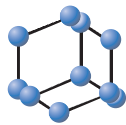
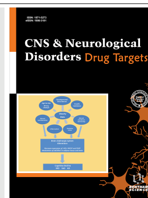
Send Orders for Reprints to reprints@benthamscience.ae

CNS & Neurological Disorders - Drug Targets, 2017, *16*, 714-723

RESEARCH ARTICLE



Demonstration of Biological and Immunological Equivalence of a Generic Glatiramer Acetate



Josephine D'Alessandro[1], Kevin Garofalo[1], Ganlin Zhao[2], Christopher Honan[1], Jay Duffner[1], Ishan Capila[1], Ian Fier[1], Ganesh Kaundinya[1], Daniel Kantor[3] and Tanmoy Ganguly[1,*]

[1]*Research Department, Momenta Pharmaceuticals, Inc., Cambridge, MA, USA;* [2]*Division of Bioequivalence I, Office of Generic Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA;* [3]*Division of Neurology, Florida Atlantic University, Boca Raton, FL, USA*

ARTICLE HISTORY

Received: December 12, 2016
Revised: January 24, 2017
Accepted: February 02, 2017

DOI:
10.2174/1871527316666170223162747

Abstract: ***Background***: In April 2015, the US Food and Drug Administration approved the first generic glatiramer acetate, Glatopa® (M356), as fully substitutable for Copaxone® 20 mg/mL for relapsing forms of multiple sclerosis (MS). This approval was accomplished through an Abbreviated New Drug Application that demonstrated equivalence to Copaxone.

Method: This article will provide an overview of the methods used to establish the biological and immunological equivalence of the two glatiramer acetate products, including methods evaluating antigen-presenting cell (APC) biology, T-cell biology, and other immunomodulatory effects.

Results: *In vitro* and *in vivo* experiments from multiple redundant orthogonal assays within four biological processes (aggregate biology, APC biology, T-cell biology, and B-cell biology) modulated by glatiramer acetate in MS established the biological and immunological equivalence of Glatopa and Copaxone and are described. The following were observed when comparing Glatopa and Copaxone in these experiments: equivalent delays in symptom onset and reductions in "disease" intensity in experimental autoimmune encephalomyelitis; equivalent dose-dependent increases in Glatopa- and Copaxone-induced monokine-induced interferon-gamma release from THP-1 cells; a shift to a T helper 2 phenotype resulting in the secretion of interleukin (IL)-4 and downregulation of IL-17 release; no differences in immunogenicity and the presence of equivalent "immunofingerprints" between both versions of glatiramer acetate; and no stimulation of histamine release with either glatiramer acetate in basophilic leukemia 2H3 cell lines.

Conclusion: In summary, this comprehensive approach across different biological and immunological pathways modulated by glatiramer acetate consistently supported the biological and immunological equivalence of Glatopa and Copaxone.

Keywords: Antigen-presenting cell, B cell, glatiramer acetate, generic, multiple sclerosis, T cell.

1. INTRODUCTION

Although no cure for multiple sclerosis (MS) exists, glatiramer acetate (GA) is an important first-line treatment for relapsing forms of MS [1]. The mechanisms of action by which GA exerts its therapeutic actions in MS are not completely understood but are thought to be primarily immunomodulatory [2, 3], targeting multiple pathways of both the innate and adaptive immune systems [3]. They fall into four broad categories: aggregate biology, antigen-presenting cell (APC) biology, T-cell biology, and B-cell biology.

The modulation of aggregate biology by GA may be responsible for its anti-inflammatory and putative neuroprotective effects, which are thought to occur through the promotion of T helper 2 (Th2)–like cells that secrete anti-inflammatory cytokines (*i.e.* "bystander" immunosuppression of pathogenic cells) [4, 5], and for the upregulation of neurotrophic factors (*e.g.* brain-derived neurotrophic factor) that can lead to reduced demyelination and also promote remyelination [3, 6]. GA may also alter APC biology through the binding of major histocompatibility complex class II molecules, resulting in the modulation of APC function and chemokine release [7-10]. The third proposed pathway that GA is thought to target involves T-cell biology, resulting in the alteration of regulatory T-cell function and subsequent cytokine release, T-cell proliferation, and Th2 polarization [7, 11-14]. Indeed, previous gene expression studies have demonstrated that GA is associated with the differential expression of genes involved in the proliferation and activation of immune cells (*e.g.* elevated FOXP3 expression in CD4+ T cells to promote conversion to CD4+CD25+ regulatory T cells) [11, 15, 16]. Finally, GA is thought to affect B-cell biology by altering B-cell function, antibody response, and immunorecognition

*Address correspondence to this author at: Momenta Pharmaceuticals, Inc., 675 West Kendall Street, Cambridge, MA 02142, USA; Tel: 617-395-2800; Fax: 617-621-0431; E-mail: tganguly@momentapharma.com

1996-3181/17 $58.00+.00
© 2017 Bentham Science Publishers

CNS & NeurologicalDisorders - Drug Targets

[17, 18]. GA is immunogenic; however, anti-GA antibodies do not impact clinical efficacy or systemic adverse events (local or adverse) [14, 17].

GA is approved under the brand name Copaxone® (Teva Pharmaceuticals USA Inc., North Wales, PA) by the United States (US) Food and Drug Administration (FDA) for the treatment of patients with relapsing forms of MS [19]. However, the use of Copaxone is associated with relatively high annual costs in the US, which are 3- to 4-fold greater than in other countries [20]. Therefore, there is an unmet need for the development of generic versions of GA, which have the potential to increase access to treatment for patients with MS.

In April 2015, the FDA approved the first generic GA, Glatopa® (M356; Sandoz Inc., Princeton, NJ), as a fully substitutable generic equivalent of Copaxone 20 mg/mL [21], utilizing the FDA approval process for generic drugs (Abbreviated New Drug Application) [22], as it is not a biologic. GA is a mixture of synthetic polypeptides of variable molecular weights (MWs) and sequences that is manufactured through chemical synthesis from the amino acids L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a specific, well-described molar ratio [10, 23]. The equivalence between the two GA drugs, Glatopa and Copaxone, has been assessed with regard to starting materials, manufacturing process signatures, and physiochemical (structural) and biological (functional) properties [24, 25].

Several of the experiments from the aforementioned biology categories were previously reported, including aggregate (MS animal models) and B-cell biology, as well as gene expression studies [24, 25]. This article re-examines those results as well as provides new examples of the remainder of the type of methods used to establish the biological and immunological equivalence of the two GA drug products—specifically, methods evaluating APC biology, T-cell biology, and other immunomodulatory effects—so that the comprehensive biological evaluation can be appreciated and discussed.

2. MATERIALS AND METHOD

All animal experiments reported here were approved and performed under the guidelines of the Institutional Animal Care and Use Committee of Momenta Pharmaceuticals, Inc. (Institutional Animal Care and Use Committee Guidebook, approval number 05-2011).

