LEADING ARTICLE

Drugs 2001; 61 (12): 1693-1703
0012-6667/01/0012-1693/$27.50/0

Interferon-β Therapy in Multiple Sclerosis

Evidence for a Clinically Relevant Dose Response

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Abstract

There have been considerable advances made recently in the treatment of multiple sclerosis (MS). In particular, interferon (IFN)β has been demonstrated in several independent, multicentre clinical trials to lower unequivocally the biological activity of this illness. The results of these trials have been remarkably consistent, demonstrating a reduction in both disease activity and cumulative disability, using a combination of clinical and magnetic resonance imaging outcome measures. Nevertheless, the importance of the total weekly IFNβ dose in the clinical management of individual patients has been controversial.

However, there is considerable information available regarding the effect of IFNβ dose on the various biochemical and clinical markers that are affected by IFNβ, which is derived both from pre-clinical studies and multicentre clinical trials. On balance, convincing evidence is provided to support the notion that there is a clinically relevant dose-response in the use of IFNβ to treat patients with relapsing/remitting MS. However, many of the clinical trials of IFNβ in MS have confounded the potential effects of dose with the possible effects of frequency of IFNβ administration. As a result, it is possible that the apparent dose-response observed in these clinical trials may be due, in part, to the more frequent dose administration schedule rather than the total weekly dose.

Interferon (IFN)β, a naturally occurring immunomodulatory glycoprotein, was the first clinically available agent demonstrated to be effective in modifying the disease course in patients with multiple sclerosis (MS). Two forms of IFNβ – IFNβ-1a (Avonex™[1], Rebif®[1]) and IFNβ-1b (Betaseron®[1], Betaferon®[1]) – have been assessed in clinical trials. Both forms have been shown to reduce the frequency and severity of relapses, and to slow the progression of neurological disability.[1-10] Magnetic resonance imaging (MRI) studies have also demonstrated that IFNβ reduces the number of active lesions and slows the increase in total MRI lesion volume over time.[1-10] Different regimens for each form of IFNβ have already been approved for the treatment of MS by the US Food and Drug Administration (FDA) and European regulatory bodies. Nevertheless, uncertainties remain about the optimal clinical application of these agents in the treatment of patients with MS. In particular, the optimal dose of IFNβ is uncertain, as is the optimal time to start therapy, and the optimal route of administration.

With regard to initiating therapy, most authorities favour early treatment and this practice is, in

1 Use of a tradename is for identification purposes only and does not imply endorsement.

fact, the position of recent consensus statements.[11,12] In addition, the recently presented trials of IFNβ-1a in patients at high risk of developing MS have shown that early treatment significantly slows the subsequent rate of conversion to clinically definite MS.[13,14] There is also now evidence showing that the inflammatory demyelination of MS is accompanied by early axonal injury,[15,16] which precedes and, presumably, underlies subsequent neurological disability.[17] This kind of evidence provides considerable empirical support for the practice of initiating disease-modifying therapies as early as possible during the course of the disease, with the aim of limiting both irreversible tissue damage and permanent disability.

However, there is less consensus on the optimal dosage and route of administration of IFNβ.[18-20] Nonetheless, there is substantial information available regarding the effect of IFNβ dose on various biochemical and clinical markers. This evidence derives both from preclinical studies (*in vitro* and in animals) as well as from large multicentre clinical trials. It is the purpose of this manuscript to review these diverse pharmacological, clinical and MRI findings as they relate to the use of IFNβ in clinical practice.

