The glatiramoid class of immunomodulator drugs

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Glatiramer acetate (GA) is a complex heterogeneous mixture of polypeptides with immunomodulatory activity approved for treatment of relapsing-remitting multiple sclerosis. GA is the first, and was until recently, the only member of the glatiramoids, a family of synthetic copolymer mixtures comprising the four amino acids, L-glutamic acid, L-alanine, L-lysine and L-tyrosine, in a defined molar ratio. Another glatiramoid, prodrimer, was recently evaluated in preclinical studies and in two small Phase II clinical trials with relapsing-remitting multiple sclerosis patients. Due to the complexity and heterogeneity of GA and other glatiramoids, the clinically active epitopes within the mixture cannot be identified and the consistency of polypeptide sequences within the mixture is dependent on a tightly controlled manufacturing process. Although no two glatiramoids can be proved identical, it is possible to differentiate among members of the glatiramoid class using analytical methods and immunological and biological markers. Even slight differences in the distribution of molecular masses or in the composition of antigenic polypeptide sequences among glatiramoids can significantly influence their efficacy, toxicity and immunogenicity profiles. Experience with GA may be instructive regarding important safety and efficacy considerations for new glatiramoid mixtures now in development.

Keywords: copolymer, glatiramer acetate, glatiramoid, pharmacology, prodrimer, relapsing-remitting multiple sclerosis


1. Introduction

In the early 1960s, scientists at the Weizmann Institute in Israel conducting basic research in the immunological properties of synthetic polymers and copolymers made a serendipitous drug discovery [1,2]. They were interested in the mechanisms involved in the induction and suppression of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Speculating that synthetic polypeptides with amino acids analogous to those found in myelin basic protein (MBP), a protein found in the CNS that seems to act as an autoantigen in MS and EAE would induce EAE, they synthesized three copolymeric mixtures of polypeptides of various chain lengths. None of the copolymers was encephalitogenic in guinea pigs; instead, all three polymers were found to be protective against EAE. Copolymer 1, now called glatiramer acetate (GA; Copaxone®. Teva Pharmaceutical Industries, Kfar Saba, Israel), proved to have the greatest protective activity in ameliorating established EAE [1]. Further testing showed GA effects were not restricted to a particular species, disease type or encephalitogen used for EAE induction [2,3]. Since this discovery 4 decades ago, extensive preclinical data, results of randomized controlled clinical studies and long-term outcomes in relapsing-remitting MS (RRMS) patients in a continuing open-label clinical trial have established the efficacy and safety of 20 mg daily subcutaneous (s.c.) GA for treatment of RRMS [4-6]. Additionally, data from animal models of other
neurodegenerative disorders [17-21] and preliminary data from small studies in RRMS patients [22,23] suggest that daily treatment with GA may have neuroprotective and/or neuroregenerative effects.

Thus, GA was the first – and until recently was the only – member of the glatiramoids. Glatiramoids are a family of synthetic copolymer mixtures comprising the four amino acids, L-glutamic acid, L-alanine, L-lysine and L-tyrosine, in a defined molar ratio. GA has a unique mechanism of action, which although not completely explained, has demonstrated effects on several different components of the immune system [24-28]. GA is an antigen-based therapy; that is, a GA-specific immune response is the sine qua non of GA efficacy [28]. Furthermore, GA is unique in that the active epitopes (amino acid sequences associated with clinical efficacy) within the polypeptide mixture cannot be completely identified or characterized using state-of-the-art multidimensional separation techniques. Therefore, no two glatiramoids can ever be proved identical; however, it is possible to distinguish among members of the glatiramoid class using analytical, biological and immunological methods.

A second glatiramoid, protiramer, was recently developed by Teva Pharmaceutical Industries and has been tested in two small Phase II clinical trials in RRMS patients [29]. Protiramer is produced by making slight changes to the GA manufacturing process. Protiramer has a higher molecular mass (MM) distribution than GA and was synthesized to determine whether the increased immunoreactivity of higher MM peptides could improve efficacy and/or decrease dosing frequency (early GA studies used a higher MM formulation than the now marketed formulation [5]). Additionally, Sigma-Aldrich Co. manufactures a glatiramoid called Poly(Ala:Glu:Lys:Tyr) (Alanine:glutamic acid:lysine:tyrosine) that is described as having an effect similar to GA in EAE; this glatiramoid is not recommended for use in humans [30]. More glatiramoids may become available as other manufacturers develop their own copolymer mixtures.

