Glatiramer acetate: Mechanisms of action in multiple sclerosis

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Abstract

Glatiramer acetate (GA) is a mixture of synthetic polypeptides composed of four amino acids resembling myelin basic protein (MBP). GA has been shown to be effective in preventing and suppressing experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. It was tested in several clinical studies and approved for the immunomodulatory treatment of relapsing-type MS in 1996. Glatiramer acetate demonstrates a strong promiscuous binding to major histocompatibility complex molecules and inhibits the T cell response to several myelin antigens. In addition, it was shown to act as a T cell receptor antagonist for the 82–100 MBP epitope. Glatiramer acetate treatment causes *in vivo* changes of the frequency, cytokine secretion pattern and effector function of GA-specific T cells. It was shown to induce GA-specific regulatory $CD4^+$ and $CD8^+$ T cells and a TH1–TH2 shift with consecutively increased secretion of antiinflammatory cytokines. GA-specific TH2 cells are able to migrate across the blood–brain barrier and cause in situ bystander suppression of autoaggressive TH1 T cells. In addition glatiramer acetate was demonstrated to influence antigen presenting cells (APC) such as monocytes and dendritic cells. Furthermore secretion of neurotrophic factors with potential neuroprotective effects was shown. © 2007 Elsevier B.V. All rights reserved.

Keywords: Glatiramer acetate; Multiple sclerosis; Experimental Autoimmune Encephalomyelitis (EAE); Neuroprotection; Myelin Basic Protein (MBP)

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1. Multiple sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory demyelinating disease affecting about 0.1% of the population in temperate climates. It varies in terms of clinical, radiological and pathological features and is characterized by physical and neuropsychological symptoms. Pathological findings include axonal damage, brain atrophy and demyelinating plaques consisting of inflammatory cells [1]. Different disease entities are known leading from relapsing–remitting (RRMS) to primary (PPMS) and secondary progressive forms (SPMS). Current concepts assume that MS occurs as a consequence of immune tolerance breakdown in genetically susceptible individuals. The major contributing factors include environmental factors and immune dysregulation.

The mechanisms of CNS inflammation involve activation of autoreactive, myelin specific T helper (TH) cells in the periphery possibly by molecular mimicry. These activated lymphocytes are able to cross the blood–brain barrier and are reactivated by recognising their specific target antigen presented by antigen presenting cells (APC) e.g. microglia. Reactivation of T cells results in increased secretion of proinflammatory cytokines and chemokines. An inflammatory cascade is initiated which causes activation and recruitment of macrophages and other inflammatory cells to the site of inflammation. Activation of B cells leads to augmented release of anti-myelin antibodies which bind complement and stimulate opsonization of myelin peptides. This ongoing destruction of the myelin sheath causes reversible and to some extent irreversible impairment of functionality of the axon by deterioration of its conduction properties. As a major consequence of inflammation, axonal loss by binding of cytotoxic $CD8^+$ T cells to demyelinated axons, release of cytotoxic factors and cytokines is proposed. In addition mechanisms not directly related to demyelination and inflammation i.e. excitotoxicity caused by glutamic acid is shown to initiate a process of neuronal cell death [1].

2. Drug development and clinical studies

Glatiramer acetate is a random mixture of synthetic polypeptides composed of four amino acids (L-glutamic acid, L-lysine, L-alanine and L-tyrosine) in a residue molar ratio 4.2 : 3.4 : 1.4 : 1.0 with an average molecular mass of 4700–11,000 Da. Myelin basic protein (MBP) is a major protein of the myelin sheath and is used as encephalitogenic protein for induction of experimental autoimmune encephalomyelitis (EAE) in animals. Glatiramer acetate was discovered in the 1960s when

