Oral Phycocyanobilin May Diminish the Pathogenicity of Activated Brain Microglia in Neurodegenerative Disorders

Mark F. McCarty, Jorge Barroso-Aranda, and Francisco Contreras,
Oasis of Hope Hospital, Tijuana, Mexico

Abstract

There is considerable evidence that activated microglia play a central role in the pathogenesis of many prominent neurodegenerative disorders, including Parkinson’s and Alzheimer’s diseases. The elevated NADPH oxidase activity of these microglia contributes importantly to their pathogenic impact, collaborating with increased iNOS activity to generate the cytotoxic oxidant peroxynitrite. Phycocyanobilin (PCB), a chromophore derived from biliverdin that constitutes up to 1% of the dry weight of spirulina, has recently been shown to be a potent inhibitor of NADPH oxidase. The possibility that orally administered PCB could reach the brain parenchyma in sufficient concentrations to influence microglial function is consistent with the findings of two rodent studies: orally administered C-phycocyanin (the spirulina holoprotein that includes PCB) suppresses the neurotoxic impact of the excitotoxin kainite in rats, and a diet high in spirulina ameliorates the loss of dopaminergic neurons in the MPTP-induced Parkinsonian syndrome in mice. Hence, supplemental PCB may have considerable potential for preventing or slowing the progression of a range of neurodegenerative disorders. Some of the central physiological effects of PCB may also reflect inhibition of neuronal NADPH oxidase, which is now known to have a modulatory impact on neuron function, and can mediate neurotoxicity in certain circumstances. Neuronal NADPH oxidase activation is an obligatory mediator of the central pressor effect of angiotensin II, and there is suggestive evidence that it may also play a role in inflammatory hyperalgesia; these findings point to possible antihypertensive and analgesic applications for PCB. The likely favorable effects of PCB on vascular health may also protect the brain by decreasing stroke risk, and inhibition of NADPH oxidase in rodents has been shown to lessen the neurotoxic impact of temporary cerebral ischemia. PCB may thus have versatile potential for preserving the healthful function of the central nervous system into advanced old age - albeit optimal neuroprotection may require more complex regimens that incorporate PCB along with other well tolerated nutraceuticals and drugs, in conjunction with prudent lifestyle modifications.

Phycocyanobilin - a Phytonutrient Inhibitor of NADPH Oxidase

Phycocyanobilin (PCB), a chromophore that plays an essential light-harvesting role in many blue-green algae and cyanobacteria, has recently been shown to be a potent inhibitor of the NADPH oxidase activity of various human cell cultures in low micromolar concentrations (Inoguchi T, personal communication). PCB is a derivative of biliverdin, and intracellular PCB can be reduced by biliverdin reductase to phycocyanorubin, which is very similar in structure to bilirubin. Recent studies have revealed that unconjugated bilirubin functions physiologically as an extremely potent
inhibitor of NADPH oxidase\textsuperscript{2-4} – a finding which rationalizes the important antioxidant activity of heme oxygenase\textsuperscript{5}, as well as the health protection associated with Gilbert syndrome, a genetic variant characterized by elevated free bilirubin levels.\textsuperscript{6-9} It thus seems likely that phycocyanorubin is the proximal mediator of PCB’s impact on NADPH oxidase activity.\textsuperscript{9} The possibility that PCB can act as an effective antioxidant when administered orally is strongly supported by Cuban studies showing that oral phycocyanin (the spirulina protein that carries PCB as its chromophore) exerts wide-ranging anti-inflammatory effects in rodents.\textsuperscript{10-12}

**A Central Role for Activated Microglia in Neurodegenerative Disorders**

Activated microglia are now suspected to play a central pathogenic role in the induction and progression of various prominent neurodegenerative conditions, including Parkinson’s and Alzheimer’s diseases, HIV-associated dementia, amyotrophic lateral sclerosis, periventricular leukomalacia (a common cause of cerebral palsy), multiple sclerosis, and subacute spinal cord injury.\textsuperscript{13-29} This conclusion is based on histological studies of clinical lesions, the neurotoxic impact of activated microglia in mixed neuron/microglia cultures, and the major pathogenic role which activated microglia play in rodent models of these neurodegenerative syndromes. Microglia are activated in response to the death of nearby neurons – a still mysterious phenomenon known as “reactive gliosis” – and/or in response to a stimulus provided by dysfunctional neurons (e.g. amyloid beta). This microglial activation can become self-sustained, and often contributes to the death or dysfunction of other nearby neurons – thereby closing a vicious cycle of neuronal death and microglial activation. In “white matter” disorders, such as periventricular leukomalacia and multiple sclerosis, activated microglia contribute to the death of oligodendrocytes that constitute the myelin lining of axons.