2.1. Evaluation of Aggregate Biology (*in vivo*)

As previously and briefly described [24], three experimental autoimmune encephalomyelitis (EAE) mouse models were used to compare the aggregate measures of efficacy of Glatopa and Copaxone. The active induction proteolipid peptide $(PLP)_{139-151}$ model of relapsing-remitting MS (RRMS) was initiated by subcutaneously immunizing female SJL/J mice at three sites on the dorsal surface with 75–100 µg of $PLP_{139-151}$ peptide emulsified in complete Freund's adjuvant. For prophylactic treatment, 500 µg of Glatopa, Copaxone, or vehicle control was included in the encephalitogenic emulsions. Symptoms are typically observed between days 11 and 13.

In the second model, active induction of the neuroantigen myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ (progressive MS) was initiated by immunizing female C57BL/6 mice subcutaneously at three sites on the dorsal surface with 50–75 µg of MOG_{35-55} peptide emulsified in complete Freund's adjuvant. In addition, the mice were injected intraperitoneally with 200 ng of pertussis toxin on days 0 and 1. For prophylactic treatment, 500 µg of Glatopa, Copaxone, or vehicle control was included in the encephalitogenic emulsions. Symptoms are typically observed between days 9 and 11.

The third EAE model involved passive induction through adoptive transfer from $PLP_{139-151}$-immunized SJL/J female donor mice, which were immunized as described earlier for the active/$PLP_{139-151}$ model. On day 10, donor spleens were removed and splenocytes were isolated for culture. The cells were cultured for 3 days at 5×10^6 cells/mL in the presence of 20 µg/mL $PLP_{139-151}$. The cells were then transferred (20-30 x 10^6 cells per mouse) intravenously to naive recipient female SJL/J mice. Mice were treated daily with Glatopa 2 mg, Copaxone 2 mg, or vehicle control given subcutaneously on days 0 to 9 or with fingolimod 3 mg given orally (used as a positive control) on days 0 to 25. Symptoms are typically observed between days 6 and 8.

Twelve animals per treatment group were included for each study. All mice were scored for disease progression using a standard scale from 0 (normal; no overt signs of the disease) to 5 (complete hind limb paralysis with front limb involvement; moribund state; euthanasia required), and score data were used to calculate disease incidence (the sum of animals that attained a score of ≥1 for two consecutive days divided by the number of animals per group), disease intensity (mean daily scores from day 7 through study completion), mean peak score (average of the highest score attained by each animal during the study), and mean day of onset (average of the first day each animal reached a score of ≥1).

Histological evaluation was also conducted for the MOG_{35-55} EAE model. On day 15, at the peak of EAE in the vehicle group, half the mice were sacrificed. On day 28 (end of study), the remaining mice were sacrificed. Mice were perfused with phosphate buffered saline and spinal cords were collected in 10% buffered formalin. For each mouse, three luxol fast blue stained sections (for demyelination) and three hematoxylin and eosin stained sections (for apoptotic cell count and inflammatory foci) from the lumbar, thoracic, and cervical spinal cord were prepared. A total of nine luxol fast blue and nine hematoxylin and eosin sections for each mouse were analyzed by a trained pathologist blinded to the experimental groups and all readouts.

Statistical comparisons of Copaxone, Glatopa, and vehicle were performed using one-way analysis of variance followed by Tukey's method for multiple comparisons.

2.2. Evaluation of APC Biology: THP-1 Chemokine Assay (*in vitro*)

The THP-1 chemokine release assay was developed to quantitatively measure antigen (Glatopa or Copaxone)-induced release of a soluble interferon-gamma (IFNγ)-mediated chemokine—monokine-induced by IFNγ (MIG)—in a THP-1

human myeloid cell line, which serves as a surrogate for APCs. One and two dilutions of Glatopa or Copaxone (diluted in assay media containing 2X IFNγ) were tested in a 10-point dose response (1:1.5 dilution series, with starting concentration at 15 µg/mL) by incubation with THP-1 cells. Assay plates were incubated for 20-24 hours at 37°C and then centrifuged at 400g for 5 minutes at 4°C for harvest. MIG release was measured using enzyme-linked immunosorbent assay (ELISA). Statistical comparisons of Copaxone *vs.* Glatopa were performed using one-way ANOVA followed by Tukey's multiple comparison test.

2.3. Evaluation of T-Cell Biology

Murine Th2-polarized T cells were generated *in vivo* and *ex vivo* to assess the effect of GA on T-cell biology. Semiquantitative testing was employed to measure the ability of GA to induce polarization of T cells toward a Th2 phenotype, which is characterized by high levels of Th2 cytokines (*e.g.* interleukin [IL]-4) and low levels of Th1 (*e.g.* IL-2) and Th17 (*e.g.* IL-17) cytokines. Balb/c mice were immunized with Glatopa 250 µg or Copaxone 250 µg, and the draining inguinal lymph nodes were harvested at day 11 postimmunization. Single-cell suspensions were made from these lymph node cells, and the CD4+ T-cell population was isolated by negative immunomagnetic selection. T cells that were reactive to GA (Glatopa or Copaxone) were generated through two rounds of restimulation *ex vivo* with either Glatopa or Copaxone, which was presented by T-cell–depleted, mitomycin C–treated naive splenic APCs. The polarization of GA-specific T cells toward a Th2 phenotype was assessed by multiplexed enhanced chemiluminescence–based assays of Th1-, Th2-, and Th17-associated cytokines. Because each polarized cell line is unique, only a semiquantitative comparison of the degree of Th2 polarization of Glatopa- and Copaxone-reactive T cells could be made. Method robustness and sensitivity were augmented using a crossover study design with Glatopa and Copaxone.

The dose-dependent release of IL-4 (a Th2 cytokine) induced by Glatopa and/or Copaxone from GA-specific murine Th2 polarized T cells) was also measured *in vitro* using ELISA as follows: murine Th2 polarized cell lines were plated with murine APCs at a 3:1 (APC:Th2) ratio in the presence of varying concentrations of Glatopa or Copaxone. After a 24-hour incubation at 37°C, ELISA was performed on the samples. To demonstrate robustness and reproducibility, this assay was repeated on several separate days to show intra- and inter-assay comparability.

2.4. Evaluation of B-Cell Biology

The effect of GA on B-cell biology was assessed *in vivo.* As previously described [24], a crossover design was used to compare the temporal generation of anti-GA (anti-Glatopa or anti-Copaxone) antibodies following multiple subcutaneous injections of Glatopa or Copaxone in SJL/J female mice (8-10 animals per arm). Antibody titers, isotype, and cross-reactivity were measured using ELISAs. Statistical comparison between Glatopa and Copaxone was performed using one-way ANOVA followed by Tukey's multiple comparison test.

The immunoreactivity of GA was evaluated by measuring the binding of Glatopa or Copaxone to a panel of murine anti–GA-specific monoclonal antibody (mAb) pairs using sandwich ELISAs. GA mAbs exhibit a high degree of specificity in their ability to bind to specific peptide sequences (epitopes) with which they can bind with varying degrees of affinity. Two different mAbs raised in mice against GA that recognized different epitopes in the peptide mixture were paired as a capture-detection pair in a sandwich ELISA format. Each specific pair is used to recognize specific molecular structure in the complex. Therefore, by using multiple sandwich ELISA with different combinations of the mAb pairs, we can map a unique "immunofingerprint" of GA. By comparing the immunofingerprints of Copaxone and Glatopa samples, structural sameness was established without the previous requirement of knowledge of the exact sequence(s) that would be recognized by these mAbs.