1. Immunomodulatory Effects of Interferon (IFN)β

The cause of MS is unknown, although considerable evidence indicates that activated T cells, autoreactive to self antigens such as myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG), proliferate, cross the blood-brain barrier and, under the influence of proinflammatory cytokines and cellular adhesion molecules, enter the central nervous system (CNS) [for reviews see[21,22]]. Other mononuclear cells such as macrophages and, to a lesser extent, B cells are also present in active MS lesions. Within the CNS, in concert with resident cells such as astrocytes and microglia, these mononuclear cells produce inflammation and, thereby, damage both to the myelin and to the oligodendrocytes, resulting in irreversible axonal transection.[23]

The mechanism by which IFNb exerts its disease-modifying effects in MS is also unknown but could potentially be through one or more of a number of immunomodulatory mechanisms.[24]

1.1 Inhibition of Gelatinase Secretion and T Cell Migration

Migration of activated T cells across the blood-brain barrier appears to be a key step in the pathophysiology of MS.[25] An important part of this process is the release by T cells of matrix metalloproteinases (MMPs) in response to stimulation by the pro-inflammatory cytokine interleukin (IL)-2. MMPs cleave type IV collagen of the extracellular matrix (a component of the blood-brain barrier), thereby facilitating the transendothelial migration of T cells into the CNS.[21,22]

Leppert et al.[26] demonstrated that pretreatment of T cells with IFNβ-1b inhibited IL-2–dependent secretion of MMP-2 and MMP-9, and reduced MMP–dependent migration across an artificial basement membrane by up to 90%, without significantly affecting normal cell locomotion. IFNβ also downregulated expression of the IL-2 cell surface receptor and the affinity of IL-2 for the T cell surface, indicating a potential further mechanism for the therapeutic effect of IFNβ. Importantly, both the effect on MMP secretion and T cell migration were dependent on the dose of IFNβ.[26] IFNβ has also been shown to dose-dependently inhibit activated leucocyte transmigration through an activated human brain microvascular endothelial cell (HB-MVEC) monolayer.[27] Prestimulation of HB-MVEC with tumour necrosis factor-α (TNFα) and IFNγ significantly promoted transepithelial migration of activated leucocytes.[27] Again, this migration is inhibited by IFNβ-1a in a dose-dependent manner, mediated through inhibition of TNFα, IL-1 and MMP-9 production.[27]

1.2 Inhibition of IFNγ -Induced Major Histocompatibility Complex Class II Expression

Vascular endothelial cells play a central role in T cell trafficking into the CNS. Under the influence of pro-inflammatory cytokines such as IFNγ and

TNFα, vascular endothelial cells express both major histocompatibility complex (MHC) class I and II molecules and adhesion molecules. These molecules help to activate and adhere leucocytes, and to facilitate their migration across the endothelium. Miller and colleagues[28] showed that addition of different doses of IFNβ to human vascular endothelial cells incubated with IFNγ led to a down-regulation of IFNγ-induced class II molecule expression in a dose-dependent manner.

1.3 Inhibition of T Cell Proliferation and IFNγ Release

Proliferation and activation of auto-reactive T cells probably represent key events in the pathogenesis of MS. Noronha et al.[29] demonstrated that IFNβ significantly reduced *in vitro* mitogen-induced proliferation of T cells from both patients with MS and healthy individuals. Significantly, this reduction was dependent on the dose of IFNβ in the concentration range 10 to 100 U/ml, and was observed irrespective of the mitogenic stimulus or the presence of IFNγ. Pre-activated T cells were down-regulated to similar extent. IFNβ 1000 U/ml also down-regulated release of IFNγ from activated T cells obtained from patients with MS and healthy controls.

1.4 Increase in Interleukin-10 Expression

Inflammatory and autoimmune responses involve a complex interplay of mediators that either promote or inhibit immunological processes, and IFNβ is known to exert inhibitory effects on immune promoters.[24] For example, IL-10 is released by activated T cells and strongly inhibits cell-mediated immune responses.[30-33] A study by Rudick et al.[34] found that incubation of peripheral blood mononuclear cells (PBMC), *in vitro*, with IFNβ-1a (varying in concentration between 0 and 1000 IU/ml) upregulated IL-10 mRNA levels in a dose-dependant manner.[34] Serum IL-10 levels in healthy individuals and patients with MS were also increased after a single intramuscular injection of IFNβ-1a and this effect was also dose-dependent at IFNβ-1a doses of 6 and 12 MIU.[34]

2. Effects of IFNβ in Experimental Allergic Encephalomyelitis (EAE)

Experimental allergic encephalomyelitis (EAE) is a T cell-mediated autoimmune CNS disease and has been used extensively as an experimental model for MS (for reviews see[35,36]).

