Adjuvant Interferon gamma in Patients with Drug - Resistant Pulmonary Tuberculosis: a pilot study.

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Abstract

Background. Tuberculosis (TB) is increasing in the world and drug-resistant (DR) disease beckons new treatments. Methods. To evaluate the action of interferon (IFN) gamma as immuno-adjuvant to chemotherapy on DR-TB patients, a pilot, open label clinical trial was carried out in the Cuban reference ward for the care of this disease. The eight subjects existing in the country at the moment received, as in-patients, $1 \times 10^6$ IU of recombinant human IFN gamma (Heberon Gamma R®, Heber Biotec, Havana) intramuscularly, daily for one month and then three times per week up to 6 months as adjuvant to the indicated chemotherapy, according to their antibiograms and WHO guidelines. Sputum samples collection for direct smear observation and culture as well as routine clinical and thorax radiography assessments were done monthly. Results. Sputum smears and cultures became negative for acid-fast-bacilli before three months of treatment in all patients. Lesion size was reduced at the end of 6 months treatment; the lesions disappeared in one case. Clinical improvement was also evident; bodyweight increased in general. Interferon $\gamma$ was well tolerated. Few adverse events were registered, mostly mild; fever and arthralgias prevailed. Conclusions. These data suggest that IFN $\gamma$ is useful and well tolerated as adjunctive therapy in patients with DR-TB who are otherwise not responding well to therapy. Further controlled clinical trials are encouraged.
Introduction

Tuberculosis (TB) is not yet a defeated affection. Although it is a controllable infection at community level and curable in an individual manner, its eradication seems distant. At present, at least one third of the world population, more than 1 500 million individuals are infected with the *Mycobacterium tuberculosis*. Every year, around 8 - 10 million new cases occur [1]. Two million people die annually due to non-AIDS related TB, which is the highest number of deaths attributable to a single infectious agent [2], and corresponds to the 7th cause of death in the world [3]. TB represents 26 % of avoidable deaths in developing countries [4]. The emergency of drug resistant (DR) or multidrug-resistant (MDR) strains has increased this world problem, leading to a high morbidity and mortality [5]. The mean survival of MDR-TB affected patients ranges from 2 to 14 months [6]. According to World Health Organization (WHO) data, the global proportion of MDR patients is 2.2 % [7].

The infection is mainly transmitted by inhalation of the bacilli coming from infected secretions of the respiratory airways. Once inhaled, the bacilli are subjected to phagocytosis within the alveolar macrophages, where they can be destroyed. Nevertheless, *Mycobacteria* have developed mechanisms to adapt to the noxious intracellular ambient. Thus, it can persist, replicate and disseminate, leading to new infectious foci. The emergence of resistance depends on several factors such as initial bacillary load, inadequate or incomplete chemotherapy administration, and the patient’s immune condition.

Chemotherapy is successful in most cases given that they thoroughly follow the treatment schedule, which is prolonged, costly, and needs to be directly observed. Otherwise it is inadequate to kill all the bacilli and drug resistance emerges. Toxicities are frequent as well [8].
The immunologic approach to TB treatment can be promising since only 10 - 20% of infected people develop the disease and many of them have spontaneous remission [9]. Interferon (IFN) gamma plays a main role in the immunity to TB. It is a glycoprotein, secreted by CD4+, CD8+ and NK cells. Nevertheless, CD4+ Th1 lymphocytes, in response to a stimulus, are the main producers [10].

Enough evidences exist related to the action of IFN γ on the immunoregulatory activity of macrophages [11-14]. Lack of production of this cytokine [15] or expression of its receptor [16,17] is associated to the most lethal forms of the infection. Interferon gamma has also a potent antifibrotic effect [18-20]. This study shows that the clinical use of this protein in eight DR-TB patients (four of them MDR) as adjuvant to second-line antibiotics had short and middle-term beneficial effects.

Materials and Methods

An open-label, non-randomized, non-controlled, pilot trial was carried out. The “Benéfico - Jurídico” Hospital, Havana is the national reference hospital for TB and other respiratory diseases. According to the national TB program, all patients with unfavorable response to treatment are remitted to this center.

The study population was constituted by eight Cuban patients, both sexes, more than eighteen years old, with diagnosis of TB without a favorable response to the usual therapy, who gave their written, informed consent to participate. The diagnosis comprised clinical findings such as cough and expectoration, pulmonary lesions at thorax radiography, and positive DR-TB sputum-smear and culture. To confirm drug resistance the Canetti’s multiple proportions method [21] was used as antibiogram. Exclusion criteria were another chronic disease, pregnancy or nursing, severe psychiatric dysfunction, multiple sclerosis or
another autoimmune disorder, other pulmonary infections, HIV co-infection, and treatment with glucocorticoids or any other immunosuppressor medication.