2.5. Evaluation of Miscellaneous Immunomodulatory Effects: Histamine Release (*in vitro*)

Release of histamine induced by an antigen (*i.e.* Glatopa/Copaxone) was assessed in an *in vitro* model (rat basophilic leukemia RBL-2H3 cell line). RBL-2H3 cells were incubated overnight at 37°C and then treated with Glatopa or Copaxone for 60 minutes. Cell supernatants were collected and histamine release was measured by ELISA. A positive control (calcium ionophore) was included to ensure that cell lines were capable of histamine release upon activation. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison test.

3. RESULTS

3.1. Aggregate Biology: EAE Models of MS

Findings in the EAE models of MS have been previously reported [24]. Briefly, all three EAE models demonstrated that Glatopa and Copaxone were equivalent with regard to the delayed onset of symptoms, disease intensity, and peak disease score (Fig. **1A-C**). Glatopa and Copaxone were both associated with significant delays in the onset of symptoms *vs*. the vehicle control (all $p < 0.05$). The mean (standard error of mean) day of onset for Glatopa and Copaxone *vs*. vehicle was 14.2 (1.3) and 14.3 (1.6) *vs.* 12.3 (0.9), respectively, in the active induction $PLP_{139\text{-}151}$ model; 28.7 (0.8) and 27.6 (3.0) *vs.* 11.8 (1.9), respectively, in the active induction $MOG_{35\text{-}55}$ model; and 18.4 (3.5) and 17.3 (3.3) *vs.* 12.4 (2.3), respectively, in the adoptive transfer model. There were no significant differences ($p > 0.05$) between Glatopa and Copaxone in all three EAE models.

Histological analysis of the $MOG_{35\text{-}55}$ model further confirmed that Glatopa and Copaxone were equivalent with regard to neuroprotective effects (reductions in demyelination and apoptotic cell counts) (Fig. **2A** and **B**) and the inhibition of inflammation (Fig. **2C**). Significant reductions of demyelination, apoptotic cell counts, and inflammatory foci were observed for both Glatopa and Copaxone relative to vehicle control on days 15 and 28 (all $p < 0.001$).

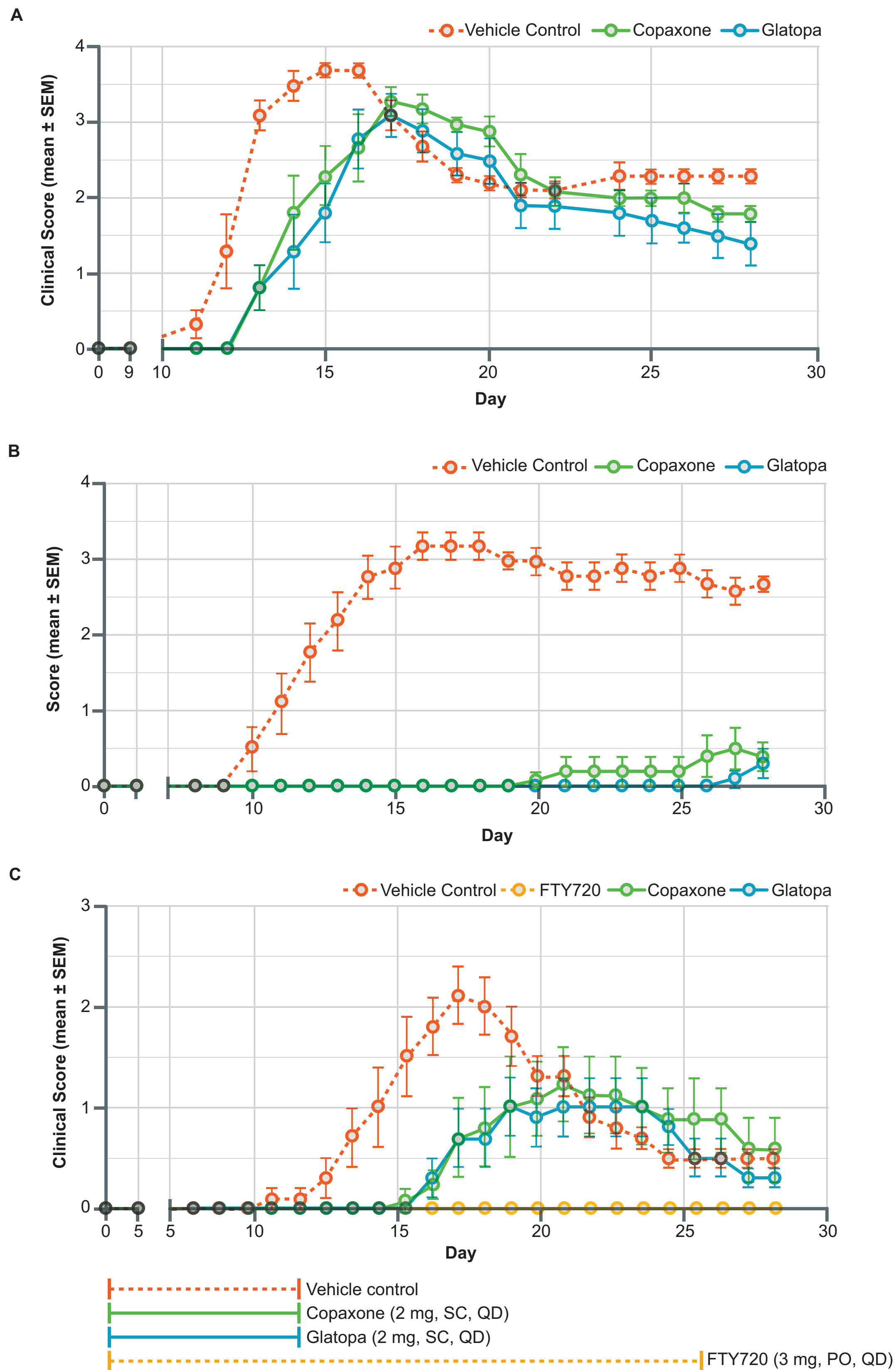


Fig. (1). Clinical scores for induction of experimental autoimmune encephalomyelitis (EAE) murine models with proteolipid peptide $(PLP)_{139-151}$ (active) (**A**), myelin oligodendrocyte glycoprotein$_{35-55}$ (active) (**B**), and adoptive transfer following $PLP_{139-151}$ immunization (passive) (**C**). Glatopa and Copaxone similarly delayed the onset of symptoms *vs.* controls in EAE models of multiple sclerosis. Reprinted with permission [24]. FTY720, fingolimod; PO, oral; QD, once per day; PBO, placebo; SC, subcutaneous; SEM, standard error of mean.

