ABSTRACT

Multiple mechanisms and pathways for neurodegeneration and neuroprotection exist in multiple sclerosis. Therapeutic interventions may therefore offer a variety of options alone or in combination. These may include anti-inflammatory agents aimed at the blood-brain barrier, localized tissue reactions, mediators of inflammation, and exotoxins (such as nitrous oxide and glutamate), and/or proteolytic enzymes. In addition, specific cells of the immune system or neurologic system may be targeted, including macrophages, CD8-positive T lymphocytes, and oligodendrocytes. Last but not least, agents known to stabilize axonal membranes, sodium channels, and destructive pathways are promising agents for both neuroprotection and intervention in early disease as well as for management of chronic pathology.

One of the key concepts in developing therapeutic strategies for multiple sclerosis (MS) is understanding that there may be different targets for neuroprotection in MS, including the various types of cells themselves—axons, oligodendrocytes, astrocytes—as well as molecules and sites within the extracellular matrix, since these are essential to the overall integrity of white matter. In addition, different stages of the disease may demand different types of therapeutic interventions. For example, one may develop a neuroprotective strategy for a clinically isolated syndrome, or for the relapsing remitting period of MS, but such a strategy may be markedly different from that required for secondary progressive disease with already established axonal injury and where regeneration of axons may be more important.

Preventive approaches for early disease include focusing on inflammation and on anti-inflammatory agents that target the blood-brain barrier, local tissue reactions, mediators of the inflammatory response (e.g., glutamate, nitrous oxide, tumor necrosis factor, interleukin-6), and proteolytic enzymes. In addition, specific cell targets may include macrophages and CD8-positive T cells, which appear to cause the tissue damage and which correlate best with the neuropathology of injury. Stabilization of the axonal membrane is also important, and research is being pursued with respect to the sodium channel and its reorganization in demyelinated axons.

Changing focus from neuroprotection to promotion of recovery, scientists are exploring the ability to enhance early remyelination before long-term, chronic changes in remodeling occur in axons or in oligodendrocytes. This also includes examining direct effects on axons themselves, be they effects on ionophore distribution, membrane remodeling,
integrity of the cytoskeleton, axonal transport, and/or proteosomal modification. It may also be important to promote neurotropic factors (those that are protective rather than deleterious) and/or reparative genes. In summary, there are many potential steps at which one can intervene to have an effect in preventing neurodegeneration, or promoting neuroprotection—some of which will be discussed in the following review.

**Noninflammatory Mechanisms of White Matter Injury**

White matter of the brain and spinal cord is susceptible to oxygen deprivation, ischemia, trauma, and autoimmune attack. Exploring noninflammatory mechanisms, and particularly ischemia and glucose deprivation, it is clear that one of the factors that initiates damage is failure of the sodium-potassium-adenosine triphosphatase (ATPase) (Na-K-ATPase) system. This leads to accumulation of axoplasmic sodium through non-inactivating sodium channels, which, together with membrane depolarization, promotes reverse sodium and calcium exchange and axonal calcium overload. The acid-base status of the tissue is also particularly important, because there is a calcium permeable acid-sensing channel that reverses itself, and pumps calcium into the cell under conditions of acidosis. This may be one of the major mechanisms by which calcium gets into a cell causing damage. Secondly, energy failure activates as non-inactivating voltage-gated sodium channel, which is particularly expressed at the node of Ranvier and this results in an influx of sodium into the axon, and an efflux of potassium, both of which result in destabilization of the membrane potential.

An equally important source of deleterious calcium originates from intracellular stores, released in part by a mechanism similar to “excitation-contraction coupling” in muscle, involving activation of ryanodine receptors by L-type Ca**++** channels. Stys demonstrated that there is depolarization of some calcium channels. This leads to calcium release from the exoplasmic reticulum, which in and of itself is damaging, and then secondarily causes activation of other calcium channels, which enhance the damage. In other words, the change of the internal sodium milieu of the axon then secondarily leads to reversal of the sodium-calcium exchanger, which again brings calcium into the cell, adding to the overload, and also has an effect on sodium-dependent glutamate transport, which actively pumps out toxic amounts of glutamate that then can become excitotoxic.

Glutamate is an excitatory neurotransmitter produced by macrophages, and is a major component of the extracellular milieu where there is inflammation in experimental autoimmune encephalomyelitis (EAE) models and in MS. Glutamate activates inotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors on glia and myelin resulting in sodium (Na) and calcium overload. It also activates metabotropic receptors triggering the intracellular calcium pool via IP3 receptors. Excessive accumulation of

**Figure 1. Noninflammatory Mechanisms of White Matter Injury**

Cytosolic calcium in turn activates various calcium-dependent enzymes such as calpains, phospholipases, and other substances, resulting in irreversible injury. Reoxygenation paradoxically accelerates injury in many axons, and promotes cytoskeletal degradation.1

Figures 1A and 1B summarize the changes that occur; some occur primarily at the node of Ranvier and some of them occur in axonal tissue itself, which does not normally express Na receptors. All of these changes lead to a massive calcium influx, which then activates many different calcium-dependent enzymes. One of these is nitric oxide synthase, which feeds back to the mitochondria and compounds the intracellular damage by creating an environment in which there is a loss of energy production. Note that there are some similarities with ischemia in the demyelinating situation that is induced by some of the same inflammatory mediators, such as nitric oxide. As illustrated, there are many different events that are either a result of inflammation or noninflammatory mechanisms that can then lead to axon, myelin, and oligodendrocyte damage.

