PROSPECTS FOR NEUROPROTECTION IN MULTIPLE SCLEROSIS

V. Wee Yong

Departments of Oncology & Clinical Neurosciences, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Pathology of MS: prevalence of axonal and neuronal injury and loss
4. Factors that contribute to the neurodegenerative components of MS
5. Can T cells injure neurons through antigen non-specific mechanisms?
6. Beneficial Aspects of Neuroinflammation
7. Neuroprotective Capacity of Currently Used MS Therapeutics
8. Other Potential Neuroprotective Agents for MS
9. Perspective
10. Acknowledgement
11. References

1. ABSTRACT

Axonal injury and neuronal loss are now recognised to be hallmarks of multiple sclerosis (MS) in addition to neuroinflammation and demyelination. This review discusses the factors that contribute to neural degeneration, and it emphasizes the need to confer neuroprotection in MS. The beneficial role of neuroinflammation is highlighted, and the possibility that glatiramer acetate enables neuroprotection in MS through beneficial inflammation is evaluated. Finally, the prospect of an experimental treatment, minocycline, in producing neuroprotection in MS is suggested.

2. INTRODUCTION

Just over ten years ago, there was no approved treatment for MS. Since then, with the introduction of the interferon-β’s (Betaseron®, Avonex® and Rebif®) and glatiramer acetate (Copaxone®), the treatment options for patients with relapsing-remitting MS have dramatically expanded. Other immunomodulators have also become available (e.g. cyclophosphamide and mitoxantrone) to add to the armamentarium and the notion of combining therapies is now realistic. The sudden wealth of effective agents in MS has engendered more forward-looking thoughts on further improving the prognosis of the disease, including the prospects of conferring neuroprotection and regeneration. It is the possibility of providing for neuroprotection in MS that will form the basis of this review. I will describe first the pathology of MS, focussing on the axonal degeneration and neuronal loss, and I will discuss the literature that detrimental aspects of neuroinflammation contribute to that pathology. I shall then review contrasting evidence that neuroinflammation has beneficial roles, including in MS, and I will further explore themes of neuroprotection in the disease. Finally, the likelihood that currently used MS drugs impact upon neuroprotection will be discussed.

3. PATHOLOGY OF MS: PREVALENCE OF AXONAL AND NEURONAL INJURY AND LOSS

The classical view of MS is that it is a disease of oligodendrocyte and myelin loss with relative sparing of axons, even though axonal degeneration was recognised from the early descriptions of the disease. In the late 1990s, exquisite microscopy techniques enabled investigators to refocus attention to the fact that MS is accompanied by substantial axonal atrophy and loss (1,2). Indeed, the extent of axonal loss in the spinal cord of non-ambulatory MS subjects can exceed 80% (3) and progression of disease may be best correlated with the continuing loss of axons (4). In addition, we now appreciate that neuronal loss in MS is also significant. This is illustrated by the report of a 30% decrease in volume of the sub-thalamic region, assessed using MRI, as well as by histological counts of autopsied specimens (5). Recently, a 19% decrease in MRI-defined bicaudate volume (6) as well as a 7% focal thinning of the cerebral cortex by MRI was also noted (7). Thus, it is clear that MS is not only an inflammatory demyelinating disease, but also a degenerative one.

Axonal injury occurs from early in the MS disease process. By N-acetyl-aspartate (NAA) spectroscopy in patients with definite MS who do not yet show clinically significant disability (EDSS <2), diffuse axonal injury in the normal appearing white matter is already evident (8). In clinically isolated syndrome (CIS), whole brain NAA is reduced compared to that in normal individuals (9).
Figure 1. The presence of β-APP-positive profiles in early active MS lesions, as well as the decrease of N-acetylaspartate signal by spectroscopy in early MS, illustrate that axonal injury occurs from early on in the disease process. Some injured axons may recover but it is likely that most will go on to degenerate to result in progressive axonal loss. In this graph, demyelination is shown to precede axonal loss but it is possible that the reverse occurs in some lesions.

Histological investigations have also confirmed that axonal injury is seen in early evolving MS lesions. The criterion of immunoreactivity for myelin oligodendrocyte glycoprotein (MOG) within macrophages in MS lesions (10) defines these as early active lesions, given that MOG is found on the outermost layer of myelin and would thus be one of the first myelin proteins to appear within phagocytic macrophages or microglia. In these early active lesions, high density of immunoreactive beta-amyloid precursor protein (β-APP) in axons, a marker of axonal injury, can already be observed. β-APP is a normal constituent of neurons that is transported down axons by fast axoplasmic flow. When an axon is injured, it accumulates to levels that then become detectable by immunohistochemistry (11). Indeed, Kuhlmann et al. (12) have reported the surprising finding that β-APP immunoreactivity is most abundant in active lesions from patients with less than 1-year duration of disease, compared to those with longer disease duration. This finding indicates that either the immune cells migrating into the CNS is most rabid in the initial stages of MS, or that the reaction of the CNS to inflammation is exaggerated early in the disease course. Nonetheless, the results do indicate the need to confer neuroprotection in MS from the very early stages in the disease.