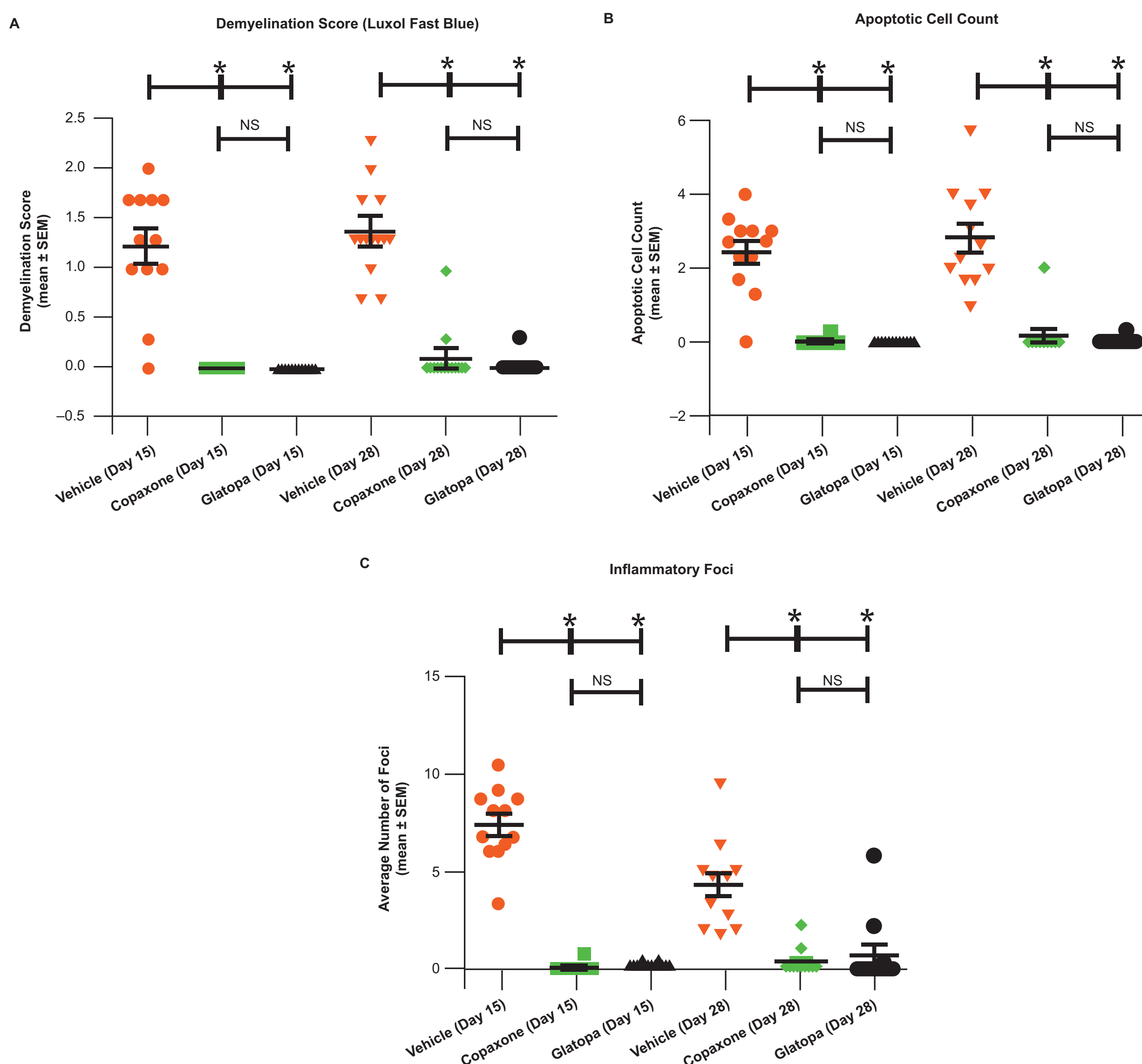


Fig. (2). Histological analysis of active induction of the experimental autoimmune encephalomyelitis model with myelin oligodendrocyte glycoprotein$_{35-55}$. Significant reductions in the extent of demyelination (**A**), apoptotic cell counts (**B**), and inflammatory foci (**C**) were observed with Glatopa and Copaxone *vs*. vehicle control. No significant differences were noted between Glatopa and Copaxone. Reprinted with permission [24]. NS, not significant; SEM, standard error of mean. *$p < 0.001$ *vs.* controls. One-way analysis of variance followed by Tukey's multiple comparison test.

3.2. APC Biology: THP-1 Chemokine Assay

The THP-1 chemokine assay was employed to evaluate APC biology. The treatment of THP-1 cells with Glatopa or Copaxone stimulated the release of MIG in a dose-dependent manner (Fig. **3**). However, no significant differences ($p > 0.05$) in GA-stimulated MIG release were seen between Glatopa and the two lots of Copaxone (Fig. **3**).

3.3. T-Cell Biology: Alterations of Regulatory T-Cell Functions

T-cell biology was assessed by the immunization of mice with Glatopa or Copaxone, followed by rechallenge *in vitro* and *ex vivo* to generate Th2-polarized T cells. This shift to a Th2 phenotype was signified by an approximately 200-fold increase in the Th2 cytokine, IL-4, and downregulation of the Th17 cytokine, IL-17 (Fig. **4A**). No significant differences ($p > 0.05$) in IL-4 or IL-17 secretion were seen between Glatopa and Copaxone (Fig. **4A**).

To further assess the biological activity of Glatopa and Copaxone, dose-dependent secretion of IL-4 was measured using sandwich ELISA *in vitro*. Dose response curves for two dilutions each of Glatopa and Copaxone indicated no significant differences ($p > 0.05$) in the secretion of IL-4 between the two versions of glatiramer acetate (Fig. **4B**).

3.4. B-Cell Biology: Anti–Glatiramer Acetate Antibody Immune Responses

As reported previously [24], formal *in vivo* testing of the immunogenic potential of Glatopa and Copaxone indicated that robust antibody titers were generated on day 28 in sera

