Journal of Immunological Methods 388 (2013) 46–48

Contents lists available at SciVerse ScienceDirect

Journal of Immunological Methods

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

Research paper

Neutralizing antibodies in multiple sclerosis patients on weekly intramuscular Avonex and biosimilar interferon beta-1a (CinnoVex): Comparing results of measurements in two different laboratories

Mohammad Amir Shahkarami [a], Behrouz Vaziri [b], Shiva Salami [c], Ali Amini Harandi [d], Joel Oger [e,*]

[a] Research Coordinator, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Protein Chemistry Unit, Biotechnology Research Center, Pasteur Institute of Iran, Iran
[c] CinnoVex Collaborating Research Group, Tehran, Iran
[d] Department of Neurology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Neuro-Immunology Laboratory, University of British Columbia, Vancouver, Canada

ARTICLE INFO

Article history:
Received 5 July 2012
Received in revised form 12 November 2012
Accepted 28 November 2012
Available online 6 December 2012

Keywords:
Multiple sclerosis
Interferon beta
Neutralizing antibody
Cytopathic effect
Luciferase

ABSTRACT

The appearance of neutralizing antibodies (NAbs) has significant clinical and regulatory consequences for interferons in patients with multiple sclerosis (MS). In a double blind, randomized clinical trial, 84 patients with relapsing remitting MS were enrolled in a 24 month study period. Patients were randomly assigned into two groups receiving 30 mcg weekly intramuscular injections of either Avonex® (Biogen Idec, USA; 42 patients) or CinnoVex® (CinnaGen Co, Iran; 42 patients). NAb titer was drawn for all patients every 6 months and assayed using cytopathic effect assay (CPE) method in Tehran, Iran. To validate the measure done in the Iranian lab, 45 sera with adequate volume and proper storing condition were selected and sent to be rechecked using luciferase reporter gene assay (LA) method for verification in 2 phases in Vancouver, Canada. The cut-off point of 20 TRU was considered for positivity. The two labs found the same three samples to be positive (2 samples from patients received Avonex and 1 received CinnoVex) and 42 to be negative. They had the following values using the Kawade formula as recommended by international standards; 2238, 89 and 302 (TRu/ml) using CPE assay versus 2464, 290 and 169 (TRu/ml) using LA method. As similar results were obtained from CinnoVex or Avonex in our study, we suggest that both medications will have a similar immunogenetic profile.

1. Introduction

IFN-beta is the recommended first-line therapy in patients with relapsing remitting multiple sclerosis (RRMS) (Compston and Coles, 2002). IFN-beta-1a is produced by recombinant DNA technology in a Chinese hamster ovary cell line (Jacobs et al., 1996). Rising of neutralizing antibodies (NAbs) to recombinant proteins is a serious problem of biotherapeutics (Schellekens, 2002). There are multiple assays to identify antibodies to biological: both binding and neutralizing have been described, assays that rely on the neutralization of the target molecules are more attractive as they must bind to active epitopes. To measure interferon neutralizing antibodies, both the induction of inhibition of cytopathic effect and luciferase reporter gene assay can be relied on (Boz et al., 2007; Files et al., 1998; Pungor et al., 1998). The antiviral activity of IFN-beta is measured by cytopathic effect (CPE) assay which detects the protection of a cell line from being lysed by an introduced virus (Grossberg et al., 2001). The luciferase reporter gene assay uses the luminescence signal from cell line HL 116 containing a plasmid holding a luciferase cDNA controlled by the immediate early IFN inducible 6–16 promoter (Uzé et al., 1994). All of these

* Corresponding author at: Brain Research Center, UBC Hospital Room S-157, 2211 Wesbrook Mall Vancouver, British Columbia, Canada V6T 2B5. Tel.: +1 604 822 7696.
E-mail addresses: Shahkarami@edc.mui.ac.ir (M.A. Shahkarami), behrouz-vaziri@pasteur.ac.ir (B. Vaziri), Shiva.salami45@yahoo.com (S. Salami), ali.amini@sbmu.ac.ir (A.A. Harandi), joel.oger@ubc.ca (J. Oger).

0022-1759/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jim.2012.11.013

assays can be rendered sensitive to very low titers of antibodies.

