International Immunopharmacology 62 (2018) 109–113

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Neutralizing antibody production against Rebif® and ReciGen® in Relapsing-Remitting Multiple Sclerosis (RRMS) patients and its association with patient's disability

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ARTICLE INFO

Keywords:
RRMS
Interferon beta
Neutralizing antibody
Rebif®
ReciGen®

ABSTRACT

Introduction: Human recombinant interferon beta (IFN-β) is one of the first line treatments for Relapsing-Remitting Multiple Sclerosis (RRMS). However, the production of neutralizing antibodies (NAb) can impair its function. The aim of this study was to investigate the production of neutralizing antibodies against Rebif® and ReciGen® (two brands of IFN-β-1a) and to evaluate its correlation with Expanded Disability Status Scale (EDSS).
Materials and methods: Serum samples of 71 RRMS patients (34 in ReciGen®, 37 in Rebif® group) were collected. Neutralizing antibody was measured by Myxo-virus resistance protein A (MxA) assay using A549 cell line. The MxA concentration was measured by enzyme-linked immunosorbent assay (ELISA) kit.
Results: The median period of treatment with IFN-β-1a was 18 months in ReciGen® and 24 months in Rebif® arms. The percentage of patients with positive titer of neutralizing antibody (NAb+) had no statistically significant difference between groups ($P = 0.6$). In both ReciGen® and Rebif® groups, the increase in EDSS score was significantly higher in NAb+ patients compared to NAb− patients ($p \leq 0.05$). The duration of using ReciGen® or Rebif® for > 24 months was influential in the NAb positivity (OR = 3.78).
Conclusion: Receiving interferon beta-1a for > 24 months is correlated with higher possibility of NAb production. The type of IFN-β used in the study had no significant impact on NAb positivity. In addition, both groups had comparable EDSS score changes, and NAb status of patients was correlated with their EDSS score.

1. Introduction

Multiple Sclerosis (MS) is a multi-organ autoimmune disease, which targets the central nervous system (CNS). The prevalence of MS in Iran is high, and nine out of every one hundred thousand people are diagnosed with MS each year [1, 2]. MS is caused by the immune system's response against myelin sheaths surrounding the neurons. In fact, lymphocytes specific response against the myelin sheath of neurons leads to nerve cell degeneration that consequently disrupts the function of neural synapses or other neural interactions [3]. Local paralysis, incoordination of walking, double vision, and physical weakness are the prevalent symptoms of MS [3]. Physical examination and MRI imaging are the main MS diagnostic methods [2, 3]. The progression of disability in patients with MS is determined by Kurtzke Expanded Disability Status Scale (EDSS) method. EDSS score categorizes patients' disability from 0 to 10 and can be used to evaluate disability progression [4, 5]. Relapsing-Remitting MS (RRMS) is the most prevalent form of MS which can be treated and recovered by early diagnosis. There is no treatment for MS. Current medications, or treatment methods attempt to improve the function of the nervous system after an attack and

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https://doi.org/10.1016/j.intimp.2018.06.032
Received 12 February 2018; Received in revised form 3 June 2018; Accepted 22 June 2018

prevent new attacks [6]. In 1993, US Food and Drug Administration (FDA) approved human recombinant interferon beta (IFN-β) as the first-line treatment for RRMS [7]. IFN-β activates the signaling pathways that inhibit T cell activation and major histocompatibility complex class II (MHCII) antigen presentation. It also leads to the activation of co-stimulators, such as CD40/CD40L and induces CTLA4 expression on the surface of the T cells [4]. Several recombinant proteins are produced in non-human expression systems, such as Chinese hamster ovary (CHO) cells. As a result, these proteins may stimulate the immune system to produce antibodies against them. These antibodies include IFN-β binding antibodies (BAb) and IFN-β neutralizing antibodies (NAb). The NAbs bind to the functional domain of IFN-β and prevent its attachment to the receptor, while binding antibodies bind to the other structural domains of the protein that does not impact its function [5]. NAbs may influence the efficacy of recombinant IFN-β by decreasing its concentration in the serum. The duration of treatment with IFN-β is an influential factor in the immune system response. In fact, repeated exposures to recombinant protein may increase the immune response and antibody production. The presence of high titers of NAbs against IFN-β can lead to decreased or lack of therapeutic response in some patients. Lack of IFN-β or its' decreased efficacy can impress the EDSS score of patients during the treatment period.

Several IFN-β products, including IFN-β1b (Betaferon® and Ziferon®) and IFN-β1a (Avonex®, Rebif®, Cinnovex® and ReciGen®) have been marketed so far [6, 7].

Recently, ReciGen® was developed as a biosimilar to the FDA approved reference product (Rebif®) produced by Merck Serono Company [8]. Two methods for measuring the titer of NAb against IFN-β in patients' sera are the cytopathic method of virus infection in interferon-sensitive cell lines and Myxovirus resistance protein A (MxA) assay [9, 10]. MxA is produced in response to IFN-β in the cytoplasm of sensitive cells, such as A549. Cost-effectiveness of the medicines depends on their quality and clinical efficacy and safety. Therefore, comparing different IFN-β products may help the physician to choose the appropriate product [11].The present study aimed at comparing two groups of patients taking Rebif® and ReciGen® regarding NAb development against recombinant IFN-β using MxA method and NAb status correlation with EDSS score of patients from diagnosis to end of the study.

