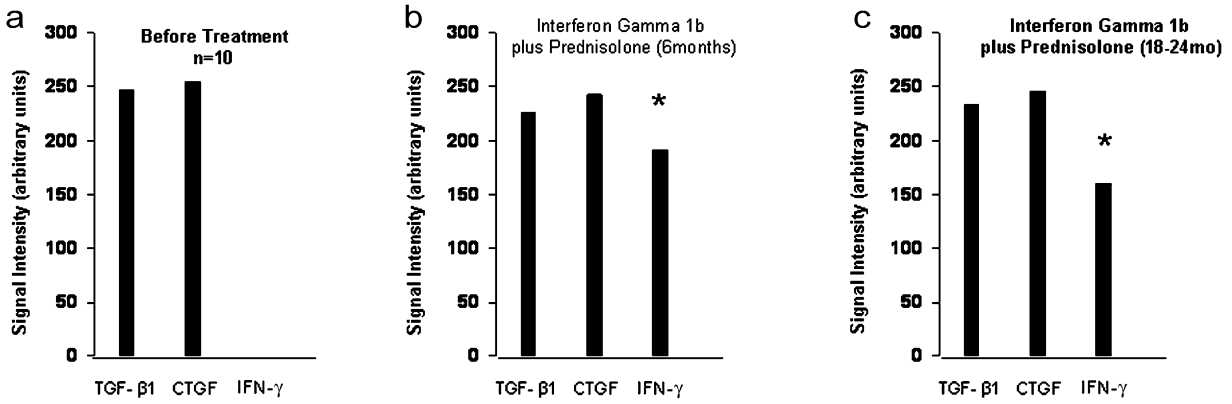

Figure 2 Bar graphs representing quantification of signal intensities (arbitrary units) for TGF-β1, *CTGF* and *IFN-γ* mRNAs before (a) and after (b, c) IFN-γ-1b treatment. * p-value <0.05.

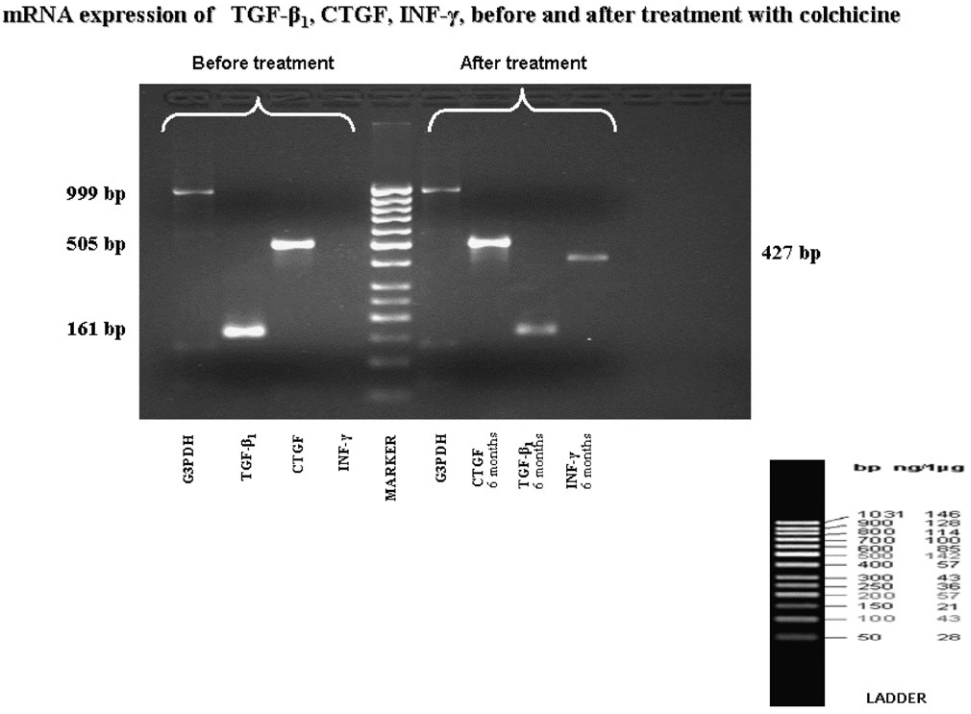

Figure 3 mRNA expression of TGF-β1, CTGF, INF-γ, before and after treatment with colchicine. The insert in the right corner pictures the DNA ladder used.

after treatment while the transcription of TGF-β1 was decreased ($p = 0.0002$) after therapy (Fig. 4a and b). The expression of G3PDH housekeeping gene remained stable before and after treatment with colchicine (Fig. 4).

