Case study of Nabs and glycosylation effects on IFN-beta drugs

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Abstract

The effect of NAb’s and glycosylation/PEGylation for the pharmacodynamics and pharmacokinetics of IFN-beta have been reviewed with more recent developments. The issue of both NAb’s and glycoslation with current pharmacokinetics in particular, and the issues with current models of NAb’s highlighted. This case study shows how the latest research particularly PEGylation can significantly improve the drug, how future developments could improve the current models of NAb’s, and what approaches have already been taken recently.

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1 Introduction

Therapy of the relapse-remitting form of Multiple Sclerosis (RRMS) includes regular injection of synthetic INF-beta. Multiple Sclerosis is itself a complex disorder, and in order to understand how the INF-beta’s work, it is necessary to breakdown the autoimmune pathways that lead to progression of the disease[1]. An antigen is bound by presenting cells, in turn signalling and activating T-cells via T-cell receptors (TCR’s). These T-cells then migrate through the blood brain barrier, mediated by adhesion molecules, proteases and chemokines, and inactivates the T-cells. Inside the brain, these T-cells are reactivated by presenting-cells bound with antigen. The T-cells then initiate the complement immune response to the myelin sheath of oligodendrocytes. The complement develops by the release of inflammatory cytokines by the T-cells. Macrophages and T-cells attack the myelin sheaths, while B-cells differentiate into plasma cells to secrete antibodies, directing the complement straight to the myelin. Synthetic INF-beta has the ability to interact with the immune system, by reducing shifting the immune response away from inflammatory and by reducing T-cell activation and mediation across the blood-brain barrier.

The three main types of INF-beta are the natural, synthetic INF-beta-1a (e.g. Avonex/Rebif) and INF-beta-1b (e.g. Betaseron). The natural form is a glycosylated 166 amino acid long protein with five domains, glycosylated at Asn80 and with a disulphide bridge between Cys31 and Cys 141[2]. The synthetic INF-beta-1a has an identical primary sequence produced from mammalian cells expressing the human gene, while INF-beta-1b has Cys17 replaced with Ser, lacks a Met1, cannot be glycosylated and is therefore produced in E.coli. The development of neutralizing antibodies (NAbs) occurs regularly in MS-patients during therapy, countering therapy efficacy.[3]. There has been controversy over the methodology of NAb assays in characterizing NAb’s, but there has been consensus that INF-beta-1a intramuscular yields much lower NAb response than INF-beta-1a subcutaneous, which in turn is lower than INF-beta-1b. There have been NAb’s found well after treatment, suggesting that NAb’s could be targeting native INF-beta well after treatment. The NAb’s are also cross-reactive to all types of INF-beta[4]. The epitopes of the mouse NAbs on the INF-beta have been identified as amino acids 39-48, whereas in humans the C-terminal and N-terminal have been suggested [6].
Figure 1: Human IFN-Alpha. The human IFNAR binding regions conserved in both INF-alpha and INF-beta, in numerous species. The dimer is chelated by the Zinc ligand. The glycosylation takes place at Asn 80 around the IFNAR1 receptor binding region. PDB File Name 1AU1[33]).

2 Discussion

2.1 INF-beta-1a Structure

The primary sequence up to quaternary structures have been well characterised for IFN-beta-1a.[7] The amino acid sequence is given:

MSYNLLGFL
QRSSNFQCQKLLWQLNGRLE
YCLKDRMNFDIPEEIKQLQQ
FKEDAALTLYEMLQNFIAL
FRQDS SSTGWNETIVENLLA
NVYHQINHLKTVLKEEKE
DFTRGKLMSLHLKRYGRI
LHYLKAKEYSHCAWTIVRVE
ILRNFYFINR
The x-ray crystallography of the natural human IFN-beta (fig1)[8] showed the glycosylation site at Asn80. The IFNAR binding regions, which allow the signalling of the Janus kinase pathway, is identified in IFN-beta. These sites have been shown through mutagenesis to be central to the binding of IFN-beta as a cytokine to the target cells for signalling.[23] The binding sites of the IFNAR1 and IFNAR2 in the human IFN-alpha have been aligned in BLAST and found to be highly conserved and very similar to IFN-beta in a number of species, and to a few other members of the interferon family.[9, 13] Recall that murine NAb’s significantly bind to amino acids 39-48, (5) and that it is shorter in length (161AA’s) compared to the human (166AA’s) but three times more glycosylated. The human NAb’s for INF-beta-1b also recognise and neutralise INF-beta-1a and vice versa, indicating that the epitope for both should be similar.[11]

