Neuroprotection in Progressive Brain Disorders

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Abstract

Progressive neurodegenerative disorders share common mechanisms of cell death, and in all likelihood multiple factors are involved in every disease. Therefore, several neuroprotective agents are being investigated with the purpose of slowing or preventing further deterioration of cell loss. These include experimental animal and clinical studies on the neuroprotective effects of caspase inhibitors, antioxidants, glutamate antagonists, anti-inflammatory agents and trophic factors in several neurodegenerative diseases. At present there is limited clinical evidence for direct neuroprotective effects against these diseases, but much effort is being invested in research on novel technologies and compounds.

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Neurodegenerative diseases are characterized by slow and progressive loss of neurons in different areas of the central nervous system, e.g., cortical (especially hippocampal) and cholinergic neurons in Alzheimer's disease, motor neurons in the anterior horn and brainstem in amyotrophic lateral sclerosis, dopaminergic neurons in the substantia nigra in Parkinson's disease, and medium spiny neurons in the striatum in Huntington's disease. Although different cell groups are affected in each disease, they probably share some common pathways involving complex molecular processes leading to degeneration. In all likelihood, multiple factors are involved in the pathogenesis of each neurodegenerative disease, and therefore, it seems likely that not one but rather several neuroprotective approaches will have to be considered. Vigorous studies utilizing experimental animal models that share morphologic, genetic, molecular biologic and clinical features of human diseases contributed tremendously to our present understanding of cell death mechanisms and opened new potential therapeutic avenues, hopefully applicable to human studies.

Neuroprotection can be defined as a therapeutic intervention that prevents death of vulnerable neurons, slows disease progression and delays transition from the preclinical to the clinical stage. In this overview we will discuss several such strategies with particular emphasis on clinical trials related to neuroprotective treatments.

Anti-apoptotic agents

Apoptosis is cell death due to an activation of genetically encoded active cell suicide programs. Morphologic signs and other indications of apoptosis have been found in brains of patients with Parkinson's disease, Huntington's disease, some of the spinocerebellar degenerations, and in anterior horn cells in spinal cords of patients with amyotrophic lateral sclerosis [1]. The same morphologic features are present in animal models of these diseases, an observation that aids in unraveling the underlying mechanisms involved in this process. The apoptotic death cascade is complex and involves many possible parallel pathways but invariably involves altered expression of the bcl_2 family members and sequential activation of inactive procaspase enzymes. Bcl2 is the founder member of a growing family composed of both suppressors (bcl_2, bcl-xl) and promoters (bax, bad) of apoptosis. Over-expression of pro-apoptotic factors accelerates neuronal death. By contrast, over-expression of anti-apoptotic factors, especially bcl_2, attenuates neurodegeneration in familial amyotrophic lateral sclerosis-linked superoxide dismutase-1 mutant mice, MPTP-treated mice and in the 3-nitropropionic treated mouse model of Huntington's disease. The bcl_2 family modulates the activation of downstream effectors of cell death such as caspases. The latter are intracellular proteases that play an important role in the pathogenesis of central nervous system diseases featuring apoptosis. There is evidence for caspase-3 activation in brains of patients with Parkinson's disease and Huntington's disease, as well as caspase-1 and caspase-9 activation in spinal cords of human and animal models of amyotrophic lateral sclerosis. *In vivo* studies indicate that caspase inhibition promotes survival and functional outcome in several neurologic disease models. Intracerebroventricular administration of a broad-spectrum caspase inhibitor (zVAD-fmk) delayed disease onset and mortality in the mutant SOD-1 transgenic model of amyotrophic lateral sclerosis [2]. Similar results were obtained in a transgenic mouse model of Huntington's disease [3]. Recently, it was shown that minocycline, by inhibiting caspase-1 and caspase-3 expression, attenuates dopaminegic cell loss and delays mortality in MPTP-treated mice and in a transgenic mouse model of Huntington's disease [4,5]. Unfortunately, the currently available caspase inhibitors are toxic in pharmacologic doses, precluding their immediate use in human studies. Another problem is that such agents may be risky as they can inhibit physiologic apoptosis (e.g., in gut and bone marrow) and promote cancer.