In summary, the transcription of the IFN-γ gene was clearly upregulated in both groups of patients receiving either IFN-γ-1b or colchicine, indicating that both agents may induce the expression of the endogenous IFN-γ.

Cloning of the human *interferon-γ* gene

The high levels of *IFN-γ* gene transcription in both groups of patients (Figs. 2b, c and 4b), after treatment, prompted us to test whether the IFN-γ bands represented an authentic cDNA product rather than being an artificially amplified PCR product of the same size of IFN-γ. To this end, and because of the very limited amount of the RT-PCR product, cDNAs were cloned and 6 positive clones were sequenced for both groups of IPF patients. The nucleotide sequences determined were identical to the published sequence of human IFN-γ, thus ensuring us that the therapeutic agents used really induced the expression of the IFN-γ gene (Figs. 5 and 6).

Pulmonary function tests (PFTs):

Comparisons in PFTs (FVC, TLC, DLCO) and PO_2 between groups were made using unpaired test, paired t-test,

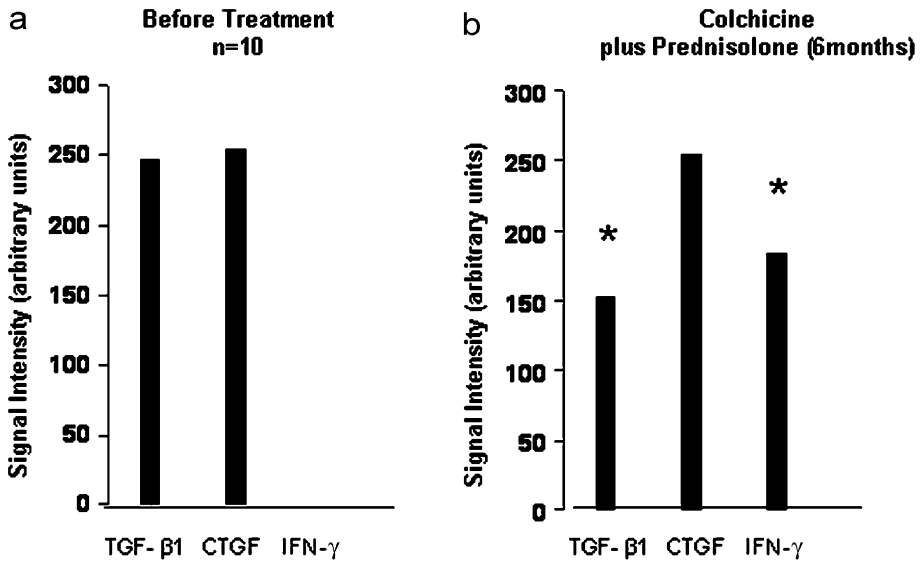

Figure 4 Bar graphs representing quantification of signal intensities (arbitrary units) for TGF-β1, *CTGF* and *IFN-*γ mRNAs before (a) and after (b) colchine treatment. * p-value <0.05.

