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Pharmacokinetics and pharmacodynamics of the interferon-betas, glatiramer acetate, and mitoxantrone in multiple sclerosis

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Abstract

Five disease-modifying agents are currently approved for long-term treatment of multiple sclerosis (MS), namely three interferon-beta preparations, glatiramer acetate, and mitoxantrone[1]. Pharmacokinetics describes the fate of drugs in the human body by studying their absorption, distribution, metabolism and excretion. Pharmacodynamics is dedicated to the mechanisms of action of drugs. The understanding of the pharmacokinetics and pharmacodynamics of the approved disease-modifying agents against MS is of importance as it might contribute to the development of future derivatives with a potentially higher efficacy and a more favourable safety profile. This article reviews data thus far present both on the pharmacokinetics as well as on the putative mechanisms of action of the interferon-betas, glatiramer acetate, and mitoxantrone in the immunopathogenesis of MS.

Keywords: Multiple sclerosis; Immunotherapy; Immunomodulation; Immunosuppression; Interferon-beta; Glatiramer acetate; Mitoxantrone

1. Introduction

Multiple sclerosis (MS) is the most common chronic central nervous system (CNS) disorder of younger adults and a major cause of lasting neurological disability [1].

Pharmacological therapeutic approaches in MS are based on three principles. Apart from (i) treatment of relapses by corticosteroids and, in some cases, plasma exchange, and (ii) symptomatic treatment, (iii) immunomodulatory or immunosuppressive long-term treatment with the aim of modifying the disease course represents the mainstay. However, as MS is a chronic disorder requiring chronic therapy, the optimal treatment choice for individual patients is often difficult to make and in many cases is discussed controversially [2].

The rationale to influence immunological activity in MS is to suppress ongoing inflammation with an aim to prevent myelin and axonal damage and thus to prevent clinical disability. For years, a number of immunosuppressive agents (*i.e.* inhibitors of crucial components of the immune system causing generalized immune dysfunction) were used off label, the potential beneficial effects of which in MS were limited by systemic adverse effects, such as increased risk of cancer or infection. Examples are azathioprine (licensed for MS treatment in some countries) or cyclophosphamide. In the 1990s, two classes of immunomodulatory agents were approved for the treatment of relapsing–remitting MS, namely interferon (IFN)-beta (IFN-β1a or IFN-β1b) as well as glatiramer acetate (GA) [3–5]. Immunomodulators – without generally suppressing immunological properties – shift immune responses from pro-inflammatory autoimmune conditions (mediated by TH-1 cytokines released from autoreactive T cells) towards a more beneficial anti-inflammatory environment (mediated through TH-2 cytokines secreted by regulatory T cells) [6]. Both IFN-β and GA have been proven at least partially effective in relapsing–remitting MS as assessed in pivotal and subsequent trials [7–9]. In

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[1] A sixth disease-modifying agent, natalizumab, has been approved for treatment of multiple sclerosis after acceptance of this manuscript. Its pharmacokinetics and pharmacodynamics are not subject of this review.

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secondary progressive MS, treatment options are limited [10]. All IFN-β preparations have been investigated in phase 3 clinical trials, but only IFN-β1b, as administered in a European Trial [11], in which many of the patients included exhibited clinical relapses, significantly delayed time to onset of sustained progression of disability [12] as measured by the Expanded Disability Status Scale (EDSS) [13]. The more recent approval of the immunosuppressant mitoxantrone in MS gave clinicians another therapeutical tool for treatment of active forms of relapsing–remitting and secondary progressive MS [14].

2. Pharmacokinetics

Pharmacokinetics describes absorption, distribution, metabolism and elimination of a given drug. The action and fate of this drug in the body over a period of time is investigated, including the processes of permeation through barriers between compartments, localization in tissues and biotransformation. The pharmacokinetics of the drug is directly related to its pharmacodynamics, *i.e.* its interaction with the body.

3. Pharmacokinetics of interferon-beta 1a and interferon-beta 1b

Human IFN-β is a polypeptide naturally produced by human fibroblasts (predominantly but not exclusively, as there are reports of other sources of natural IFN-β, such as dendritic cells or retinal glial cells; upon stimulation, virtually all mammalian cells can produce IFN-β). IFN-β is highly species-specific, *i.e.* it is effective only in the human organism. It consists of 166 amino acids forming five α helices (Fig. 1). IFN-β1a is produced in Chinese hamster ovary cells and is pharmacologically identical with the natural form, *i.e.* it is glycosylated by oligosaccharides at Asn 80; IFN-β1b is produced in *E. coli* and has slight differences compared to the natural form: it is not glycosylated, Met 1 is lacking (*i.e.* IFN-β1b has 165 amino acids), and Cys 17 is replaced by Ser. In both IFN-β forms, a disulphide bridge extends between Cys 31 and Cys 141. The optimal dosage and route of administration (subcutaneously *vs.* intramuscularly) and the resulting pharmacodynamic properties of IFN-β are subjects of controversial discussion [15–19].