Antioxidants

Oxidative stress, energy depletion, and mitochondrial dysfunction are believed to be intimately involved in the pathogenesis and progression of neurologic diseases [6]. The CNS contains excitatory

SOD = superoxide dismutase
CNS = central nervous system

amino acids, as well as dopamine, which generate reactive oxygen species during their metabolism. Impairment of mitochondrial function contributes to generation of free radicals; oxidative stress is also a cause for mitochondrial DNA mutations. These related processes converge into a common pathway leading to apoptosis. Therefore, much effort is being invested to develop potent antioxidants and energy-yielding compounds. Crucial properties of an antioxidant include the ability to cross the blood-brain barrier following systemic administration, removal of O_2, scavenging reactive oxygen species or their precursors, inhibition of reactive oxygen species formation, and up-regulation of endogenous antioxidant defenses. Antioxidant agents can be classified into two groups: enzymes and low molecular weight antioxidants. The enzymes include SOD, catalase and peroxidase. The second group includes compounds that are synthesized by the cell itself such as glutathione, nicotinamide, urate and co-enzyme Q10 or those obtained from dietary sources (vitamin E, vitamin C, creatine and lipoic acid). Creatine functions as an antioxidant through an enhancement of energy transduction. It attenuates the inactivation of mitochondrial creatine kinase and the opening of the mitochondrial permeability transition pore. Creatine supplementation was found to reduce the accumulation of oxidative stress in animal models of Huntington's disease, amyotrophic lateral sclerosis and Parkinson's disease [7]. This compound is currently being tested in a randomized, double-blind trial in Huntington's disease [8]. Based on the fact that co-enzyme Q10 is a co-factor of the electron transport chain, it was assumed that it might be used as an antioxidant agent [9]. Thus far there is no solid clinical evidence to support this view. Its efficacy in animal models, such as striatal necrosis caused by malonate and 3-nitropropionic acid, MPTP toxicity and transgenic amyotrophic lateral sclerosis mice, could not be replicated in a large clinical study in Huntington's disease [10]. Vitamin E was tested as a neuropotective agent as part of the DATATOP study in Parkinson's disease (in combination with deprenyl) [11], and in one relatively large and well-designed clinical trial of Alzheimer's disease [12]. Although vitamin E supplementation increased the concentration of the vitamin in plasma and cerebrospinal fluid of Alzheimer's disease and Parkinson's disease patients, its limited clinical benefits and lack of detection in slowing disease progression did not justify recommendation of its use on a regular basis. A double-blind, placebo-controlled study of treatment with vitamin E for 1 year in amyotrophic lateral sclerosis patients already treated with riluzole found that patients in the vitamin E arm remained longer in the milder stages of the disease, but there was no change in survival rates [13]. Vitamin C (ascorbic acid) protects cortical neurons in culture from the toxic effects of NMDA [14]. Urate, a naturally occurring product of purine metabolism, is a scavenger of biological oxidants implicated in numerous disease processes. Uric acid protects cultured rat hippocampal neurons against cell death induced by insults such as exposure to the excitatory amino acid glutamate and the metabolic poison cyanide [15]. Uric acid suppresses the accumulation of reactive oxygen species (hydrogen peroxide and peroxynitrite) and lipid peroxidation, and prevents mitochondrial dysfunction induced by the excitotoxic and metabolic insults. It also attenuated delayed elevations of intracellular free calcium levels induced by glutamate and cyanide. These data demonstrate a neuroprotective action of uric acid that involves suppression of oxyradical accumulation, stabilization of calcium homeostasis, and preservation of mitochondrial function [15]. *In vitro* studies provided evidence that the neuroprotective effect of deprenyl goes beyond its ability to block monoamine oxidase-B and might involve up-regulation of anti-apoptotic molecules or by binding to a pro-apoptotic molecule, GAPDH (glyceraldehyde-phosphate dehydrogenase), thereby reducing its toxic effect. Deprenyl was also assumed to block the formation of free radicals derived from the oxidative metabolism of dopamine. However, two major clinical studies in Parkinson's disease (the DATATOP studies) [11,16] and several small clinical trials in Alzheimer's disease [17] suggested a mild symptomatic effect in untreated Parkinson's disease patients and some beneficial effect in the treatment of cognitive deficits. It seems that deprenyl does not prevent disease progression. Another MAO-B inhibitor with prominent anti-apoptotic properties in animal models, rasagiline, is currently being tested in clinical trials in Parkinson's disease and Alzheimer's disease [18]. It is hoped that this compound will truly provide protective effects in these diseases.