A key feature of microglial activation is an amplification of NADPH activity, which contributes in at least two ways to the pathogenicity of these microglia – it often up-regulates microglial expression of enzymes and cytokines that can mediate cytotoxicity – such as iNOS, Cox-2, TNF-alpha, and IL-1\textsuperscript{30-35}; but it also contributes more directly to the pathogenic impact of microglia by promoting production of hydrogen peroxide and, more importantly, peroxynitrite, thought to be a key mediator of neurotoxicity in many neurodegenerative disorders.\textsuperscript{36-42} One recent study in vitro concludes that microglia are neuroprotective in the absence of oxidative stress, but become neurotoxic once superoxide production via NADPH oxidase is stimulated.\textsuperscript{43} Other recent research concludes that microglia are only notably cytotoxic if NADPH oxidase and iNOS are concurrently induced – thus enabling rapid production of peroxynitrite.\textsuperscript{26} Microglia tend to be relatively resistant to oxidant damage, owing to their superior antioxidant defenses, but the peroxynitrite they produce is readily cell permeable, and is far more destructive to neurons.

**PCB May Have Access to Brain Parenchyma**

These considerations suggest that PCB may well have potential for preventing or slowing the progression of neurodegenerative syndromes via its impact on oxidant generation in
microglia. However, can safe and feasible intakes of this agent could get through the blood-brain barrier in sufficient concentration to achieve a meaningful inhibition of oxidant stress in brain microglia? Two studies suggest that the answer to this crucial question may be yes.

In 1999, Spanish and Cuban researchers reported that oral administration of C-phycocyanin – a spirulina protein which contains PCB as a covalently bound chromophore – protects rats from the neurotoxicity of kainite, a glutamate analog that induces excitotoxicity and epileptic seizures. C-phycocyanin was repeatedly administered orally in a dose of 100 mg/kg (roughly equivalent to 5 mg/kg PCB), 24 hours, 16 hours, and 1 hour prior to injection of kainite. The rats were sacrificed one week later, and indirect markers of hippocampal neuronal death were assessed – markers indicative of microglial and astroglial activation. These markers were notably elevated in the control rats treated with kainite, but pre-administration of C-phycocyanin almost wholly prevented this elevation. Moreover, the phycocyanin-treated rats were significantly less prone to the neurobehavioral effects of kainite – seizures, tremors, and “wet dog shakes” – and, as contrasted with the decrease in weight gain noted in the rats treated with kainite alone, the C-phycocyanin-treated rats maintained normal weight gain.

These findings can be viewed in the context of a recent report that kainite administration evokes oxidant stress in the hippocampus that is associated with membrane translocation of the cytoplasmic components of NADPH oxidase (indicative of the activation of this enzyme complex). Ex vivo, this excess oxidant stress was suppressed by DPI, a potent inhibitor of NADPH oxidase. Furthermore, kainite-evoked neuronal damage was substantially mitigated in transgenic mice expressing increased levels of extracellular superoxide dismutase (which would be expected to suppress peroxynitrite generation). In light of these findings, a reasonable interpretation of the previous Spanish study is that PCB derived from digested C-phycocyanin (or perhaps a digestive product containing PCB) was absorbed, passed through blood-brain barrier, and was able to suppress the microglial peroxynitrite generation evoked by kainite administration – thereby diminishing the neuropathological impact of the kainite.

Very recently, additional pertinent observations have been reported by Mexican researchers. Diets highly enriched in whole spirulina (providing up to 200 mg/kg/day) were found to have a marked ameliorative effect on the loss of striatal dopamine in MPTP-treated mice. The neurodegeneration associated with MPTP administration is considered to represent a model for human Parkinsonism. Previous research has established that microglial NADPH activation is a prominent mediator of the neural damage triggered by MPTP in this syndrome. Although whole spirulina contains a range of phytochemicals that have radical-scavenging potential, the most parsimonious explanation of the protection afforded by spirulina in MPTP-treated mice is that PCB derived from digested spirulina was absorbed, gained access to brain microglia, and suppressed their production of oxidant stress.