IFN-β1a is a lyophilized glycoprotein produced in mammalian cells using the natural human gene sequence [20]. Two preparations are licensed for treatment of MS, 30 μg once a week administered by intramuscular injection, and 22 or 44 μg administered three times a week subcutaneously. In a comparative study of three routes of administration of 60 μg IFN-β1a in healthy donors, the intramuscular route was observed to induce the highest area under curve (AUC) for serum IFN activity as compared to subcutaneous and intravenous routes [20]. This putative disadvantage of the subcutaneous route is thought to be circumvented by the higher weekly dose. However, the results of direct dosing comparison studies are contradictory: in the pivotal trial of IFN-β1a administered subcutaneously, the 44 μg three times a week group was shown to exhibit more beneficial effects as compared to the 22 μg three times

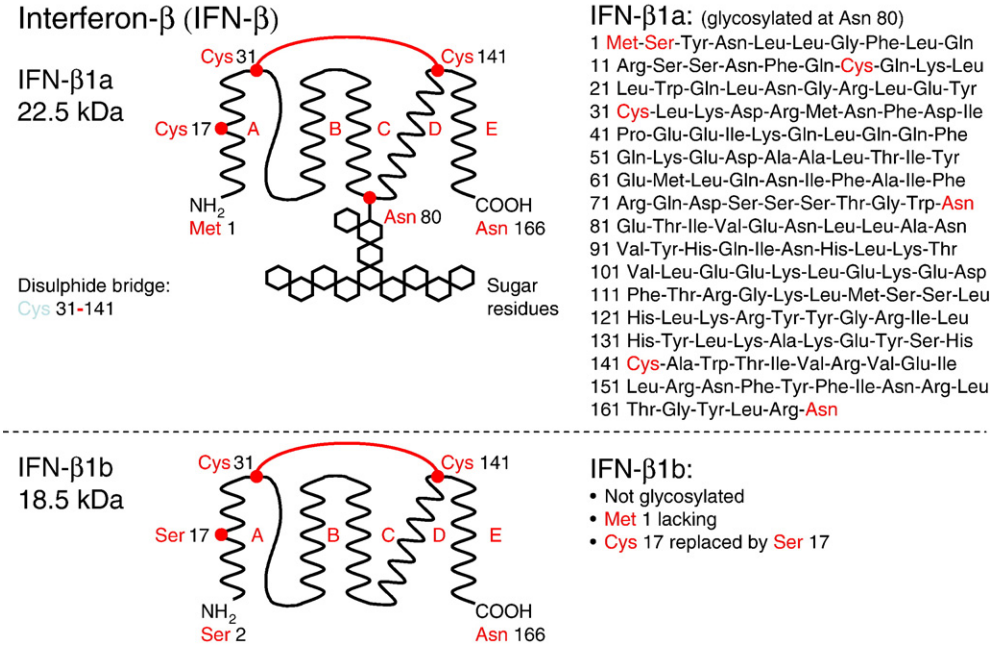


Fig. 1. Structure of interferon-beta. Two recombinant interferon (IFN)-β preparations are approved for MS treatment. IFN-β1a is pharmacologically identical with the natural form, *i.e.* it is glycosylated by oligosaccharides at Asn 80; IFN-β1b has slight differences compared to the natural form: it is not glycosylated, Met 1 is lacking (*i.e.* IFN-β1b has 165 amino acids), and Cys 17 is replaced by Ser. In both IFN-β forms, a disulphide bridge extends between Cys 31 and Cys 141. Structurally important amino acids are highlighted in red.