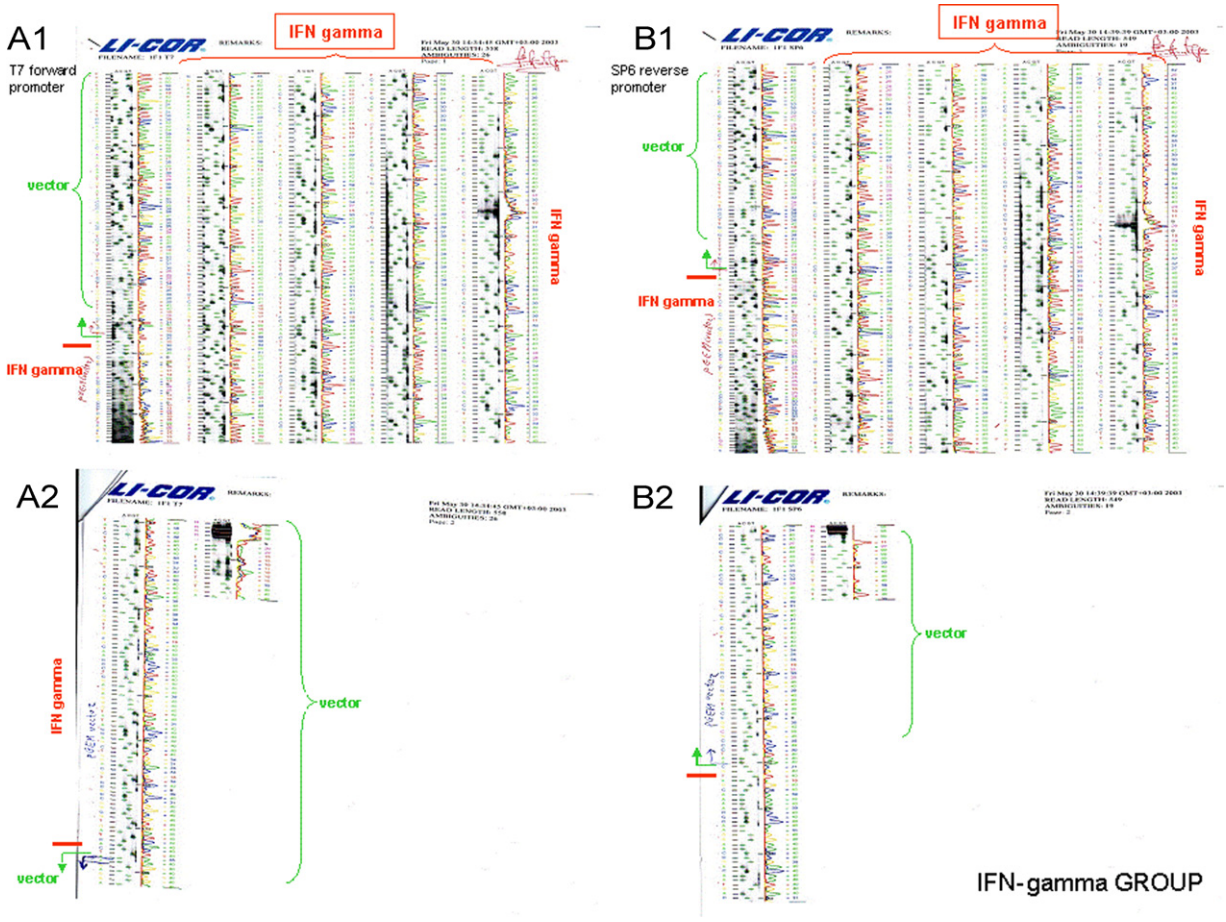

Figure 5 Sequencing of *IFN-*γ gene in a IFN-gamma treated IPF patient. A1 and A2 show sequencing results of IFN-γ gene within T7 forward promoter of pGEM-T Vector. B1 and B2 show sequencing results of IFN-γ gene within SP6 reverse promoter of pGEM-T Vector. Green braces and arrows show pGEM-T Vector sequence, and red braces show the IFN-γ gene sequence.