The trial was done in compliance with the Helsinki Declaration. The protocol was approved by the hospital Ethics Committee and by the Cuban Regulatory Authority. Data from nineteen historical control cases were obtained from the hospital’s archives.

Patients received 1 000 000 IU of human recombinant IFN gamma (Heberon Gamma R®, Heber Biotec, Havana), intramuscularly, daily during 4 weeks and then 3 times per week for the next 20 weeks. Participants stayed as in-patients during the study period. They received second-line anti-TB drugs (WHO schemes) [22], according to the resistance detected in each case by the antibiogram (Table 1). Drugs were given as follows: rifampin 10 mg/Kg (maximum 600 mg), ethambutol 20 mg/Kg (max. 1500 mg), ethionamide 10 mg/Kg (max. 750 mg), pyrazinamide 15-30 mg/Kg (max. 2000 mg), ciprofloxacin 15-20 mg/Kg (max. 1500 mg), amikacin 15 mg/Kg (max. 1000 mg), and kanamycin 15 mg/Kg (max. 1000 mg) daily. After the end of the 6-months IFN gamma treatment period, chemotherapy continued up to 9 months if the scheme included rifampin and 18 months otherwise.

Follow-up was carried out at entry and monthly during IFN gamma treatment. A complete physical examination was done. Sputum samples were taken for acid-fast-bacilli smear and culture, as well as blood samples for hematological counts, globular sedimentation rate, alanine aminotransferase, and creatinine determinations. Thorax radiographies were also recorded. Afterwards, patients were followed up with half-yearly evaluations during one year.

Treatment efficacy evaluation included clinical, bacteriological and radiological outcomes. Complete response was defined as total disappearance of all signs and symptoms,
negative sputum acid-fast-bacilli smear and culture, and pulmonary lesions improvement at X-ray. Partial response included signs and symptoms decrease, negative sputum smear and culture and stable X-ray lesions. No response consisted in signs and symptoms persistence, positive bacteriological examinations, and lesions stabilization or progression. Safety and tolerability of the IFN $\gamma$ treatment were monitored by means of a rigorous control of the adverse reactions that could be presented.

**Results**

Eight patients were enrolled in the study. Those were all the DR-TB cases in the country during the inclusion period, from December 1999 to February 2002. They had not responded to the usual Directly Observed Treatment Short-course (DOTS) chemotherapy regime. Their demographic and baseline characteristics are shown in Table 1. Five of them were men, six of them non-white. The age ranged between 23 and 54 years old, and bodyweight between 36 and 65 Kg. Their main symptoms were cough, expectorations, dyspnea, stertors, distal cyanosis, and finger clubbing. Bacteriological tests codification was mostly high and all patients showed active lesions at thorax radiography. Most of them had accelerated globular sedimentation rates (GSR). Anemia or other hematological alterations were not recorded.

A rapid favorable evolution was obtained after treatment with IFN gamma (Table 2). Clinical improvement was evident since the first month of treatment, when all signs and symptoms (except for finger clubbing) had disappeared in all patients and body weight increased in all but one patient. Sputum acid-fast-bacilli smears and cultures were negative since the 1 - 3 months of treatment. The eight patients had radiological improvement, with lesions size reduction (total disappearance in one case) (Figure 1). GSR decreased in five out of 6 patients who had abnormal values at inclusion. After six
additional months follow-up, patients # 3 and 4 normalized it to 10 and 15 mm/h, respectively. At the end of the IFN gamma treatment all the patients were evaluated as complete responders.

The treatment with IFN gamma was safe and well tolerated. Four patients presented at least one adverse reaction. These reactions were arthralgias, fever, headache and asthenia. All adverse reactions were mild, except for a moderate event of fever, which was efficiently controlled with acetaminophen. Significant differences were not detected in other clinical laboratory tests.

After completion of the IFN gamma 6-months treatment the patients continued with the corresponding chemotherapy schedule. Seven of the eight patients remained bacteriologically, clinically and radiologically negative at least twelve months after the treatment with IFN gamma concluded. However, patient number five relapsed six months after the end of IFN therapy. He developed additional resistance to rifampin and ethionamide and a chronic obstructive respiratory disease that contributed negatively to his evolution.