2.1 Histological Impact of IFN in EAE

In a study by Abreu and colleagues,[37] rats with a passively transferred form of EAE were treated with placebo or purified rat IFN at doses of 12 000, 40 000 or 120 000 U/kg. A clear dose response was observed on the number of lesions within the brain. The lowest IFN dose had no effect on the number of lesions (perivascular accumulation of mononuclear cells), but the 40 000 U/kg dose significantly reduced the number of these mononuclear cuffs and the 120 000 U/kg dose provided a further reduction, indicating a significant effect of IFN dose on the EAE disease process.

2.2 Effect of IFNβ on Clinical Parameters in Mice with EAE

A later study by Yu et al.[38] demonstrated that IFNβ also exerts a therapeutic effect on the clinical manifestations of EAE. Mice with EAE received placebo or IFNβ 5000 or 10000IU at the onset of clinical disease. Dose-dependent benefits were observed in several clinical parameters, including survival at 14 days, frequency of relapses, number of multiple relapses and time to first relapse. There was also a delay in the progression of disability to day 56. These clinical benefits were accompanied by a dose-dependent inhibition of *in vitro* proliferation of autoreactive T cells in the mice, as well as a reduction in inflammation and demyelination within the CNS.

3. Effects of IFNβ on Non-Immunological Mediators

Although the mechanisms of action suggested for IFNβ in the treatment of MS have emphasised its immunumodulatory effects, the influences of

IFNβ on non-immunological processes may also be important.

3.1 Inhibition of Inducible Nitric Oxide Synthase

Nitric oxide generated by the inducible enzyme, nitric oxide synthase, has been implicated in damage to oligodendrocytes, contributing to the pathogenesis of MS.[39] An *in vitro* study by Hua et al.[40] showed that IFNβ produced dose-dependent, selective and potent inhibition of IL-1β/IFNγ–induced nitric oxide synthase expression in cultured human astrocytes. This observation suggests a potential mechanism by which IFNβ may ameliorate inflammation and cytotoxicity within the CNS of patients with MS.

3.2 Promotion of Neurotropin Nerve Growth Factor Release by Astrocytes

Growth factors released by astrocytes are important for oligodendrocyte development, maturation and survival; nerve growth factor (NGF) in particular stimulates adult porcine oligodendrocytes to extend processes and proliferate, and may also promote CNS remyelination.[41] Boutros and colleagues[42] showed that incubation of murine astrocytes in the presence of murine IFNβ (concentration range 10 to 1000 U/ml) induced a dose-dependent release of NGF, up to 40 times that of untreated controls. If a similar effect occurs in humans, it may be one mechanism of IFNβ action in MS. Interestingly, administration of NGF has also recently be shown to delay the onset and lessen the severity of EAE in marmosets.[43] In this marmoset model, intrathecal administration of NGF seems to produce this anti-inflammatory effect by both the down-regulation of IFNγ and the up-regulation of IL-10 production by glial cells.[43]

4. Pharmacodynamic, Pharmacological and Biological Effects of IFNβ

IFNβ is known to induce the expression of IFN-specific markers such as 2′,5′-oligoadenylate synthetase (2′,5′-OAS), neopterin, tryptophan, β_2-microglobulin and Mx protein.[44-46] These markers reflect several biological activities of IFNβ, such as MHC class I gene expression, antiviral and antiproliferative actions, and monocyte activation. These markers have been used to measure the pharmacodynamic response in clinical studies.[47,48]

4.1 Dose Response: Pharmacodynamics After Single and Multiple Doses

Witt et al.[49] examined the pharmacodynamic effects of IFNβ-1b in 32 healthy individuals. Four groups of volunteers received 0.09, 0.9, 9 or 45MU ('old standard' MIUs – equivalent to the 'current standard' 0.018, 0.18, 1.8, and 8 MIU) as a single dose, then on 4 alternate days after a 7-day washout. Significant ($p < 0.02$) increases in b2-microglobulin and cellular 2′,5′-OAS activity were observed at 24 hours for the 0.9MU dose. The 9 and 45MU doses were associated with significant increases in β_2-microglobulin and cellular 2′,5′-OAS, neopterin and tryptophan levels ($p < 0.01$). A clear dose response ($p < 0.01$) was evident for all markers over the IFNβ-1b concentration range 0.09 to 45MU, and changes in all markers correlated with each other.