Axonal Na Channel Expression in MS

It has been observed in MS that following demyelination some axons recover their ability to conduct impulses (leading to clinical remission of symptoms) whereas other axons, stripped of their protective sheath, degenerate, and this results in chronic clinical deficits and disability. Waxman et al discovered that altered expression of 2 distinct isoforms of Na channels underlies these 2 events.2 In the normal organization of the node of Ranvier, the Na 1.6 channel (Na1.6) is selectively expressed at the node of Ranvier, and this channel allows normal propagation of the axon potential. In the setting in which there is demyelination, but retention of the axon itself, there is a loss of a normal structural organization. There is extension of the Na1.6 ionophores that go beyond the area in which they are normally restricted. In addition, there is re-expression of the Na1.2 channel, which normally is only present in developing, but not in mature, axons. The continued presence of Na1.6 is highly associated with other ionophore changes, which then produce some of the damage previously discussed that potentially can truncate axons. For example, Na1.2 marked axons, unlike those with the Na1.6 sodium channel, are not associated with the presence of Alzheimer precursor protein.

With respect to recovery, Waxman et al has postulated a model whereby if there is a demyelinated axon, but expression of Na1.2, there is recovery of conduction in that axon over the segments that are demyelinated (Figure 2).2 However, if there is demyelination, and there is increased expression of Na1.6 extending beyond the nodal area, there is then activation of the Na-potassium exchanger with reverse influx of calcium into the cell with its associated damage. In addition, a combination of these 2 events may lead to a chronic excess of Na influx into the axon that not only may mediate the acute demyelinating-associated changes, but more chronic degenerative changes that can occur in the axon as well. These phenomena have been demonstrated both in EAE animal models and in MS itself.

Therapeutic Applications of Na-Channel Expression in MS

The question is whether it is possible to take the information that has been learned from these particular models of variations in Na-channel expression and apply them to create useful therapy for patients.
with MS. A number of existing medications currently in use for other conditions are known to block the Na-channel activation that occurs. These include anticonvulsants such as phenytoin, local anesthetics such as lidocaine, and certain antiarrhythmics, including flecainide.

Bechtold et al treated rats with EAE with flecainide or vehicle, and upon morphometric examination of neurofilament-labeled axons in the spinal cord, it was demonstrated that flecainide regimens resulted in significantly higher numbers of axons surviving the disease (83% and 98% of normal) compared with controls (62% of normal, Figure 3). Specifically, impairment of a compound action potential in the spinal cord was protected by the administration of flecainide, whereas there was no effect on peripheral nerve stimulation. In addition, the investigators looked at neurofilaments, and in the untreated animals there was a marked loss of neurofilaments compared with the flecainide-treated animals. Thus, this medication is clinically protective not only in its capacity to generate an action potential, but it is neuroprotective as far as axons are concerned as well, as it protects against massive loss of axons. This is in agreement with the current notion of neuronal injury. For example, in Parkinson’s disease, patients do not present with clinical symptoms until the vast majority of neurons have actually degenerated, particularly in the substantia nigra, if not in other areas. Finally, there is a positive correlation between the degree of myelin loss and the degree of axonal loss in examining results in vehicle-treated animals versus drug-treated animals. Thus, flecainide appears to be myelin-protective as well as neuroprotective in axons as well. Lo et al has shown similarly that phenytoin is a neuroprotective agent, and the degree of neurologic impairment in these animals can be reduced without the added addition of any anti-inflammatory agents.

However, several limitations exist with these medications. For example, the cardiac antiarrhythmic medications that inhibit the Na channel often have unacceptable levels of toxicity, as do local anesthetics like lidocaine, which has the added complication of being difficult to introduce into the nervous system. By contrast, phenytoin, and other anticonvulsants that are Na-channel inhibitors, may be useful as therapeutic approaches for neuroprotection.

ADDITIONAL NEUROPROTECTIVE AGENTS

PHOSPHOLIPASE A2 ENZYMES AND INHIBITORS

Phospholipases comprise a large family of enzymes. One of their main functions is to hydrolyze cell membrane phospholipids to produce lysophospholipids and free fatty acids. These enzymes have a number of characteristics, but the ones that are of particular interest are calcium dependent and are secreted by mononuclear cells, and it is probably by their action on these cells that one may see a neuroprotective effect. Tissue injury is produced by phospholipase metabolites including lipid mediators, free radicals, and reactive oxygen species. Interest has been focused in particular upon secretory phospholipase A2 (sPLA2), because its inhibitor appears to be quite promising as a neuroprotective agent. The expression of metabolites by phospholipase A2 enzymes is actually intimately regulated by a number of immune mediators, including interleukin (IL) 1, tumor necrosis factor alpha, and IL-6.