The work by Runkel et al[1998] clearly showed structural and functional differences between glycosylated and non-glycosylated forms of human-IFN-beta[24]. The question remains that do the NAb epitopes have anything to do with glycosylation. The human glycosylation site and IFNAR1 site are adjacent, but the NAb’s have been suggested to bind to the N and C termius further away from the active sites of the interferon-beta. Also worth remembering is that patients under therapy of INF-beta-1b, which is not glycosylated, have a significantly higher development level of NAb in serum than INF-beta-1a derivatives.

2.2 Pharmacodynamics and Pharmacokinetics

The binding of INF-beta to the cell-receptor initiates the Janus-kinase pathway[12], leading to modulation of gene transcription, upregulating and downregulating a large complex of immune responses. B. Weinstock-Guttman et al. [2008] showed that as little as 2-4 hours after the first exposure to IFN-beta therapy there was significant increase levels of mRNA’s associated with a variety of immune responses. Factors affected by IFN-beta include MxA, Stat1, TRAIL (tumour necrosis factor-related apoptosis-inducing ligand), IRF-7 (interferon regulatory factor-7), HLA-C and B2 myoglobulin mRNA’s. After first dose, the main pathways affected are the Transforming growth factor beta (TGF-beta) and the Retinoic acid Pathways, both affecting the cell life cycle. Under regular therapy, Akt/Pkc(s) is altered, further affecting T-cell proliferation. IFNG/STAT3 levels and TNF/beta estradiol are also significantly altered. V. Wee Yong et al [1998] summarised the general mechanisms of IFN-beta as follows.[14] T-cell proliferation is reduced, also IFN-gamma production and its proinflammatory effects are reduced, as shown just before. IFN-beta also reduces antigen presentation by the MHC to the T-cells.
Figure 2: Representation by Oliver Neuhaus et al, [2003,2007][2, 17] showing the various mechanisms of INF-beta and other pharmaceuticals against T-cell mediated demyelination.
The origin of the NAb’s in question have been traced via the MHC Class II presentation to the HLA genes DRB1*0401 and HLA-DRB1*0408[5]. Therefore the association of NAb’s and IFN-beta to the MHC Class II presentation has been established. The reduction of T-cell-antigen binding, T-cell proliferation, IFN-gamma and inflammation respectively also reduce the T-Helper 1 activity shifting the response to TH2 anti-inflammatory response which significantly improves the condition of MS. [14] IFN-beta reduces the production of adhesion molecules, solubilizes VCAM-1 at the endothelial wall and reduces MMP transcript levels, each step being a direct interference of T-cell migration into CNS at the blood-brain barrier. T-Cell reactivation is also reduced in the CNS by IFN-beta [15].

The precise difference between beta-1a and beta-1b pharmacodynamics is the of shift from TH1 to TH2 response [16]. INF-beta-1a increases TH2 cytokines IL-4 and IL-10 whereas beta-ab reduces TH1 cytokine IFN-gamma. Therefore the therapy of synthetic IFN-beta acts primarily to shift the immune response from a TH1 to TH2 anti-inflammatory response and reducing the onset of demyelination. The pharmacokinetics of IFN-beta have shown various results. For IFN-beta-1a Alam J, McAllister A et al [1997] compared the subcutaneous, intramuscular and intravenous routes of administration and found intramuscular to have the greatest effect per dosage on a weekly basis. Subcutaneous routes with dosages of 44ug and 22ug show the former has a greater beneficial effect than the latter, whether once a week or three times a week. However intramuscular routes are seemingly indifferent to 30ug or 60ug dosages, both resulting in the same effect.