CinnoVex® is a biosimilar form of intramuscular IFN-beta-1a manufactured by CinnaGen Co (SiminDasht Industrial Park, Tehran, Iran). Since the appearance of NAbs has significant clinical and regulatory consequences for interferons, a measurement of NAbs in RRMS patients receiving Avonex or CinnoVex in a double blind randomized study has been undertaken with measurement of antibodies done in 2 different laboratories using two different methods.

1.1. Material and methods

1.1.1. Samples

In a double blind, randomized clinical trial 84 patients with RRMS were enrolled in a 24 month study period. They were recruited from all MS clinics affiliated to the three main medical universities in Tehran and Isfahan, Iran. Patients were randomly assigned into two groups receiving weekly intramuscular (IM) injections of either Avonex® (Biogen Idec, USA; 42 patients) or CinnoVex® (CinnaGen Co, Iran; 42 patients). Both Avonex® and CinnoVex vials contained 30 mcg lyophilized IFN-beta-1a and were same in shape and route of administration.

NAb titer was drawn for all patients every 6 months and assayed in Iran. NAbs were found to be negative in 6th and 12th month of study. However, 2 samples (1 sample from patients who received Avonex and 1 sample from patients on CinnoVex) at 18th month and 3 samples (2 samples from patients on Avonex and 1 sample from patients on CinnoVex) at 24th month were positive for NAbs.

To validate the measure done in the Iranian lab, 45 sera with adequate volume and proper storing condition were selected and sent to be rechecked for verification in 2 phases in the Neuro-Immunology Laboratory, Brain Research Centre, University of British Columbia, Vancouver, Canada. All samples were drawn from patients who had completed 24 months of therapy (23 on Avonex and 22 on CinnoVex).

A further 42 NAB positive samples from patients having received Beta-1a interferon (either Avonex® or Rebif®) were blindly sent from the UBC MS serum bank to be assayed in Institute Pasteur Teheran and the correlation coefficient "r" was calculated.

1.1.2. Laboratory methods

For measuring Nabs titer, luciferase reporter gene assay (LA) was used in Vancouver and the cytopathic effect assay (CPE) was used in Tehran. LA was done as previously described (Lam et al., 2008). The recommended CPE assay by World Health Organization was used according to the standard procedure (Antonelli et al., 1998). In both methods, signals above 20 TRU/mL were considered positive. Positive samples were serially diluted and titrated.

1.2. Results

1.2.1. Qualitative assay

The mean study duration for 45 assessed samples from the Iranian trial was about 24 months (mean ± SD: 23.4 ± 1.7 months). The samples were assayed by both methods i.e., CPE in Teheran and LA in Vancouver, with a cut-off point of 20 TRU for positivity. The two labs found the same three samples to be positive (2 samples from patients received Avonex and 1 received CinnoVex) and 42 to be negative. The 3 samples which were positive by both techniques had the following values using the Kawade formula as recommended by international standards; 2238, 89 and 302 (TRu/ml) using CPE assay versus 2464, 290 and 169 (TRu/ml) using LA method.

1.2.2. Quantitative assay

Details of NAb titers of 42 positive samples which were sent from UBC MS serum bank to Pasteur institute of Iran are shown in Table 1.

The correlation coefficient (Pearson product-moment correlation coefficient PMCC) between the Canadian and Iranian quantitative results was found to be $R = 0.96$ for the titres of these 42 samples ($P < 0.001$). The corresponding scatter plot represented in a log10 transformation is shown in Fig. 1. The correlation coefficient for logarithm was similar to that previously calculated from NAb titers tested by LA in UBC with the results obtained by the CPE assay method as assayed

Table 1
The details of Nab titers and log (TRU/ml) using cytopathic effect assay in Iran Pasteur Institute and luciferase reporter gene assay in UBC center, Canada in all 42 samples. Spearman coefficient $r = 0.946$, $P < 0.001$.