studies on the immunological properties of a series of polymers and copolymers developed to resemble MBP were conducted. Instead of EAE induction several of these polypeptides were able to decrease the severity or prevent EAE [2]. Copolymer 1, later known as glatiramer acetate, was shown to be the most effective polymer. It took several years until first exploratory open studies on patients with either relapsing remitting or secondary progressive multiple sclerosis were performed in the late 1970s and early 1980s. However the results of these studies must be interpreted with caution as drug production was not standardized before 1991. In 1991 a phase III multicentre, double-blind, placebo-controlled trial with standardized glatiramer acetate preparation was conducted at 11 US medical centres with 251 RRMS patients receiving treatment for two years. Relapse rate decreased about 30% which was statistically significant ($P=0.007$) [4]. In 1996 GA was approved by the US Food and Drug Administration (FDA) as a treatment for patients with active relapsing–remitting MS. Since then, studies using an oral formulation of GA (CORAL) and applying GA to primary progressive multiple sclerosis patients (PROMISE) failed to demonstrate significant treatment effects. There are promising new data on using a higher GA dose (40 mg) which are currently studied in a multi-centre, double-blind, controlled trial (FORTE).

3. GA as antigen-based therapy

In contrast to other immunomodulatory MS therapies glatiramer acetate seems to preferentially affect immune cells in an antigen-specific way. The peptide mixture is applied to patients with a putative autoimmune disease over many years by daily injection. From a broader perspective GA is one of the few practical examples of therapeutic vaccination distinct from prophylactic vaccination against infectious diseases. The precise mechanism of action of glatiramer acetate is not fully understood yet. The mechanisms discussed include competition with myelin peptides especially MBP for binding to major histocompatibility molecules (MHC) on APC, antagonism at the T cell receptor of myelin specific T cells and induction of GA-specific suppressive/regulatory T cells secreting potentially antiinflammatory cytokines upon activation (Table 1). The vast majority of studies performed initially in EAE and subsequently in MS have focused on evaluating and targeting T cell responses especially the role of $CD4^+$ T cells. Nevertheless there is increasing evidence for immunomodulating effects of GA on $CD8^+$ T cells but also on B cells and APC.

Table 1
Immunological effects of glatiramer acetate

	Effect
MHC II-complex	● Promiscuous binding to various HLA-DR alleles [5]
MBP–MHC-complex	● Displacement of already bound MBP from binding site of MHC II-complex
T cell receptor	● T cell receptor antagonism to the 82–100 MBP epitope [15]
T cell proliferation *in vivo* after treatment	● Decreased GA specific T cell frequency in patients on GA treatment [15,18]
T cell proliferation *in vitro*	● Suppression of MBP specific T cell lines [6] ● Reduced proliferation of GA-specific CD4$^+$ T cells [23]
T cell migration	● Reduced, mechanism unclear
T cell features	● Shift from TH1 to TH2 cells [6,14,16,17] ● Bystander suppression [22] ● Induction of suppressive/regulatory CD4$^+$ and CD8$^+$ T cells [25–27]
Effects on monocytes	● Reduced activation upon stimulation [7] ● Increased IL-10, IL-4 secretion [9] ● Inhibition of TNF-α secretion *in vitro* [8]
Effects on dendritic cells	● Inefficient maturation [12] ● Reduced ability to stimulate T-cells [10,11] ● Increased IL-10 secretion [9,10] ● Controversial findings regarding TNF-α [8,9] ● Decreased IL-12 secretion [10]
Effects on macrophages	● Increased IL-10 secretion ● Decreased production of IL-12/TNF-α [11]
Astrocyte/microglia	● Enhanced secretion of IL-10 and TGF-β [14] ● Inhibition of transformation to an activated microglia form [13]
Neuroprotection	● Enhanced secretion of neurotrophic factors (BDNF, NT-3/4) [34] ● Decreasing neuronal damage [35] ● Increased neuronal proliferation [35]