In summary, there is increasing appreciation that MS is not only a degenerative disease involving loss of axons and neurons in addition to myelin and oligodendrocytes, but that the axonal perturbations occur from early on in the disease process (Figure 1). Indeed, in some lesions, axonal loss may even precede the demyelination (13). The long-term consequence of the early axonal injury is as yet unclear; some perturbed axons may have intrinsic repair capacity to recover fully but it is likely that most will go on to degenerate to account for the progressive axonal loss noted in MS.

4. FACTORS THAT CONTRIBUTE TO THE NEURODEGENERATIVE COMPONENTS OF MS

There are multiple factors that may result in the injury to axons and neurons in MS and almost all these are associated with neuroinflammation. The CNS is normally a region where immune reactivity is kept at a low basal surveillance level, so that a substantial elevation of various inflammatory components as occurs in MS then renders the tissue susceptible to injury. Several studies of autopsied specimens have indicated a good correlation between expression of β-APP or transected axons with the number of activated microglia/macrophages and T cells (1,2,10,14). The products of activated leukocytes include inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), which have been reported to be toxic to oligodendrocytes and neurons under some conditions (15-17). This is controversial, however, since protective effects of cytokines against neuronal injury have also been observed in certain situations (18,19). Other leukocyte products that may be damaging to neurons and axons include free radicals, nitric oxide, matrix metalloproteinases (MMPs) and cell surface associated molecules such as FasL (20-23). Complement activation and the deposition of membrane attack complex is also a potent means to induce axonal injury (24) and microglia...
5. CAN T CELLS INJURE NEURONS THROUGH ANTIGEN NON-SPECIFIC MECHANISMS?

Although there is good evidence for the initiation of neuroinflammation through antigen-specific mechanisms in models of MS, including experimental autoimmune encephalomyelitis (EAE), many of the T cells that subsequently enter into the CNS may be antigen non-specific. In EAE where antigen-specific cells can be detected on neurons in brains from Rasmussen encephalomyelitis and these appear in close apposition to T cells, indicating that potential toxicity of T cells to neurons can occur in an MHC dependent manner in vivo (30).

6. BENEFICIAL ASPECTS OF NEUROINFLAMMATION

Despite the well-documented detrimental consequences of neuroinflammation, it has become evident in recent years that neuroinflammation has beneficial properties as well. In the situation of CNS trauma, such as in spinal cord injury models, the implantation of macrophages or microglia post-injury can alleviate the degenerative process to result in some regeneration of axons (35-37), although the converse has also been found (38,39). Similarly, an initial microglia activation in brain trauma could be a beneficial response, since microglial-derived IL-1β regulates the subsequent production of a growth factor, ciliary neurotrophic factor (CNTF) (40). Many cytokines are recognized to have direct neurotrophic properties, including the induction of neurite extension by neurons in culture (41).

Schwartz and colleagues have championed the concept that T cells can also have beneficial properties (42). The model that these investigators have used involves a crush injury to structures such as the optic nerve. In animals that are not treated following a crush injury to the optic nerve, there is the primary loss of cells in the immediate period at the epicentre of the injury. Over time, however, there is progressive loss of structures, including axons and projection neurons, presumably because these have not been able to recover from the original injury (Figure 2). This delayed death is referred to as secondary degeneration and its extent is substantial. Indeed, in the optic nerve, there is a 25% loss of projection retinal ganglion neurons at 1 week following the crush of the optic nerve, but this increases to 55% by the second week of injury (43). Because secondary degeneration occurs over a period of time, there is optimism that a time window of opportunity exists to alleviate secondary degeneration or to confer neuroprotection following an insult.

Schwartz et al. reported that following a crush injury to the optic nerve, mice injected with myelin-
reactive T cells had lower loss of retinal ganglion neurons resulting from the trauma compared to untreated animals (44) (Figure 2). Similar neuroprotection by myelin-reactive cells has also been observed after a spinal cord crush in rats (45). Presumably, these myelin reactive T cells undergo reactivation at the site of the injury by virtue of antigen (myelin components) presentation by microglia or macrophages that have engulfed degenerating myelin. The concept that some T cells can confer neuroprotection is referred to as protective autoimmunity (46) although some have preferred the term neuroprotective immunity (47). It highlights the concept that not all activated T cells are necessarily harmful, but that some of these are even neuroprotective in disease states. Indeed, the suggestion has been put forth that MS is contributed by the malfunctioning of a physiological autoimmune response whose purpose is protective (48). Other groups have since confirmed the beneficial properties of T cells in a variety of conditions, including in facial nerve resection (49), or in revascularization following an aseptic cerebral injury induced by a cold probe to the mouse cerebral cortex (50).

