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Effect of gamma interferon on lung function of mustard gas exposed patients, after 15 years☆

Mostafa Ghanei[a,*], Yoones Panahi[a], Mojtaba Mojtahedzadeh[b], Ali reza Hosseini Khalili[a], Jafar Aslani[a]

[a]*Research Center of Chemical Injuries, Baqiyatallah Medical Sciences University, Mollasadra Street, P.O. Box 19945-546, Tehran, Iran*
[b]*Tehran University of Medical Sciences, Tehran, Iran*

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Abstract

Background: Bronchiolitis has been known to be among the main the pathological features of lung lesions in Mustard Gas (MG) exposed patients. The purpose of this research was to evaluate the efficacy of interferon gamma-1b on the lung function in MG exposed patients with bronchiolitis.

Method: Thirty-six bronchiolitis patients, whose lung lesion had been diagnosed through High Resolution Computerized Tomography (HRCT) of the chest and also pathological study, were divided into two 18-member case and control groups. Both groups were receiving their conventional treatment (inhaled Felixotide and Servent). The case group were treated for 6 months with a combination of 200 μg of interferon gamma-1b (given three times per week subcutaneously) plus 7.5 mg of prednisolone (given once a day), while the control group received their previous conventional medications. Lung function was measured at base line and after 1, 3 and 6 months of treatment.

Results: In case and control groups, Forced Expiratory Volume in first second (FEV1) did not have statistical differences at the base line (49.3 ± 2.9 and 48.7 ± 4.1, respectively $= 0.6$), whereas a significant increase was seen in the case group (66.3 ± 5.4) compared control group (57.3 ± 8.6) at the subsequent months ($P = 0.001$ for the difference between the groups). Similar pattern of increase was observed in Forced Vital Capacity (FVC).

Conclusion: The findings of this study indicate that a 6-month treatment with interferon gamma-1b plus a low-dose prednisolone is associated with an improvement in the lung function in mustard-gas exposed patients with bronchiolitis.

1. Introduction

Thousands of Iranians were exposed to Mustard Gas (MG) during Iran–Iraq war (1980–1988) [1]. Currently, 34,000 of survivors, with different degrees of involvement, are suffering from respiratory complications [2]. Chronic bronchitis, bronchiectasis and lung fibrosis have been reported as late complications of MG exposure [3]. Moreover, according to newly published studies, Bronchiolitis Obliterans (BO) should be considered as a major long-term sequel following MG exposure [4–6].

TGF-β is one of the well-characterized growth factors and is one of the most important regulators of inflammation in connective tissue synthesis in vivo and in vitro. TGF-β1, an isoform of TGF-β, has been described to play an important role in the pathogenesis of progressive inflammatory and fibrotic diseases such as idiopathic pulmonary fibrosis (IPF) and BO [7,8]. Recent studies of our team show that the level of TGF-β1 is significantly higher in Broncho-Alveolar Lavage (BAL) of patients with MG exposure, compared with veterans not exposed to MG [9].

Gamma-interferon (IFN-γ) and TGF-β are known as exerting antagonistic effects on the process of inflammation in many aspects: It has been shown that IFN-γ inhibits the transcription of collagen in fibroblasts independent of Stat1-promoter interactions and abrogates its stimulation induced

☆ This work has been done in: Baqiyatallah Medical Sciences University, Research Center of Chemical Injuries, Tehran, Iran.

* Corresponding author. Tel.: + 98 21 8053770; fax: +98 21 8040106.
E-mail addresses: m.ghanei@bmsu.ac.ir (M. Ghanei), yunespanahi@yahoo.com (Y. Panahi), mmojtahedzadeh@yahoo.com (M. Mojtahedzadeh), drreza_hosseini@hotmail.com (A. Hosseini Khalili), aslani@bmsu.ac.ir (J. Aslani).

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by TGF-β1 [10–14]. IFN-γ shows its anti-inflammatory effect via down-regulating TGF-β and procollagen I and III gene expression [15]. Gamma-interferon (IFN-γ) and TGF-β may also provide opposing signals to macrophages [16].