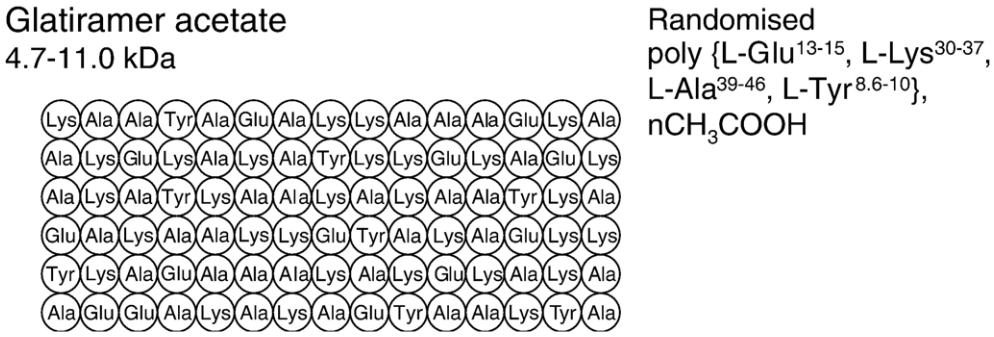


Fig. 2. Structure of glatiramer acetate. Glatiramer acetate (GA) is the acetate salt of a standardized, randomized mixture of synthetic polypeptides consisting of L-glutamic acid, L-lysine, L-alanine, and L-tyrosine with a defined molar residue ratio of 0.14 : 0.34 : 0.43 : 0.09 and an average length of 45 to 100 amino acids. One possible example sequence is shown here.

a week group [9]. A similar dose-dependent effect was observed in a study comparing 44 μg *vs.* 22 μg subcutaneously once a week (a dose that is not licensed for treatment of MS) [21]. In contrast, a study comparing 30 μg *vs.* 60 μg (double dose) once a week intramuscularly did not reveal any differences [22]. Taken together, the optimal route, dose and frequency of IFN-β1a in MS remains elusive.

IFN-β1b is a lyophilized protein produced by DNA recombinant technology using *E. coli* and has a molecular weight of 18.5 kDa. It is combined with mannitol and human albumin in order to have a neutral pH of 7.2. The approved dose is 250 μg every other day administered by subcutaneous injection [23]. A comparative phase III trial assessing the double dose of 500 μg every other day subcutaneously is currently underway.

4. Neutralizing antibodies to interferon-beta

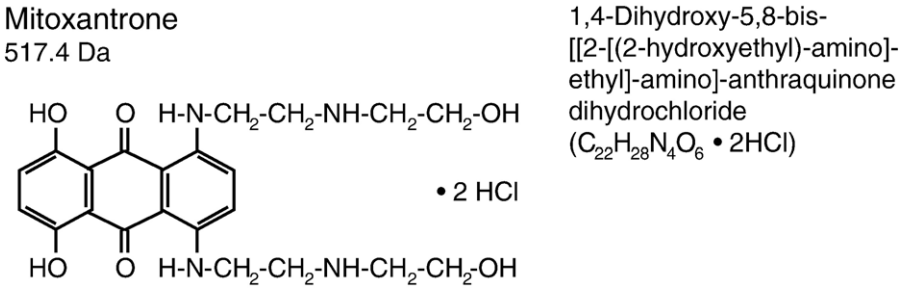
A major issue of the pharmacokinetics of IFN-β (and also affecting its pharmacodynamics) is the relevance of neutralizing antibodies [15,24–27]. Although it is established that the induction of neutralizing antibodies in patients treated with IFN-β reduces its clinical effects and may accelerate disease progression [27], the mode of measuring the presence of neutralizing antibodies as well as the optimal time point (after a given treatment duration *vs.* when clinical non-responsiveness to IFN-β occurs) and the clinical consequences are still a matter of debate [26,28].

5. Pharmacokinetics of glatiramer acetate

GA is the acetate salt of a standardized, randomized mixture of synthetic polypeptides consisting of L-glutamic acid, L-lysine, L-alanine, and L-tyrosine with a defined molar residue ratio of 0.14:0.34:0.43:0.09 and an average length of 45 to 100 amino acids (Fig. 2). The approved dose is 20 mg applied subcutaneously every day. After subcutaneous administration, GA is quickly degraded to free amino acids and small oligopeptides with only 10% remaining at the injection site after 1 h [29]. No systemic plasma concentrations nor any urinary or faecal excretion are detectable. Due to its high polarity and hydrophilic nature, the penetration of GA through the blood–brain barrier is impeded [29]. Thus, GA is most unlikely to reach the central nervous system and most probably initiates its major immunological effects in the periphery [30].

6. Pharmacokinetics of mitoxantrone

Mitoxantrone has a molecular mass of 517.4 Da and is a synthetic anthracenedione derivative related to the anthracyclins doxorubicin and daunorubicin (Fig. 3). The agent interacts with topoisomerase-2 and causes single and double strand breaks by intercalating the DNA. Due to the rapid diffusion of mitoxantrone into different tissue compartments followed by a relatively slow elimination phase, its reported maximum plasma half-life varies between 25 and 215 h



[31–36]. Mitoxantrone is eliminated both by the kidney and the liver, although less than 11% of mitoxantrone was shown to be recovered in urine and less than 25% in faeces within 5 days after administration [31]. Furthermore, it has been reported to persist in the body for up to 9 months [37]. Reports on the ability of mitoxantrone to cross the blood–brain barrier are contradictory [37–39]. After intravenous administration, there is a linear relationship between the dose and the AUC. Mitoxantrone is 78% bound to plasma proteins [40].