Sample	Iran titers (TRU/ml)	UBC titers (TRU/ml)	Log Iran	Log UBC
1.	83977	13523	4.924	4.131
2.	3751	1262	3.574	3.101
3.	4406	916	3.644	2.962
4.	63702	5793	4.804	3.763
5.	16000	8770	4.204	3.943
6.	44000	7308	4.643	3.864
7.	10572	1664	4.024	3.221
8.	941	358	2.974	2.554
9.	94224	3014	4.974	3.479
10.	33431	5374	4.524	3.730
11.	3192	401	3.504	2.603
12.	4208	1429	3.624	3.155
13.	253	113	2.403	2.053
14.	167	61	2.223	1.785
15.	13936	2034	4.144	3.308
16.	133095	11979	5.124	4.078
17.	73141	16726	4.864	4.223
18.	22088	2614	4.344	3.417
19.	2979	1018	3.474	3.008
20.	152	68	2.182	1.833
21.	878	200	2.943	2.301
22.	16754	2300	4.224	3.362
23.	210	82	2.322	1.914
24.	1492	256	3.174	2.408
25.	17544	2781	4.244	3.444
26.	365	160	2.562	2.204
27.	21093	3976	4.324	3.599
28.	2014	1136	3.304	3.055
29.	55483	6194	4.744	3.792
30.	6670	1515	3.824	3.180
31.	50601	10159	4.704	4.007
32.	334	29	2.524	1.462
33.	21093	3237	4.324	3.510
34.	30490	4332	4.484	3.637
35.	3500	683	3.544	2.834
36.	167	42	2.223	1.623
37.	33431	3694	4.524	3.567
38.	230	51	2.362	1.708
39.	3977	872	3.600	2.941
40.	16754	1813	4.224	3.258
41.	20144	4148	4.304	3.618
42.	26555	4724	4.424	3.674

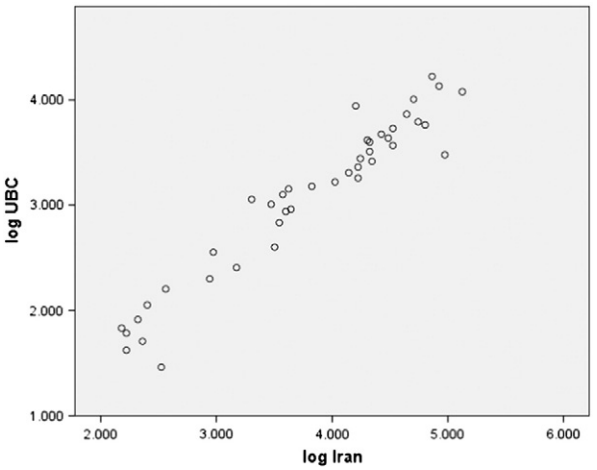

Fig. 1. The scatter plot for logarithm of NAb titers measured by luciferase (UBC) vs. CPE (Iran). Titers are expressed in log10. Spearman coefficient $r = 0.946$, $P < 0.001$.

by Grossberg's lab to validate the first UBC center. In addition, as the assumption of normal distribution was violated in non-transformed data, Spearman's correlation coefficient was calculated for original values ($r = 0.946$, $P < 0.001$).

1.3. Discussion

According to our results we found that the parameters that optimize assay sensitivity and the accuracy of results have been considered and organized satisfactorily in Iran comparing to the methods of gold standard laboratory in UBC. This study helps to qualify the Institute Pasteur of Iran as being able to generate quality results using the CPE assay.

Although the principle of NAb measurement is similar among different clinical trials, the reported results have varied from study to study. While up to 80% of treated patients have been reported to become binding antibodies (BAbs) positive over time, NAbs were found in 2% to near 50% of patients. Phase III studies with IM IFNβ-1a have shown consistently lower levels of immunogenicity, with incidences of NAbs ranging from 2% to 5.8% (Cohen et al., 2002). In PRISM study, 23.7% of patients who received subcutaneous (SC) IFNβ-1a 22 mcg and 14.3% of patients who received SC IFNβ-1a 44 mcg had a positive test result for NAbs (Compston and Coles, 2002). Interestingly, in the earlier pivotal phase III trial of IM IFNβ-1a, NAbs were detected in 14% of patients treated with IM IFNβ-1a 30 mcg once weekly at week 52, 21% at week 78, and 22% at week 104 (Clanet et al., 2002; Jacobs et al., 2000). This implies that the majority of antibodies to IFN-beta do not interfere with the biological action of the drug. In our study, the presence of NAbs was time dependent, with most positivity occurring after the first 6 months of treatment.