As these two cytokines are secreted by inflammatory cells at the sites of tissue injury, their antagonistic interactions are likely to be of great importance in the maintenance of connective tissue homeostasis. Hence, treatment with IFN-γ may be effective in pathological conditions where an increase in the level of TGF-β is encountered. In fact, IFN-γ has been shown to be effective in treatment of patients with IPF [17]. Moreover, MG exposed patients with moderate clinical manifestations experience a shift from Th1 to Th2 cytokine patterns since leukocyte cultures from these patients show a decrease in IFN-γ levels [18]. In the light of these observations, we assume that 6 months of treatment with interferon gamma-1b could improve the lung function of MG exposed patients, whose bronchiolitis is one of the pathological features of their lungs.

2. Method and materials

Based on a randomized case-control clinical trial study, which was done from March 2001 to September 2002 among subjects having been exposed to MG, a total of 36 male patients (mean age: 38 ± 2) with bronchiolitis obliterans, whose treatment with beonchodilator and corticosteroid was ineffective, were selected. All of these subjects were exposed to MG from 1985 to 1987 during Iran–Iraq war. The documentation of MG exposure was based on official certification issued by The Veterans' (Janbazan) Foundation

2.1. Inclusion criteria

Patients with the following characteristics were included in our study: pathological findings compatible with bronchiolitis in Trans Bronchial Lung Biopsy (TBLB) specimens and radiological evidence of BO in chest HRCT, which was the presence of air trapping equal or more than 25% of the cross-sectional area of an affected lung on at least one scan level and at least 3 months free of immunosuppressive drug use.

2.2. Exclusion criteria

These criteria consisted of depression, uncontrolled vomiting, diabetes mellitus, uncontrolled hypertension and multiple sclerosis.

Having obtained additional written consents from the patients, we performed fiberoptic bronchoscopy and obtained specimens of the lung during peripheral transbronchial biopsy for the assessment of lung pathology. The protocol was approved by the ethics committee of the Pharmaceutical Faculty of Tehran University.

Table 1
Dyspnea Index (SEPAR)

0	No dyspnea except for very intense efforts
1	Dyspnea with accelerated walking or when climbing a hill
2	The patient walks more slowly than people his age
3	The patient has to stop after walking for 5 minutes
4	Dyspnea at dressing or undressing; cannot leave home

The effectiveness of therapeutic effects of the regime was assessed during drug administration. The clinical and paraclinical evaluation of the data monitored is as follows: dyspnea indices (defined in Table 1), hospitalization (the admission days in hospital over a 6 months period), Pulmonary Function Test (PFT) and Arterial Blood Gas (ABG) changes at rest [19]. The subjects in both groups were receiving their conventional drug regimen, including felixotide 2 puffs (125 mg/puff) twice a day and servent 2 puffs (125 mg/puff) twice a day, over the study period. The patients were randomly assigned to case and control groups to receive either 200 μg of IFN-1b (Imukin, Boehringer Ingelheim, Vienna, Austria) subcutaneously three times per week and 7.5 mg of oral prednisolone daily for 6 months or, just their previous drug regimen, for their respiratory disorder, respectively. Oral prednisolone was administered for them only when their symptoms were exacerbated.

2.3. Pulmonary function test

Lung function was measured at base line and after 1, 3 and 6 months of treatment. The PFT tests were performed using Pneumotachography with a computerized analysis of spirogram (Autobox DL 6200,SensorMedics, Austria). The tests were performed by experienced lung-function technicians who were blind to our study, and all the patients were familiar with the equipment and experienced in performing maneuvers. While seated with a nose clip in place, the subjects were asked to perform at least three forced expiratory maneuvers with verbal encouragement to blow maximally throughout until they felt there was no air to expel. Both the patients and the technicians received visual feedback from a monitor screen during the test, which was repeated until three technically satisfactory curves with reproducible contours were obtained [20]. All the indices used for analysis were derived from the same maneuver, which was the one with the largest Forced Vital Capacity (FVC) and FEV1.

2.4. Arterial blood gas evaluation

Arterial-blood gases at rest were measured with a gas analyzer (model ABL 510, Radiometer, Copenhagen, Denmark).

2.5. Chest high resolution computerized tomography

Chest HRCT examinations were obtained on one scanner (Hi Speed Advantage; General Electric Medical Systems).