As described below, preclinical experience with protiramer demonstrates that glatiramoids that are quite similar to each other, but not identical, cannot be presumed to have comparable safety and efficacy profiles. Even slight differences in MM distribution or in the primary, secondary or tertiary polypeptide structure of different glatiramoids can significantly alter their pharmacologic activity, as was illustrated by the results of preclinical studies of protiramer (described below). This review describes experience with GA, the best-studied member of this growing therapeutic class, and the relevance of GA findings as they relate to important safety and efficacy considerations for new glatiramoid mixtures in development.

2. Chemical characterization

Glatiramoids are complex polypeptide mixtures that share a specific molecular formula. GA is a glatiramoid prepared from N-carboxy-α-amino acid anhydrides (monomers) with diethylamine as the polymerization initiator. The bifunctional amino acids are protected (the α-NH2 of lysine is protected by a trifluoroacetyl group and the γ-COOH of glutamic acid is protected by a benzyl group); therefore, the polymerization occurs through the growth of linear chains from monomers, with no crosslinking between the polymer chains. Polymerization is followed by polymer cleavage and deprotection. The amino acid sequences and the size of the resultant polypeptides are dependent on factors such as the relative reactivity of the activated amino acid monomers and reaction conditions such as temperature and duration of cleavage process. As a result, the sequences of polypeptides in GA, although not uniform, are not entirely random and are highly reproducible under strictly controlled reaction conditions.

Glatiramoids are characterized by the molecular formula below, in which \( X \) represents an anion (e.g., acetate or any other pharmaceutically acceptable salt). The superscripts represent the relative molar ratios of amino acids and the subscript, \( n \), relates to the polymeric chain length, and \( m \) is the molar quantity of counter-ions.

\[
(L\text{-Glu}^{15 - 15}, \ L\text{-Ala}^{39 - 46}, \ L\text{-Tyr}^{6 - 10}, \ L\text{-Lys}^{30 - 37})_n \ mX
\]

GA is composed of Glu, Ala, Tyr and Lys in an approximate molar ratio of 0.14:0.43:0.09:0.34 and the average MM of GA is 5000 – 9000 daltons (Da) [31]. Most of the polymers and copolymers of amino acids in GA have an MM distribution of ~2500 – 20,000 Da. The glatiramoid, protiramer (formerly known as TV-5010), which is produced by making slight changes to the GA manufacturing process (e.g., temperature, reaction time) following the polymerization reaction has the same molar ratio of amino acids as GA and the average MM is 13,500 – 18,500 Da. Sigma reagent Poly(Ala:Glu:Lys:Tyr) has a molar ratio of 0.14:0.42:0.07:0.36 of Glu, Ala, Tyr and Lys, respectively, and the average MM is 10,000 – 20,000 Da [30].

GA and other glatiramoids contain an almost incalculably large number of amino acid sequences (>10^6) possible theoretical sequences in GA). It is at present impossible to isolate and identify active amino acid sequences (i.e., those acting as epitopes), even using the most technologically sophisticated multidimensional separation techniques. The consistency of polypeptide sequences within GA is dependent on a well-controlled proprietary manufacturing process. Therefore, no two glatiramoid mixtures prepared by different manufacturers can be shown to be ‘identical’ and new glatiramoids must be considered distinct members of the class. There are means by which to differentiate glatiramoid mixtures. Members of this class can be distinguished by the following characteristics: MM distribution profile, peptide mapping by capillary electrophoresis profile, certain nonrandom and reproducible patterns in amino acid sequences, secondary and tertiary structures, specific hydrophobic interactions owing to unique charge dispersion, characteristic ratio between molecules with C-terminal carboxylates.
Figure 1. Molecular mass distribution by gel permeation chromatography of: (A) GA and protiramer; (B) GA and Sigma Poly(Ala:Glu:Lys:Tyr).


and C-terminal diethylamides, and proteolytic enzymatic digestion profile.

