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The glatiramoid class of immunomodulator drugs

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Glatiramer acetate (GA) is a complex heterogenous 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, protiramer, 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, protiramer, relapsing-remitting multiple sclerosis

Expert Opin. Pharmacother. (2009) 10(4):657-668

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 [4-16]. Additionally, data from animal models of other

10.1517/14656560902802877 © 2009 Informa UK Ltd ISSN 1465-6566

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 neurogenerative 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 δ-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.

$(\text{L-Glu}^{13-15}, \text{L-Ala}^{39-46}, \text{L-Tyr}^{8.6-10}, \text{L-Lys}^{30-37})_n$ 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 0.14:0:0.42:0.07:0.36 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^{36} 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

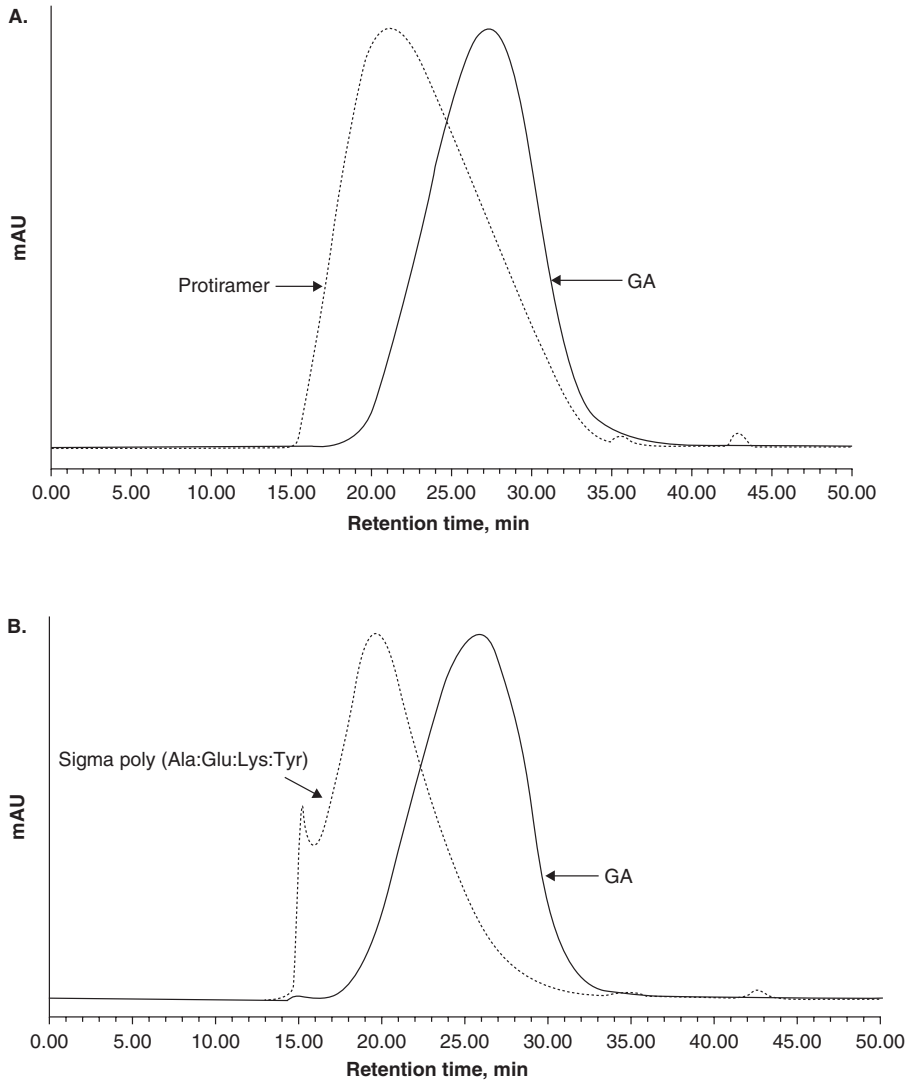

Figure 1. Molecular mass distribution by gel permeation chromatography of: (A) GA and protiramer; (B) GA and Sigma Poly(Ala:Glu:Lys:Tyr).

Ala:Glu:Lys:Tyr: Alanine:glutamic acid:lysine:tyrosine; GA: Glatiramer acetate.