Similar results were reported recently by Stürzebecher et al.[50] These authors contrasted the effects of IFNβ-1a given subcutaneously or intramuscularly and IFNβ-1b given subcutaneously to 75 healthy volunteers on the levels of human Mx protein, neopterin and 2′,5′-OAS. Each patient in the study received a single dose, which was administered for IFNβ-1a at doses of 1, 3, 6, 9 and 12 MIU and for IFNβ-1b at doses of 2, 4, 8, 12 and 16 MIU. The production of all 3 markers was induced in a dose-dependent manner for both IFNβ-1a and IFNβ-1b. Moreover, although earlier authors had suggested that intramuscular administration produced greater IFNβ activity than subcutaneous administration,[47] this study found no differences in any of these biological effects between the 2 types of IFNβ or between the different routes of administration. However, with regard to their effect on these biologic markers, intramuscular IFNβ-1a 6 MIU was found to be approximately equal to sub-

cutaneous IFNβ-1b 8 to 9 MIU.[50] Importantly, even at the highest doses used in this study, these biological effects were not sustained for a week after a single injection.

4.2 Effect of Dose Frequency on Biological Effect Markers and Cytokine Release

Findings pertinent to the dosage schedule for IFNβ were reported in a comparative study by Williams and Witt,[51] in which healthy volunteers received either a single 6 MIU intramuscular dose of IFNβ-1a (Avonex™) or 8 MIU IFNβ-1b (Betaseron®) subcutaneously every other day for 1 week. Biological response parameters reached similar maximum concentrations in both treatment groups. However, serum neopterin and β_2-microglobulin levels remained significantly above baseline throughout the 7-day study among those receiving IFNβ every other day, whereas this effect was sustained only to day 5 in the group given IFNβ weekly. Furthermore, the overall induction of neopterin, β_2-microglobulin and IL-10 levels, as assessed by area under the concentration-time curve (AUC), was significantly greater in the alternate-day group compared with the weekly ($p = 0.018$, $p = 0.031$, and $p = 0.014$, respectively). These data suggest that more frequent IFNβ administration provides a more continuous biological response than once weekly administration.

In another study, 24 healthy individuals received IFNβ-1a (Rebif®) subcutaneously at doses of 22μg once weekly, 66μg once weekly, 22μg 3 times a week or placebo for 4 weeks.[52] Blood samples were obtained to assess pharmacodynamic responses; PBMCs from each sample were cultured and cytokine release measured with and without mitogenic stimuli. The immunomodulatory action of IFNβ was found to be dose dependent. Moreover, the biological effect of the 66μg total weekly dose of IFNβ was 2 to 3 times greater when divided into 3 injections compared with when it was administered as a single weekly injection, suggesting that more frequent administration may improve the biological effects of IFNβ therapy.

A recent study investigated the levels of the anti-viral protein MxA following administration of the IFNβ in 237 patients with clinically definite MS.[53] 78 patients received IFNβ-1b (Betaseron®) at a dosage of 8 MIU (250μg) every other day; 71 patients received IFNβ-1a (Rebif®) at a dosage of 6 MIU (22μg) subcutaneously either weekly or 3 times weekly; and 21 patients received IFNβ-1a (Avonex™) at a dosage of 6 MIU (30μg) intramuscularly once weekly. Samples were drawn 1 to 2 days after IFNβ administration, although, unfortunately, the time of MxA sampling was significantly delayed (by a half a day) in the Betaseron® group compared with the groups receiving other IFNβ preparations. MxA stimulation in the untreated blood from 10 healthy volunteers was also studied. These authors reported that the levels of MxA were 2.29 ng/10^5 peripheral blood lymphocytes (PBLs) in the Betaseron®–treated patients, 1.00 ng/10^5 PBLs in the Rebif®–treated patients, and 0.57 ng/10^5 PBLs in the Avonex™–treated patients. *In vitro* stimulation of PBLs with all 3 of these agents resulted in a dose-dependant increase in MxA levels that was approximately equivalent (on an MIU for MIU basis) for each of the compounds. Although this trial seems somewhat poorly designed, these results, nevertheless, suggest that the biological activity of these different IFNβ preparations are similar and that increasing the total weekly IFNβ dose is associated with an increasing biological effect.

