A Role for Activated Microglia and Peroxynitrite in Lewy Body Diseases – Implications for Prevention and Control

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Abstract

Lewy body diseases – encompassing Parkinson's disease, dementia with Lewy bodies, and Parkinson's disease with late dementia – may reflect a vicious cycle of neuroinflammation in which aggregated alpha-synuclein promotes microglial activation, and the peroxynitrite and cytokines produced by activated microglia in turn promote intraneuronal alpha-synuclein aggregation and neuronal death. If this model is correct, practical measures which dampen microglial activation and lessen peroxynitrite toxicity may aid the prevention and treatment of these disorders. Spirulina, a host of food polyphenols, DHA, astaxanthin, caffeine, a Mediterranean or plant-based diet, and exercise training have potential for blunting microglial activation. Measures which increase intraneuronal levels of urate and of glutathione – such as supplemental inosine, N-acetylcysteine, and phase 2 inducers – should mitigate the toxicity of peroxynitrite. Astaxanthin may suppress intraneuronal generation of superoxide and peroxynitrite by protecting the structure of mitochondrial inner membranes. Hence, it may eventually prove feasible to control or at least slow the progression of these devastating disorders with complex nutraceutical/lifestyle strategies.

Synucleopathies – A Vicious Cycle of Microglial Activation and Neuronal Damage?

The Lewy body diseases – a.k.a. synucleopathies, characterized by the intraneuronal and extraneuronal accumulation of protein aggregates rich in alpha-synuclein – include dementia with Lewy bodies (DLB), Parkinson's disease (PD), and Parkinsons's disease with late dementia (PDLD), seem likely to reflect a common pathogenetic mechanism that emerges initially in different parts of the brain – DLB in cortical regions, PD in the substantia nigra. But DLB is commonly complicated by Parkinsonian symptoms as it progresses, and PD patients are prone to progress to dementia. Hence, the synucleopathies tend to converge to a common clinical picture over time. Complicating the picture is the fact that dementias associated with Lewy bodies can be associated to a greater or lesser degree with amyloid beta-mediated pathology suggestive of Alzheimer's disease.

Despite the fact that DLB is now thought to be responsible for about 20% of dementing illness, there is essentially no epidemiology extant pertaining specifically to this disorder, and relatively few studies with transgenic mouse models overexpressing wild type or mutant alpha-synuclein have focused on cortical or cognitive function. Hence, efforts to postulate measures which might aid prevention or control of DLB might reasonably look to the ample research literature on PD for guidance – on the admittedly hypothetical but not unreasonable premise that the pathogeneses of DLB and PD are substantially homologous, differing primarily in the region of the brain that is first afflicted.

On the basis of PD research, it is reasonable to suspect that Lewy body diseases are driven by a vicious cycle in which extracellular alpha-synuclein-rich protein aggregates, derived from necrotic neurons, promote the inflammatory activation of microglia, and the products of these microglia – peroxynitrite,

pro-inflammatory cytokines (such as interleukin-1 and tumor necrosis factor), and prostanoids – in turn act on neurons to further induce aggregation of alpha-synuclein while promoting neuronal dysfunction and death.[1-4] There is indeed a growing consensus that microglial inflammation plays an essential role in the pathogenesis of PD[5-8] – and it is reasonable to suspect that this extends to other synucleopathies. Indeed, a recent PET scan study detected signs of microglial activation in the substantia nigra, putamen, and cortices of patients with early DLB.[9] If this model is reasonably accurate, it can be predicted that nutraceuticals, drugs, or lifestyle measures which blunt cerebral microglia activation, or that promote scavenging of peroxynitrite-derived radicals, should aid the control of synucleopathies.

Strategies for Quelling Microglial Activation

Intriguingly, studies demonstrating the capacity of alpha-synuclein aggregates to activate microglia have concluded that stimulation of microglial NADPH oxidase activity is an obligate mediator of this activation.[1-3] Hence, one such study concludes that "NADPH oxidase could be an ideal target for potential pharmaceutical intervention, given that it plays a critical role in alpha-synuclein-mediated microglial activation and associated neurotoxicity."[1] In this regard, it is now appreciated that the key intracellular antioxidant activity of heme oxygenase-derived bilirubin reflects its ability to suppress the activity of certain NADPH oxidase complexes.[10-12] Moreover, the phycocyanobilin (PhyCB) found in cyanobacteria such as spirulina has the potential to mimic this inhibitory impact on NADPH oxidase.[13] This effect seems likely to rationalize the profound and versatile anti-inflammatory impact of oral phycocyanin (the spirulina protein that contains PhyCB as a chromophore) or of whole spirulina in rodents.[13, 14] Notably, oral spirulina confers partial protection in mouse models of PD triggered by administration of MPTP or by intra-nigral injection of a viral vector for alpha-synuclein.[15, 16] Moreover, exposure to phycocyanin opposes lipopolysaccharide-induced activation of microglia in vitro.[17] Hence, oral administration of spirulina or PhyCB may have potential for preventing or treating neurodegenerative conditions whose pathogenesis is at least partially driven by microglial activation – likely including the synucleopathies.[18]