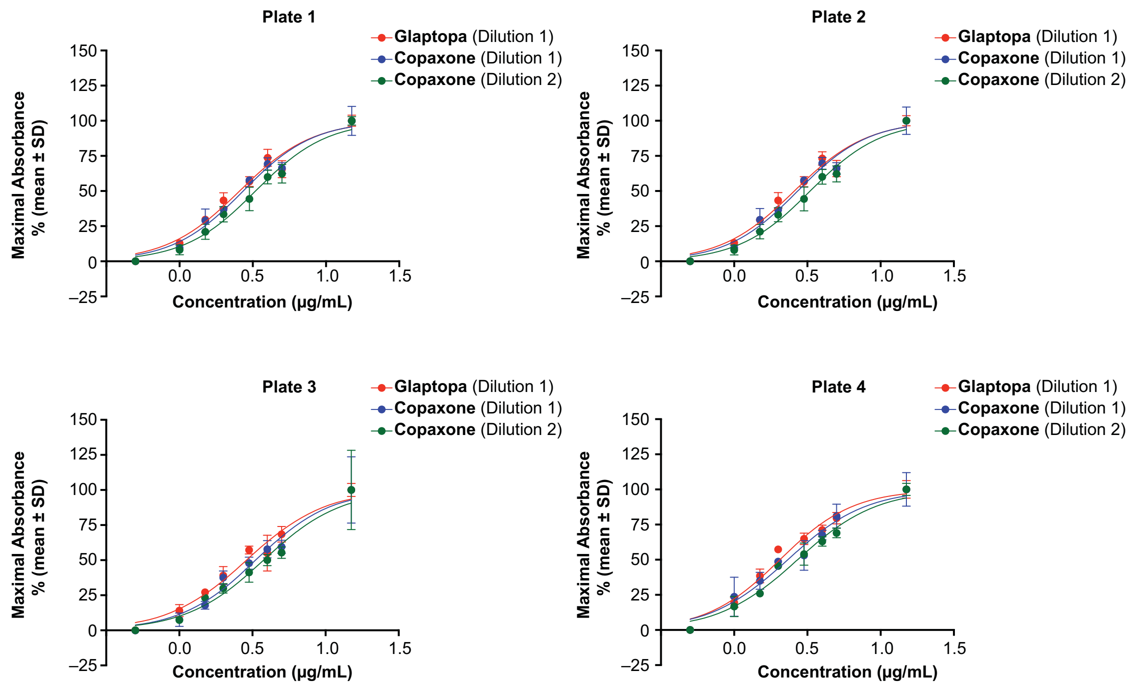


Fig. (3). Evaluation of antigen-presenting cell biology using the THP-1 chemokine assay. Plates 1 and 2 were duplicates of one dilution of Glatopa and two dilutions of Copaxone. Plates 3 and 4 were the same experiment run separately to demonstrate reproducibility. In all plates, Glatopa and Copaxone stimulated the release of monokine induced by interferon gamma in a similar fashion. SD, standard deviation.

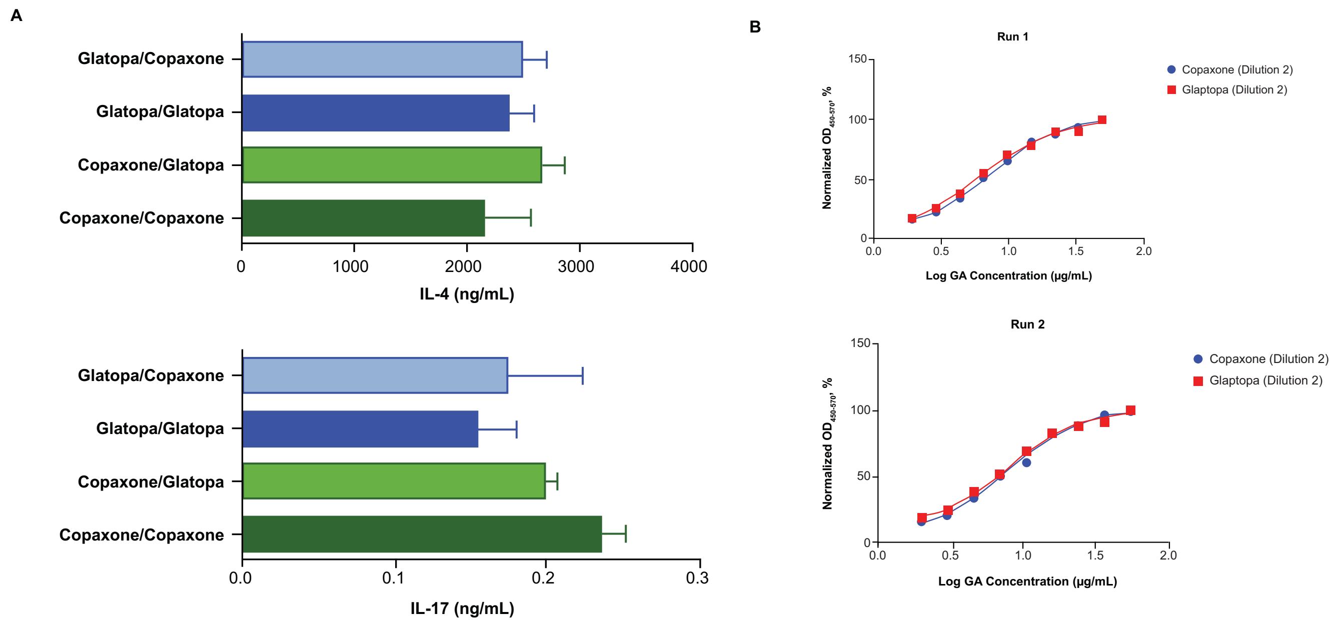


Fig. (4). T-cell biology. Glatiramer acetate (Glatopa or Copaxone)–induced release of the T helper (Th) 2 cytokine, interleukin (IL)-4 (top), and Th17 cytokine, IL-17, following rechallenge (**A**). Dose-dependent secretion of IL-4 in Th2-polarized cell lines, measured using sandwich enzyme-linked immunosorbent assay (**B**). There were no significant differences in IL-4 and IL-17 levels observed between Glatopa and Copaxone during rechallenge, and there were no differences in dose-dependent IL-4 release between the two versions of GA. GA, glatiramer acetate; IL, interleukin; OD, optical density.

samples from mice immunized with either agent, which cross-reacted equally (*i.e.* no statistically significant differences) ($p > 0.05$) with both antigens (Glatopa/Copaxone) within each individual animal (Fig. **5**). Glatopa and Copaxone were equivalent with regard to antibody titers, independent of the capture antigen.

An immunorecognition assay was also conducted using a panel of GA-specific mAbs to map epitopes. Sandwich ELISA of the GA-specific mAb pair shown in Fig. (**6**) demonstrated that EC_{50} values of the three lots of Glatopa and three lots of Copaxone were equivalent. The epitopes recognized by this mAb pair were in the same abundance in both

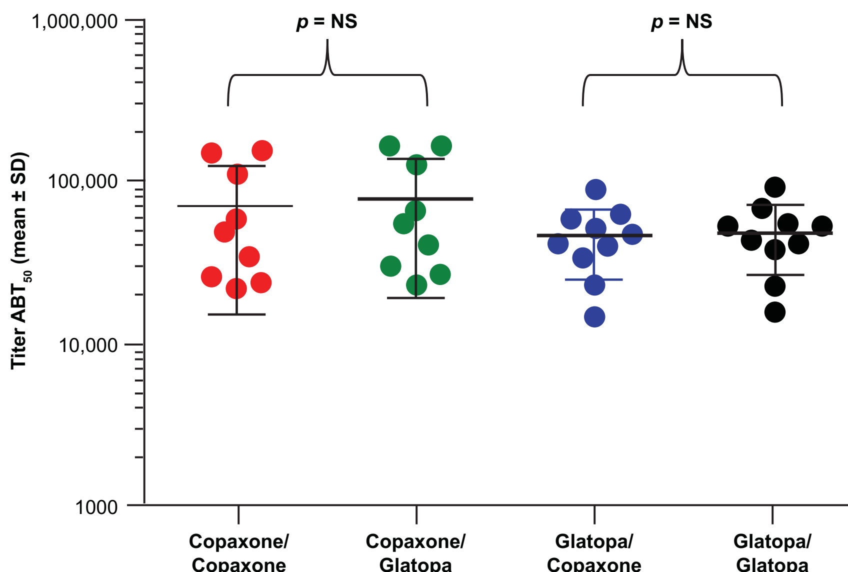


Fig. (5). B-cell biology. Anti–glatiramer acetate antibody response was similar with Glatopa and Copaxone. There were no statistically significant differences in the antibody titers obtained in mice immunized with Copaxone or Glatopa independent of the capture antigen. Reprinted with permission [24]. ABT_{50}, titers at which 50% binding occurs; NS, not significant; SD, standard deviation.