For GA, the MM distribution profile, based on the separation of polypeptides according to size, is determined using a gel permeation column calibrated using a set of sequence-defined, well-characterized proprietary linear polypeptide markers selected based on certain nonrandom patterns of amino acid sequences. There is some overlap in MM distribution between GA and protiramer (Figure 1A), and between GA and Sigma Poly(Ala:Glu:Lys:Tyr) (Figure 1B). Polypeptide mapping using capillary electrophoresis separation of polypeptide fragments obtained after digestion with trypsin and mapping based on the proteolytic hydrolysis by carboxypeptidase P followed by separation of the fragments by reverse-phase HPLC are methods of discerning sequence differences among GA structures and those of other glatiramoids (Figures 2 and 3).

The sequence of amino acids (primary structure) of the polymer obtained at the first stage of the synthesis in a bulk solution is governed mainly by the homopolymerization rate constants of each of the activated amino acids (monomers) present and by reaction conditions (e.g., temperature and concentration). The size of the GA mixture components and the nature of the terminal
Figure 2. Typical electropherogram of GA fragments after exposure to trypsin.

amino acids are dependent on the acetyloytic cleavage conditions.

Among tests to explain the primary structure are spectroscopic techniques (Fourier transform infrared, ultraviolet, proton and carbon\textsuperscript{13} NMR) and enzymatic hydrolysis followed by chromatographic separation of the fragments to demonstrate the characteristic composition of the obtained mixture. Another test of the primary polypeptide structure is Edman degradation, a step-wise sequential hydrolysis of amino acids starting from the N-terminal end of the polypeptide. In this method, the characteristic sequence of amino acids in the polypeptide chain at the N-terminal end is determined by step-by-step cleavage of amino terminal residues without disrupting other polypeptide bonds. The GA polypeptide mixture exhibits a consistent and characteristic average order of amino acids in the N-terminal region. Additionally, GA has a certain fixed ratio of molecules with C-terminal carboxylic acids to those with C-terminal diethylamide (originating from the polymerization reaction initiator, diethylamine).

Information on the secondary structure of GA can be obtained by circular dichroism measurements showing that GA possesses relatively stable secondary structures with substantial \(\alpha\)-helical content. These results were supported by evaluating the denaturation energy of GA drug substance (by measuring circular dichroism at different temperatures) and demonstrating the presence of a specific absorbance by second derivative Fourier transform infrared that is characteristic of \(\alpha\)-helical structures.

Information on GA tertiary structure can be obtained by comparing the size of the GA molecules before and after denaturation with guanidine HCl and by measuring the migration time on a gel permeation column, expressed in \(K\alpha\) (the smaller the \(K\alpha\) value, the larger the molecule size). Glatiramer acetate possesses a small degree of tertiary structure.

Together, these tests indicate GA contains certain nonrandom sequences, is characterized by partial \(\alpha\)-helical structure and has a small degree of tertiary structure.

It is known that proteins tend to form quaternary structures resulting in formation of high MM aggregates. Although polypeptides are less likely to aggregate, their presence is monitored in GA when it is produced and in stability studies. Quantitation of stable high MM species indicates that levels are typically quite low.

3. Mechanisms of action

After extensive study in laboratories worldwide focusing on the mechanism of action of GA, the active epitopes in the GA mixture and their specific effects on the immune system are still not fully understood. Preliminary data suggest protiramer has a similar, but not identical, mechanism of action to that of GA.

3.1 Experience with GA

Mechanisms that are thought to contribute to GA effects include: i) high affinity binding to MHC class II
Figure 3. Comparative reverse-phase HPLC chromatograms of GA (the top five chromatograms show different batches of GA drug product) and Sigma Poly(Ala:Glu:Lys:Tyr) (bottom chromatogram) proteolytic digests by carboxypeptidase P.


molecules on antigen presenting cells (APCs) and competition with MBP at the APC level for binding to MHC; ii) inhibition of MBP-specific T-cell activation through competition with MBP/MHC complexes for the T-cell receptor; iii) induction and activation of glatiramer acetate-reactive T cells and a shift from a type-1 T helper (TH1) phenotype, which tends to promote inflammation, to a type-2 T helper (TH2) phenotype, which typically promotes an anti-inflammatory environment; iv) preferential migration of GA TH2 cells into the CNS leading to decreased local inflammation through ‘bystander suppression’; and v) neuroprotection and axonal protection related to GA-stimulated secretion of brain-derived neurotrophic factor, an important factor for neuronal survival, neurotransmitter release and dendritic growth [24-28].