The mechanism of neuroprotective immunity is still unclear although a favored hypothesis is that these leukocytes bring with them neurotrophins and other growth factors into the CNS (Table 2). It is now clear that leukocytes that accumulate in human MS brain lesions (51) or in experimental models of injury (15) express brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and nerve growth factor (NGF). These neurotrophins are survival factors that have the capacity to promote the well being of injured axons and neurons, and they may also facilitate the survival of oligodendrocytes and thereby preserve myelin (47,52). Another advantage of neurotrophins is their ability to modulate immune reactivity (53). Some neurotrophins have been shown to decrease antigen presentation (54) and thereby potentially reduce detrimental inflammation in the CNS. NGF has been shown to reduce the transmigration of monocytes across a model of the blood-brain barrier (55) and also to promote a Th2 milieu within the CNS of marmosets (56).

The concept of neuroprotective immunity to treat diseases of the CNS is still in its infancy. It can be a dangerous manoeuvre, particularly since the arrival of many activated T cells can destroy neurons, as pointed out earlier. In the reports that the administration of autoreactive T cells conferred neuroprotection to some axonal tracts, a side effect was the development of EAE in animals (44,45). Others have also noted that the massive accumulation of MBP-specific T cells in experimental models of trauma is degenerative (57,58).

The postulate that neuroprotective immunity involves neurotrophic factors is by no means resolved. Also, whether particular T cell subsets, including the T helper 1 or 2 effectors, are more neuroprotective than others, remain unclear. There have been other potential explanations for why leukocytes could be neuroprotective (Table 2), including that these cells deliver molecules such as anti-thrombin-3 into the CNS to detoxify thrombin with potential neurotoxic properties (59).

There has also been interest in other neurotrophic factors in the context of EAE and MS. In EAE, mice genetically deficient for CNTF (60) and leukemia
glatiramer acetate or glatiramer acetate-reactive T cells (67) showed that the treatment of animals with a variety of degenerative processes including in MS. Schori et al. (68) reported that the mean NAA:Cr ratio by spectroscopy increased by 5.5% in 10 patients on interferon-β after 1 year of treatment compared to pre-treatment values. However, this suggestion of neuroprotection was not supported by a subsequent report by Parry et al. (73) in which the mean NAA:Cr ratio decreased by 6.2% after 1 year of treatment. Finally, with respect to the proportion of contrast enhancing lesions that involved into “black holes”, Bagnato et al. (74) found no evidence of a reduction in this parameter in 10 relapsing-remitting MS patients given interferon-β.

On balance, it appears that glatiramer acetate may have the capacity to confer neuroprotection. The case for interferon-β is less strong, although it may be argued that decreasing pathogenic inflammation from entering the CNS (even at the cost of blocking the entry of some beneficial ones) would ultimately lead to resolution of damaging inflammation, and thereby neuroprotection. However, this indirect mode may not offer protection to neural elements already undergoing degeneration, as might be conferred by the physical presence of glatiramer acetate-reactive cells

868
EAE. For this reason, we have used minocycline in a to address possible neuroprotection of minocycline in spinal cord is not consistent across animals, it is difficult disease in which the inflammation across a section of the excitotoxicity (82). Because of these multiple activities, Minocycline has also been reported to inhibit glutamate activities, including the inhibition of microglial activation, the inhibition of activity of caspases-1 and -3, which are involved in the regulation of apoptosis (79-81). It turns out that minocycline has a myriad of activities, including the inhibition of microglial activation, the inhibition of activity of caspases-1 and -3, which are involved in the regulation of apoptosis (79-81). Minocycline has also been reported to inhibit glutamate excitotoxicity (82). Because of these multiple activities, we have asked whether minocycline might be able to confer neuroprotection. Because EAE is a variable disease in which the inflammation across a section of the spinal cord is not consistent across animals, it is difficult to address possible neuroprotection of minocycline in EAE. For this reason, we have used minocycline in a compression injury to the spinal cord of mice. We have determined that the administration of minocycline from 1 hour after the injury resulted in the attenuation of the lesion size in the spinal cord, axonal sparing, and also improves the motor behaviour of animals (83). These results suggest the prospects of minocycline not only as an immunomodulator in MS, but also as a potential neuroprotective agent in the disease (Table 3).