Glatopa and Copaxone, which suggests that the two versions of GA have a similar "immunofingerprint" and equivalent amino acid compositions and sequences. Similarly, there were no significant differences ($p > 0.05$) in the immunoreactivity between Glatopa and three lots of Copaxone with the other five monoclonal antibody pairs [26].

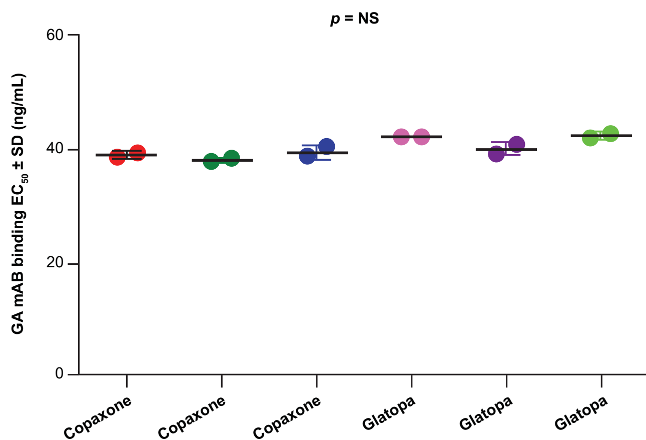


Fig. (6). Sandwich enzyme-linked immunosorbent assay. An immunorecognition assay using a panel of GA-specific monoclonal antibodies (mAbs) to map epitopes showed that the EC_{50} values of three lots each of Glatopa and Copaxone were not significantly different for the mAb pair shown. EC_{50}, concentrations at which 50% of binding occurs; GA, glatiramer acetate; mAb, monoclonal antibody; NS, not significant; SD, standard deviation.

3.5. Additional Immunomodulatory Effects: Histamine Release

There was an equivalent amount of histamine release following treatment of RBL-2H3 cell lines with Glatopa or Copaxone (Fig. 7). The positive control, calcium ionophore, was associated with a pronounced histamine release.

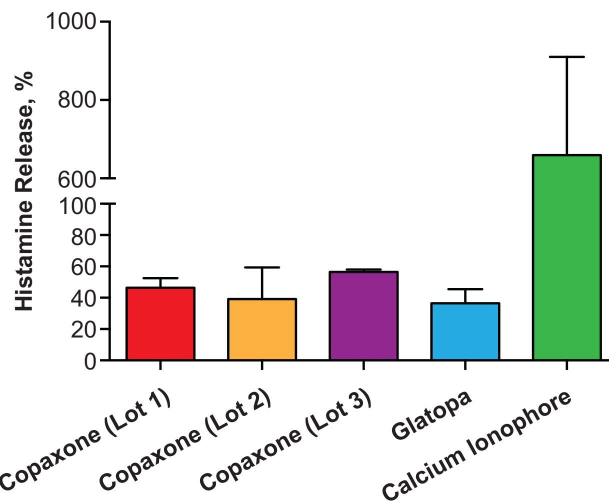


Fig. (7). Percentage of histamine release from rat basophilic leukemia 2H3 cells after 1 hour of incubation with three lots of Copaxone, Glatopa, or calcium ionophore (positive control). Each bar represents the mean triplicate test runs of histamine released as a percentage over background control. There were no significant differences in histamine release seen between Glatopa and Copaxone.

4. DISCUSSION

Glatopa is the first FDA-approved generic disease-modifying therapy for MS and remains the sole FDA-approved generic GA as of November 2016. Our report describes a comprehensive approach across different categories of biological and immunological pathways modulated by GA, confirming the biological and immunological equivalence of Glatopa and Copaxone with regard to aggregate biology, APC biology, T-cell biology, and B-cell biology (Table **1**).

In vivo animal models, such as EAE, are commonly used to mimic MS in humans, with EAE models of RRMS and

Table 1. Summary of findings from experimental biological and immunological studies used to establish the equivalence and active ingredient sameness of Glatopa and Copaxone.

	Model/Assay	Main Findings	References
Aggregate biology	$PLP_{139\text{-}151}$ EAE model of RRMS (*in vivo*)	• Time to onset of symptoms for both Glatopa and Copaxone *vs*. vehicle control: 14.2 and 14.3 *vs*. 12.3 days, respectively (both $p < 0.05$). • Glatopa and Copaxone were equivalent with regard to time to onset of symptoms, disease intensity, and peak disease score.	[24]
	$MOG_{35\text{-}55}$ EAE model of progressive MS (*in vivo*)	• Time to onset of symptoms for both Glatopa and Copaxone *vs*. vehicle control: 28.7 and 27.6 *vs*. 11.8 days, respectively (both $p < 0.05$). • Glatopa and Copaxone were equivalent with regard to time to onset of symptoms, disease intensity, and peak disease score. • Equivalent improvements in neuroprotective effects were noted for Glatopa and Copaxone *vs*. vehicle control: reductions in demyelination, apoptotic cell counts, and inhibition of inflammatory foci.	[24]
	$PLP_{139\text{-}151}$ EAE adoptive transfer EAE model (*in vivo*)	• Time to onset of symptoms for both Glatopa and Copaxone *vs*. vehicle control: 18.4 and 17.3 *vs*. 12.4 days, respectively (both $p < 0.05$). • Glatopa and Copaxone were equivalent with regard to time to onset of symptoms, disease intensity, and peak disease score.	[24]
APC biology	THP-1 chemokine assay (*in vitro*)	• No difference in GA-stimulated MIG release in THP-1 cells when treated with Glatopa and Copaxone.	–
T-cell biology	Immunization of mice with Glatopa or Copaxone to generate Th2-polarized T-cells (*in vitro, ex vivo*)	• Immunization resulted in a 200-fold increase in IL-4 secretion and downregulation of IL-17. • No differences in IL-4 and IL-17 secretion were seen between Glatopa and Copaxone.	–
	Dose-dependent release of IL-4 in Th2-polarized T-cell lines (*in vitro*)	• Dose-dependent secretion of IL-4 (measured by sandwich ELISA); no differences between Glatopa and Copaxone	
B-cell biology	Immunization of mice with Glatopa or Copaxone (*in vivo*)	• Robust antibody titers generated on day 28 following immunization with Glatopa or Copaxone. • Antibody titers were equivalent for Glatopa and Copaxone. • Antibodies cross-reacted equally with both antigens (Glatopa/Copaxone).	[24]
	Immunorecognition and immunoreactivity assays (*in vitro*)	• Glatopa and Copaxone had a similar “immunofingerprint” and equivalent amino acid compositions and sequences. • No differences in immunoreactivity between Glatopa and Copaxone.	
Immunomodulatory effects	Histamine release assay (*in vitro)*	• Equal amounts of histamine release in RBL-2H3 cell lines following treatment with Glatopa and Copaxone.	–

APC, antigen-presenting cell; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; GA, glatiramer acetate; IL, interleukin; MIG, monokine-induced by interferon-gamma; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid peptide.

