Use of Multiple, High Resolution, Orthogonal Assays for Demonstration of Biological and Immunological Equivalence of Glatopa™ and Copaxone® 20 mg

P647

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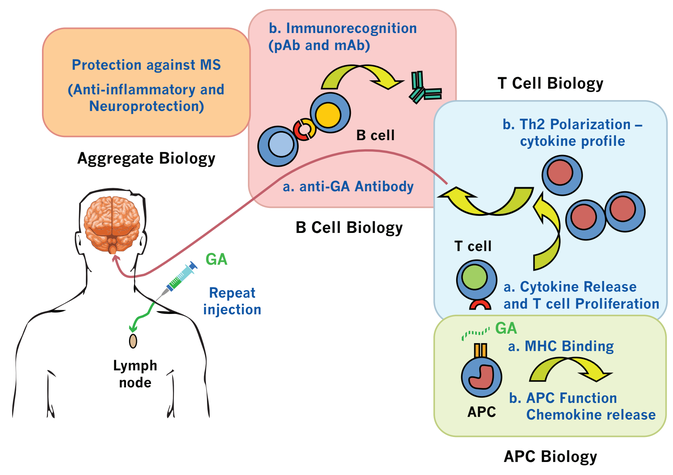
INTRODUCTION AND PURPOSE

Glatiramer acetate (GA) is a mixture of synthetic polypeptides of variable molecular weights and sequences, and is manufactured entirely through a chemical synthesis from the amino acids L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a specific well-described molar ratio [1-3]; thus, it is not a biologic product. Glatopa™ (glatiramer acetate injection; Sandoz, Inc.; M356) is the first and only FDA-approved, substitutable generic equivalent of Copaxone® 20 mg. Equivalence between these two GA drugs was assessed in terms of starting materials, manufacturing process signatures, physicochemical/structural properties, and biological and immunological properties.

GA is believed to exert its biological effects as an antigen-based immunomodulatory agent by targeting multiple pathways on both the innate and adaptive arms of the immune system. These immunological pathways fall into 4 broad categories of biological effects, which are described below and shown in Figure 1.

Figure 1: Immunological Pathways modulated by Glatiramer Acetate



1) **Antigen Presenting Cell (APC) Biology**
   a) **MHC Class II Binding:** GA is taken up by APCs in the subcutaneous space or in the local draining lymph node following injection. GA is presented in the context of major histocompatibility complex (MHC) class II antigen by APCs to modulate T cell responses [4].

   b) **APC Function / Chemokine Release:** GA modulates the profile of cytokines and chemokines produced by myeloid cells, and other APCs, through an MHC class II-independent pathway [2,5].

2) **T Cell Biology**
   a) **Cytokine Release and T Cell Proliferation:** Naïve T cells initially respond to GA through polyclonal, antigen-specific cytokine production and proliferation [6].

   b) **Th2 Polarization:** With repeated exposure to GA, the T cell response to GA is modulated over time towards a tolerogenic Th2-like phenotype [7].

3) **B Cell Biology**
   a) **Antibody Response:** GA induces a robust antibody response. Anti-GA antibodies are non-neutralizing and do not appear to contribute to efficacy and are not associated with any side effects [8,9].

   b) **Immunorecognition:** Due to the immunogenic nature of GA, reagents such as polyclonal antibodies and monoclonal antibodies can be raised in laboratory animals. GA is then identified by specific immunoreactivity to these antibodies.

4) **Aggregate Biology**
   Anti-inflammatory Effect and Neuroprotection: The GA-reactive Th2-like cells are thought to circulate from the periphery to the central nervous system (CNS) [10] and exert an immunosuppressive effect on the local pathogenic inflammatory response through the secretion of anti-inflammatory cytokines (increases in IL-4 and IL-5, and decrease in IFN ɣ). The broad (antigen non-specific) suppression of pathogenic cells by GA-specific T cells has been termed "bystander suppression" [11]. The EAE model is used as a disease-relevant animal model to capture the "aggregate" biological effects of GA in the CNS that lead to protection against MS such as T (Th2) cell trafficking to CNS and anti-inflammatory and neuroprotective effects.

The strategy to establish biological and immunological equivalence involved the development of multiple, redundant, orthogonal assays within each biology category described above. In addition, GA also has the potential to mediate other immunomodulatory effects not illustrated in Figure 1, such as stimulating histamine release from basophils. Examples from each biology category are shown below.

METHODS

THP-1 chemokine assay: The antigen (Copaxone/Glatopa)-induced release of the CXCL9/MIG chemokine in a dose dependent manner from human monocytic cell line (THP-1) was compared using ELISA.

Generation of murine Th2 polarized T cells: The antigen (Copaxone/Glatopa)-induced ex vivo polarization of CD4+ T cells (from a Th1 to a Th2 phenocyte) of murine lymphocytes isolated following single immunization step was compared in a crossover design using multiplexed ECL based assays.

Murine Th2 polarized T cell IL-4 ELISA: The antigen (Copaxone/Glatopa)-induced release of IL-4 (a Th2 cytokine) in a dose dependent manner from GA specific murine Th2 polarized T cells was compared using ELISA.