Researchers continue to investigate and discover novel mechanisms of GA activity. Recently, scientists at the Weizmann Institute demonstrated that GA treatment interferes with demyelination directly at the myelin and stimulates remyelination in an EAE model [32]. These effects were attributed not only to reduced inflammation, but also to a GA effect on the proliferation, differentiation and survival of oligodendrocyte progenitor cells and their recruitment to injury sites, thereby enhancing repair in situ.

A substantial fraction of the therapeutic GA dose is hydrolyzed locally at the site of injection [31,33]. GA interacts with peripheral blood lymphocytes locally at the site of injection, and the immune response is secondarily manifested as a systemic distribution of activated GA-specific T cells. T cells produced in the periphery cross the blood–brain barrier and accumulate in the CNS [27,28]. Thus, systemic distribution of the drug is irrelevant to effects following s.c. administration and systemic concentrations of GA or its metabolites are not indicative of drug activity or exposure to the immune system.
GA affects immune cells in an antigen-specific way; that is, GA administered subcutaneously daily over many years works as an antigen-based vaccine [28]. On repeated exposure, GA-specific T cells shift from a TH1 to a TH2 phenotype. After several months of treatment, GA-reactive T-cell proliferation declines [33]. Despite the decline, during long-term treatment there is no decrease in magnitude of cross-reactivity between GA-reactive T cells and MBP, and the cytokines released by GA-reactive T cells remain TH2-biased [34,35]. This effect is sustained in patients taking daily GA injections for > 6 – 9 years [34]. Because T cells responsive to myelin antigen epitopes and GA-reactive T cells seem to represent the same or overlapping T-cell populations, long-term chronic administration of GA may restore immunological tolerance in MS patients by sustained deletion of, or anergy induced toward, myelin-antigen-specific T cells [34,35].

3.2 Considerations for new glatiramoids

Because the active epitopes of GA and other glatiramoids have not been identified, it is impossible to predict whether they have comparable pharmacologic and immunological activity. Moreover, unlike most conventional drugs, glatiramoids are not amenable to typical pharmacokinetic profiling, eliminating this approach to establishing comparability between two glatiramoids. Several approaches have been used to determine the bioavailability of GA with little success. The use of radiotracers in animal models was uninformative owing to the extremely rapid breakdown of GA after s.c. injection and attempts to assess bioavailability by urinary measurements were not effective for the same reason. More importantly, preclinical studies suggest that the GA-induced T-cell mediated immune response, not systemic concentrations of GA or its metabolites, is associated with drug efficacy. The immunomodulating activity of GA can be adoptively transferred to recipient mice by GA-specific T cells and not by the drug or by the serum [36]. Therefore, measuring concentrations of a new glatiramoid or its metabolites in the systemic circulation would not be indicative of drug activity at the site of action (i.e., the CNS).

T-cell receptors (TCR) respond to antigenic portions of peptides in a characteristic way and modifications to the amino acids in the peptide, for example, a substitution or deletion, create an altered peptide ligand (APL). Thus, differences in amino acid sequences of the polypeptides of different glatiramoids alter the interaction at the TCR. APLs can affect the repertoire and specificity of T cells induced, which could influence the strength and the nature of the immunological response [37]. As noted above, GA activity depends on antigen (epitope) presentation by APCs to T cells followed by T-cell activation. T-helper cells bind to complexes of short contiguous amino acid sequences (about 13 – 17 amino acids) of the antigen protein bound to MHC class II molecules present on the surface of the APCs. The TCR interacts with both the MHC molecule and the polypeptide fragment. Thus, the repertoire of activated T-cell clones following exposure to the polypeptide antigen and the nature of the T-cell response are driven by the specific set of short contiguous amino acid sequences following antigen presentation by the APCs. The TCR can be exquisitely sensitive to changes in the structure presented by an APL. Studies have shown that interactions with APLs can result in dramatically different phenotypes of induced T cells and mutation of even a single amino acid is sufficient to alter the T-cell response from strong killing to no response at all [37-43]. In a study investigating the basis by which a TCR can discriminate between two polypeptides differing only at a single MHC anchor residue, it was shown that the residue substitution did not significantly alter binding of the polypeptide to the MHC class II molecule; however, it reduced the specific T-cell response ∼ 1000-fold [39].