5. Clinical and Magnetic Resonance Imaging Evidence for a Dose Response in Patients with Multiple Sclerosis

Several multicentre clinical trials in patients with MS have demonstrated beneficial effects of IFNβ on each of the 4 outcome measures in current clinical use, including clinical MS attack rate, clinical disability progression, MRI lesion activity and MRI lesion burden.[1,5,7-9] Key results from the 4 principal phase III clinical trials of IFNβ in relapsing-remitting MS (RRMS) are summarised in table I.

Table I. Findings from 4 clinical trials of interferon-β (IFNβ) in patients with relapsing remitting multiple sclerosis during years 1 and 2 by increasing IFNβ dose. All comparisons are versus placebo in the same study[a]

Study parameter	Betaseron® Trial[1-3]	Rebif® Trial[9]	Avonex™ Trial[4,5]	Rebif® Trial[9]	Rebif® Trial[6,7]	Betaseron® Trial[1-3]	Rebif® Trial[6,7]
Dosage and administration information							
Total weekly dose of IFNβ (MIU)[b]	5.6	6	6	12	18	28	36
weekly dose of IFNβ (μg)	175	22	30	44	66	875	132
Mean EDSS at entry	2.9	2.7	2.4	2.6	2.5	3.0	2.5
EDSS range at entry	0-5.5	0-5.0	1-3.5	0-5.0	0-5.0	0-5.5	0-5.0
Dose schedule (route)	qod (SC)	qw (SC)	qw (IM)	qw (SC)	tiw (SC)	qod (SC)	tiw (SC)
Measures of disease activity (%)							
Relapse rate, 1y	–15	0	–10[c]	–19	–33****	–33****	–37****
Relapse rate, 2y (ITT)	–13*		–18*		–29**	–34***	–32****
Median time to first relapse	+18		+31		+70*	+86*	+113*
Relapse-free patients, 1y		0		–11	+59		+87
Relapse-free patients, 2y	+29		+42		+69*	+95**	+100**
Median MRI attack rate, 2y[d]	–67*		–33*		–67***	–83**	–78****
Measures of disease severity (%)							
Confirmed progression (1 EDSS point), 2y			–37*		–19*	–29	–30*
Final EDSS ≥1 point more than baseline	0		–32			–31*	
Median change in MRI BOD, 2y	–5.1		–6.7		–12.1****	–17.3***	–14.7****

a Percentage reductions (or increases) were calculated by dividing the reported rates in the treated group by the rates in the placebo group, except for MRI disease burden (BOD) which was calculated as the difference in % change between the treated and placebo groups.

b Dosages of IFNβ as expressed in millions of international units (MIU) of IFNβ activity, as reported in the published papers. However, each company used a slightly different assay to measure IFNβ activity and, therefore, the MIU scales are not directly comparable between the different publications. Approximate conversion formulas to reflect the different IFNβ doses used are: (i) 6 MIU of Avonex™ (30μg) is approximately equal to 9 MIU of Betaseron® (280μg). (ii) Avonex™ and Rebif® are approximately equal in activity on a μg for μg basis.

c Summary basis for US Food and Drug Administration approval.[9,54]

d MRI attack rate (activity) was measured differently in different trials. The Betaseron®[1-3] and Rebif®[6,7] trials include the number of new, recurrent and enlarging T2 lesions but not gadolinium (Gd) enhancement; The Avonex™[4,5] trial used only the number of Gd enhancing lesions.