Mann–Whitney *U* tests or chi-squared statistics as appropriate, and comparisons in PFTs within groups in each time point were made by ANCOVA test for repeated measurements. Significant difference in DLCO (% pred), was found between the two treatment groups (mean+SD, 71.2+19.8 to 75.1+14.5 in IFN-γ patients versus 57.2+18.7 to 56.0+14.0 in

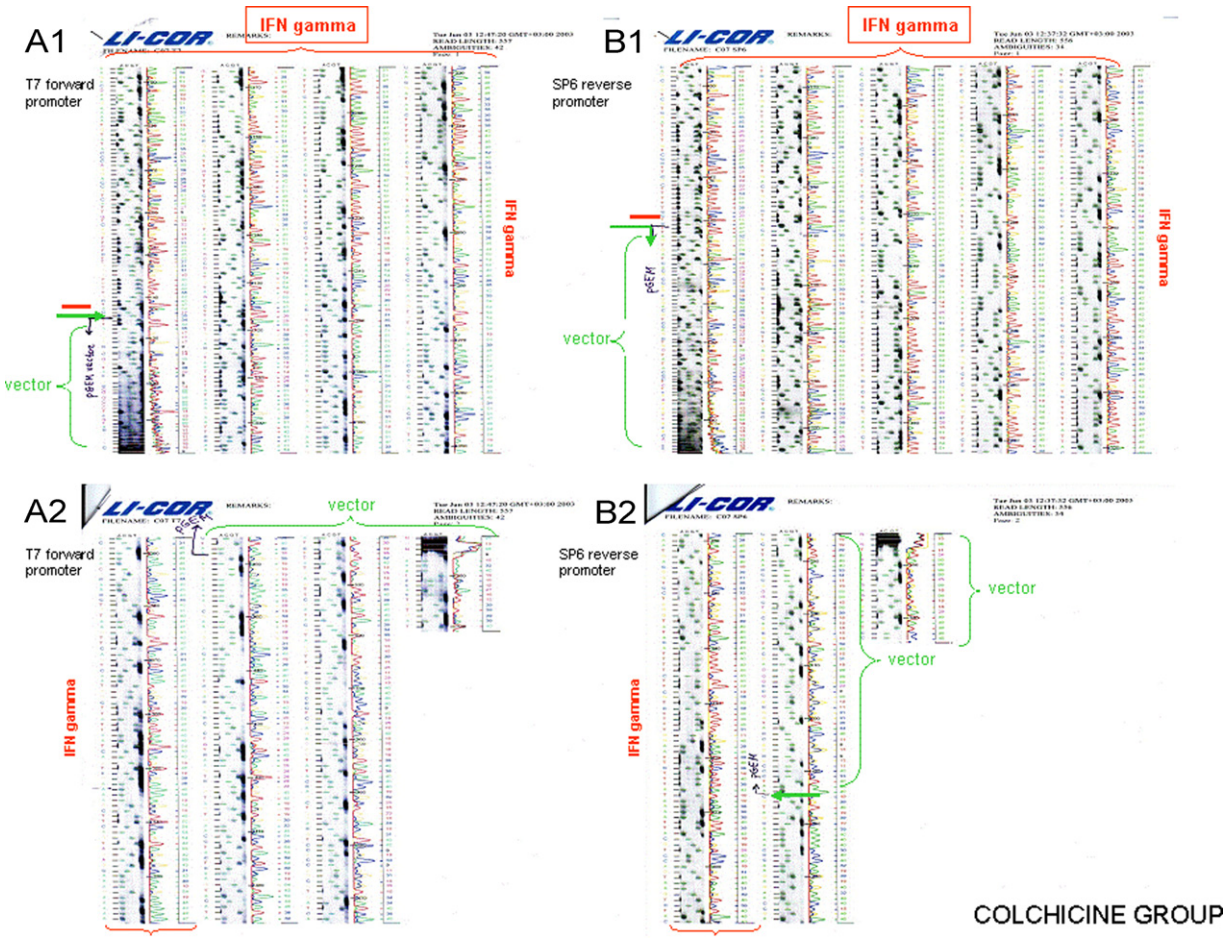

Figure 6 Sequencing of IFN-γ gene in a colchicine treated IPF patient. A1 and A2 show sequencing results of IFN-γ gene within T7 forward promoter of pGEM-T Vector. B1 and B2 show sequencing results of *IFN-γ* gene within SP6 reverse promoter of pGEM-T Vector. Green braces and arrows show pGEM-T Vector sequence, and red braces show the *IFN-γ* gene sequence.