Hence, two experimental studies, one in rats and one in mice, are consistent with the proposition that feasible oral doses of PCB can gain access to brain parenchyma in
sufficient concentration to suppress the generation of oxidant stress by activated microglia. Unless humans are notably different from rodents in regard to PCB transport, it thus appears likely that PCB has great clinical potential as a neuroprotective agent. With respect to safety considerations, it may be noted that spirulina is a food that was a staple of the pre-Columbian Aztec diet, and that spirulina-derived phycocyanin is an approved food additive, employed as food colorant owing to the intense blue color imparted by its chromophore PCB. The safety of high spirulina intakes in mice and rats – up to 30% of diet - has been established.51,52

NADPH Oxidase in Neurons is Potentially Pathogenic

Recent studies reveal that many if not most neurons, centrally and peripherally, express the proteins that constitute NADPH oxidase, and that under certain circumstances these proteins congregate to generate NADPH oxidase activity.53-62 Although neuronal NADPH oxidase activity is never as intense as that expressed by activated microglia, it can have a sufficient impact on neural redox status to influence the function and survival of neurons. Thus, activation of NADPH oxidase in sympathetic neurons is an obligate mediator of the apoptosis evoked by nerve growth factor withdrawal.53,59 A pro-apoptotic or pro-necrotic role for neural NADPH oxidase has also been demonstrated in neurons exposed to fibrillar amyloid-beta, HIV-derived gp120, brain-derived neurotrophic factor, toxic concentrations of zinc, and a pathogenic prion peptide, or that are subjected to anoxia and reoxygenation.54-56,63-67 Glutamate excitotoxicity in neuroblastoma cells likewise requires activation of NADPH oxidase.68 These considerations suggest that neural NADPH oxidase activation may contribute to neuronal loss in certain pathologies. With respect to ischemia-reperfusion damage, rodents that are genetically deficient in NADPH oxidase activity, or that are pre-treated with the NADPH oxidase inhibitors apocynin or atorvastatin, experience a lesser infarct volume when their brains are subjected to transient ischemia.69-71 While these latter studies do not clarify the chief source of the oxidant stress that is most neurotoxic in ischemia-reperfusion syndrome – neurons, activated microglia, or infiltrating leukocytes – they do point to the likelihood that supplementation with PCB could improve the tolerance of the brain to transient ischemic episodes.

Sub-lethal levels of oxidant stress attributable to NADPH oxidase activation in neurons may play a role in certain pathologies. Thus, angiotensin II acting in the rostral ventrolateral medulla (RVM) exerts a sympathetic pressor effect that presumably contributes to clinical hypertension in many cases; this effect of angiotensin II is contingent on activation of NADPH oxidase in RVM neurons, and it is markedly attenuated by the antioxidants DPI or tempol, as well as by dominant-negative Rac1.72,73 Increased oxidative stress mediated by NADPH oxidase is also observed in the cortex and hippocampus of salt-fed stroke-prone spontaneously hypertensive rats, and in the postganglionic sympathetic neurons of DOCA-salt hypertensive rats;62,74 whether this excess neural oxidative stress plays a pathogenic role in the associated hypertension is not clear. NADPH oxidase is also responsible for increased oxidative stress in the sympathetic ganglia of apolipoprotein E-deficient mice (a model of atherogenesis).75 It seems likely that PCB could act both centrally and peripherally to ameliorate
hypertension – by boosting the vasorelaxant bioefficacy of nitric oxide in resistance arteries (consequent to suppression of arterial superoxide production), and by antagonizing the central pressor actions of angiotensin II. Indeed, it is notable that Gunn rats, a genetic variant in which free bilirubin levels are constitutively elevated, are much less responsive to the longterm hypertensive impact of angiotensin II infusion, and that the prevalence of hypertension in middle-aged subjects with Gilbert syndrome appears to be anomalously low. Moreover, it seems likely that long term use of PCB could lower stroke risk – both through an antihypertensive action, and by protecting the bioefficacy of the nitric oxide produced by cerebrovascular endothelium.

There is also growing evidence that increased oxidant stress in both primary and secondary sensory neurons is a key mediator of inflammatory as well as diabetic hyperalgesia. Whether NAPDH oxidase is the primary source of this stress has not yet been established, but it is reasonable to suspect that it is. Indeed, nerve growth factor – known to be a mediator of inflammatory hyperalgesia – promotes thermal hyperalgesia in small diameter sensory neurons by boosting the expression and axonal transport of TRPV1 (the heat receptor responsive to capsaicin); this effect has been shown to be contingent on sequential activation of Rac1, NADPH oxidase, and p38 MAP kinase. The cold receptor, TRPA1, is also up-regulated by p38 MAP kinase activation in these neurons, so it is reasonable to suppose that NADPH oxidase activation can likewise contribute to cold hyperalgesia. Hence, it will be of interest to explore the impact of PCB on inflammatory hyperalgesia.