Table 1. Current state of neuroprotective strategies in clinical trials in humans

	Neuroprotective agents [ref]	
Parkinson's disease	Vitamin E [11]	Symptomatic
	Deprenyl [11,16]	Symptomatic
	Rasagiline [18]	In clinical trial
	Riluzole [20]	Negative
	GDNF [29]	Negative
	Estrogens [37]	Doubtful
	Dopamine agonists [39,40]	Symptomatic
Huntington's disease	Co-enzyme Q10 [10]	Negative
	Remacemide [10]	Negative
	Riluzole [21]	Negative
	Creatine [7]	In clinical trial
Alzheimer's disease	Vitamin E [12]	Probably negative
	Selegiline [12,17]	Probably negative
	Statins [35]	Possible protection
	Estrogens [36]	Doubtful
	NSAID [23]	Possible protection
Amyotrophic lateral sclerosis	Trophic factors (CNTF, IGF-1) [25,27]	Negative
	Riluzole [19]	Neuroprotective
	NSAID [24]	Negative
	Vitamin E [13]	Negative
	Creatine [7,8]	Negative
	Co-enzyme Q10 [8]	Negative

Glutamate toxicity-blocking agents

Glutamic acid, the precursor of glutamate, is a potent excitatory neurotransmitter. Accumulation of extracellular glutamate, either by loss of glutamate transporters or by activation of its receptors,

MAO-B = monoamine oxidase

might lead to a mode of cell death known as excitotoxicity. Glutamate toxicity blocking includes glutamate antagonism, direct or indirect growth factor activity, as well as GABA agonism and interaction with calcium channels. Two drugs were already tested in clinical trials: riluzole, which attenuates glutamate release by interacting with sodium channels, and remacemide, a non-competitive NMDA receptor antagonist. Both were effective in delaying clinical signs of disease and delaying death in several animal models. Riluzole is the only drug that was found effective in delaying motor deterioration and prolonging life in patients with amyotrophic lateral sclerosis, especially those with bulbar-onset disease [19]. In this group, 73% survived after 1 year compared to 35% in the placebo group. Riluzole and remacemide are currently being tested in open-label clinical trials for Parkinson's disease and Huntington's disease. A preliminary study on 20 patients with early untreated Parkinson's disease failed to show a meaningful symptomatic effect of riluzole on motor function [20]. Long-term treatment with riluzole in patients with Huntington's disease did not improve the functional capacity of these patients [21]. In a randomized, placebo-controlled trial in 340 patients with Huntington's disease, the remacemide-treated patients showed no improvement in total functional capacity or in abnormal movements [10]. Although these studies are discouraging, more extensive studies are needed to determine whether these agents have neuroprotective effects.

Anti-inflammatory agents

Levels of pro-inflammatory cytokines including tumor necrosis factor-alpha, interleukin-1 beta, IL-2 and IL-6 were found to be increased in postmortem brains of patients with Parkinson's disease and Alzheimer's disease and in spinal cords of amyotrophic lateral sclerosis patients [22]. This observation, together with the presence of reactive inflammatory cells, especially microglia and other immune-associated proteins, in affected CNS areas, provided the basis of association of inflammation in the pathogenesis of neurodegenerative diseases. Yet, it is still unclear whether the inflammatory reaction represents an attempt to repair neurons or further contributes to their injury. It is also possible that the increased immune reactivity causes increased vulnerability of neuronal cells to potential neurotoxic factors. Activated microglia promote production of cytotoxic mediators such as TNFα, free radicals and quinolinic acid that might damage and kill neurons.

Exposure of dopaminergic primary cultures to TNFα resulted in a dose-dependent decrease in neuronal viability. The hypothesis in Alzheimer's disease is that IL-1β promotes neuronal synthesis of beta amyloid precursor protein with continuing deposition of beta amyloid, activation of astrocytes and microglial cells and release of cytokines that injure the cells. The neuronal injury can further activate microglia and create a feedback amplification process of overproduction of IL-1. Motor neuron death in amyotrophic lateral sclerosis may also be triggered or amplified by inflammatory reactions through the action of cytokines, immunoglobulins and activated microglia. Along with these observations, several epidemiologic and prospective studies found a correlation between the use of non-steroidal anti-inflammatory agents with reduced risk of Alzheimer's disease [23]. There is a critical period for the neuroprotective capacity of these drugs and they should perhaps be taken at least 2 years before the onset of dementia. Clinical trials with NSAIDs in amyotrophic lateral sclerosis were negative [24], but this could be due to delayed treatment. These drugs act by inhibition of cyclooxygenase 1 and 2. COX-2 has been implicated in several animal models as a death-promoting factor. It will be interesting to study the effects of selective COX-2 inhibitors (celecoxib) in delaying or preventing disease symptoms.