Table 3 shows the results obtained at the same hospital with DR-TB cases during the five years prior to the present study. Management was essentially the same as for the patients included in the study, except for IFN gamma treatment. None of these DR-TB cases reached culture conversion at three months of treatment with chemotherapy and less than half had converted at six months. Their clinical outcome was also worse.
Discussion

In spite of the reduced size of the population studied, the results suggest the efficacy of IFN gamma on DR-TB patients, when used as adjuvant to second-line chemotherapy. All eight patients were considered as complete responders at the end of IFN treatment with disappearance of the disease signs and symptoms, sputum tests conversion, and pulmonary lesions improvement. Bacteriological and radiological improvement correlated with the clinical evolution; body weight increased and manifestations as cough and expectoration did not recur after IFN $\gamma$ treatment. Clinical practice demonstrates that these results are very difficult to obtain in such a short period of time with the chemotherapy alone.

Any conclusion from this study is also limited by the fact that it was not a controlled trial. This was not possible due to the low incidence of TB (7.6/100,000 inhabitants in 2002) [23] and DR-TB in Cuba [7]. Therefore a historical control with the results obtained at the same hospital by the same investigators in patients treated only with chemotherapy is used for comparison. A clear difference is shown regarding patients´ performance. Literature reports on chemotherapy-treated DR-TB patients show similar unfavorable outcome [6]. The fact that four patients received rifampin, according to the strain sensitivity, which is one of the election drug for the treatment of TB with excellent results in patients treated with DOTS regime, does not diminish the consideration regarding the possible benefit exerted by IFN gamma since these same patients had already not responded to this antibiotic as part of the DOTS. Moreover, one patient recurred after he had finished IFN gamma treatment for 6 months, despite still being under chemotherapy regime. This suggests that in his case a new cycle of IFN gamma combined to the second line antibiotics would have been necessary, but this was not previewed in the protocol.
However, only a controlled trial can definitely clarify the roles of IFN gamma and second line antibiotics in this kind of patients’ improvement.

The radiological results demonstrated the antifibrotic properties of IFN gamma as well. All patients had a reduction in the size of the pulmonary lesions, while one showed a complete resolution. This effect cannot be attributable to the antibiotics, since it is well known that DR-TB patients only develop radiological improvement long time after sputum smears and culture become negative. In many cases extensive fibrotic lesions never improve, and stay stable for life. This antifibrotic action agrees with that obtained with IFN gamma in idiopathic lung fibrosis [24] and suggests that IFN gamma can have future indications in other pulmonary diseases where fibrosis is present.

Therapy was well tolerated. It was not necessary to suspend the combined treatment due to adverse events; mostly mild. Adverse reactions such as arthralgias, fever and headache coincide with those reported for interferons [25].

Immunity to TB depends on the development of CD4+ cells- and macrophages-mediated Th1 response. The role of IFN gamma as the main macrophage–activator Th1 cytokine has been clearly established in animal models infected with M. tuberculosis [15,26,27]. IFN gamma action on the macrophages leads to kill intracellular Mycobacteria. It stimulates macrophages to produce tumor necrosis factor alpha (TNF α), oxygen free radicals and nitric oxide, increase surface display of MHC antigens and Fc receptors, decrease lysosomal pH, and increase the intracellular concentration of some antibiotics [11-14,28].

Regarding its antifibrotic effect, IFN gamma inhibits lung fibroblast proliferation and chemotaxis in a dose dependent manner, and reduces collagen synthesis [18,19,29]. Furthermore, this protein is a potent inhibitor of the transforming growth factor β (TGF-β) [20], involved in the pathogenesis of many fibrotic lung diseases [30-32].
On the other hand, mutations in the IFN gamma gene [15], or in the IFN gamma receptor alpha chain gene [16,17], increase susceptibility to develop the disease. Patients with disseminated BCG and other infections present defects in IFN gamma and other cytokines secretion and action [33-35].

IFN gamma therapy has been shown effective for cerebral tuberculosis caused by a multidrug-resistant strain [36]. The aerosol route of administration has been proposed as organ specific delivery method, obtaining a high release to infected alveoli [37]. Condos et al. reported clinical and bacteriological improvement and tolerability with aerosolized IFN γ in five patients with MDR-TB [38]. In addition, other previous trials demonstrated promising results in patients infected with other Mycobacteria [10,39-42].

**Conclusions**

These results can suggest a beneficial effect of IFN gamma when it is used as adjuvant in the treatment of tuberculosis patients that have resistance to standard chemotherapy, and encourage carrying out more extensive, controlled studies. Combination with second-line drugs can reduce the time of treatment, diminishing toxicities and possible relapses; in many cases could reduce the application of recessional surgery. Further controlled clinical trials are needed to confirm these results.
Authors’ contributions

RSM conceived the study and carried out the bacteriological determinations. IGG participated in the study design and coordinating, and wrote the manuscript draft. NFO, MVQ, MTM, DC, and DMM took care of patient recruitment, management, and follow-up. CVS participated in the study design and result analysis. PLS took part in the design, results analysis and manuscript writing. All authors read and approved the final manuscript.

