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IFNβ bioavailability in multiple sclerosis patients: MxA versus antibody-detecting assays ☆

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Abstract

Anti-IFNβ antibodies are related to IFNβ bioavailability loss in multiple sclerosis. We investigated the reliability of radioimmunoprecipitation and cytopathic assays in detecting binding (BAbs) and neutralizing (NAbs) antibodies and the correlation of these antibodies to MxA mRNA production. Eleven percent of IFNβ-treated patients showed a lack of MxA induction, with an inverse correlation between MxA mRNA and the presence of BAbs and NAbs. Some patients had contemporary MxA induction in the presence of high NAb titres, thus calling into question the reliability of cytopathic assay. Since anti-IFNβ antibodies well correlated with MxA induction loss, MxA assay is an appropriate test to determine IFNβ bioavailability.

Keywords: Interferons; Multiple sclerosis; MxA; Bioavailability; NAbs; BAbs

1. Introduction

Some patients with multiple sclerosis (MS) develop antibodies to interferon beta (IFNβ) because the recombinant IFNβ preparations are immunogenic and may elicit an antibody response, primarily of IgG isotype (Pachner, 2003b), that targets the bioactive protein (PRISMS Study Group, 1998; IFNB Multiple Sclerosis Study Group, 1996; Rudick et al., 1998; Deisenhammer et al., 2000; Polman et al., 2003; Pachner, 2003b). Antibody generation can be related to several factors, including duration and dose of treatments, frequency of administration, and route of injection. Some other aspects, such as the formulation of commercial IFNβ, method of production, glycosylation status, and formation of aggregates could also be responsible for the degree of antibody response to the injected drug (Ross et al., 2000; Larocca et al., 1989). An optimal assay or approach to identifying the presence of anti-IFNβ antibodies has not yet been determined. Two different types of assays are preferentially used: those identifying antibodies that bind to the molecule (binding antibodies [BAbs]) (Ross et al., 2000; Brickelmaier et al., 1999; Pachner et al., 2003b) and those measuring antibodies that interfere with the in vitro biologic activity of IFNβ (neutralizing antibodies [NAbs]) (Malucchi et al., 2004; Grossberg et al., 2001; Pachner, 2003a). At present, the most commonly used technique is based on the virus-induced cytopathic effect (CPE) assay, which quantifies NAbs (Sorensen et al., 2005a). However, the NAb assay has some considerable disadvantages: NAb titer may vary depending on the sensitivity of the assay, which in turn depends on the type and amount of cells and viruses used for the cytopathic assay, dilutions of the sera, and concentration of IFNβ added to the assay. Furthermore, the level at which NAb titer is considered biologically relevant is arbitrary, and finally the assay does not measure antibodies per se, thus leading to possible false-positive

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or false-negative results induced by mechanisms other than antibody-mediated neutralization, such as the serum cytotoxicity (Ross et al., 2006).

For all these reasons, until NAb assays are better standardized, this test is not easily incorporated into clinical practice. Enzyme-linked immunosorbent assays (ELISA) that identify BAbs are usually performed as initial screening for anti-IFNβ antibodies (Sorensen et al., 2005a) and may detect an immune response against IFNβ before clinically relevant NAbs are recorded. However, although all BAbs bind to IFNβ, not all of them will neutralize its activity, unless they bind biologically relevant protein domains or inactivate IFNβ by conformational changes. Commercial direct and capture ELISA for BAbs are easy to perform and useful for screening for the presence of antibodies, but false-positive and false-negative results are common if specific precautions are not taken (Bendtzen, 2003). BAb detection can also be done with an alternative approach based on the use of radiolabeled IFNβ and radioimmunoprecipitation assay (RIPA), which has been shown to be sensitive and easy to perform (Lawrence et al., 2003). Recently, some authors pointed out that the separate identification of BAbs and NAbs may not detect different biologic phenomena but rather an artificial feature produced by the use of two different assays measuring the same population of molecules; they guess that all "true" BAbs would cause neutralization if the neutralization assays were adequately sensitive (Pachner, 2003b; Bendtzen, 2003).