As reviewed recently, a number of other nutraceuticals have potential for dampening microglial activation.[19] These include a range of plant-derived polyphenols – flavones and flavonols (such as luteolin, fisetin and quercetin), catechins (notably the epigallocatechin gallate of green tea), anthocyanins (as found in berries and berry juices), and tannins (as in pomegranate) – the omega-3 fatty acid DHA, astaxanthin, and the adenosine receptor antagonist caffeine.[20-36] Exercise training can also diminish microglial activation.[37] Conversely, dietary saturated fat, acting via promotion of de novo ceramide synthesis, appears to up-regulate microglial activation.[38, 39] Consistent with a key role for microglia in the genesis of PD, ample intakes of caffeinated coffee or green tea, as well as regular exercise, good vitamin D status, and "Mediterranean" or quasi-vegan diets low in saturated fat, have been linked to reduced risk for PD in epidemiological studies.[40-48] And studies with MPTP-treated mice likewise suggest that many of these measures may have potential for preventing or controlling PD.[49-66]

A Central Role for Peroxynitrite

The possibility that peroxynitrite may be a key mediator of the pathogenic impact of activated microglia in synucleopathies is consistent with the observation that sustained intracerebral infusion of an iNOS inhibitor to transgenic mice overexpressing a pathogenic variant (human A53T) of alpha-synuclein, prevents the microglial activation triggered by a few i.p. injections of LPS from inducing a sustained

syndrome of neuroinflammation and dopaminergic neurodegeneration in the substantia nigra.[4] Moreover, in the murine MPTP-induced model of PD, deficient activity of either iNOS or nNOS is protective, likewise consistent with a pathogenic role for peroxynitrite in this disorder.[67-72] Conversely, nigral infusion of a peroxynitrite-releasing drug causes profound damage to dopaminergic neurons.[73] Nitration of tyrosine in alpha-synuclein – which can be induced by peroxynitrite exposure – is commonly observed in Lewy bodies and increases the propensity of alpha-synuclein to aggregate; moreover, nitrated alpha-synuclein is directly toxic to dopaminergic neurons.[74-82] Peroxynitrite exposure can also induce dimerization of alpha-synuclein via dityrosine formation.[81] Importantly, nitrotyrosine is observed in cortical Lewy bodies and alpha-synuclein in patients with DLB – suggesting that the role of peroxynitrite in synucleopathies is not just confined to the substantia nigra.[74, 75, 83]

Boosting Urate and Glutathione Levels May Afford Protection

The proposition that peroxynitrite plays a mediating role in PD could help to rationalize the very substantial evidence that high-normal serum levels of urate – a potent scavenger of peroxynitrite-derived radicals[84] – is associated with lower risk for PD as well as a slower clinical deterioration in PD patients.[85] Moreover, two studies suggest that risk for subsequent dementia in PD may be lower in patients with high-normal urate – consistent with a role for peroxynitrite in synucleopathies per se.[86, 87] These findings are particularly intriguing in light of the fact that urate levels can be readily raised by ingestion of supplemental inosine – a strategy which is now being tested in the management of multiple sclerosis.[88-90] A dose of 1-3 g daily, titrated to keep serum urate in the safe range of 6-9 mg/dL, appears to be reasonably safe, aside from an increased risk for urate kidney stones that presumably would be minimized by concurrent alkalinization of the urine. While inosine supplementation requires too much clinical supervision to be practical for primary prevention of synucleopathies, it should be noted that a diet which avoids dairy products (which acutely lowers serum urate[91]) and includes alcohol (a risk factor for gout – particularly beer[92]) and fruit (a source of fructose[93]) tends to elevate serum urate – and indeed such as dietary pattern has been linked to decreased risk for PD.[47, 85, 94-99] White meats and fish – rich in purines, and part of a Mediterranean diet pattern – also raise urate levels and are linked to decreased Parkinson's risk;[47] although red meats increase urate, their saturated fat content may exert a countervailing adverse impact on this risk.[47]

Tyrosine nitration mediated by peroxynitrite can also be opposed by the physiological antioxidant glutathione, which can reduce the intermediate tyrosyl radicals which give rise to nitrotyrosine.[100, 101] Intracellular levels of glutathione can be increased to some degree by increasing intracellular levels of cysteine, the rate-limiting substrate for glutathione synthesis; clinically, this can be achieved by supplementation with N-acetylcysteine or cystine.[102, 103] The ability of N-acetylcysteine administration to boost cerebral glutathione levels and mitigate cerebral peroxynitrite toxicity has been demonstrated in mice.[104] Not surprisingly, oral or parenteral administration of N-acetylcysteine has been shown to be protective in both the transgenic alpha-synuclein and MPTP models of PD in mice.[105-109] Hence, it is reasonable to suspect that supplementation with ample doses of N-acetylcysteine or cystine should have some value for suppressing the contribution of peroxynitrite to human synucleopathies. Co-administration of nutraceutical phase 2 inducers with access to the brain, such as lipoic acid, has the potential to amplify the impact of cysteine supplementation on cerebral glutathione levels, as gamma-glutamylcysteine synthase, the rate-limiting enzyme for glutathione synthesis, is phase 2 inducible.[110, 111] Various phase 2 inducers, including lipoic acid, shown utility in MPTP-induced PD; conversely,