Acknowledgments

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References


2. Tuberculosis. WHO information, April 2000, Fact Sheet No 4.


30. Broekelmann TJ, Limper AH, Colby TV, McDonald JA: **Transforming growth factor beta is present at sites of extracellular matrix gene expression in human pulmonary fibrosis.** *Proc Natl Acad Sci USA* 1991, **88**:6642-6647.


34. Levin M, Newport M. **Understanding the genetic basis of susceptibility to mycobacterial infection.** *Proc Assoc Amer Physicians* 1999, 111:308-312.


**Figure legends**

**Figure 1.** Radiological improvement with IFN gamma treatment (ray-x of two patients are shown).

Legend: Patient 4 (A), left-lung fibroexudative lesions, and (B) complete resolution after IFN gamma treatment. Patient 2 (C), bilateral moderate exudative lesions before IFN gamma treatment, and (D) important improvement of the lesions afterwards. Patients’ identification data were removed from the figure.
### Table 1. Demographic and baseline characteristics of patients with DR-TB treated with IFN gamma

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28</td>
<td>50</td>
<td>54</td>
<td>27</td>
<td>44</td>
<td>50</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Race</td>
<td>Mestizo</td>
<td>White</td>
<td>Mestizo</td>
<td>Mestizo</td>
<td>White</td>
<td>Mestizo</td>
<td>Black</td>
<td>Black</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>65.0</td>
<td>49.0</td>
<td>36.0</td>
<td>54.0</td>
<td>42.2</td>
<td>54.5</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Sputum smear status*</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Sputum culture status **</td>
<td>7</td>
<td>9</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Resistance to drugs</td>
<td>INH, RIF, STR</td>
<td>INH, STR</td>
<td>INH, RIF, STR</td>
<td>INH, RIF</td>
<td>INH, STR</td>
<td>INH, RIF, STR</td>
<td>INH</td>
<td>INH</td>
</tr>
<tr>
<td>GSR (mm/h)</td>
<td>5</td>
<td>66</td>
<td>53</td>
<td>53</td>
<td>68</td>
<td>101</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

Legend:


* Number of bacilli: 0: 0 in 4 lines; 8: 25 or more in 1 line; 9: bacilli in most of the fields.

Table 2. Six months follow-up data of DR-TB patients treated with IFN gamma

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug regimen</td>
<td>ETB, ETN, PRZ, CPF, KAN</td>
<td>RIF, ETB, PRZ, KAN</td>
<td>ETB, ETN, PRZ, CPF, KAN</td>
<td>ETB, ETN, PRZ, CPF, AMK</td>
<td>RIF, ETB, PRZ, CPF, KAN</td>
<td>ETB, ETN, PRZ, CPF, KAN</td>
<td>RIF, ETB, PRZ, KAN</td>
<td>RIF, ETB, PRZ, KAN</td>
</tr>
<tr>
<td>Gain body weight (Kg)</td>
<td>6.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>5.5</td>
<td>5.0</td>
<td>-8.0</td>
</tr>
<tr>
<td>Sputum smear status</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Sputum culture status</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Conversion time</td>
<td>2 months</td>
<td>3 month</td>
<td>3 months</td>
<td>1 months</td>
<td>3 months</td>
<td>2 months</td>
<td>2 months</td>
<td>3 months</td>
</tr>
<tr>
<td>Thorax X-ray</td>
<td>Residual fibrosis</td>
<td>Reabsorption and residual fibrosis</td>
<td>Residual bilateral fibrosis</td>
<td>Resolution of the lesions</td>
<td>Residual fibrosis</td>
<td>Reduction of magnitude of lesions</td>
<td>Residual fibrosis</td>
<td>Reduction of magnitude of lesions</td>
</tr>
<tr>
<td>GSR (mm/h)</td>
<td>5</td>
<td>48</td>
<td>26</td>
<td>40</td>
<td>42</td>
<td>20</td>
<td>23</td>
<td>77</td>
</tr>
</tbody>
</table>

Legend: see Table 1

! Lost body weight
Table 3. Bacteriological evolution of DR-TB historical controls at “Benéfico Jurídico” Hospital (1994-1999) under specific chemotherapy

<table>
<thead>
<tr>
<th>Trimester</th>
<th>Sputum acid-fast-bacillus smear status</th>
<th>Sputum culture status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>89.5</td>
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<tr>
<td>2</td>
<td>5</td>
<td>26.3</td>
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<tr>
<td>3</td>
<td>2</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Five patients died and other three interrupted the chemotherapy before concluding it.

Percents are calculated considering initial DR-TB population (19 patients)