Anti-GA antibody response: The temporal generation of anti-GA (Copaxone/Glatopa) antibodies following multiple injections was compared in a crossover design. The antibody titers, the isotype, and cross reactivity was measured using ELISAs.

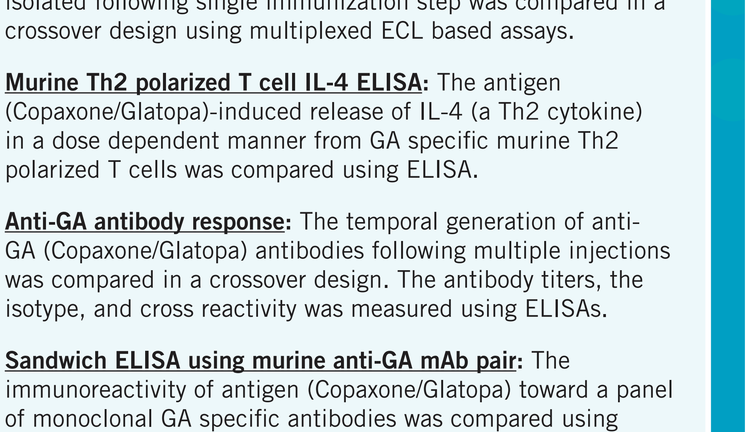
Sandwich ELISA using murine anti-GA mAb pair: The immunoreactivity of antigen (Copaxone/Glatopa) toward a panel of monoclonal GA specific antibodies was compared using sandwich ELISAs.

Experimental autoimmune encephalomyelitis (EAE) model: please refer to methods in Ref. 12.

Histamine release assay: The antigen (Copaxone/Glatopa)-induced release of the histamine from RBL-2H3 (human mast cell line) was compared using ELISA.

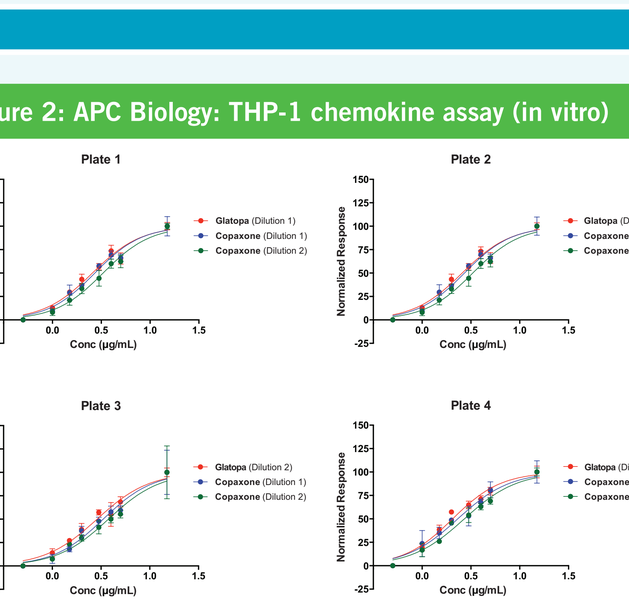
RESULTS AND DISCUSSION

Figure 2: APC Biology: THP-1 chemokine assay (in vitro)



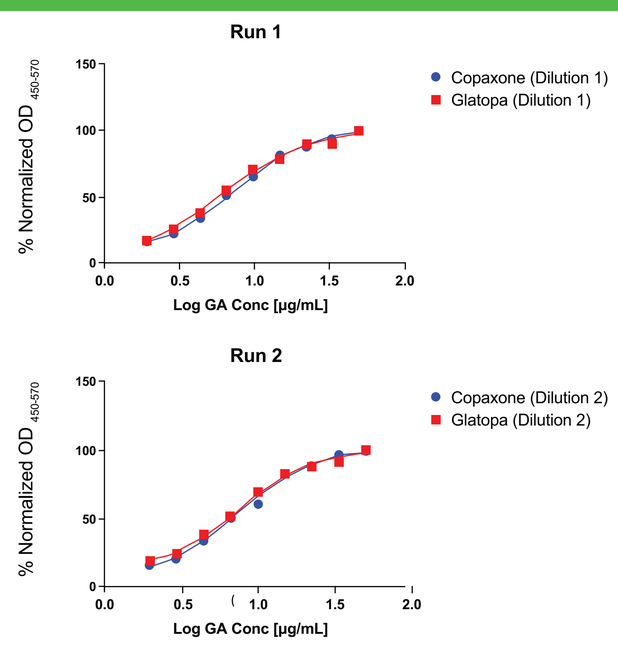
- The GA-stimulated release of monokine induced by interferon-gamma (MIG) from THP-1 cells was used as a measure of equivalence between the 2 products.
- There were no statistically significant differences between Glatopa and the 2 lots of Copaxone.