BOD = burden of disease; **EDSS** = Kurtzke Expanded Disability Status Scale; **IM** = intramuscular; **ITT** = intention to treat analysis; **MRI** = magnetic resonance imaging; **qod** = every other day; **qw** = once per week; **SC** = subcutaneous; **tiw** = 3 times per week.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$ compared with controls.

5.1 Dose-Finding Studies Using IFNβ

One of the earliest clinical trials of IFNβ in patients with MS was that of Knobler et al.[54] in which 30 patients (5 groups) with RRMS were administered either placebo or IFNβ-1b (Betaseron®) at doses of 0.8, 4, 8, or 16 MIU subcutaneously 3 times weekly for 24 weeks. This trial demonstrated a clear trend in favour of higher dosages of IFNβ in the ability to reduce attack frequency over this short interval. Indeed, although the highest dosage used (48 MIU per week) was poorly tolerated and several patients required dose reductions because of adverse effects, the observed attack rate at this IFNβ dose was actually zero. A similar dose-effect also seems to be present using intramuscular IFNβ-1a (Avonex™).[47,55,56] Thus, following treatment with IFNβ-1a, 6 MIU intramuscularly, the reported maximum concentration (C_{max}) for IFN activity was 34 U/ml and the AUC was 824 U/ml □ h.[47] Following treatment with 12 MIU, these values were 44 and 1352, respectively,[56] whereas fol-

lowing 15 MIU, they were 103 and 2242, respectively.[55]

5.2 Phase III Clinical Trials of IFNβ

The multicentre study of IFNβ-1b (Betaseron®) in MS included 372 patients with RRMS who had scores on the extended disability status scale (EDSS) of 0 to 5.5.[1,2] Patients were randomised to receive placebo, low dose (1.6 MIU; 50μg) or high dose (8 MIU; 250μg) IFNβ-1b, subcutaneously, every other day for 2 years. Primary endpoints of the trial were relapse rate and proportion of patients remaining relapse free. Secondary outcomes included clinical disease progression, number of active MRI lesions (new, recurrent, or enlarged) and the total disease burden as measured by the volume of T2 white matter disease seen on MRI. After 2 years, compared with placebo, treatment with high dose IFNβ-1b reduced both the clinical attack rate (–34%; $p < 0.0001$) and the MRI attack rate as measured by median number of T2 active lesions (–83%; $p < 0.009$). The median volume of T2 white matter disease seen on MRI (burden of disease) increased by 16.5% compared with baseline among placebo-treated patients, whereas with IFNβ-1b treatment it decreased by 0.8% (i.e. a 17.3% difference compared with placebo; $p = 0.001$). The high dose also reduced the rate of confirmed 1-point EDSS progression but this was not significant (–29%; $p = 0.16$). In this trial, treatment with low dose IFNβ-1b (5.6 MIU per week) was also better than placebo on some outcomes, although it was not as effective as the higher dose.

This study extended for up to 5 years in some patients[3] and the data demonstrated a persistent dose-dependent reduction in relapse rate (approximately 30% reduction in 8 MIU group compared with placebo in all 5 years). Furthermore, in the high dose group there was no significant increase in MRI disease burden compared to baseline, either yearly or for the entire duration of the study, whereas among patients receiving the low dose there was a significant increase each year ($p < 0.05$), with the exception of year 5.

The multicentre trial of IFNb-1a (Avonex™) included 301 patients with RRMS treated with placebo or intramuscular IFNβ-1a 6 MIU per week (30 μg per week).[4] After 2 years, treatment with Avonex™ resulted in marginally significant reductions in the clinical attack rate (–18%; $p = 0.04$), the MRI attack rate as measured by the median number of gadolinium enhancing lesions (–33%; $p = 0.05$), and the rate of confirmed 1-point EDSS progression (–37%; $p = 0.02$) compared with placebo. The total volume of MRI T2 disease burden was also reduced but this was not significant (–6.7 %; $p = 0.36$). Because the design of this trial only included a single treatment arm, the question of the proper administration of IFNβ-1a cannot be addressed from these results. Nevertheless, the generally greater effect sizes and the greater statistical significance of the Betaseron® trial compared with the Avonex™ trial, together with the fact that dose seemed to be an important consideration in the Betaseron® trial, suggests that dose may also be an important therapeutic consideration when using IFNβ-1a in clinical practice.