7. Pharmacodynamics

Pharmacodynamics assesses the action of a given drug in an organism, *i.e.* the interaction between a drug and the body. It is the study of the biochemical and physiological effects and the mechanisms of drug action as well as the relationship between drug concentration and effect.

On the molecular level, the binding to and activation or deactivation of receptors, signal transduction, induction of biosynthesis, impact on transport processes contribute to the pharmacodynamics of a drug.

8. Current view on the immunopathogenesis of multiple sclerosis

In order to understand the pharmacodynamics of disease-modifying treatments in MS, the current concept of its pathogenetic background needs to be highlighted. Deviations of immune responses play a central role in the pathogenesis of MS [41] by contributing to the formation and perturbation of the MS lesion [42]. The initial inflammatory phase is characterized by selective demyelination, and eventually subsides to a neurodegenerative phase with axonal loss and gliosis [41,43]. With ever novel scientific information emerging, concepts regarding the pathogenesis of MS are in constant flux and shown in Fig. 4 [44]. In genetically susceptible individuals an activation of antigen-specific, encephalitogenic T cells occurs in response to a yet unidentified trigger, probably in the periphery. Once activated, T lymphocytes are able to migrate across the blood–brain barrier and invade the CNS . Reactivated there, these T cells release pro-inflammatory cytokines and orchestrate destruction of the myelin sheath by various immune cell types. Pathologically, four different pathologic patterns have been described [42]: (i) T-cell and macrophage-mediated demyelination; (ii) antibody-mediated demyelination involving complement activation; (iii) distal oligodendrogliopathy and oligodendrocyte apoptosis; (iv) primary oligodendrocyte degeneration. Interestingly, the presence of pattern (iii), oligodendrocyte apoptosis, has recently been demonstrated in very early MS lesions [45]. In parallel to the autoaggressive inflammatory phase causing demyelination, recent evidence suggests that axonal loss responsible for irreversible disability occurs already early in the disease course and – as the disease evolves – predominates in the underlying pathogenetic mechanisms [41,46,47].

9. Pharmacodynamics of interferon-beta 1a and interferon-beta 1b

IFN-β exerts its immunological effects antigen-independently through reducing the secretion of proteolytic matrix metalloproteinases that mediate the migration of T cells across biological barriers [48,49], as well as possibly downregulating MHC class II on various antigen presenting cells (APC) [50,51]. IFN-β has immunomodulatory properties, antiviral and antiproliferative effects and promotes cell differentiation [52]. Although the mechanisms of action of IFN-β are not yet finally understood, there is agreement that the major effects are mediated by a transmembranous IFN receptor leading to upregulation or downregulation of target genes [53,54]. IFN-β shares the receptor with IFN-α as so-called "type I interferons" (in contrast to the "type II interferon", IFN-γ). The receptor consists of two chains, IFN-αR1 and IFN-αR2, or several subvariants. Ligand binding of IFN-β to the extracellular domain of the receptor induces an intracellular signal transduction cascade by (i) recruitment and activation of the cytoplasmic tyrosine kinase (Tyk)-2 (by IFN-αR1) and Janus kinase (Jak)-1 (by IFN-αR12); (ii) subsequent phosphorylation and recruitment of "signal transducers and activators of transcription" (Stat)-1 and Stat-2 forming a Stat-1/Stat-2 heterodimer; (iii) migration of the Stat-1/Stat-2 heterodimer to the nucleus; (iv) association with the p48 protein forming the active "IFN-stimulated gene factor-3"; (v) binding to promoter elements and initiation of the transcription of target genes. The variations of this rough scheme of signal transduction – that are based on the wide complexity in the single interaction steps – are still not completely identified [53].

IFN-β exerts a wide and ever broadening range of immune effects [52,55]. Among other effects, it

- suppresses T-cell proliferation [56,57];
- diminishes IFN-γ-induced upregulation of MHC class II expression [58,59];
- downregulates matrix metalloproteinases (MMP) [48,49,60]; decreases surface-expressed adhesion molecules [61] and increases release of soluble adhesion molecules [60] (the latter three actions in concert reduce the T-cell migratory potential);
- induces the production of TH2 cytokines and conversely reduces synthesis of TH1 cytokines [62,63];
- inhibits activation of monocytes [64].