progressive forms of MS being used to test or predict the potential clinical efficacy of novel therapies for MS [27, 28]. The EAE model, which captures many of the biological and immunological processes associated with MS, is currently used as a release test to confirm the biological activity of Copaxone and is considered by the FDA to be the most useful biological assay for confirmation of active ingredient sameness between GAs [29]. EAE can be induced using various immunogenic myelin neuroantigens either directly by immunization with these agents or passively following the transfer of lymphocytes specific to these neuroantigens. The $PLP_{139\text{-}151}$ model is a well-established model of RRMS that simulates immunological processes, including autoimmune antigen recognition and presentation, T-cell activation and polarization, trafficking of autoreactive inflammatory cells, initiation of inflammation in the central nervous system, and resolution of disease [30, 31]. The $MOG_{35\text{-}55}$ model is a widely accepted simulation of primary progressive MS that also mimics the various autoimmune response processes, with a focus on neurodegeneration [27, 32]. Finally, the adoptive transfer $PLP_{139\text{-}151}$ model is another well-established simulation of RRMS that focuses on lymphocyte trafficking to the central nervous system and resolution of inflammation, and it was chosen to evaluate these aspects of autoimmune response, using a different daily therapeutic-dosing regimen [31]. Taken together, the use of these animal models of MS enabled us to evaluate the aggregate measures of efficacy of Glatopa and Copaxone, thus confirming the equivalence and active ingredient sameness of these two versions of GA.

A series of *in vitro* and *in vivo* experiments was performed to look more closely at the individual biological and immunological processes that are thought to be targeted by GA. GA has been shown to stimulate the release of soluble factors from APCs that are thought to alter immune function in MS [3]. Therefore, we modeled this proposed mechanism

of action of GA *in vitro* using a THP-1 human myeloid cell line and demonstrated similar dose-dependent increases in Glatopa- and Copaxone-induced MIG release from THP-1 cells. These findings indicate that both products are equivalent in terms of APC biology.

GA has also been shown to activate T cells by inducing a shift from a Th1 to Th2 phenotype, which results in the increased secretion of anti-inflammatory Th2 cytokines (*e.g.* IL-4) [10, 13]. EAE models have also demonstrated that GA downregulates expression of the pro-inflammatory Th17 cytokine IL-17 [33, 34], which plays a role in chronic inflammation and has been linked to autoimmune diseases, including MS [35]. Our findings showed that both Glatopa and Copaxone induced the secretion of IL-4 and downregulated IL-17 release to a similar extent, indicating that both versions of GA are equivalent in terms of their immunomodulatory actions on regulatory T-cell function.

Although repeated treatment with Copaxone has been shown to activate the humoral immune system and result in the development of anti-Copaxone antibodies [14, 36], the anti-Copaxone antibodies are considered to be non-neutralizing and do not adversely affect the safety and efficacy of Copaxone. Furthermore, a correlation between high anti-Copaxone antibody titers and improved clinical response has been previously shown [14], and animal models suggest that anti-GA antibodies may promote the repair of demyelinated lesions [37]. Therefore, we used antibody response to GA as a potential biomarker for GA activity *in vivo*. Our experiments showed no differences in antibody titers between animals immunized with Glatopa and those immunized with Copaxone, confirming the immunogenic equivalence of the two products. Results of immunorecognition assays reported here and by Anderson *et al*. [24] further supported these findings, indicating that the two versions of GA have similar immunofingerprints.

Finally, the assay on histamine release showed that neither Glatopa nor Copaxone induced histamine release in RBL-2H3 cell lines. High MW copolymers induce histamine degranulation in RBL-2H3 cell lines, which are considered to be a surrogate model of human mast cells. Therefore, our results indirectly demonstrate that the high MW copolymer content of these two versions of GA is similar.

The findings of this set of experiments further builds on the rigorous scientific program that has been undertaken to establish the equivalence of a generic GA, Glatopa, with the reference listed drug, Copaxone, across the various biological and physiochemical aspects of GA [24]. Recently, the use of gene profiling as an analytic tool was also employed to demonstrate the equivalence of Glatopa and Copaxone [25]. Copaxone induced expected changes in the expression of genes related to the mechanism of action of GA in MS, and there were no differences in gene expression profiles between Glatopa and Copaxone [25].

Evidence from the literature highlights the potential for differences to arise between branded complex mixtures and generic versions [16, 38, 39]. By undertaking a rigorous scientific approach, we were able to establish the biological and immunological equivalence of Copaxone 20 mg/mL and Glatopa.

CONCLUSION

The findings presented here were obtained from a set of physicochemical and biological assays, providing supportive evidence that was used to establish the equivalence of Glatopa with Copaxone. The findings affirm that a rigorous scientific approach can be undertaken to establish the equivalence of a branded complex mixture product and a generic version, and they have important implications for the development of other generic complex mixtures and biosimilars. Multiple ANDAs for a generic of the 40 mg/mL three-times-weekly dose of a Copaxone are currently under active review by the US FDA, including a 40 mg/mL formulation of Glatopa.

LIST OF ABBREVIATIONS

ANDA = Abbreviated New Drug Application
ANOVA = Analysis of Variance
APC = Antigen-presenting Cell
EAE = Experimental Autoimmune Encephalomyelitis
ELISA = Enzyme-linked Immunosorbent Assay
FDA = US Food and Drug Administration
GA = Glatiramer Acetate
IFNγ = Interferon Gamma
IL = Interleukin
MIG = Monokine-induced by Interferon Gamma
mAb = Monoclonal Antibody
MOG = Myelin Oligodendrocyte Glycoprotein
MS = Multiple Sclerosis
PLP = Proteolipid Peptide
RRMS = Relapsing-remitting Multiple Sclerosis
Th = T helper Cell
US = United States

CONFLICT OF INTEREST

J. D'Alessandro, K. Garofalo, G. Zhao, C. Honan, J. Duffner, I. Capila, I. Fier, G. Kaundinya, and T. Ganguly are employees of and stockholders in Momenta Pharmaceuticals. D. Kantor has received consulting fees from Momenta Pharmaceuticals.

ACKNOWLEDGEMENTS

The authors thank Paul Miller for his writing and editorial support, which was funded by Momenta Pharmaceuticals. All authors were involved in the design of the study, data collection and analysis, decisions to publish, and preparation of the manuscript.

REFERENCES

[1] Scott LJ. Glatiramer acetate: a review of its use in patients with relapsing-remitting multiple sclerosis and in delaying the onset of clinically definite multiple sclerosis. CNS Drugs 2013; 27: 971-88.
[2] Arnon R, Aharoni R. Mechanism of action of glatiramer acetate in multiple sclerosis and its potential for the development of new applications. Proc Natl Acad Sci U S A 2004; 101(Suppl 2): 14593-8.
[3] Lalive PH, Neuhaus O, Benkhoucha M, *et al*. Glatiramer acetate in the treatment of multiple sclerosis: emerging concepts regarding its mechanism of action. CNS Drugs 2011; 25: 401-14.

[4] Aharoni R, Teitelbaum D, Leitner O, Meshorer A, Sela M, Arnon R. Specific Th2 cells accumulate in the central nervous system of mice protected against experimental autoimmune encephalomyelitis by copolymer 1. Proc Natl Acad Sci U S A 2000; 97: 11472-7.

[5] Aharoni R, Teitelbaum D, Sela M, Arnon R. Bystander suppression of experimental autoimmune encephalomyelitis by T cell lines and clones of the Th2 type induced by copolymer 1. J Neuroimmunol 1998; 91: 135-46.