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

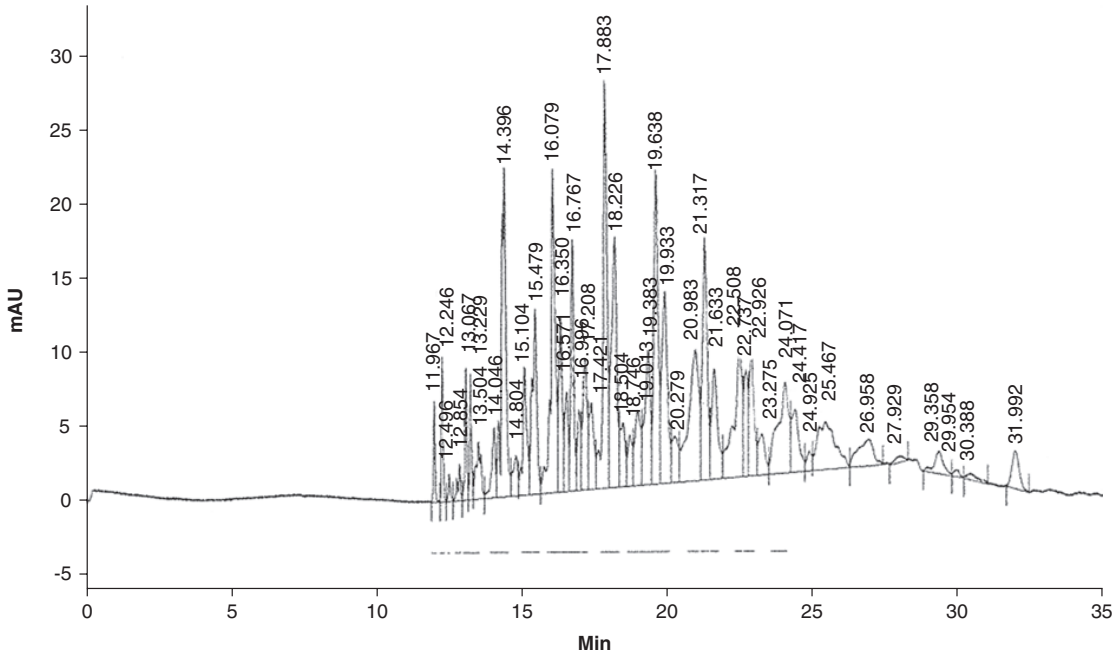

Figure 2. Typical electropherogram of GA fragments after exposure to trypsin.

amino acids are dependent on the acetolytic cleavage conditions.

Among tests to explain the primary structure are spectroscopic techniques (Fourier transform infrared, ultraviolet, proton and carbon[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 α-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 α-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 Kav (the smaller the Kav 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 α-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

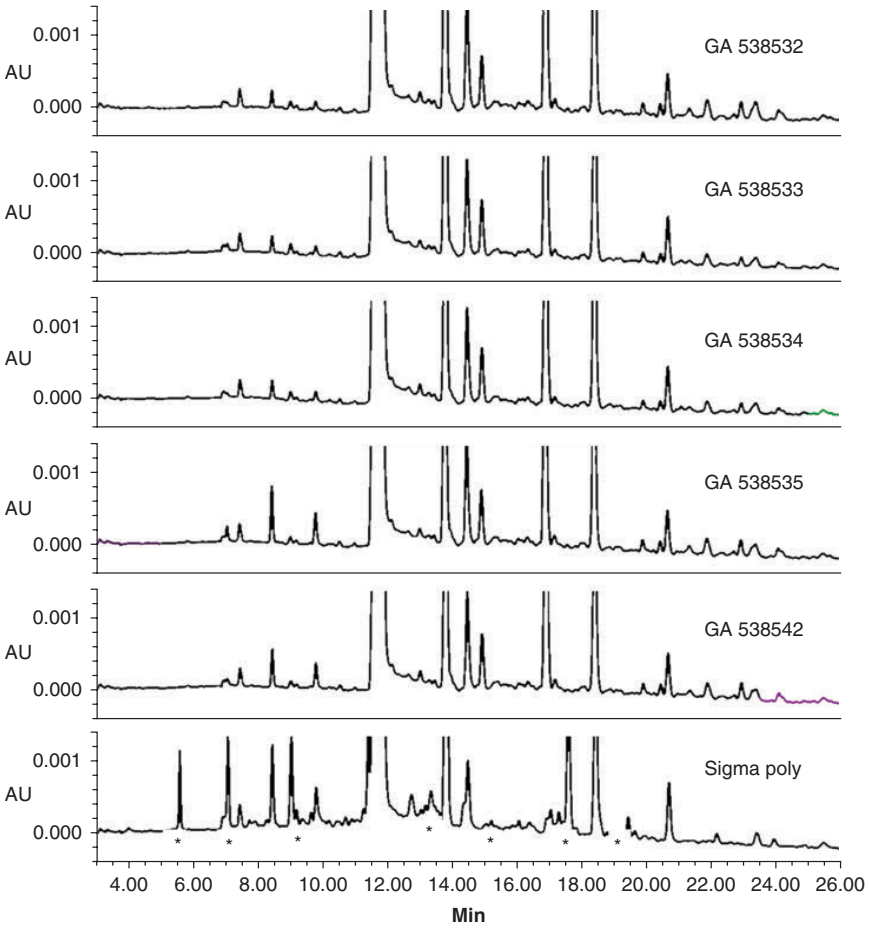

Figure 3. Comparative reverse-phase HPLC chromatograms of GA (the top five chromatograms show different batches of GA drug product) and Sigma Poly(Ala:Glu:Lys:Tyr) (bottom chromatogram) proteolytic digests by carboxypeptidase P.
Ala:Glu:Lys:Tyr: Alanine:glutamic acid:lysine:tyrosine; GA: Glatiramer acetate.

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.

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