Comparing the three interferon preparations, apart from clinical parameters as assessed in the pivotal and subsequent trials [7–9], some pharmacodynamical differences have been demonstrated. In an open trial comparing the cytokine profiles of short-term peripheral blood lymphocyte cultures in patients treated with IFN-β1a i.m. and IFN-β1b, respectively, IFN-β1a has been shown to increase the concentrations of the TH2 cytokines IL-4 and IL-10 whereas IFN-β1b reduced the concentration of the TH1 cytokine IFN-γ [65]. However, the TH1 net effect of both treatments putatively remained comparable

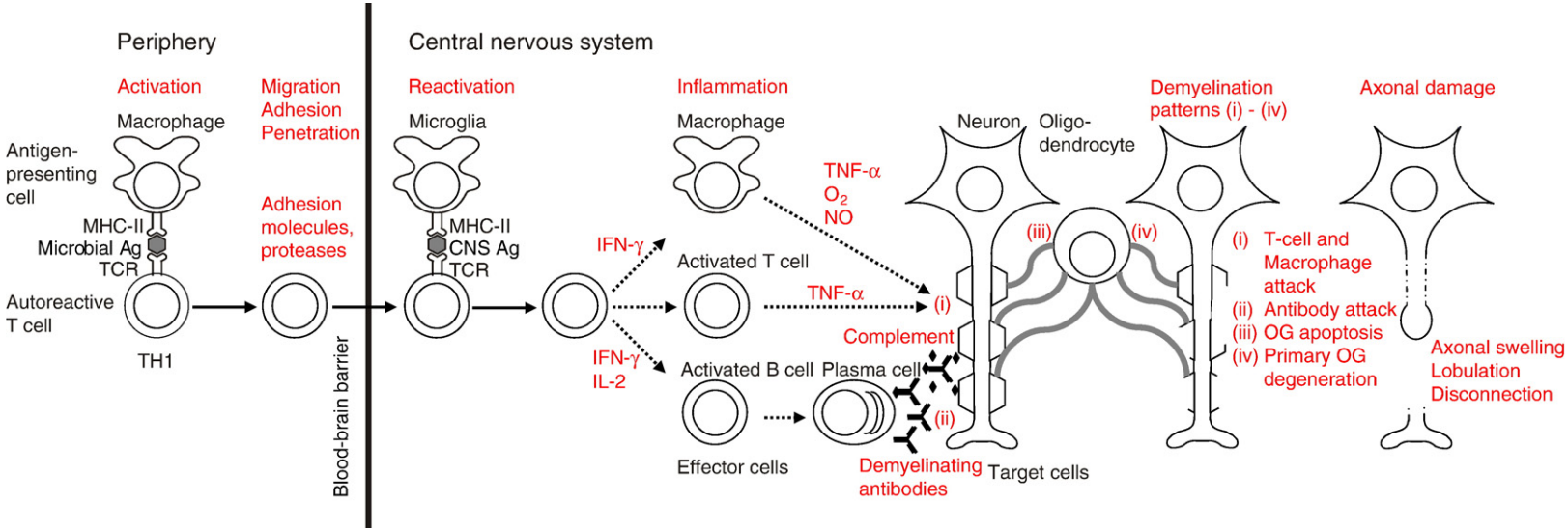


Fig. 4. Hypothetical pathogenesis of MS. Via their T-cell receptor (TCR), pro-inflammatory T cells are activated in the periphery by foreign or self antigens (Ag) presented on major histocompatibility complex class II (MHC-II) by antigen-presenting cells (APC), including dendritic cells and B cells. Activated T cells migrate to, adhere at and penetrate through the blood–brain barrier, a step mediated by adhesion molecules, proteases and chemokines. Inside the central nervous system (CNS), T cells are reactivated in the context of MHC-II on resident APC, predominantly microglia cells. These reactivated T cells secrete pro-inflammatory cytokines, such as interferon (IFN)-γ or interleukin (IL)-2 and induce CNS inflammation by subsequent activation of macrophages, other T cells and B cells as effector cells. Macrophages and T cells attack the oligodendrocytic myelin sheath by cytotoxic mediators, mainly tumor necrosis factor (TNF)-α, oxygen (O_2) radicals and nitric oxide (NO). B cells differentiate to plasma cells that secrete demyelinating antibodies. They can guide and activate macrophages or ignite the complement cascade with assembly of the membrane attack complex which causes pore formation in myelin membranes. Modified from Ref. [44], with permission from Elsevier.

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