4. Immunogenicity

Glatiramoids affect both cellular and humoral immunity and there are consistent pharmacodynamic effects associated with their administration [28,29,33]. In addition to stimulating peripheral blood lymphocytes, both GA and protiramer induce the production of antibodies in MS patients. Anti-drug antibodies should be characterized by their ability to neutralize drug efficacy, cause serious adverse events or bind to endogenous proteins that are crossreactive with the drug.

4.1 Experience with GA

GA induces the formation of circulating anti-GA-specific antibodies in all treated animals and patients [33,44-46]. These antibody levels peak between 3 and 6 months of treatment and then gradually decline (Figure 4A) [33]. GA-reactive antibodies are mainly of the IgG class. These antibodies tend to shift from an IgG1 toward IgG2 and IgG4 isotypes, which correlates with the GA-mediated shift in T-cell phenotype from a TH1 to a TH2 milieu [33,44,45].

Anti-GA antibodies do not seem to be neutralizing. Several preclinical and clinical studies have evaluated the effect of GA-reactive antibodies on biological activity and clinical efficacy [33,46]. In vivo and in vitro studies using serum samples from patients with the highest titers of GA-reactive antibodies indicate that these antibodies do not interfere with: i) the ability of GA to block EAE induction in mice in vivo; ii) activation of GA-specific T cells in vitro; iii) the ability of GA to inhibit the activation of MBP-specific T-cell lines in vitro; or iv) binding of GA polypeptides to MHC class II molecules in vitro [46]. Additionally, in the 35-month pivotal trial, clinical benefits of GA were apparent early and maintained throughout the treatment period, regardless of changes in GA-reactive antibody levels in RRMS patients [8].

The safety implications of GA-reactive antibodies in MS patients receiving chronic treatment have also been evaluated. So far, no correlation has been found between anti-GA
antibodies and the development of local or systemic adverse effects in RRMS patients. Moreover, no risk of immune-complex formation or kidney damage was detected in a long-term (> 4 years) study that monitored kidney function of RRMS patients treated daily with GA [47].

4.2 Experience with protiramer
Like GA, patients treated with protiramer once-weekly developed primarily IgG class anti-protiramer antibodies [50]. However, the antibody profile induced with weekly injections of protiramer differs from that observed with daily injections of GA, in that, after peaking at ~ 2 – 3 months, antibody levels begin to plateau and at 9 months continue to remain at peak levels (Figure 4B). This same pattern was also observed in monkeys treated with protiramer for 12 months.

Differences between GA and protiramer in antibody profiles may reflect differences in dosing frequency (GA is administered once-daily; protiramer once-weekly) or differences in the composition of the polypeptide mixtures; for example, the different distribution of MM peptides in the two glatiramoids. Whether these differences have safety or efficacy implications is unknown; however, as described in the following section, preclinical testing in animals demonstrated toxicity with protiramer that has never been observed with GA.

4.3 Considerations for new glatiramoids
An important consideration regarding the immunogenicity of new glatiramoids is their potential to cause neutralizing antibodies. Neutralizing antibodies that compromise efficacy have especially important implications when treating a progressive and irreversible disease. Several factors contribute to a higher risk of developing neutralizing antibodies against therapeutic protein products. These risk factors include generation of a high titer binding antibody response, sustained treatment and treatment by a more immunogenic route (i.e., subcutaneously), concomitant treatment with immune modulators, and genetic deficiency of the relevant therapeutic factor [48]. Thus, glatiramoid mixtures, which are therapeutic polypeptide mixtures administered chronically and subcutaneously, which elicit a high IgG response (binding antibodies) and which have immunomodulatory activity, possess a high potential risk for the development of neutralizing antibodies, although GA does not seem to cause them.