In fact, this point is underscored by the results of the IFNβ-1a (Rebif®) trial,[6,7] in which a total of 560 patients with RRMS were treated for 2 years with placebo or IFNβ-1a at doses of either 22μg (6 MIU) or 44μg (12 MIU) subcutaneously 3 times weekly. This trial is the first to report a statistically significant benefit on each of the 4 major outcome measures that are in current clinical use. Thus, compared with placebo, treatment with IFNβ-1a 132 μg per week (36 MIU per week) reduced the clinical relapse rate (–32%; $p < 0.005$), the MRI attack rate as measured by median number of T2 active lesions (–78%; $p < 0.0001$) and the MRI T2 volume of white matter disease (–14.7%; $p \leq 0.0001$; i.e. an increase of 10.9% *versus* baseline for placebo, compared with a decrease of 3.8% for IFNβ-1a), and a reduction in the 1-point EDSS progression rate (–30%; $p < 0.05$). Treatment with IFNβ-1a (66 μg per week), subcutaneously was also highly effective on each of these outcome measures.

However, with the exception of the outcome of T2 active lesions, high dose IFNβ-1a was not sta-

tistically better than the low dose on these clinical parameters at the 2-year time-point. Nevertheless, there was a trend favouring high dose on each of these other outcome measures.[6,7] In addition, combined analysis of the results of the Avonex™[4] and Rebif®[6,9] trials demonstrates a marked difference in efficacy (as measured by clinical relapse rate at 1 year) across the spectrum of administration from 22 to 132μg weekly.[9]

The IFNβ-1a (Rebif®) trial[6] was continued for an additional 2 years.[8] Placebo-treated patients were re-randomised in a blinded fashion to receive either IFNβ-1a 22 or 44μg three times weekly. At the 4-year time-point a statistically significant dose response was apparent for selected clinical and MRI outcome measures.[8] Thus, the high dose (132 μg per week) reduced the relapse rate during years 3 to 4, prolonged the time to second relapse and increased the proportion of relapse-free patients compared with the low dose of 66 μg per week ($p < 0.05$). Similarly, high dose IFNβ-1a was significantly better than low dose at reducing the MRI disease burden and T2 lesion activity ($p < 0.001$).

Recently, two head-to-head comparative trials have been reported in preliminary form.[57,58] The first non-blind trial[57] compared IFNβ-1b (Betaseron®; 28 MIU per week subcutaneously) to IFNβ-1a (Avonex™; 30μg per week intermuscularly) in 188 patients with RRMS over 2 years. After the first year of treatment, a significantly greater clinical benefit in the IFNβ-1b group (compared to the IFNβ-1a group) was reported for the clinical outcomes of relapse-free status and sustained progression, and for the MRI outcomes of new T2 lesions and gadolinium-enhancing lesions. The second was a randomised, single-blind, multicentre trial[58] that compared high-dose IFNβ-1a (Rebif®; 132μg per week subcutaneously) to low-dose IFNβ-1a (Avonex™; 30μg per week intermuscularly) in 677 patients with RRMS. After only 6 months of therapy, results for the high-dose group were significantly superior to the low-dose group on every clinical and MRI outcome measure related to attack rate examined in the trial, including the odds of remaining attack-free, the attack rate, the time to first exacerbation, the odds of having no new T1 or T2 lesions, the total number of new lesions and the cumulative number of new active lesions.[58] However, each of these trials varied both the total IFNβ dose and the frequency of IFNβ administration so that the relative contribution of each factor to the final reuslts cannot be easily judged. Indeed, a recently presented comparative trial[59] of IFNβ-1a (Avonex™) at doses of 30μg or 60μg, intramuscularly, once weekly, found no significant difference on any outcome measure over 3 years between the two dosages studied. This result suggests that the frequency of IFNβ administration (in addition to total dose) may be an important therapeutic consideration.

