Journal of Neuroimmunology 290 (2016) 84–95

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Functional effects of the antigen glatiramer acetate are complex and tightly associated with its composition

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ARTICLE INFO

Article history:
Received 30 September 2015
Received in revised form 16 November 2015
Accepted 23 November 2015

Keywords:
Multiple sclerosis
Gene expression
Glatiramer acetate
Microarray
Therapeutic equivalence

ABSTRACT

Glatiramer acetate (Copaxone®; GA) is a non-biological complex drug for multiple sclerosis. GA modulated thousands of genes in genome-wide expression studies conducted in THP-1 cells and mouse splenocytes. Comparing GA with differently-manufactured glatiramoid Polimunol (Synthon) in mice yielded hundreds of differentially expressed probesets, including biologically-relevant genes (e.g. *Il18*, adj $p < 9e-6$) and pathways. In human monocytes, 700+ probesets differed between Polimunol and GA, enriching for 130+ pathways including response to lipopolysaccharide (adj. $p < 0.006$). Key differences were confirmed by qRT-PCR (splenocytes) or proteomics (THP-1). These studies demonstrate the complexity of GA's mechanisms of action, and may help inform therapeutic equivalence assessment.

1. Introduction

Glatiramer acetate (GA; Copaxone®) has been safely used to treat multiple sclerosis (MS) patients for almost two decades. Compositionally, it is a heterogeneous colloidal mixture of polymeric molecules, each up to 300 amino-acids long. Up to 10^{29} variants are present in the mixture, subsets of which exist at subpicogram quantities and thus remain unidentifiable with current state-of-the-art methodologies. Upon subcutaneous injection, GA is immediately hydrolyzed at the site of the injection, resulting in an undetectable systemic pharmacokinetic profile. Furthermore, the therapeutic properties of GA have yet to be correlated with any type of validated pharmacodynamic biomarker. Indeed, GA's active moiety(ies), ie, the specific amino acid sequences (acting effectively as immunological "epitopes", or antigenic motifs that uniquely activate certain aspects of the immune system) responsible for its clinical efficacy, have yet to be identified. Harnessing high resolution, comprehensive, and unbiased methods is essential to furthering the scientific understanding of the complex mode of action of GA.

The clinical effects of GA are believed to result from its functioning as an antigen modulating the immune system. GA was designed to mimic the autoantigen myelin basic protein (MBP), which is attacked by the immune system in multiple sclerosis. After degradation at the injection site, GA is thought to bind MHC Class II molecules on antigen-presenting cells (APCs), which interact with naïve T-cells, generating GA-specific T-cells and shifting their phenotype from pro-inflammatory helper-T types 1 and 17 (Th1/Th17) to anti-inflammatory regulatory T cells (Tregs) and helper-T type 2 (Th2) cells (Duda et al., 2000; Neuhaus et al., 2000; Arnon and Aharoni, 2004). GA-specific T-cells are able to migrate through the blood–brain barrier (BBB) and some of them cross-react with the similarly-structured MBP or other myelin associated antigens inducing local secretion of anti-inflammatory cytokines at the site of the lesions, shifting the balance from a pro-inflammatory (Th1/Th17) to anti-inflammatory (Th2/Treg) phenotype (Neuhaus et al., 2000; Arnon and Aharoni, 2004; Aharoni et al., 2010). GA also induces type-II monocytes, which direct differentiation of Th2 and protective Tregs (Kim et al., 2004; Weber et al., 2007), an effect that is independent of antigen specificity; thus cross-reactivity of T cells with myelin antigen is not required for therapeutic benefit (Weber et al., 2007). In addition, GA promotes production of neurotrophic factors such as BDNF by T cells (Arnon and Aharoni, 2004), and induces B-cell activation, which appears necessary for GA response in animal models (Jackson et al., 2014). Data from GA-treated MS patients indicate that GA also modulates CD8+ T cell

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http://dx.doi.org/10.1016/j.jneuroim.2015.11.020

Table 1
Numbers of differentially expressed probesets in each model system.

a) Mouse splenocyte data:

Immunization	Treatment comparison	Total	Up-regulated	Up, FC ≥ 2	# pathways	Down-regulated	Down, FC ≤ −2	# pathways
Copaxone	Copaxone-mannitol	16,647	8342	411	76	8305	485	56

Immunization	Treatment comparison	Total	Up-regulated	Up, FC ≥ 1.2	# pathways	Down-regulated	Down, FC ≤ −1.2	# pathways
Copaxone	(Polimunol-mannitol)-(Copaxone-mannitol)	223	208	73	22	15	6	0
Polimunol	(Polimunol-mannitol)-(Copaxone-mannitol)	431	301	77	10	130	22	0
Corresponding to treatment	$(\text{Polimunol-mannitol})_{\text{Pol}}$-$(\text{Copaxone-mannitol})_{\text{Cop}}$	462	362	206	25	100	30	3

b) THP-1 data:

	Treatment comparison	Total	Up-regulated	Up, FC ≥ 1.3	# pathways	Down-regulated	Down, FC ≤ −1.3	# pathways
Pre-filtering	Copaxone-mannitol	12,115	5296	456	180	6819	183	6
Post-filtering	Copaxone-mannitol	12,001	5227	456	180	6774	183	6

	Treatment comparison	Total	Up-regulated	Up, FC ≥ 1.1	# pathways	Down-regulated	Down, FC ≤ −1.1	# pathways
Pre-filtering	(Polimunol-mannitol)-(Copaxone-mannitol)	807	518	241	177	289	80	0
Post-filtering	(Polimunol-mannitol)-(Copaxone-mannitol)	779	494	218	137	285	78	0

activity (Karandikar et al., 2002). Additional mechanisms may also be involved in GA action.