Each HRCT examination consisted of five 1.0 mm collimation images obtained during both deep inspiration and full expiration, respectively, with the patient lying in a supine position. Images were obtained at the levels of the aortic arch, midway between the aortic arch and the tracheal carina, midway between the tracheal carina and the right hemi diagram and finally, 1 cm above the right hemi diagram No. 4 contrast was administered. All the images were reconstructed using a high-spatial-resolution algorithm and displayed at standard (level −700,width 1500)and narrow (level 700,width 1000)lung window settings. The HRCT scans were reviewed by a radiologist and a pulmonologist. The only data available to the HRCT reviewers were the patients' age, sex and the history of exposure to MG. The images were interpreted simultaneously, and consensus for air trapping and mosaic parenchymal attenuation was registered. The expiratory images were assessed for the presence and lobar distribution of air trapping. The criteria used to diagnose the presence of air trapping were the alteration of normal anterior posterior lobar attenuation gradients and /or a lack of homogeneous increase in lung attenuation resulting in persistent areas of decreased attenuation. The extent of air trapping was qualified and classified using the same system as defined for hyperlucent regions on inspiratory images, considering that limited air trapping has been reported in normal individuals. The presence of air trapping was considered indicative of BO only if it exceeded 25% of the cross-sectional area of an affected lung on at least one scan level. Expiratory images displayed at standard and narrow window settings were directly compared to determine the differences in the conspicuity of air trapping.

The value of these measurements was assessed based on the following definitions: improvement, stabilization, and failure. Improvement was defined when FVC and Pao2 increased more than 12 and 10% [21], respectively, and

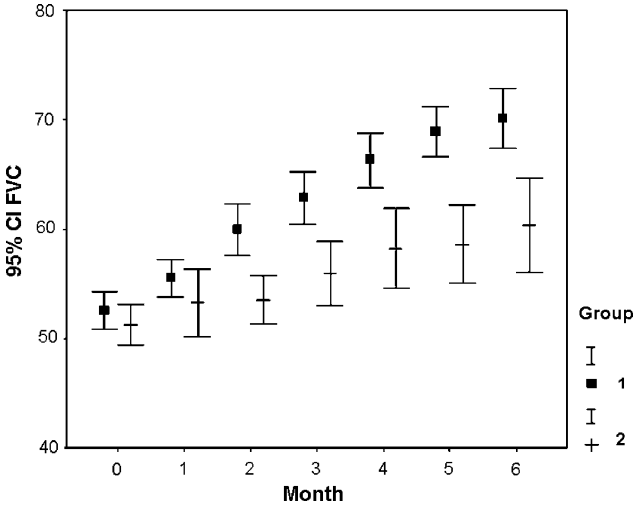

Fig. 1. Distribution and regression line of %FEV1 over a 6-month period in case and control groups.

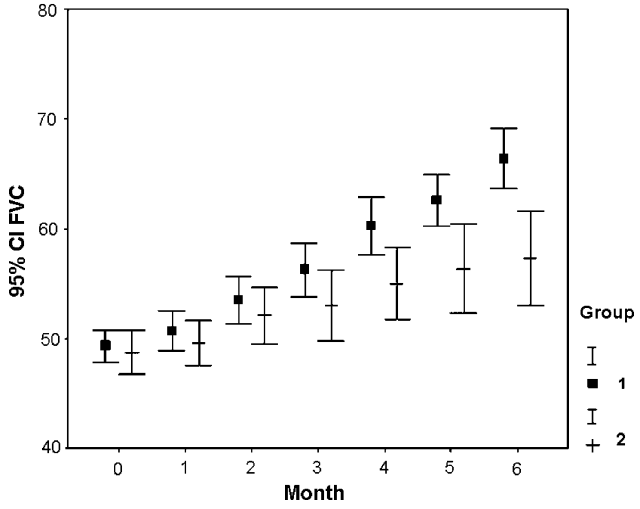

Fig. 2. Distribution and regression line of %FVC over a 6-month period in case and control groups.

dyspnea indices declined one stage. Stabilization was defined if FVC and Pao2 increased less than 12 and 10%, respectively, and dyspnea indices had no changes. And finally, failure was defined by any decrease in the baseline of FVC and Pao2 and a one-stage increase in dyspnea index. All the data were analyzed by a single investigator in a blind fashion.