Trophic factors

There is no doubt that apart from their role in development, differentiation and survival of surrounding tissues, trophic factors have neurotropic and neuroprotective effects on neural tissues. Among the trophic factors that were mostly studied in neurodegenerative diseases are ciliary neurotrophic factor, insulin-like growth factor-1, glial cell-derived factor, vascular endothelial growth factor, and erythropoietin. Despite their potential role as survival and tissue growth-promoting factors, the results of clinical trials were rather disappointing. CNTF is a potent survival factor found in Schwann cells and is released in response to nerve injury. Treatment with recombinantly produced human CNTF in patients with amyotrophic lateral sclerosis had no effect on muscle strength or mortality rates [25]. IGF-1 is a pleiotropic protein. The widespread distribution of its receptors allows IGF-1 to affect the survival of numerous populations of neurons and glial cells. Applying IGF-1 to already injured motor neurons provided neuroprotection from slow glutamate toxicity in organotypic spinal cord [26]. A clinical trial has revealed beneficial effects of IGF-1 in patients with amyotrophic lateral sclerosis [27]. Another growth factor that might be involved in the pathogenesis of amyotrophic lateral sclerosis is VEGF. Reduced expression of VEGF in the spinal cord caused adult-onset progressive motor neuron degeneration in rodents, mimicking the pathologic findings of amyotrophic lateral sclerosis. Moreover, administration of VEGF165 to neuronal cultures promoted their survival [28]. GDNF is one of the most promising neuroprotective factors of the dopaminergic system. It has been shown to restore dopaminergic function in MPTP-treated monkeys. There has been only one attempt to inject GDNF intraventricularly; this was in a young patient with Parkinson's disease, but it failed, probably due to lack of diffusion from the ventricles to brain parenchyma [29]. Encouraging results were obtained with its delivery via Lenti virus vector, enabling direct intra-parenchymal application, but so far only in primate models of Parkinson's disease [30]. Neurotrophic factor gene therapy with

IL = interleukin
TNF = tumor necrosis factor

NSAID = non-steroidal anti-inflammatory drug
COX = cyclooxygenase
CNTF = ciliary neurotrophic factor
IGF = insulin-like growth factor
VEGF = vascular endothelial growth factor
GNDF = glial cell-derived factor

GDNF gene delivery into muscle, where motor neurons have access to secreted GDNF, might also be useful in amyotrophic lateral sclerosis patients similar to the positive results in animal models. Erythropoietin is another cytokine-hormone that potentially exerts neuroprotective effects. Treatment with this agent improved locomotor activity and rescued nearly all of the dopaminergic cells in MPTP-treated mice [31].

Antiprotein aggregation agents

Many neurodegenerative diseases are defined histopathologically by intracellular inclusions composed of ubiquitinated proteins and abnormal deposition of aggregated proteins. The mechanism by which protein aggregates can kill cells, however – whether the presence of such inclusions are a cause or a consequence of the cellular pathology – is not yet established. Lewy bodies, the pathologic hallmark of Parkinson's disease, are composed of proteins that are linked to mutations in familial Parkinson's disease, i.e., mainly α-synuclein and parkin. Mutations in α-synuclein cause the protein to missfold, aggregate, and form insoluble amyloid fibrils. Normally, α-synuclein is polyubiquitinated by parkin, which acts as ubiquitine ligase. It is postulated that the mutated parkin has lost its capacity to ubiquitinate α-synuclein, and therefore, the latter is accumulated in the cell. Inclusion bodies containing aggregates of unstable proteins were found also in Huntington's disease and several other triplet repeat expansion diseases. Mutant superoxide dismutase-1 mice develop prominent cytoplasmic inclusions immunoreactive to SOD in motor neurons, even before onset of clinical disease, supporting the view that aggregates might also contribute to the pathogenesis of amyotrophic lateral sclerosis. Therefore, an attractive neuroprotective strategy would be to prevent protein missfolding, accumulation and aggregation. Administration of creatine monohydrate to a transgenic mouse model of Huntington's disease caused reduction of neuronal atrophy and the formation of Huntingtin-positive aggregates, along with improvement of motor performance [32].