Immunogenic potential is influenced by many factors, such as the nature of the active drug, product- and process-related impurities, excipients and stability of the product, route of administration, dosing regimen and target patient population [49]. The distribution of MM and the presence and activity of polypeptide fractions unique to each glatiramoid mixture will influence the immunogenicity of each. Similarly, factors introduced during the manufacturing process may produce aggregates, dimers, impurities or leachables in glatiramoid mixtures with immunogenic consequences. Owing to its structure, GA tends to form high MM aggregates during production and during long-term or improper storage conditions. However, strict process control parameters and appropriate storage conditions are used to limit the formation of aggregates to a minimally acceptable level.

Finally, an important feature of GA activity, and presumably of other glatiramoids, is its crossreactivity with MBP [5]. However, the similarity to MBP raises the risk that encephalitogenic sequences may reside within the polypeptide sequences of glatiramoid mixtures (GA has never shown encephalitogenic activity). Thus, unless strict manufacturing controls and specifications are in place, a glatiramoid mixture could potentially have encephalitogenic effects.
5. Preclinical and clinical experience

5.1 Experience with GA

The literature is replete with preclinical data and results of clinical efficacy trials of GA in RRMS patients. A detailed discussion is beyond the scope of this article and the reader is referred to primary publications of these data [1-16]. Briefly, in preclinical studies, GA prevented onset of EAE or ameliorated the symptoms of existing EAE effects irrespective of animal species, disease type or encephalitogenous used for EAE induction [1,2]. The most frequent adverse effects in preclinical studies were injection site reactions, including mild fibrosis. In RRMS patients, extensive clinical data demonstrate the beneficial effects and safety of GA on the clinical indices of MS – relapse rate and progression of disability – as measured by the Expanded Disability Status Scale [7-10,15,16]. Supportive evidence for the anti-inflammatory and neuroprotective effects of GA was demonstrated by conventional and more advanced MRI techniques. MRI scans of the brain show that GA treatment reduces the number of enhancing lesions, decreases lesion load, inhibits new lesions from developing into permanent ‘black holes’ (areas of severe and permanent tissue damage) and reduces brain atrophy [11-14].

Finally, studies show that GA efficacy and safety are sustained with long-term continuous use (> 12 years) [16]. In MS patients, the most frequent adverse events observed with GA therapy are injection site reactions and symptoms associated with an immediate, transient post-injection reaction, which may include vasodilation, chest pain, palpitation, tachycardia or dyspnea [7-10,16,31].

5.2 Experience with protiramer

Protiramer was tested in preclinical safety evaluations in monkeys, rats and swine in studies ranging in duration from 6 days to 52 weeks. Repeated injections (twice weekly) in monkeys and rats resulted in severe injection-site lesions with disseminated necrosis and inflammation of dermal structures, including muscle, nerves and blood vessels. Extensive fibrosis was observed in all treated animals. Although GA is administered more frequently than protiramer, only mild and well-tolerated inflammation with slight fibrosis has been seen with GA at comparable doses.

On chronic administration of protiramer in Sprague-Dawley rats (0, 2.5, 40 and 300 mg/kg s.c. protiramer twice weekly for 26 weeks), several treatment-related mortalities occurred in rats treated at the middle (40 mg/kg) and high (300 mg/kg) protiramer dose levels (300 mg/kg is ~ 70-fold greater than the estimated concentration of protiramer used in human clinical trials[47]). Local reactions to treatment (e.g., induration, erythema, hematoma) were dose-dependent. Some hematology and serum clinical chemistry parameters were affected, including reductions in prothrombin time/activated partial thromboplastin time and red blood cell counts, increased platelet and neutrophil counts, decreased total protein and albumin concentrations, increased globulin concentration, increased total cholesterol levels, and slightly decreased serum sodium and creatinine levels. There was a marked increase in protein levels in the urine of animals treated with 40 and 300 mg/kg. Treatment-related changes seen in histopathology examinations were confined to the injections sites, liver and kidneys of animals treated with 2.5, 40 and 300 mg/kg. The incidence and severity of lesions at the injection site were dose-related and consisted of a thick fibrotic layer associated with necrosis in the deep dermis. Liver lesions consisted mainly of bridging fibrosis with bile duct cell proliferation and lymphoid cell infiltration in the periportal area, leading to restricted vascular perfusion of the liver. Kidney lesions indicated progressive nephropathy composed of fibrosis, lymphoid cell infiltration, tubular basophilia and tubular dilatation.