The complexity of GA's mechanism of action (MoA) cannot be fully captured by any one in vitro or in vivo system. Genome-wide expression profiling of splenocytes extracted from GA-treated mice serves to capture elements of the biological impact in T cells, given the observed physicochemical differences between GA and other glatiramoids (Weinstein et al., 2015). This model system has demonstrated utility as a means to model GA's interaction with lymphocytes (Bakshi et al., 2013; Towfic et al., 2014); and similar studies in a human monocyte cell line have been used to study GA's impact on APCs (Kolitz et al., 2015). Together, these studies represent all three key elements required for GA's activation of "the immunological triad" — including lymphocytes, immunogenic epitopes, and APCs (Jackson et al., 2014; Sellebjerg et al., 2013), while also accounting for some of the factors that further modulate therapeutic response in vivo. Utilizing learnings from previous studies of GA in these cell types, a reciprocal-control experimental design was applied in order to further elucidate GA's MoA and to assess the degree to which these mechanisms are sensitive to differences in composition and manufacturing of the therapeutic antigen. Namely, mouse splenocytes were utilized to model three likely clinical scenarios in a single experiment. The first scenario models a patient treated initially with GA and then switched to a follow-on glatiramoid (FOGA), which is modeled by immunizing mice with GA and then comparing the gene expression profiles when extracted splenocytes are activated ex vivo with either GA or FOGA. The second scenario is one in which a patient is treated initially with a FOGA and then switched to GA, which is modeled by immunizing mice with FOGA and then comparing the gene expression profiles when extracted splenocytes are activated ex vivo with either GA or FOGA. The third scenario models a setting in which a patient is treated purely with either GA or FOGA, such that comparison of the gene expression profiles derived from the activated splenocytes in each experiment may reflect long-term treatment-induced functional differences. This experimental design was combined with mRNA and proteomic studies conducted with the same glatiramoids in a human monocyte cell line, previously published as consistent with human primary monocyte studies in glatiramoids (Kolitz et al., 2015). Taken together, these orthogonal experimental models, biomarker technologies, and functional analyses provide important insights into GA's mode of action and differences with FOGAs.

The analysis of multiple lots of GA was conducted in parallel to Synthon's FOGA (Polimunol®), marketed in Argentina as a purported clinical equivalent to GA (as of May 2014). Polimunol is believed to be the same Synthon FOGA (entitled "GTR") used in the only bridging clinical trial conducted to date with the objective of demonstrating surrogate equivalence to GA in terms of MRI measurements (GATE study, NCT Number NCT01489254). The authors report that for T1-GdE lesions during months 7–9 the "GTR/GA ratio was within the pre-defined margins". However, according to results reported from the placebo-controlled phase of the study (Cohen et al., 2014), clinical equivalence in terms of annualized relapse rate (ARR), as well as correlation between MRI-lesion predictors and ARR reductions, have not been met, indicating lack of sameness in therapeutic effect. It is for these reasons that studies comparing Synthon's FOGA to GA using high-resolution physicochemical tests, as well as genome-wide expression studies in immunologically relevant model systems, are of keen interest to the scientific and medical community worldwide, considering that no traditional therapeutic equivalence methods have been shown to be clinically validated for this class of compounds. Thus, careful investigation and establishment of novel preclinical and clinical standards are needed in evaluating therapeutic equivalence between FOGAs and GA.

2. Materials and methods

2.1. Reciprocal mouse splenocyte study: experimental design and methods

2.1.1. Mice

All experimental procedures conformed to accepted ethical standards for use of animals in research and were in accordance with Committee for the Care and Use of Experimental Animals guidelines and approved by the Teva Institutional Animal Care and Use Committee. 8- to 12-week-old female (Balb/c X SJL) F1 mice (Janvier, France) were purchased, and kept at 21 ± 3 °C with relative humidity 30%–70%, and light/dark cycle 12:12 h. Animals were maintained on a standard rodent pellet diet and sterile filtered tap water available ad libitum.

2.1.2. Immunization of mice and preparation of ex vivo spleen cell cultures

To stimulate induction of GA and FOGA-reactive T cells, twelve mice in each treatment group were injected subcutaneously with 100 μL of a 2.5 mg/mL solution of either Copaxone (GA drug product, Teva Pharmaceutical Industries, Petach Tikva, Israel) or Polimunol (FOGA drug product, Synthon, Nijmegen, Netherlands) in phosphate-buffered saline (250 μg GA per mouse). Mice were housed for 3 days after immunization, then sacrificed. Spleens were aseptically removed and placed on ice in RPMI 1640. A single cell suspension was prepared. After red blood cell lysis, splenocytes from the same immunization group were pulled and resuspended to a final concentration of 10×10^6 cells/mL in defined cell culture medium (DCCM1) (Biological Industries, Beit