Figure 3: T Cell Biology: Generation of murine Th2 polarized T cells (in vivo/ex vivo)



- T cells generated with Copaxone as the immunizing antigen were challenged with either Copaxone or Glatopa. Similarly, T cells generated with Glatopa as the immunizing antigen were challenged with either Glatopa or Copaxone.
- There were no statistically significant differences in the secretion of Th2 (IL-4 shown) and Th17 cytokines between Glatopa and Copaxone.

Figure 4: T Cell Biology: Murine Th2 polarized T cell IL-4 ELISA (in vitro)

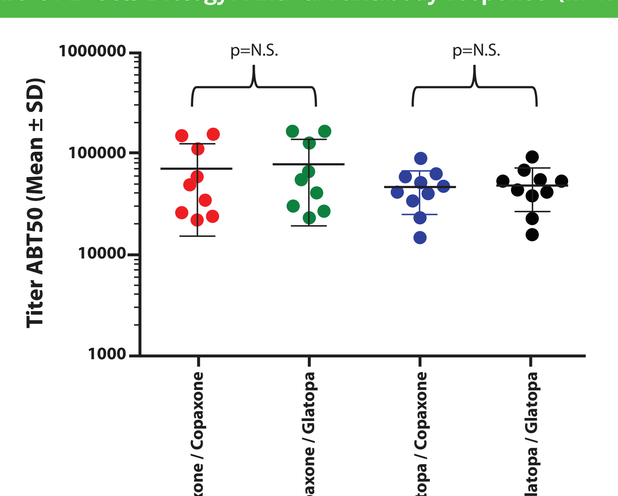


- Shown are representative IL-4 response curves for 2 dilutions of each GA.
- There were no statistically significant differences between Glatopa and Copaxone.

Figure 5: B Cell Biology: Anti-GA antibody response (in vivo)

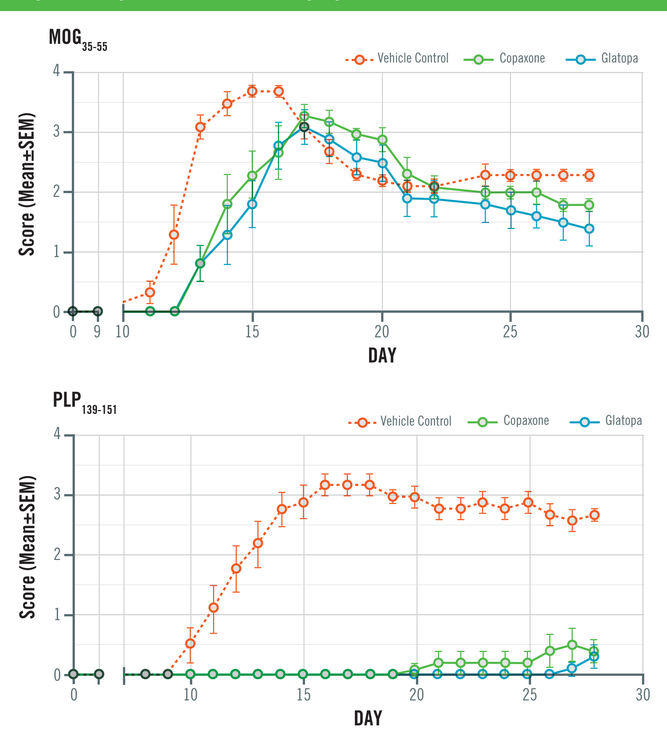
- Sera samples from mice immunized with Glatopa or Copaxone generated a robust antibody titer at Day 28, which cross-reacted equally with both antigens within each individual animal.
- There were no statistically significant differences in the antibody titers obtained in the treatment groups immunized with Glatopa or Copaxone independent of the capture antigen.

Figure 6: B Cell Biology: Sandwich ELISA using murine anti-GA mAb pair (immunological)



- EC_{50}s of 3 lots of Glatopa were not statistically significantly different from 3 lots of Copaxone for the monoclonal antibody pair shown above.
- The epitopes recognized by this monoclonal antibody pair were in the same abundance in both Glatopa and Copaxone, evidence of a similar "immunofingerprint" between these 2 versions of GA.

Figure 7: Aggregate Biology: Experimental Autoimmune Encephalomyelitis (EAE) Prophylaxis Model (in vivo)



- In both PLP and MOG versions of the EAE model, Glatopa and Copaxone delayed symptom onset and reduced the magnitude of "disease" intensity.
- There were no statistically significant differences between Glatopa and Copaxone.

Figure 8: Miscellaneous: Histamine Release from RBL-2H3 Cells

- The positive control (calcium ionophore; A23187) shows histamine levels 6 times greater than media control (not shown; p<0.0001).
- There was no significant difference between Glatopa and any of the Copaxone lots tested.

CONCLUSIONS

- This comprehensive approach across different categories of biological and immunological pathways modulated by GA establishes the biological equivalence of Glatopa and Copaxone 20 mg.
- These results were supportive of and consistent with results from a larger program to demonstrate equivalence of Glatopa and Copaxone 20 mg across biological and physiochemical aspects of GA (see Posters P641 and 1143 and Reference 12).

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