A 52-week study was conducted to determine the toxicity and immunotoxicity of protiramer in the cynomolgus monkey. Protiramer was administered to monkeys at dose levels of 0, 2, 10 and 60 mg/kg twice weekly. Two deaths occurred in the highest (60 mg/kg) protiramer dose group at 24 weeks and at 40 weeks of treatment. In both cases, pathological examination revealed fibrosis, lymphoid and eosinophilic infiltrates, as well as s.c. and/or vascular necrosis at the injection site. Although these signs were considered to be factors contributing to death, other causes of death cannot be excluded.

Again, these toxicity signs were not observed in chronic toxicity studies of GA in rats and monkeys.

Importantly, the serious toxic effects of protiramer only became apparent after > 3 months of chronic administration; short-term (3 month) toxicity studies in rats and monkeys were completed successfully, with no serious adverse effects detected. Therefore, longer preclinical toxicity testing is warranted to ensure the safety of new glatiramoid drugs for chronic use.

Based on generally favorable results of the short-term toxicity studies, and before serious toxic effects in preclinical testing were observed, protiramer was approved for testing in two small, short-term clinical studies with RRMS patients [29]. One study evaluated a 15 mg once-weekly dose and the other evaluated a 30 mg once-weekly dose; both studies comprised a 10-week pretreatment phase followed by a 36-week treatment phase. Once-weekly s.c. protiramer injections were apparently well tolerated. The most common adverse events in both studies were injection site reactions (erythema, pain, induration) and transient immediate post-injection site reactions. MRI outcomes showed that treatment with a 15 mg/week protiramer dose was suboptimal, but 30 mg/week protiramer significantly reduced gadolinium (Gd)-enhancing and T2-weighted lesions compared with pretreatment values. However, study results should be weighed cautiously because there was a large reduction in Gd-enhancing and T2-weighted lesions in these patients during the 10-week pretreatment period. Moreover, MRI changes do not necessarily predict clinical effects. For example, increasing the dose of GA from 20 to 40 mg daily in RRMS patients showed a trend for better efficacy with
the higher GA dose, as indicated by a borderline significant reduction in the number of Gd enhancing lesions on T1-weighted MRI, but the reduction was not accompanied by a clinically meaningful reduction in relapse rate [50].

Three patients (two in the 15 mg/week and one in the 30 mg/week) had anti-protiramer IgE antibodies that were slightly above the limit of detection; none of these patients developed a hypersensitivity reaction [29]. As noted above, the typical (IgG) antibody profile with protiramer was different from that observed with GA (Figure 4A and B). Whether continued treatment with protiramer would have led to other adverse effects, or whether altered immunogenic responses to the drug would have eventually compromised clinical efficacy is unknown. Drug development of protiramer has been terminated owing to the serious adverse events observed in chronic toxicity studies.

5.3 Considerations for new glatiramoids

As was done with GA and protiramer, and should be done for any new chemical entity, a careful prospective development program for new glatiramoids is required from preclinical toxicology and pharmacology studies, through well-controlled preclinical and clinical trials, using sensitive and validated procedures. MRI outcomes are not validated or accepted surrogate endpoints for clinical efficacy [51]; therefore, glatiramoid pivotal studies should measure clinical end points (relapse rate and disability progression). Glatiramoids require long-term chronic administration and clinical studies must have sufficient power and duration to adequately assess the safety, efficacy and immunogenicity of each new member of the class. Experience with natalizumab and reports of progressive multifocal leukoencephalopathy emphasize the need for longer-term safety data for immunomodulatory therapies [52,53]. MRI is a useful tool for MS diagnosis and is widely used in Phase II proof of concept studies; however, monitoring only CNS lesions using conventional MRI is neither indicative of complete disease burden nor predictive of clinical events, and, therefore, clinical outcomes are preferred [54,55].

Another important consideration for new glatiramoids is the risk of potentially detrimental immunological consequences when introducing a new glatiramoid with altered epitopic sequences to patients previously treated with GA. Similarly, the likelihood of a potentially dangerous immunologic reaction in a patient switching to GA from another glatiramoid is unknown. Therefore, a crossover clinical study, in which patients who have been treated with GA are switched to the new glatiramoid mixture under evaluation, and patients exposed to the glatiramoid are subsequently treated with GA, may be necessary to adequately address this safety concern.