Table 3 Sequence of PCR primers and internal control, the product size and the GenBank Accession number of each gene studied.

Gene	Upstream primer	Downstream primer	Product size	GenBank
TGF-β1	5′-GCCCTGGACACCAACTATTGC-3′	5′-AGGCTCCAAATGTAGGGGCAG-3′	161	NM_000660
CTGF	5′-CCGACTGGAAGACACGTTTGG-3′	5′-TCATGCCATGTCTCCGTACATCTT-3′	505	NM_001901
IFN-γ	5′-GCATCGTTTTGGGTTCTCTTGGCTGTTACTGC-3′	5′-CTCCTTTTTCGCTTCCCTGTTTTAGCTGCTGG-3′	427	J00219
G3PDH	5′-GGGGAAGGTGAAGGTCGGAGT-3′	5′-TTGGAGGCCATGTGGGCCAT-3′	999	NM_002046

colchicine patients, $p = 0.04$) at three different time points (baseline, 6 and 12 months) in favour of IFN-γ-1b group (Table 2). The IFN-γ-1b group showed stability in arterial PO_2 (mean+SD, 80+5.8 to 80.1+4.7, p = NS) while the colchicine group significantly deteriorated (71.2+13.4 to 62.8+11.1, $p = 0.02$). No differences were detected for FVC% pred, TLC% pred and DLCO within both treatment groups over time (Table 2).

Discussion

To our knowledge this is the first study to report the effect of IFN-γ-1b versus colchicine on specific biomarkers expression (TGF-β, CTGF, IFN-γ), in a well defined population of IPF/UIP patients. Fourteen newly diagnosed, untreated, patients with final histopathological diagnosis of IPF/UIP according to ERS/ATS Consensus Classification of the Idiopathic Interstitial Pneumonias criteria,[2] with surgical lung biopsy, were included. Multiple transbronchial-biopsies specimens (3 biopsies each from the right, middle, and lower lobes) were further obtained at 6 and 18–24 months interval, after therapy. A concern could be that pre- and post-treatment lung biopsies were delivered by different methods. However, recent studies in the literature[20] suggest that characteristic histologic features of UIP can be identified on transbronchial biopsies specimens and TBB may be more useful in UIP than previously recognized.[20] Moreover, in this study, a pathologist (A.V.K.)

evaluated the transbronchial-biopsies specimens. All samples were adequate, containing features considered diagnostic of UIP (the typical patchwork pattern of interstitial fibrosis with alternating areas of interstitial fibrosis and normal alveolar septa).

Marked mRNA expression of TGF-β1, but no transcription of IFN-γ mRNA was detected in fibrotic lung tissue before therapy. The expression of CTGF gene remained almost stable before and after treatment while the transcription of TGF-β1 was statistically decreased ($p = 0.0002$) after 6 months of therapy, only in the colchicine group. Colchicine is a potent in vitro inhibitor of fibroblast functions in terms of proliferation and collagen synthesis.[21] On the other hand, although colchicine is known to inhibit many leukocyte functions, it is a poor inhibitor of cytokines known to be important for fibrogenesis (e.g. IL-6, TNF-alpha, IL-1), as concluded from in vitro studies.[21,22] After treatment, both groups exhibited increased expression of IFN-γ gene at 6 months, finding that was sustained at 24 months in the IFN-γ-1b group. A third transbronchial biopsy after 18–24 months of treatment was performed in the IFN-γ-1b group in order to see any significant over time changes in the mRNA levels of TGF-β1, CTGF and IFN-γ genes. Unfortunately, this practice was not possible for the colchicine group since two patients died within twelve months of therapy and the rest did not consent.