In addition to the BAb and NAb detection assays, other markers for IFNβ activity can be used to assess bioactivity of injected IFNβ, including neopterin, β2 microglobulin, 2′–5′ oligoadenylate synthetase, and myxovirus resistance protein A (MxA). Among these proteins, MxA has the highest specificity in detecting IFNβ-receptor (IFNAR) stimulation because it is induced by the interferon-1 class in a dose-dependent manner (Deisenhammer et al., 2000; Pachner et al., 2005; Bertolotto et al., 2001). MxA-based assays can therefore assess the response of IFNβ-treated patients reflecting the presence of NAbs (Vallittu et al., 2002; Pachner et al., 2003a; Gilli et al., 2005). Whereas patients without anti-IFNβ antibodies respond to IFNβ with an increase in MxA mRNA concentrations, which peaks 12 h following injection (Pachner, 2003a; Pachner et al., 2005), this rise does not occur in patients with high levels of anti-IFNβ antibodies. In this study, we quantified MxA mRNA in patients treated with IFNβ and correlated the lack of IFNAR activation with the presence of BAbs and NAbs as determined by RIPA and CPE.

2. Materials and methods

2.1. Patients

Ninety-nine patients, 36 men and 63 women, >18 years of age, with relapsing–remitting or secondary progressive MS according to the McDonald criteria (McDonald et al., 2001), were included in the study (Table 1). Patients had MS for at least 8 months before treatment, and all of them received intramuscular (IM) IFNβ-1a (Avonex®), subcutaneous (SC) IFNβ-1a (Rebif®) 22 μg and 44 μg, or IFNβ-1b (Betaferon®) for >24 continuous months. Their Kurtzke Expanded Disability Status Scale (EDSS) score ranged from 0 to 6.5. All patients provided written informed consent. Patients were excluded from the study if they previously received treatment with immunosuppressive drugs or therapy of any kind or had poor IFNβ therapy compliance (<95% of injections carried out). Furthermore, in our study, patients' blood has been drawn only after careful clinical evaluation in order to exclude the presence of any concurrent viral disease and, therefore, to avoid false-positive MxA results. The patients were also asked to report any change in their health status within the next few days immediately following the examination. There were no differences in mean disease duration before therapy initiation or in treatment disease duration within patients treated with different IFNβ preparations. The age of patients treated with IFNβ-1b was significantly higher than the mean age of patients in the other groups. To establish the basal level of MxA in patients with MS, samples were collected before IFNβ injection and after 1 week of IFNβ washout in a subgroup of 20 patients randomly chosen from the study population. To investigate the stability of MxA induction in a short period of time, two consecutive samples were also obtained at 3- to 6-month intervals from 18 patients. Samples were taken for a 1-week period, before and after each drug injection, from four patients for each therapy group to study whether all drug injections were effective; this group of patients were MxA-induced and without BAbs and NAbs.

Table 1
Patient demographics and clinical features

Therapy	Patients, *n*	Mean age, years (range) ***	Relapsing–remitting MS, *n*	Secondary progressive MS, *n*	Mean disease duration before therapy, months (range) **	Mean treatment duration, months (range) *	Mean EDSS score
IFNβ-1b	23	49 (24–66)	12	11	92.8 (18–239)	60.2 (24–114)	4
SC IFNβ-1a 44 μg	28	37 (19–63)	27	1	89.1 (23–216)	45.9 (26–67)	3
SC IFNβ-1a 22 μg	22	37 (23–55)	22	0	58.31 (18–149)	52.4 (24–112)	2
IM IFNβ-1a	26	39 (22–60)	26	0	95.9 (18–294)	53.15 (27–96)	2
Total	99	40 (19–66)	87	12	85.12 (18–294)	53.01 (24–114)	3

n=number; MS=multiple sclerosis; SC=subcutaneous; IM=intramuscular; EDSS=Expanded Disability Status Scale.
* $p=0.28$ (Kruskal–Wallis).
** $p=0.10$ (Kruskal–Wallis).
*** $p=0.0005$ (Kruskal–Wallis).