3.1. Effects of GA on APC

In vitro studies have shown that GA competes with myelin peptides from binding to MHC molecules and is able to displace MBP peptides from APC. GA binds to many different alleles of MHC class II molecules without antigen processing and without any obvious preference ("promiscuous binding") [5]. Thus GA inhibits MHC II assisted activation of myelin specific T cells [6]. GA treatment leads to a generalized, antigen-non-specific modulation of APC function i.e. monocytes and macrophages resulting in a reduced reactivity towards proinflammatory stimuli [7]. First *in vitro* experiments demonstrated that GA blocked the activation of a monocytic cell line in the absence of T cells by inhibiting the induction of HLA-DR and HLA-DQ proteins leading to a reduction of Tumor Necrosis Factor α (TNF-α)

release and decline of cathepsin B activity [8]. GA-treated monocytes and macrophages also produced increased amounts of Interleukin-10 (IL-10) [9]. GA-treated dendritic cells were able to shift the phenotype of naive T cells towards TH2 like T cells. In addition GA inhibited the secretion of IL-12 and TNF-α by human dendritic cells and macrophages [10]. In contrast to these findings GA exposed bone-marrow-derived dendritic cells produced increased amounts not only of IL-10 but also of TNF-α upon stimulation maybe due to a less effective autoregulation via IL-10 [9]. Sanna et al. [11] recently demonstrated that in MS patients pretreatment with GA significantly decreased the proliferative effect of dendritic cells on lymphocytes *in vitro*. Furthermore GA treated MS patients showed a significant reduction of GA induced proliferation of peripheral blood mononuclear cells. Plasmacytoid dendritic cells (pDCs) from multiple sclerosis patients were shown to exhibit an inefficient maturation process after stimulation compared to controls. GA treatment partially restored phenotype and function of pDCs in these patients [12]. Chabot et al. [13] demonstrated a reduced ability of GA-treated T cells to interact with microglia. Consecutively the secretion of proinflammatory cytokines was reduced in microglia-T cell-coculture experiments. Furthermore CNS infiltration of GA-specific T cells resulted in increased expression of Transforming Growth Factor β (TGF-β) and IL-10 by resident astrocytes and microglia [14].

3.2. Effects of GA on T cells

In addition to competition for binding to MHC molecules and thus inhibiting the T cell response to several myelin antigens, GA is proposed to act as an antagonist to MBP/MHC at MBP-specific T cell receptors (TCR). It was demonstrated to operate as an "altered peptide ligand" to the 82–100 epitope of MBP [15]. This effect presumably occurs in the periphery at the injection sites or in the corresponding draining lymph nodes. The immunomodulatory effect of GA is primarily attributed to its ability to induce *in vivo* changes of the cytokine secretion pattern and the effector function of GA-reactive T helper cells. Modulation of the immune response with glatiramer acetate leads to deviation of cytokine production in response to MBP from so-called TH1 cytokines to TH2 cytokines [6,16,17]. This so called TH1–TH2 shift is characterized by increased secretion of antiinflammatory cytokines like IL-4 and decreased TNF-α and Interferon-γ (IFN-γ) production [18]. After prolonged GA treatment T cell response of MS patients remains TH2 biased [19]. In EAE experiments GA-reactive TH2

human TH1 and TH2 GA-reactive T cells were shown to migrate across an artificial blood–brain barrier *in vitro* [21]. So it can be concluded that activated TH2 cells are able to cross the blood–brain barrier (BBB), accumulate in the CNS [20] and express *in situ* antiinflammatory cytokines like IL-4, IL-10, TGF-β and IL-5, which not only modulate the local milieu but are able to suppress the activity of encephalitogenic TH1 cells [16–18]. This so called bystander suppression is regarded as essential part of the mechanisms of action of GA [22] (see Fig. 1). It seems very unlikely that sufficient amounts of GA reach the CNS to compete with locally degraded myelin antigens. So it might be speculated that GA-specific TH2 cells are reactivated in the CNS when crossreacting with locally presented myelin autoantigens.