[6] Aharoni R. Immunomodulation neuroprotection and remyelination - the fundamental therapeutic effects of glatiramer acetate: a critical review. J Autoimmun 2014; 54: 81-92.

[7] Racke MK, Lovett-Racke AE, Karandikar NJ. The mechanism of action of glatiramer acetate treatment in multiple sclerosis. Neurology 2010; 74 (Suppl 1): 25-30.

[8] Fridkis-Hareli M, Strominger JL. Promiscuous binding of synthetic copolymer 1 to purified HLA-DR molecules. J Immunol 1998; 160: 4386-97.

[9] Jung S, Siglienti I, Grauer O, Magnus T, Scarlato G, Toyka K. Induction of IL-10 in rat peritoneal macrophages and dendritic cells by glatiramer acetate. J Neuroimmunol 2004; 148: 63-73.

[10] Vieira PL, Heystek HC, Wormmeester J, Wierenga EA, Kapsenberg ML. Glatiramer acetate (copolymer-1, copaxone) promotes Th2 cell development and increased IL-10 production through modulation of dendritic cells. J Immunol 2003; 170: 4483-8.

[11] Hong J, Li N, Zhang X, Zheng B, Zhang JZ. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proc Natl Acad Sci U S A 2005; 102: 6449-54.

[12] Aharoni R. The mechanism of action of glatiramer acetate in multiple sclerosis and beyond. Autoimmun Rev 2013; 12: 543-53.

[13] Neuhaus O, Farina C, Yassouridis A, *et al*. Multiple sclerosis: comparison of copolymer-1- reactive T cell lines from treated and untreated subjects reveals cytokine shift from T helper 1 to T helper 2 cells. Proc Natl Acad Sci U S A 2000; 97: 7452-7.

[14] Brenner T, Arnon R, Sela M, *et al.* Humoral and cellular immune responses to Copolymer 1 in multiple sclerosis patients treated with Copaxone. J Neuroimmunol 2001; 115: 152-60.

[15] Achiron A, Feldman A, Gurevich M. Molecular profiling of glatiramer acetate early treatment effects in multiple sclerosis. Dis Markers 2009; 27: 63-73.

[16] Bakshi S, Chalifa-Caspi V, Plaschkes I, *et al*. Gene expression analysis reveals functional pathways of glatiramer acetate activation. Expert Opin Therap Targets 2013; 17: 351-62.

[17] Teitelbaum D, Brenner T, Abramsky O, Aharoni R, Sela M, Arnon R. Antibodies to glatiramer acetate do not interfere with its biological functions and therapeutic efficacy. Mult Scler 2003; 9: 592-9.

[18] Karussis D, Teitelbaum D, Sicsic C, Brenner T. Long-term treatment of multiple sclerosis with glatiramer acetate: natural history of the subtypes of anti-glatiramer acetate antibodies and their correlation with clinical efficacy. J Neuroimmunol 2010; 220: 125-30.

[19] Copaxone (glatiramer acetate injection) for subcutaneous use [package insert]. North Wales, PA: Teva Pharmaceuticals USA Inc; 2014.

[20] Hartung DM, Bourdette DN, Ahmed SM, Whitham RH. The cost of multiple sclerosis drugs in the US and the pharmaceutical industry: too big to fail? Neurology 2015; 84: 2185-92.

[21] Glatopa (glatiramer acetate injection), for subcutaneous use [package insert]. Princeton, NJ: Sandoz Inc; 2015.

[22] US food and drug administration. Code of federal regulations title 21. part 320 bioavailability and bioequivalence requirements. April 1, 2015. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm? fr=320.1. Accessed February 22, 2016.

[23] Farina C, Then BF, Albrecht H, *et al*. Treatment of multiple sclerosis with Copaxone (COP): elispot assay detects COP-induced interleukin-4 and interferon-gamma response in blood cells. Brain 2001; 124: 705-19.

[24] Anderson J, Bell C, Bishop J, *et al*. Demonstration of equivalence of a generic glatiramer acetate (Glatopa™). J Neurol Sci 2015; 359: 24-34.

[25] D'Alessandro JS, Duffner J, Pradines J, *et al*. Equivalent gene expression profiles between Glatopa and Copaxone®. PLoS One 2015; 10: e0140299.

[26] D'Alessandro J, Garofalo K, Zhao G, *et al*. Use of multiple, high resolution, orthogonal assays for demonstration of biological and immunological equivalence of Glatopa and Copaxone 20 mg/ml. Presented at: ACTRIMS 2016 Forum; February 19, 2016; New Orleans, LA.

[27] Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 2006; 129: 1953-71.

[28] Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 2006; 60: 12-21.

[29] US Department of Health and Human Services. Citizen petition denial letter from CDER to Teva Pharmaceuticals. Docket No. FDA-2015-P-1050-012. https://www.pharmamedtechbi.com/~/media/Supporting%20Documents/The%20Pink%20Sheet/77/17/FDA_response_copaxone_citizen_petition.pdf. Posted April 16, 2015. Accessed March 8, 2016.

[30] McRae BL, Kennedy MK, Tan LJ, *et al*. Induction of active and adoptive relapsing experimental autoimmune encephalomyelitis (EAE) using an encephalitogenic epitope of proteolipid protein. J Neuroimmunol 1992; 38: 229-40.

[31] Teitelbaum D, Fridkis-Hareli M, Arnon R, Sela M. Copolymer 1 inhibits chronic relapsing experimental allergic encephalomyelitis induced by proteolipid protein (PLP) peptides in mice and interferes with PLP-specific T cell responses. J Neuroimmunol 1996; 64: 209-17.

[32] Ben-Nun A, Mendel I, Bakimer R, *et al.* The autoimmune reactivity to myelin oligodendrocyte glycoprotein (MOG) in multiple sclerosis is potentially pathogenic: effect of copolymer 1 on MOG-induced disease. J Neurol 1996; 243: 14-22.

[33] Begum-Haque S, Sharma A, Kasper IR, *et al*. Downregulation of IL-17 and IL-6 in the central nervous system by glatiramer acetate in experimental autoimmune encephalomyelitis. J Neuroimmunol. 2008; 204: 58-65.

[34] Aharoni R, Eilam R, Stock A, *et al*. Glatiramer acetate reduces Th-17 inflammation and induces regulatory T-cells in the CNS of mice with relapsing-remitting or chronic EAE. J Neuroimmunol 2010; 225: 100-11.

[35] Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerg Microbes Infections 2013; 2: e60.

[36] Conner J. Glatiramer acetate and therapeutic peptide vaccines for multiple sclerosis. J Autoimmun Cell Resp 2014; 1-3.

[37] Ure DR, Rodriguez M. Polyreactive antibodies to glatiramer acetate promote myelin repair in murine model of demyelinating disease. FASEB J 2002; 16: 1260-2.

[38] Towfic F, Funt JM, Fowler KD, *et al*. Comparing the biological impact of glatiramer acetate with the biological impact of a generic. PLoS One 2014; 9: e83757.

[39] Kolitz S, Hasson T, Towfic F, *et al*. Gene expression studies of a human monocyte cell line identify dissimilarities between differently manufactured glatiramoids. Sci Rep 2015; 5: 10191.