2. Materials and methods

2.1. Patients and study design

Patients who received either ReciGen® or Rebif® were selected with random sampling with no background information on their NAb status. After obtaining the written informed consent, patients were evaluated regarding their baseline characteristics. The mean age of the patients was 34.91 ± 7.73 years in ReciGen® and 33.97 ± 10.63 years in Rebif® group. Serum samples were collected from the patients and stored at −20 °C. The EDSS score of patients was calculated using the questionnaire. In addition, physical examination by the neurologist and MRI imaging was conducted in three-time points: 1. at the time of diagnosis, 2. at the time of NAb assay, and 3. one year after the NAb assay (current stage). The difference in EDSS score was calculated for NAb+ and NAb− patients in two-time intervals: between the first and third-time point ($EDSS_{current\ stage} - EDSS_{First\ diagnosis}$) and between the second and third-time point ($EDSS_{current\ stage} - EDSS_{NAb\ assay}$).

2.2. Cell culture

The IFN-sensitive cell line, A549 (human lung carcinoma cells), was purchased from the Iranian Biological Resource Center (IBRC, Iran) and was cultured according to an existing protocol in IBRC [12]. When the cells formed up to 80% confluent monolayer, they were detached by adding 2 ml trypsin 0.25%/EDTA 0.05% (Gibco, USA) and washed. The cells were counted by Trypan-blue exclusion test of cell viability. 100 µl supplemented medium (A549 conditioned media DMEM-high glucose containing 10% FBS (Gibco-USA), 1% Penicillin-streptomycin [12] containing 5000 cells were seeded in each well of a 96-well plate. The plates were incubated for 24 h in the 37 °C incubator with 5% CO2 and 95% humidity to allow cells to attach to the well bottom. Two IFN-β1a products, ReciGen® (CinnaGen, Iran) and Rebif® (Merck, Germany) were purchased. Then serial dilutions of these interferons, with the following concentrations 0.125, 0.25, 0.5, 1, 2.5, 5, 10, 15 and 20 IU/ml were prepared in 100 µl medium.

Briefly, serum samples were initially diluted 1:5 by medium, containing 37.5 IU/ml of IFN-β and prepared in three dilutions: 1/160, 1/320, 1/640. Three dilutions of serum were chosen based on IFN dilution in NAb related protocols in similar studies such as “Blind-Panel Serum Samples Tested in the MxA Assay” in Wadhwa et al. study and other literatures [9, 13].

After 1 h of incubation at room temperature, 50 µl of each medium, containing diluted patient serum was added to the plates. Then 50 µl of conditioned medium without serum was added per well and the plates were incubated overnight at 37 °C. The final concentration of IFN-β was measured traditionally according to Kawade titration and set to 18.75 IU/ml [9, 14]. To achieve this concentration, the conditioned medium of A549 cell line was prepared by adding 37 IU/ml of either ReciGen® or Rebif®.

2.3. Enzyme-linked immunosorbent assay (ELISA)

A serum sample of each patient was collected and stored at −20 °C. All the samples were then delivered to the Immunology Research Laboratory of Semnan University of Medical Sciences. After 24 h of incubation at 37 °C, the supernatant or media of the cells were aspirated gently and cells were washed with phosphate buffer saline (PBS; Sigma, Germany). The cells were lysed by the addition of 100 ml of lysis buffer (25 mm Tris-HCl–pH 7.2, 0.02% sodium azide, and 2% NP-40) for approximately 2 h at room temperature. The cell lysates were frozen and MxA protein was determined by MxA sandwich ELISA kit (Biovendor, Czech) according to the kit's protocol. The standard curve of ELISA was plotted after the test. The positive and negative controls were tested by serial dilutions of IFN-β and cytokine-free medium. The Optical Density (OD) value is directly proportional to the concentration of MxA in each well. The patients' sera were analyzed in three dilutions (1/160, 1/320 and 1/640). The titer of serum dilution that reduced cytokine activity up to 50% was considered as NAb positive titer [9]. The cytokine concentration which was enough for the saturation of specific receptor activity was considered as saturation threshold of that cytokine. The Optical Density or absorbance of MxA is proportional to receptor activity.

2.4. Statistical analysis

All the analyzes were performed by SPSS software, version 22 (Chicago, USA) after data collection (including group coding, age, gender, duration of drug usage, EDSS score in three time points, delta EDSS in two time points, NAb status and NAb positive titer). The age and duration of receiving the drug were tested for normal distribution using one-sample Kolmogorov-Smirnov test. Normally distributed data were analyzed using the t-Student test to compare the mean of two independent groups. For data without normal distribution, Mann-Whitney *U* test was utilized to compare the median of two independent groups. In some comparisons we included all the patients, while in some cases data were analyzed by subgroup approach. The odds ratio between the EDSS score and group while controlling NAb status, was calculated using Mantel-Haenszel test. The Spearman test was used to evaluate the correlation between NAb status and other parameters. Since Rebif® and ReciGen® arms had the statistically significant difference regarding the duration of using IFN-β1a and this may be as a confounding factor, another set of analyses was conducted by omitting