6. Discussion

In summary, on the basis of several independent multicentre clinical trials, IFNβ therapy has demonstrated unequivocal efficacy in modifying the disease course in relapsing MS. Presumably, this therapeutic effect is mediated through one or more of a wide a range of immunomodulatory and anti-inflammatory actions of IFNβ, although the actual mechanism of action remains to be elucidated. In addition, presumably the mechanism of action for IFNβ-1a and IFNβ-1b is the same and that the differences in outcome between the various clinical trials reflects both random error and dosage differences rather than a true difference in therapeutic effect between products.

Evidence now supports the value of early intervention with disease-modifying therapies in the hope of preventing the axonal injury that presumably precedes functional loss. As a result, it seems that the optimal therapy for MS should be that which provides maximum modulation of its pathogenetic mechanisms and minimises damage to oligodendrocytes and axons, while, at the same time, produces the least adverse reactions. In the case of IFNβ, this would seem to translate into a recommendation that patients be given the highest dose that can be tolerated.

Importantly, evidence from clinical studies demonstrate a dose-response relationship for IFNβ.[1-3,6-9,57,58] The *in vitro* and animal data also support a dose response for many biological actions of IFNβ that are of potential relevance to its clinical therapeutic effect.[25-29,34,37,38,40,42] Moreover, pharmacodynamic studies indicate that the biological effects of IFNβ are relatively short-lived, suggesting that the maximum clinical benefit of IFNβ therapy may be achieved with administration that is more frequent than once weekly.[47,49,50,52,56] Moreover, the clinical data suggest that, using the same dose schedule, higher doses are generally more effective than lower doses.[1-3,6-9]

The concept of a dose response for IFNβ in patients with MS is not surprising. A similar dose response has been reported in other conditions for which IFN therapy has been used, such as chronic[60] and acute[61] hepatitis, various cancers[62-64] and anogenital warts.[65]

However, as with any therapy, tolerability and the risk of toxicity place a limit on dose levels, and avoidance of adverse effects has been a significant concern in clinical trials with IFNβ. As discussed in section 5.1, a pilot study with IFNβ-1b reported that a dose of 16 MIU 3 times weekly was associated with unacceptable levels of adverse events including chills, fever and injection site reactions.[55] However, more recently the prophylactic use of ibuprofen and paracetamol (acetaminophen) in trials with IFNβ have proved useful in minimising unwanted effects. Indeed, in a recent comparison of IFNβ-1b (Betaseron®) administered 3 times weekly versus IFNβ-1a (Avonex™) once weekly, the incidence, duration and severity of adverse effects (mainly fever, chills and myalgia for 6 to 12 hours after first dose of either agent) did not differ between treatments.[51] Moreover, the major IFNβ clinical trials have generally found that treatment is well tolerated irrespective of dose.[1-10]

Nevertheless, it seems that, at least for some of the outcomes, the therapeutic benefits of IFNβ are approaching a plateau at the highest doses in current use.[9] As a result, it seems unlikely that IFNβ (by itself) will eliminate the clinical activity of MS, even if substantially higher doses could be tolerated by patients.

7. Conclusion

There seems to be compelling evidence from multiple sources (including both clinical trials and pharmacodynamic data) to support the notion that there is a clinically relevant dose-response in the use of IFNβ to treat patients with RRMS. Nevertheless, many of the clinical trials of IFNβ in MS have confounded the potential effects of dose with the possible effects of frequency of IFNβ administration. As a result, it is possible that a portion of the apparent dose-response observed in these clinical trials may be due to the more frequent dose administration schedule rather than the total weekly dose.

Acknowledgements

The author of this manuscript has participated (or is currently participating) in several industry-sponsored clinical therapeutic trials in multiple sclerosis. The sponsoring pharmaceutical companies for these trials have included (or do include): Biogen, Inc; Berlex Laboratories; Immunex Corp; Serono, Inc; and Teva Marion Partners. In addition, the author has lectured extensively at both medical conferences and in public on various aspects of the diagnosis and management of multiple sclerosis. In many cases these talks have been sponsored by non-restricted educational grants from one or another of each of the above-listed companies or by Athena Neurosciences.

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