A Functional Role for Neuronal NADPH Oxidase

It should be acknowledged that modest levels of oxidant stress evoked by activation of NADPH oxidase in neurons are likely to play a useful physiological role in some circumstances – implying that complete abrogation of neural NADPH oxidase activity could have some adverse impacts and would be inappropriate. For example, transient activation of NADPH oxidase appears to play a role in the phenomenon of long term potentiation (LTP) in hippocampal neurons, a process that is crucial for memory formation. On the other hand, excessive oxidant stress is detrimental to LTP, and this phenomenon may contribute to impaired memory function during the early stages of Alzheimer’s that precede the major loss of hippocampal neurons. In mice, various antioxidant measures tend to have a slightly detrimental impact on learning in young mice, but a positive impact in aging mice; this suggests that hippocampal oxidant stress is quite low in young mice, but gradually increases until it has a negative impact on LTP. Minor learning defects have been observed in young mice that are genetically incapable of producing one or more of the essential components of NADPH oxidase; that these defects are quite subtle may reflect the fact that mitochondria and cyclooxygenase can serve as alternate sources of superoxide in neurons.

Clearly, our understanding of the role of NADPH oxidase in neural function and neuropathology is still in its infancy. Nonetheless, we know enough to be confident that excess activity of this complex, whether in microglia or neurons, plays an important role in many common neuropathologies. Thus, in light of suggestive evidence that orally
administered PCB has access to brain parenchyma, it is reasonable to suspect that PCB may have a bright future in the prevention or amelioration of a wide range of neurologic disorders reflecting neuronal death or dysfunction.

**PCB in the Context of Broader Neuroprotective Regimens**

It must however be acknowledged that, in light of the physiological role which NADPH oxidase plays in the immune system, neurons, and other tissues, it will not be safe and appropriate to achieve more than partial inhibition of this enzyme complex. Moreover, NADPH oxidase overactivity is only one aspect – albeit a crucial one - of pathological neurodegeneration. It is likely that the most effective neuroprotective regimens will entail the concurrent use of several nutraceuticals and/or drugs that address complementary aspects of the neurodegenerative process\textsuperscript{15,96,97} – preferably in conjunction with prudent lifestyle measures.\textsuperscript{98} Thus, measures which boost the antioxidant defenses or bioenergetic capacity of neurons, which provide protection from excitotoxicity, which lessen production of amyloid beta, which increase brain production of protective neurotrophic factors, or which work in complementary ways to dampen microglial activation, could be expected to amplify the protection afforded by PCB.

Vitamin D may be particularly appropriate as a complement to PCB, in light of evidence that activated microglia can convert circulating 25-hydroxyvitamin D to the active hormone calcitriol,\textsuperscript{99} which in turn can down-regulate expression of iNOS in microglia.\textsuperscript{100-102} The efficacy of this effect will presumably be proportional to the concentration of 25-hydroxyvitamin D in brain parenchyma; there is some evidence that risk for Alzheimer’s and Parkinson’s correlates positively with latitude, and poor vitamin D status is a frequent concomitant of these disorders.\textsuperscript{103-105} To the extent that optimal vitamin D status can decrease, if perhaps only to a minor degree, microglial iNOS activity, it should collaborate with PCB in suppressing peroxynitrite production in regions of the brain challenged by inflammatory neuropathology.

The particular merit of supplemental PCB and vitamin D – and other potentially neuroprotective nutrients, such as creatine, taurine, selenium, melatonin, lipoic acid, soy phytoestrogens, and fish oil\textsuperscript{15,106} - is that they may be suitable for the primary prevention of neurodegenerative disorders in healthy subjects, particularly since these agents seem likely to confer a host of other preventive health benefits. Lifestyle measures such as ample coffee (caffeine) consumption, regular physical and mental exercise, a high potassium/low-salt diet, and possibly intermittent fasting, may also be helpful for staving off neurodegeneration.\textsuperscript{15,98,107} In individuals who show early signs of neurodegenerative disorders, or who are known to be at high genetic risk for such disorders, the use of certain drugs – minocycline, dextromethorphan, cox inhibitors, PPAR-gamma agonists, statins, and memantine are currently credible candidates\textsuperscript{15,108} – might provide amplified protection; future clinical research should establish which of these have truly worthwhile efficacy in doses that avoid unacceptable side effects.
References


9. McCarty MF. "Iatrogenic Gilbert syndrome" - a strategy for reducing vascular and cancer risk by increasing plasma unconjugated bilirubin. Medical Hypotheses 2007; accepted for publication.


104. Newmark HL, Newmark J. Vitamin D and Parkinson's disease-A hypothesis. Mov Disord. 2007;..