The overall molecular analysis did not show any consistent effect of IFN-γ-1b treatment on mRNA expression of TGF-β1 or CTGF, which are in accordance with the recent report by Strieter et al.[23] but in contrast to Ziesche et al.[13] findings. Lack of effect on transcription levels of those profibrotic cytokines in the IFN-γ-1b group, however, does not negate preclinical data showing that IFN-γ modifies collagen synthesis by human lung fibroblasts through other pathways.[23,24] A current hypothesis proposes that the aberrant neovascularization that occurs in pulmonary fibrosis results from the imbalance of angiogenic versus angiostatic CXC chemokines.[25] Recently, Strieter et al.[23] in a randomized double-blind placebo-controlled trial with 32 IPF patients supported that IFN-γ upregulates molecules associated with antimicrobial defence and antiangiogenesis. Changes in angiogenic/angiostatic cytokines after 6 months treatment with IFN-γ-1b, as compared with placebo, suggest beneficial effects of IFN-γ-1b on the process of fibrosis.[23] Strieter et al.[23] findings could provide a basis for the results from a large clinical trial consisted of 330 IPF patients in which a trend toward prolonged survival was observed in IFN-γ-1b treated group.[12]

However, our findings could not be comparable with previous studies,[13,23] because of differences in the study population and/or study design. Firstly, Ziesche's results[13] have been revaluated and it has been demonstrated that only some of the patients included, were IPF patients.[26,27] Secondly, we performed this study using the VATS biopsy as baseline tissue sample, while Strieter et al.[23] have used the transbronchial biopsies in two different time points. To the best of our knowledge, our study is the first to result long-term effects of two different treatment arms in a well-defined IPF/UIP population and it is complementary in the current experience in this field.[13,23]

Another treatment strategy might propose the administration of IFN-γ-1b as a promoter of T helper 1 (Th1) response which facilitates cell-mediated immunity, decreases fibroblast proliferation and angiogenesis and promotes restoration.[25] Interleukin-18 (IL-18) has been identified as an interferon-gamma (IFN-γ) mediator, promoting a Th1 response. Th1 response is characterized by increased expression of IFN-γ, interleukin-12 (IL-12) and interleukin-18 (IL-18). In this context, we have measured the levels of IL-12 and IL-18, in the bronchoalveolar lavage fluid (BALF) in a subgroup of 10 IPF/UIP patients, participating in this study, before and after 6 months of treatment with IFN-γ-1b (5 patients) or colchicine (5 patients).[28] BALF IL-18 levels were significantly decreased after treatment with IFN-γ-1b ($p<0.05$). A significant difference was also found after treatment with colchicine ($p<0.01$). A significant correlation was found between IL-18 BALF levels and the BALF neutrophils ($r = 0.75$, $p = 0.024$). Our data suggested the potential role of IL-18 as an inflammatory marker in the pathogenetic pathway of IPF.[28]

An additional finding of our study is the significant difference of DLCO (%pred) and pO_2 in favor of IFN-γ-1b treatment after 12 months of treatment. For DLCO (%pred) this difference was obvious even after 6 months of therapy. A similar trend has been reported by Strieter et al.[23] in the IFN-γ-1b group for gas exchange and for FEV_1 after six months of treatment.[23] In addition, a statistical significant difference has been detected in FVC (%pred) in favour of IFN gamma group after two years of treatment in 50 IPF patients in a prospective randomized study.[17] However, smaller studies of IFN-γ therapy in IPF have shown controversial results. Kalra et al.[29] observed symptomatic and functional improvement only in one of the 21 patients treated. Similarly, Prasse et al.[30] found improvement in PFTs only in one among five patients. In contrast, Nathan et al. reported that, paradoxically, patients with advanced disease appear to derive the most benefit from IFN-γ therapy.[31]

In conclusion, our data showed that the transcription of the *IFN-γ* gene was clearly upregulated in both groups of IPF/UIP patients, indicating that IFN-γ-1b is effective at least, as well as conventional colchicine, in inducing the expression of the endogenous IFN-γ. Moreover, IFN-γ-1b treatment could improve outcome and survival in a well-defined population of IPF patients if given early and for long period of time.[17,30,32,33] These results suggested that systemic administration of IFN-γ could favorably affect various biological pathways in IPF patients. However, extended studies are needed.

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