2.6. Statistical analysis

Numeric data were expressed as mean values ± standard deviation. SPSS soft ware was used to calculate the differences between the case and control groups. *P*-values <0.05 were considered statistically significant.

3. Results

The mean age of the case and control groups were 38 ± 5 and 38 ± 1, respectively. The means of exposure lapse until the time of study for the case and control groups were 14 ± 5 and 14 ± 6, respectively. In both groups, FEV1 did not have

Table 2
Pre-treatment and post treatment indexes of Dyspnea, Hospitalization and Pao2 in case and control groups

	Case group	Control group	*P*
Dyspnea indices			
Pre-treatment	5.3 ± 0.4	4.9 ± 0.7	>0.05
Post treatment	1.2 ± 0.4	3.2 ± 07	<0.05
Hospitalization			
Pre-treatment	4.2 ± 0.8	4.5 ± 0.1	>0.05
Post treatment	1.1 ± 0.8	4.7 ± 1	<0.05
Pao2			
Pre-treatment	56.1 ± 5	58.3 ± 5.9	>0.05
Post treatment	65 ± 5.2	59.5 ± 4.3	<0.05
Dyspnea indices			
Pre-treatment	5.3 ± 0.4	4.9 ± 0.7	>0.05
Post treatment	1.2 ± 0.4	3.2 ± 07	<0.05

Table 3
The assessment of response to treatment based on improvement, stabilization and failure definitions

	Case group			Control group		
	FVC	Dyspnea	Pao2	FVC	Dyspnea	Pao2
Improvement	17 (94.4%)	18 (100%)	12 (66.7%)	4 (22.3%)	6 (33.3%)	5 (27.05%)
Stabilization	1 (5.6%)	–	6 (33.3%)	13 (72.2%)	7 (38.9%)	8 (44.4%)
Failure	–	–	–	1 (5.6%)	5 (27.8%)	5 (27.5%)

statistical differences at base line (49.3±2.9 and 48.7±4.1, respectively, $P=0.6$). Nevertheless, these figures increased significantly in the case group (66.3±5.4) when compared with those in the control group (57.3±8.6) at the subsequent months ($P=0.001$ for the difference between the groups) (Fig. 1). On the other hand, there was a considerable increase in the FVC of the case group (77.7± 10) when compared with that of the control group (60.6± 10.9, $P<0.0001$) (Fig. 2). Other parameters of response to treatment are indicated in Table 2. Table 3 provides a breakdown of the value of changes in the case and control groups according to the following definitions: improvement, stabilization and failure ($P<0.05$). Figs. 3 is sample picture of chest HRCT in a chemically induced bronchiolitis obliterans patient. Histological view of one of the specimens obtained by TBLB is shown in Fig. 4.

4. Discussion

To date, according to the available clinical data, there have been few immunosuppressive strategies showing significant and reliable improvement of lung function in patients with BO syndrome [22]. In other words, stabilization and decline in lung function are the only outcomes following augmented immunosuppressive regimens.

The results of our study supported the hypothesis that the pulmonary function tests of patients with MG induced BO could be improved with the administration of interferon gamma-1b. Furthermore, decrease in hospitalization, increase in arterial oxygenation and improvement in dyspnea indices are other benefits that could be reaped with interferon gamma 1-b administration. Since a newly published study shows that TGF-β1 plays a pivotal role in BO pathogenesis [8], we assume that lung function improvement in our patients can be ascribed to the down-regulating effects of interferon gamma 1-b on TGF-β1.

Differentiation and proliferation of a wide variety of cells are controlled by TGF-β1, which is an important component of the cytokines family [23,24]. Although TGF-β1 was identified and named based on its ability to induce and transform rat fibroblasts, it is now clear that it exerts multiple effects on different types of cells [25]. It modulates some inflammatory parameters which have an important role in the genesis and maintenance of fibrotic reactions of the lung, including chemotaxis of macrophages, suppression of macrophage and lymphocyte function, chemotaxis and proliferation of fibroblasts and modulation of collagen synthesis [26,27]. Moreover, TGF-β1 is a strong stimulator of extra cellular matrix synthesis; it is synthesized and released in inflamed sites by a variety of inflammatory cells (including activated macrophages, lymphocytes and