In the setting of autoimmunity, one of the goals of successful therapeutic immune modulation is the induction of peripheral tolerance, a large part of which is mediated by regulatory T cells. GA treatment gradually reduces the proliferative reactivity of GA-specific CD4$^+$ T cells [23] probably by inducing apoptosis in some T cells [24]. Karandikar et al. [25] demonstrated

that during GA treatment proliferative CD4$^+$ T cell responses were comparable in healthy individuals and MS patients, whereas untreated MS patients showed a deficit in CD8$^+$ T cell-mediated proliferation towards GA compared with healthy subjects. GA-specific treatment enhanced the frequency and suppressive ability of GA specific CD8$^+$ T cells to those seen in healthy individuals. CD8$^+$ T cells from GA treated patients and healthy subjects, but not those from untreated patients with MS, exhibited potent, HLA class I-restricted, GA-specific cytotoxicity [26]. In addition to these regulatory CD8$^+$ T cells Hong et al. [27] demonstrated that expression of Foxp3 in CD4$^+$ T cells was significantly increased in MS patients treated with GA. This induction of regulatory CD4$^+$ T cells was mediated by IFN-γ and to a lesser degree TGF-β1. Adoptive transfer of GA-specific T cells suppresses induction of EAE by different encephalitogens, but cannot cure ongoing EAE, which shows that T cells alone have only a limited role when disease is established [15,22]. By contrast GA injections not only prevent but also suppress established EAE [2].
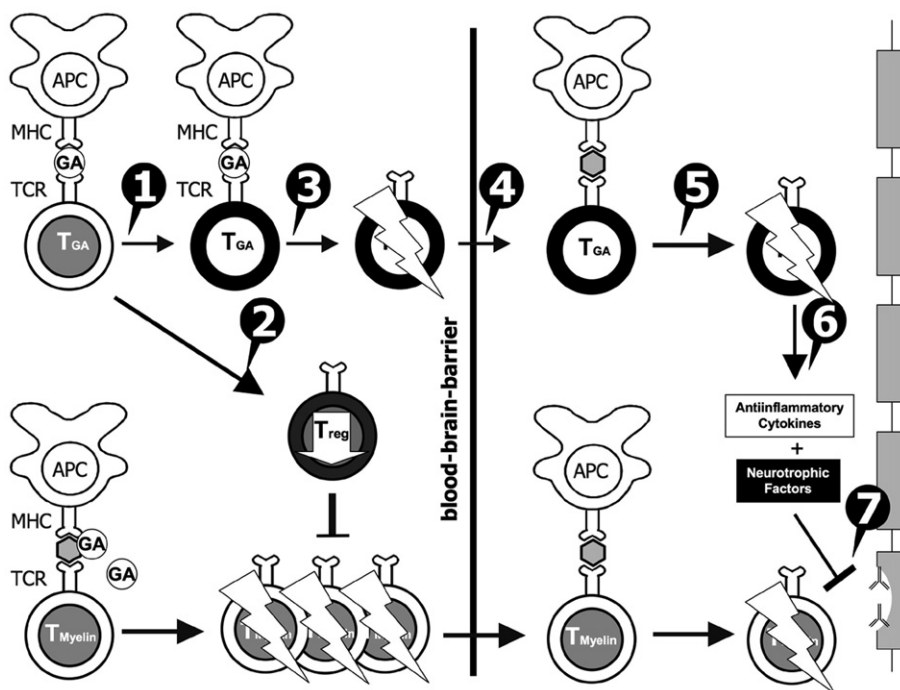

Fig. 1. Mechanisms of action of glatiramer acetate in multiple sclerosis. 1. GA exhibits competitive binding at the MHC-II complex and TCR-antagonism. In addition GA is able to displace MBP from the binding site on MHC-II molecules. Treatment with GA leads to the induction of antigen specific TH2 T cells in the periphery. 2. In addition CD8$^+$ and CD4$^+$CD25$^+$ regulatory T cells are induced by GA therapy. 3. The constant activation seems to have an important impact on the induction and maintenance of the regulatory/suppressive immune cells. 4. Because of the daily activation, GA T cells are believed to be able to cross the blood–brain barrier. 5. Inside the CNS, some GA-specific T cells cross-react with products of local myelin turnover presented by local APCs. 6. In response anti-inflammatory cytokines are secreted which dampen the local inflammatory process