The next issue regards NAb’s effect on the pharmacokinetics. Since we now know that NAb’s are related to MHC class II presentation, any levels of NAb’s will reduce the effect of IFN-beta in shifting the TH1 to TH2 response and in reducing T-cell proliferation, for all routes of administration. This will make quantifying dose-effect of IFN-beta difficult since it is necessary to quantitate the effects of NAb’s on IFN-beta. NAb’s will also reduce the half-life of the IFN-beta since by definition IFN-beta is neutralised.

There have been successful attempts already with PEGylation of IFN-alpha, and there is now two main recombinant forms available: ADAGEN, PEGASYS, Pegintron to name a few. Ceaglioia et al.[2010] showed the half life of IFN-alpha has been extended by glycosylation[19]. Doing so resulted in an increased size, reducing anti-viral and antiproliferative activity in some cases. PEGylation of IFN-alpha has been described as showing a dramatic increase of in-vivo half life accompanied by a remarkably improved in-vivo function. The half-life is determined by both metabolism mainly in the kidney, and the clearance in the liver.[20] The renal filtering and redirection from the kidney to the liver is limited by the size and branching of the PEG-IFN. In the
case of the IFN-alpha again, the negative charge from the sialic acid contributed to the reduced renal clearance. The glycosylated and PEGylated IFN was also more resistant to serine proteases.

The concept of glycosylation and PEGylation can be considered for IFN-beta here, knowing that if the pharmacokinetics and pharmacodynamics of IFN-alpha can be improved here, and knowing that they are structured similarly, especially the IFNAR receptor, and share the Janus-Kinase pathways to an extent, then the implications for IFN-beta could be significant. Should the immune system attempt to neutralize and clear the IFN-beta by protease activity, perhaps these methods could improve the pharmacodynamics and pharmacokinetics of IFN-beta by reducing the effect of NAb's, also increasing the half-life of IFN-beta and prevent the metabolism of active IFN-beta. It has been recently discovered that viruses act in many ways to interfere with the IFN system, including proteolytic cleavage of IFN[21]. This has implications for the development and therapy of Multiple Sclerosis, and its suggestable that viral proteolysis of IFN-beta could also be reduced by glycosylation and PEGylation.

Amartya Basu et al. [2006][22] extensively analysed the effect of PEGylation on IFN-beta-1b and found improved stability, Solubility, Potency, immunogenicity, and Pharmacokinetics by site selective mono-PEGylation. n a logical manner they chose the PEG binding sites to IFN-beta-1b such as to avoid the IFNAR receptor regions, choosing three specific regions on the protein: 1) site-specifically at the N-terminus, 2) site-selectively on lysines in a stochastic mixture of mono-PEGylated products, and 3) site-specifically in engineered attachment sites. In mice and rats the pharmacokinetics were shown to be significantly improved after PEGylation. Knowing that IFN-beta is highly species specific, it is still a very promising result for betaseron. This was particularly acknowledged when quantifying NAb production against the commercial beta-1b and the PEGylated versions. Their work also identified antibodies screened for binding to PEGylated IFN-beta-1b actually had significant binding to native IFN-beta. This was already shown previously where NAb’s remain long after therapy is ended, due to NAb’s being able to recognise native IFN-beta. [25] Similar work was carried out on IFN-beta-1a by PEGylating the N-terminus with minimal effect of receptor binding. [26, 27] Monkeys, rats and mice were tested in their work. Greatly improved pharmacokinetics and pharmacodynamics were also found after PEGylation.