1.4. Conclusion

As similar results were obtained from CinnoVex or Avonex in our study, we suggest that both medications will have a similar immunogenetic profile.

References

Antonelli, G., Bagnato, F., Pozzilli, C., Simeoni, E., Bastianelli, S., Currenti, M., De Pisa, F., Fieschi, C., Gasperini, C., Salvetti, M., Dianzani, F., 1998. Development of neutralizing antibodies in patients with relapsing-remitting multiple sclerosis treated with IFN-beta1a. J. Interferon Cytokine Res. 18, 345.

Boz, C., Oger, J., Gibbs, E., Grossberg, S.E., 2007. Reduced effectiveness of longterm interferon-ß treatment on relapses in neutralizing antibody positive multiple sclerosis patients: a Canadian multiple sclerosis clinic-based study. Mult. Scler. 13, 1127.

Clanet, M., Radue, E.W., Kappos, L., Hartung, H.P., Hohlfeld, R., Sandberg-Wollheim, M., Kooijmans-Coutinho, M.F., Tsao, E.C., Sandrock, A.W., European IFNbeta-1a (Avonex) Dose-Comparison Study Investigators, 2002. A randomized, double-blind, dose-comparison study of weekly interferon beta-1a in relapsing MS. Neurology 59, 1507.

Cohen, J.A., Cutter, G.R., Fischer, J.S., Goodman, A.D., Heidenreich, F.R., Kooijmans, M.F., Sandrock, A.W., Rudick, R.A., Simon, J.H., Simonian, N.A., Tsao, E.C., Whitaker, J.N., IMPACT Investigators, 2002. Benefit of interferon beta-1a on MSFC progression in secondary progressive MS. Neurology 59, 679.

Compston, A., Coles, A., 2002. Multiple sclerosis. Lancet 359, 1221.

Files, J.G., Gray, J.L., Do, L.T., Foley, W.P., Gabe, J.D., Nestaas, E., Pungor Jr., E., 1998. A novel sensitive and selective bioassay for human type I interferons. J. Interferon Cytokine Res. 18, 1019.

Grossberg, S.E., Kawade, Y., Kohase, M., Klein, J.P., 2001. The neutralization of interferons by antibody. II. Neutralizing antibody unitage and its relationship to bioassay sensitivity: the tenfold reduction unit. J. Interferon Cytokine Res. 21, 743.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., Alam, J.J., Bartoszak, D.M., Bourdette, D.N., Braiman, J., Brownscheidle, C.M., Coats, M.E., Cohan, S.L., Dougherty, D.S., Kinkel, R.P., Mass, M.K., Munschauer III, F.E., Priore, R.L., Pullicino, P.M., Scherokman, B.J., Whitham, R.H., et al., 1996. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis: the Multiple Sclerosis Collaborative Research Group (MSCRG). Ann. Neurol. 39, 285.

Jacobs, L.D., Beck, R.W., Simon, J.H., Kinkel, R.P., Brownscheidle, C.M., Murray, T.J., Simonian, N.A., Slasor, P.J., Sandrock, A.W., 2000. N. Engl. J. Med. 343, 898.

Lam, R., Farrell, R., Aziz, T., Gibbs, E., Giovannoni, G., Grossberg, S., Oger, J., 2008. Validating parameters of a luciferase reporter gene assay to measure neutralizingantibodies to IFN beta in multiple sclerosis patients. J. Immunol. Methods 336, 113.

Pungor Jr., E., Files, J.G., Gabe, J.D., Do, L.T., Foley, W.P., Gray, J.L., Nelson, J.W., Nestaas, E., Taylor, J.L., Grossberg, S.E., 1998. A novel bioassay for the determination of neutralizing antibodies to IFN-beta1b. J. Interferon Cytokine Res. 18, 1025.

Schellekens, H., 2002. Bioequivalence and the immunogenicity of biopharmaceuticals. Nat. Rev. Drug. Discov. 1, 457.

Uzé, G., Di Marco, S., Mouchel-Vielh, E., Monneron, D., Bandu, M.T., Horisberger, M.A., Dorques, A., Lutfalla, G., Mogensen, K.E., 1994. Domains of interaction between alpha interferon and its receptor components. J. Mol. Biol. 243, 245.