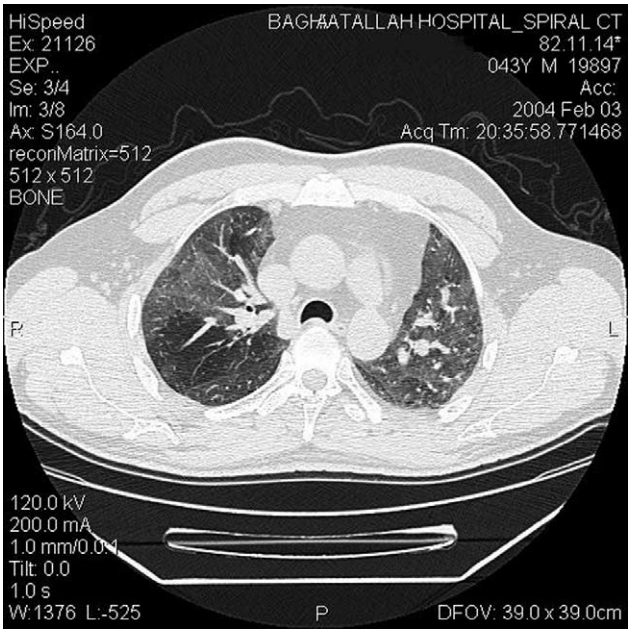

Fig. 3. Air trapping in chest HRCT in expiratory phase in a mustard gas exposed case with bronchiolitis obliterans.

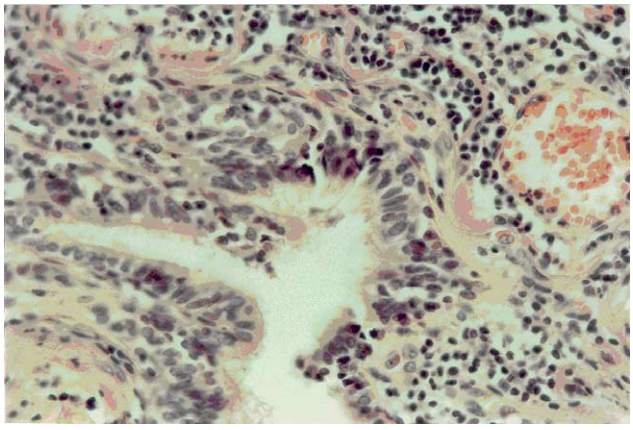

Fig. 4. Photomicrograph of non-specific chronic bronchiolitis showing smooth muscle hyperplasia in bronchiole wall associated with mild luminal narrowing and mild chronic inflammatory cell infiltration.

platelets) which contribute to inflammatory processes of the lung [28].

Several animal studies have indicated that neutralized TGF-β1 antibodies and natural TGF-β1 inhibitors (e.g. decorin) can block the effects of excessive TGF-β1 activity through the inhibition of TGF-β1 binding to its receptors. Moreover, gene therapy, by inhibiting Smads proteins or dominant negative TGF-β1 receptors, can block TGF-β1 signaling and improve the process of fibrosis in the liver and kidney [29,30]. Another animal study, carried out on mice, has demonstrated that in bleomycin induced pulmonary fibrosis, the expression of inhibitory Smad7 decreases lung fibrosis [31].

In human studies, the successful treatment of diabetic nephropathy by angiotensin-converting-enzyme inhibitors, hepatic fibrosis by INF-α, autoimmune hepatitis by azathioprine and prednisone and finally, pulmonary fibrosis by cyclosporine or interferon gamma 1-b is in part due to the ability of these drugs and cytokines to reduce TGF-β1 serum levels [32–34]. In fact, the efficacy of INF-α in treating hepatic fibrosis directly correlates with decline in serum TGF-β1 level [35].

Although we did not measure the TGF-β1 level in our cases in this study, we have recently demonstrated that TGF-β1 target protein is substantially increased in BAL aspirates and target tissues of MG exposed patients, compared with non-exposed individuals [9]. According to the findings of this study, we assume that the response to treatment in our patients can be justified with down-regulating mechanisms of TGF-β1 gene expression by INF γ-1b. The results of this research open a new window for investigators studying the pathogenesis of bronchiolitis obliterans. Future immunobiologcal progresses will bring about satisfactory solutions to this devastating process.

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