suppression of phase 2 induction by nrf2 knockdown sensitizes mice to MPTP or to a stereotaxically injected viral vector for alpha-synuclein expression.[112-117] Induction of gamma-glutamylcysteine synthase can also be achieved with the hormone melatonin, via a mechanism independent of nrf2;[118, 119] melatonin likewise has shown utility in rodent models of PD.[120] It should be noted that measures which increase glutathione synthesis also have potential for blunting microglial activation.[113, 121]

Quelling Mitochondrial Oxidative Stress with Astaxanthin

There is reason to suspect that, at least in PD, peroxynitrite may also arise within neurons, reflecting an interaction of nNOS activity and excessive mitochondrial superoxide production. Pathology studies have demonstrated that mitochondrial complex I activity tends to be deficient in the nigra of PD patients; such a deficiency would be expected to boost superoxide production at this complex.[122-124] Moreover, the ability of rotenone or MPTP administration to induce a Parkinsonian syndrome in rodents has been traced to the fact that these agents can inhibit complex I, and thereby promote oxidative stress within dopaminergic neurons.[125, 126] These agents are far less neurotoxic if nNOS is inhibited concurrently, consistent with the possibility that peroxynitrite is a major downstream mediator of their cytotoxicity.[70-72] The ability of alpha-synuclein overexpression to trigger mitochondrial damage and oxidative stress in hypothalamic cell cultures[127] suggests that possibility of a vicious cycle in which alpha-synuclein accumulation and/or aggregation damages mitochondria, promoting oxidative stress than in turn amplifies the pathogenicity of alpha-synuclein.

Intriguingly, if neuroblastoma cells are chronically exposed to a low concentration of rotenone (5 nM) too low to impair oxygen consumption or bioenergetics, there is no immediate detectible increase in oxidative stress; however, after incubation for several weeks, clear indices of oxidative stress are seen.[128] Arguably, this may reflect the fact that low level oxidative damage to the inner mitochondrial membrane results in gradually accumulating structural lesions that potentiate the impact of low-dose rotenone on superoxide production. The same phenomenon occurs, but much more rapidly, in ischemia reperfusion damage.[129, 130] Remarkably, this chronic low-dose rotenone model recapitulates many of the effects observed in dopaminergic neurons in Parkinson's disease – increased levels of both soluble and insoluble alpha-synuclein (apparently stemming from decreased clearance), glutathione deficiency, and increased apoptosis.

These considerations suggest that lipid-soluble antioxidants capable of protecting the mitochondrial membrane might have potential for alleviating any contribution of mitochondrial dysfunction to synucleopathies. Astaxanthin may be of particular interest in this regard, as it substantially more effective than alpha-tocopherol as a membrane antioxidant.[131-133] Indeed, the ability of astaxanthin pre-administration to markedly prevent ischemia-reperfusion damage likely reflects important protection of oxidatively stressed mitochondria.[134-137] It is therefore not surprising that a recent study finds that pre-incubation with astaxanthin can protect neuroblastoma cells from subsequent exposure to the active metabolite of MPTP, and that astaxanthin pre-treatment (30 mg/kg for 28 days) likewise is protective in vivo in MPTP-treated mice.[138] Astaxanthin also protects neuroblastoma cells exposed to the oxidant 6-hydroxydopamine, a likely adjunctive source of oxidative stress in PD;[139] it also protects a dopaminergic cell line from either 6-hydroxydopamine or peroxidized DHA, blocking the induction of mitochondrial damage and an increase in superoxide generation.[140, 141] To the extent that mitochondrially-generated

oxidative stress plays a pathogenic role in synucleopathies, astaxanthin may have useful protective potential, and nicely complement the antioxidant effects of urate, glutathione, and PhyCB.

Overview

In aggregate, these considerations suggest that, in the absence of more definitive evidence, a lifestyle regimen incorporating a Mediterranean or quasi-vegan diet, regular exercise, spirulina, caffeine, moderate alcohol, and ample dietary or supplemental intakes of green tea polyphenols, berry anthocyanins, flavonols, tannins, fish oil, astaxanthin, and vitamin D, may be a prudent strategy when attempting to prevent or control synucleopathies. Supplementation with inosine, N-acetylcysteine/cystine, phase 2 inducers such as lipoic acid, and melatonin may also be considered for the management of these disorders. Such recommendations are of course highly provisional, but are likely to be harmless, and indeed may promote overall health in a number of additional ways. Development of innovative functional foods or complex nutraceuticals could make implementation of such a strategy more feasible.

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