Another deciding factor on the pharmacokinetics is the route of administration and formulation [28]. The pharmaceutical preparation of the cytokine involves a combination of serum albumin, Mannitol and various other ingredients. Another issue is the aggregation of ingredients in the formulation.[29] These two papers highlight the issue of neutralizing antibodies and attempt to
quantitate the levels of NAb's as related to drug type. When aggregates are removed from solution, the molecular ratio of NAb's to IFN-beta has been found to be 1:1. It becomes clear that NAb's bind to one region on the molecule at a time. There have been a number of markers developed to assay NAb's, including the MxA mRNA already mentioned previously.[30] It is also clear that NAb's must reduce the binding efficiency of the IFNAR regions to the cell-surface-receptors by altering some physiochemical property such as the three-dimensional structure. It has also been shown that even taking one drug with its one type of formulation, the assay can produce significant variations. This is due to the methods of analysis and threshold classification used. Guidelines have been drawn up regarding the measurement of anti-IFN-beta antibodies[31], and a critical approach has been described to the interpretation of certain bioassays[3]. Overall the consensus from the task forces of european and american neurologists on NAb's is that: 1). The presence of NAb, especially in persistent high titers, is associated with reduction of clinical and magnetic resonance imaging-based effectiveness of IFN-beta treatment. 2). All currently available IFN-beta formulations induce NAb in IFN-betatreated persons with MS. 3). Regarding current formulations, once-weekly intramuscular IFN-beta-1a is less immunogenic than either subcutaneous IFN-beta-1a or IFN-beta-1b multiple times per week, and subcutaneous IFN-beta-1a is less immuno-

2.3 Further Research

There have been the arrival of oral pharmaceuticals such as Fingolomod onto the market, welcoming the significant increase in MS relapse reduction rates[32]. The convenience of oral therapy compared to regular injections coupled with the higher success rates of these brings multiple-research therapy to a new era. There is ongoing research into the comparison of these oral drugs to immunomodulatory drugs like interferon and glatiramer acetate, which will be published soon. There are many side effects to some of these drugs. It is also interesting to see if combination therapies between INF-beta and these new drugs will emerge. Even more interesting is how stem cells have been found to repair demyelinated neurons to become healthy in rats, opening up many more possible research directions for the now-possible recovery from MS-lesioning[34].

The press conference from Biogen Idec announced in 2009 the opening of PEG-INF-beta-1a clinical trials for MS.[35] The time taken to get through the various stages is unclear, but given the amount of data already available especially on the biosimilars, the discretion of the FDA regarding the difference and the detail needed will ultimately decide how quickly this project gets through. This project is now in Phase III clinical trials.

There is also the potential for homology studies on the NAb-IFN-beta bind-
ing. There has been similar work done on IFN-gamma (Pubmed 9367779), including the FAB fragment (Pubmed 15388922). Epitope mapping coupled with sequencing of the NAb would establish the binding model. The model would be central to whether PEGylation of IFN-beta would reduce NAb binding, and this question is likely to be covered in the clinical trials by Biogen Idec in the future. The results have already shown improved pharmacokinetics and pharmacodynamics, and the future research might relate this to reduced NAb binding. The sequence alignment of IFN-beta in numerous species have shown the IFNAR conserved binding region. This has also been elucidated from the site-directed mutagenesis of the cytokine. However, improvements can also be made by the insertion of Asn for glycosylation or for PEGylation the amino acids that could be added by site-directed mutagenesis are: lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, tyrosine. Lysine was seen earlier to be used in the previous PEGylation of IFN-beta trials. The structure of IFN-beta could be modified also to shift the internal amino acids to the exterior of the molecule for more efficient binding, especially those outside the IFNAR regions. The studies on these modified structures would detect for NAb’s, and especially the pharmacodynamics and pharmacokinetics of the drug itself. The added advantage of modelling the IFN-beta-NAb binding allows for better assay development, improving the ELISA’s already available for example, and removing the need for an indirect marker such as MxA.

3 Conclusion

The case study of the NAb’s effect on MS therapy with IFN-beta has been highlighted again in this paper. The very latest developments in NAb research have been reviewed, especially its pharmacodynamics and pharmacokinetics for MS therapy. On a separate but not independant case the study of glycosylation and PEGylation effects on pharmacodynamics and pharmacokinetics have been covered. The issues of NAb quantification on PEGylated drugs was noted, and the half-life of the drug was determined by both NAb’s and PEGylation for example. The case that both NAb and glycosylation/PEGylation are essential for a thorough investigation of IFN-beta therapy was described.

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