A Phase I trial of Minocycline in MS has been concluded and the results have been encouraging (84; Metz et al., manuscript in preparation). Future studies will clarify whether this potentially useful agent will have utility in MS, either when used in isolation, or when combined with existing MS therapeutics.

Finally, the sodium channel blocker, phenytoin, has been found to be neuroprotective in EAE (85); whether this will have utility in MS deserves attention. It is likely that the literature on neuroprotection in EAE, and hopefully in MS, will expand significantly in the near future.

9. PERSPECTIVE

As the pathology of MS becomes increasingly understood, it has become clear that this is not only an inflammatory demyelinating disease, but also a degenerative one. Thus, useful therapies must combine immunomodulatory actions with neuroprotective ones. Of the current available immunomodulators, there is stronger evidence for possible neuroprotective efficacy of glatiramer acetate compared to other drugs. It is also becoming clear that not all aspects of neuroinflammation are necessarily harmful and that the non-specific inhibition of inflammation may be detrimental. There are potential new therapies in MS and the example of minocycline has been noted. The prospect of conferring neuroprotection in MS is good, and this bodes well for the prognosis of the disease.

10. ACKNOWLEDGEMENTS

I wish to thank the past and present trainees in my laboratory who have collaborated with me on the various roles of neuroinflammation in MS: Amit Bar-Or, Veronika Brundula, Sophie Chabot, Fabrizio Giuliani, Peter Larsen, Olaf Stuve, Jennifer Takahashi, Joon Uhm, Andrew Weaver, Jennifer Wells and Rana Zabad. I would also like to acknowledge the consistent and superb technical assistance offered by Yan Fan, Tammy Wilson and Fiona Yong. Finally, I wish to thank the many clinical collaborators, and in particular, Luanne Metz, who have taught me much about MS.

<table>
<thead>
<tr>
<th>Mechanisms of protection</th>
<th>Glatiramer acetate</th>
<th>Interferon-β</th>
<th>Minocycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect Mechanisms (e.g. decrease of CNS inflammation)</td>
<td>Likely</td>
<td>Likely</td>
<td>Likely</td>
</tr>
<tr>
<td>Direct mechanisms within the CNS (e.g. by production of growth factors within the CNS)</td>
<td>Likely</td>
<td>Not Likely</td>
<td>Likely</td>
</tr>
</tbody>
</table>

Table 3. A summary of potential neuroprotection by established or experimental MS therapeutics and their mechanisms

that are directly within the CNS to elaborate their beneficial contents (Table 3).

8. OTHER POTENTIAL NEUROPROTECTIVE AGENTS FOR MS

As yet, there has been no evidence of neuroprotective roles for other immunomodulators that are employed in MS, including mitoxantrone, cyclophosphamide, IVIG and others. Experimental therapies offer a more optimistic future. Minocycline is a tetracycline antibiotic that is taken orally for the treatment of conditions such as acne. It is a small molecule that diffuses well into the CNS. It produces a low frequency of antibiotic resistance and therefore has been used in the long-term management of patients with acne. In the United Kingdom alone, over 6.5 million individuals have been treated for an average of 9 months with minocycline. It has many activities, including the potential to inhibit the activity of the matrix metalloproteinases (MMPs) (75).

The MMPs are a family of proteolytic enzymes that are implicated in the physiological remodelling of the extracellular matrix (ECM). While having many physiological functions, the MMPs are also upregulated in a variety of CNS disorders including MS. In the context of MS, upregulated MMPs may be utilized by leukocytes to infiltrate into the CNS; MMPs may also produce encephalogens from myelin proteins or it may be directly or indirectly cytotoxic (76). We have used minocycline, originally because of its capacity to inhibit the activity of the matrix metalloproteinases (MMPs) (77).
11. REFERENCES

31. Cross AH, B Cannella, CF Brosnan & CS Raine: Homing to central nervous system vasculature by antigen-
specific lymphocytes I Localization of 14C-labeled cells during acute, chronic, and relapsing experimental allergic encephalomyelitis. *Lab Invest* 63, 162-170 (1990)


81. Tikka T, BL Fiebich, G Goldsteins, R Keinanen & J Koistinaho: Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. J Neurosci 21, 2580-2588 (2001)


**Key Words:** Nervous system, Brain, Glatiramer Acetate, Interferon-β, Minocycline, Multiple sclerosis, Neuroprotection, Review

**Send correspondence to:** Dr V. Wee Yong, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada, Tel:403-220-3544, Fax:403-283-8731, E-mail: vyong@ucalgary.ca