3.3. Effects of GA on B cells

A report including data from three different therapeutic trials demonstrated that all GA treated patients developed anti-GA antibodies which declined 6 months after treatment initiation but were still detectable. Anti-MBP-antibody titers were low and remained unchanged during treatment. Antibodies were of the IgG class with IgG1 isotype levels two- to threefold higher than those of IgG2 [3]. Farina et al. showed GA-specific IgG4 antibodies in GA-treated MS patients in a cross-sectional study. In addition they showed the occurrence of GA antibodies of the IgM, IgG1 and IgG2 class in some untreated patients [28]. Also in GA-treated primary progressive MS patients GA-reactive antibodies of the IgG1 subclass predominated [29]. IgG1 subsequently decreased while anti-GA antibodies of the IgG4 subclass increased and remained high for the 3 years of follow-up. This isotype switch to IgG4 seems to reflect the interaction of B cells with TH2 lymphocytes [30]. The presence of GA-reactive antibodies in some untreated control subjects suggests that such antibodies, which are mainly of the IgM isotype, are present in the common B cell immune repertoire. One might speculate that they are T cell independent, polyspecific "natural" antibodies produced by so called B1 cells.

Teitelbaum et al. did not observe any neutralizing activity in sera of GA-treated patients. GA antibodies did not interfere with GA action neither in terms of MHC binding and T cell stimulation nor regarding suppression of EAE [31]. However *in vitro* experiments by Salama et al. demonstrated inhibition of GA-specific T cell lines by purified GA antibodies [32]. No clear evidence has yet been found that GA antibodies inhibit clinical efficacy *in vivo*. Moreover relapse-free patients seem to develop higher antibody titers, which would be consistent with a beneficial rather than neutralizing activity of the antibodies [3]. In a murine model of demyelinating disease induced by Theiler's virus remyelination of spinal cord axons was enhanced by GA antibodies [33]. These results support the hypothesis that the antibody response in GA treated patients may be beneficial by facilitating repair of demyelinated lesions.

3.4. Neuroprotection

Human GA-specific TH1-, TH2-, and TH0 cells all showed low levels of basal secretion of Brain Derived Neurotrophic Factor (BDNF) and an increase of BDNF production after stimulation [34]. This ability of GA-specific cell could also be demonstrated in situ in the mouse model of MS (EAE) resulting not only in de-

proliferation [35]. Gilgun-Sherki et al. demonstrated a significant reduction of axonal loss and neuronal damage in myelin oligodendrocyte glycoprotein (MOG)-induced EAE, a model characterized by a chronic disease course [36]. Kipnis et al. showed that in a model of traumatic injury of the optic nerve the posttraumatic spread of degeneration could be attenuated by either active immunization with GA on the day of injury or by adoptive transfer of GA-specific T cells [37]. After restimulation by cross-reactive myelin antigens in the CNS, GA-reactive T cells seem to secrete not only immunomodulatory cytokines but also neurotrophic factors. Therefore these T cells might confer neuroprotection in addition to bystander suppression.

3.5. Biomarkers

Similar to effects seen in patients treated with other immunomodulatory drugs some GA-treated patients do not respond to therapy. Early identification of such non-responders would be very useful for adjusting therapy regimens. For this reason there has been much interest in the development of appropriate biomarkers. We are just running a study (COPIMMUNONET) which aims at finding a link between clinical outcome and immunological parameters specific for GA.

Take-home messages

- Glatiramer acetate is an approved drug for the treatment of relapsing–remitting multiple sclerosis, a chronic inflammatory disease of the central nervous system.
- Treatment with glatiramer acetate results in reduction of clinical exacerbations and progression rate in multiple sclerosis patients as well as a decrease of burden lesion shown on MRI scans.
- Its proposed mechanisms of action include competition with myelin antigens for binding to APC, antagonism at specific T cell receptors, induction of GA-specific regulatory CD4$^+$ and CD8$^+$ T cells which downregulate inflammation by secretion of antiinflammatory cytokines and cause bystander suppression of proinflammatory TH1 cells in the CNS.
- In addition, neuroprotective effects as well as regulatory effects on B cells, monocytes and dendritic cells have been shown.

References

[1] Steinman L. Multiple sclerosis: a two-stage disease. Nat

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