Miscellaneous

Several epidemiologic studies found neuroprotective effects of dietary compounds and drugs in Parkinson's disease and Alzheimer's disease. Few studies have linked coffee drinking and cigarette smoking to a reduced risk of developing Parkinson's disease [33,34]. The CNS effects of caffeine are mediated primarily by antagonistic actions at the A_1 and A_{2A} subtypes of adenosine receptors.

In Alzheimer's disease, there is epidemiologic evidence that long-term use of statins (cholesterol-lowering drugs that inhibit the enzyme HMG-CoA reductase) lowers the likelihood to develop dementia in 70% compared to untreated subjects [35]. The rationale behind the use of statins is that by lowering the level of brain cholesterol there is inhibition of the A_β-forming amyloidogenic pathway, thus reducing the ability of A_β to form fibrils.

Estrogens were associated with a reduction in risk of Alzheimer's disease and, less convincingly, of Parkinson's disease. In experimental animals, estrogens attenuated neuronal death in models of ischemia, trauma and Parkinson's disease. The postulated mechanisms of protection include activation of the nuclear estrogen receptor, altered expression of bcl2, activation of kinase pathway, activation of signal transduction pathways, or direct antioxidant activity. In humans, the results are much less impressive. In Alzheimer's disease, more clinical trials failed to prove any effect on attention, verbal memory or visual memory than trials that did find improvement in these parameters. Epidemiologic and observational clinical data on the use of estrogen to maintain cognitive function with normal aging have been promising, however studies involving women with established Alzheimer's disease showed no benefit of long-term estrogen replacement therapy [36]. Neurons and pathways that mediate cognitive function may have already been damaged or lost due to the cascade of neurodegeneration. It may be that the window of opportunity has been missed in these women. Alternatively, would a woman who has not been exposed to estrogens for many years benefit from estrogen replacement therapy? In Parkinson's disease, there are a few reports of clinical benefits as a symptomatic therapy in advanced stages, as well as a recent report providing indirect evidence of possible protection, i.e., increased risk of Parkinson's disease in early post-menopausal women and in women not treated with hormonal therapy [37]. Therefore, in light of the troubling results of the study by Viscoli et al. [38] who found that replacement therapy with estrogens in post-menopausal women with previous strokes increased the risk of fatal stroke, designing further studies with estrogens should be considered cautiously.

Several experimental studies demonstrated that treatment with dopamine agonists such as apomorphine, ropinirole or pramipexole prevented nigrostriatal cell loss in animal models of Parkinson's disease. It is suggested that this neuroprotective effect is achieved by these drugs acting as antioxidants, anti-apoptotics or by inhibition of excitotoxicity [39]. Clinically, it is very difficult to dissociate the symptomatic and neuroprotective effects of the dopamine agonists. Clinical trials with dopamine agonist monotherapy did not confirm this effect and the results reflect the natural course of the disease. The REAL PET study demonstrates a possible (though not yet proven) neuroprotective effect of ropinirole by demonstrating slower rates of disease progression in patients taking this agonist compared to treatment with levodopa [40]. Similar results were obtained with dopamine transporter imaging using sequential SPECT studies with β-CIT [39].

Conclusion

The above data highlight the diversity and interrelations of many key factors that eventually may lead to progressive neuronal loss. Understanding all these different mechanisms will allow us to develop effective and curative therapies for neurodegenerative diseases. Currently, industrial effort is focused mainly on the development of antioxidants, free radical scavengers, anti-ecitotoxic and anti-apoptotic neuroprotective agents. Certainly, *in vitro* studies and even *in vivo* animal studies cannot mimic the complex human brain, therefore it is likely that many compounds that show promising results in these studies will not reach clinical trials, and probably a cocktail of neuroprotective agents will be needed to produce beneficial effects in humans. However, based on the strategies reviewed here, there are many

compounds in the pipeline awaiting clinical trials. Ideally, a neuroprotective agent should be administered during the critical narrow vulnerable period, when the events begin to snowball, leading to the cascade of cell death. Since this critical period may well occur before the appearance of clinical signs, we must discover the means for early detection.

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