6. Conclusion

Glatiramoids are quite complex mixtures of copolymers with significant and varied effects on the human immune system. The novel immunomodulatory mechanisms of action of glatiramoids and their potential to prevent neuronal damage and promote neuroregeneration make these medications highly attractive for future drug development for the treatment of MS and perhaps other neurodegenerative disorders.

GA, the first and best-studied glatiramoid, has been shown to reduce relapse rate, delay progression of disability and ameliorate MRI indices of disease in RRMS patients. Although GA polypeptide sequences are not entirely random, it is impossible to identify all of the active epitopes associated with clinical efficacy. The consistent safety and efficacy of GA are dependent on its well-controlled proprietary manufacturing process. Likewise, new glatiramoids must be manufactured using a well-controlled process and have proven stability, and a battery of sensitive and validated quality assurance tests are necessary to ensure drug consistency and safety.

Differences among glatiramoids are detectable using a variety of analytical, biological and immunological methods. It is impossible to predict whether or which of these differences will produce unwanted pharmacologic effects. As experience with protiramer illustrates, even small differences in MM and in the immunological properties among glatiramoid mixtures may have significant safety and efficacy implications. The benefit:risk ratio of new glatiramoids must be established in well-controlled preclinical, clinical and immunological studies.

7. Expert opinion

MS is a life-altering progressive irreversible disease for which there is no guarantee that once neurological damage is sustained, damage can be repaired or reversed. Given that an ineffective or unsafe product can lead to irrevocable neurologic and axonal damage, the manufacture, chemical composition and clinical activity of any glatiramoid to be used for MS (or any other progressive CNS disorder, for that matter) warrant rigorous scrutiny.

Decades of experience with GA show it is safe and effective with long-term chronic daily use. However, even if other glatiramoids are shown to be similar to GA, they cannot be presumed to confer comparable therapeutic and safety effects in RRMS patients, as experience with protiramer demonstrates. Careful prospective preclinical toxicology and pharmacology studies are needed to ascertain the safety of new glatiramoids. Furthermore, clinical studies with adequate power and of sufficient duration to evaluate clinical efficacy (at least 1 year) and safety (minimum 2 years) in MS patients, with GA as an active comparator, are required. Establishing drug efficacy in MS is not an easy task. Inter- and intra-patient variability of disease symptoms and disease course make efficacy evaluations meaningful only in the aggregate. MRI outcomes are often used in Phase II clinical studies to establish proof of concept for new treatments in MS and changes in MRI parameters associated with treatment may be detected relatively rapidly (in months
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rather than years). However, the exact relationship between MRI findings and the clinical status of patients is unknown. The prognostic significance of MRI findings in clinical studies has not been evaluated, and MRI measures are not accepted by regulatory authorities as surrogate end points for drug efficacy. Experience with natalizumab and reports of progressive multifocal leukoencephalopathy emphasize the need for longer-term safety data for immunomodulatory therapies.

Because they alter immune function, it is also essential to devise an appropriate risk-assessment strategy for any new glatiramoid that includes functional evaluation of its immunogenic properties. Although anti-GA antibodies are not neutralizing, there is no guarantee that antibodies to other glatiramoids will share this property; the immunogenicity of each glatiramoid must be evaluated during preclinical and clinical development. Anti-drug antibodies should be characterized by their ability to neutralize drug efficacy, to cause serious adverse events or to bind to endogenous proteins that are crossreactive with the drug. Moreover, correlation studies should be planned to assess the relationships between immunogenicity and efficacy and safety results over time.

An important feature of immunogenicity studies of new glatiramoids is determining the risk of potentially detrimental immunological consequences of introducing a different glatiramoid with altered epitopic sequences to patients previously treated with GA (and vice versa).

As increasing data explain the multi-faceted immunomodulatory mechanisms of action of complex copolymer mixtures, and as technology evolves to the extent that these substances can be more fully characterized, more effective, safe and convenient glatiramoid mixtures may improve therapeutic alternatives for patients with MS or other neurodegenerative disorders.

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