Figure 4 illustrates a chain of events that proceed from activation of macrophages or monocytes, with modulation by a number of different mediators, including activation of secretory phospholipase and of
many other compounds that are either directly damaging to axons, or indirectly damaging to axons. In the central nervous system, it is known that phospholipase, in and of itself, has a number of deleterious effects. It produces conduction block, causes myelin damage via lysophosphatidylcholine, causes neuronal apoptosis, worsens axotomized motoneuron damage, and has a synergistic effect with glutamate, thus adding another pathway to injury. sPLA₂ also has a receptor on cells that can activate cytosolic phospholipase A₂, which is, again, a major means of signal transduction into the cell causing intracellular damage, apoptotic and other forms of injury.

In a model using C-57 black mice, Kalyvas et al have shown that cytosolic phospholipase A₂ (cPLA₂) is a “central mediator” in evoking the complex pathological changes seen in MS and EAE. One of the metabolic products of this enzyme is proinflammatory, while the other induces myelin breakdown, demyelination, and chemokine/cytokine expression. The authors provided evidence not only that the enzyme is highly expressed in EAE lesions, but that blocking this enzyme leads to a reduction in the onset and progression of EAE. Likewise, Pinto et al synthesized extracellular cPLA₂ inhibitors, which do not penetrate the cell and interfere with vital phospholipid metabolism or cell viability. These inhibitors reduced inflammation and the occurrence of EAE in rats and mice.

The difficulty with some of these agents that are available is that they may not enter the central nervous system readily, nor are they necessarily completely stable. Thus, work had been ongoing to develop a survival peptide that also would have neuroprotective effects. A molecule called “CHEC-9” has these qualities and also demonstrates marked inhibition of sPLA₂. In fact, this compound has the potential not only to have an effect on the inflammatory component of demyelination, but also may potentially act directly in the brain to stabilize axons both in animals and humans. Its mechanism of action is illustrated in Figure 5.

**Monoclonal Antibodies**

One final approach to neuroprotection and subsequent development of effective therapies for MS involves the use of monoclonal antibodies combined with neuroprotective agents. Kanwar et al attempted a multifaceted technique to ameliorate chronic EAE by blocking inflammation with an antimucosal address in cell adhesion molecule-1 mon-
clonal antibody along with inhibitors of glutamate excitotoxicity. The fact that neuroprotection was observed even at an advanced stage of unremitting EAE, with repair of the central nervous system taking place, as assessed by increased oligodendrocyte survival and remyelination, along with corresponding decreased paralysis, inflammation, central nervous system apoptosis, and axonal damage was impressive. Each treatment reduced the expression of nitric oxide and a large panel of proinflammatory and immunoregulatory cytokines, including IL-6. There was also improvement in physical symptoms, but the disease relapsed when treatment was suspended, suggesting treatment must be maintained to be effective.

These findings would suggest that if one combines an anti-inflammatory therapy with a neuroprotective therapy, one may have additional and potentially synergistic benefits that may be therapeutically useful in the clinical management of MS. In terms of clinical management of MS, clinical trials are ongoing to test agents developed based upon various theories and scientific models for mechanisms of MS and EAE.

**Clinical Trials for Neuroprotective Therapies**

One current trial uses a combination of interferon beta-1A and topiramate, an anticonvulsant, in a potentially neuroprotective approach. Recall that blockers of persistent Na channels represent an attractive therapeutic target because of their ability to simultaneously interfere indirectly with several calcium sourcing pathways.

Topiramate blocks sodium channels, and it does so for the persistent channels as well as transient ones. In addition, it inhibits signal transduction from the AMPA/kainate receptor and it may in fact have multiple potential modes of action likely to be desirable in a neuroprotective agent.

The trial involves patients with early relapsing/remitting MS, with a primary endpoint of safety as required by the US Food and Drug Administration. However, other parameters are also being examined, including brain parenchymal fraction/brain atrophy as well as other radiologic and clinical outcomes, as evidenced by criteria established in the Expanded Disability Standard Scale and Multiple Sclerosis Functional Composite.

**Conclusion**

There is overwhelming data demonstrating many different pathways that might, in fact, produce neurodegeneration in MS. Thus, future directions with anti-inflammatory and neuroprotective therapies should be aimed at these multifaceted and varied mechanisms that seem to be at play in MS. In the future, selection of appropriate anti-inflammatory agents, including selective adhesion molecules and other mechanisms of immune-modulation, in combination with neuroprotective agents that target ion channels, might be the best strategy to effect acute and/or chronic change in this complex disease.

**References**
