Inflammation in Parkinson´s Diseases and Other Neurodegenerative Diseases: Cause and Therapeutic Implications

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Abstract: Agents suppressing microglial activation are attracting attention as candidate drugs for neuroprotection in Parkinson´s disease (PD): While different mechanisms including environmental toxins and genetic factors initiate neuronal damage in the substantia nigra and striatum in PD, there is unequivocal evidence that activation of neuroinflammatory cells aggravates this neurodegenerative process. It was shown that following an acute exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and other toxins the degenerative process continues for years in absence of the toxin. Reactive microglia has been observed in the substantia nigra of patients with PD, indicating that this inflammatory process might aggravate neurodegeneration. By releasing various kinds of noxious factors such as cytokines or proinflammatory molecules microglia may damage CNS cells. The stimuli triggering microgliosis in Parkinsonian syndromes are unknown so far: However, analysis of neuronal loss in PD patients shows that it is not uniform but that neurons containing neuromelanin (NM) are predominantly involved. We hypothesized that extraneuronal melanin might trigger microgliosis, microglial chemotaxis and microglial activation in PD with subsequent release of neurotoxic mediators. The addition of human NM to microglial cell cultures induced positive chemotactic effects, activated the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) *via* phosphorylation and degradation of the inhibitor protein κB (IκB), and led to an upregulation of TNF-α, IL-6 and NO. These findings demonstrate a crucial role of NM in the pathogenesis of Parkinson´s disease by augmentation of microglial activation, leading to a vicious cycle of neuronal death, exposure of additional neuromelanin and chronification of inflammation. Antiinflammatory drugs may be one of the new approaches in the treatment of PD.

Key Words: Microglia, Parkinson´s disease, neuroinflammation

INTRODUCTION

Idiopathic Parkinson's disease (PD), first described by James Parkinson in 1817, is the second most common neurodegenerative disorder after Alzheimer´s disease. It affects 1- 2 % of the general population over the age of 65. The disease predominantly affects dopaminergic neurons of the substantia nigra pars compacta, culminating in their demise with subsequent depletion of dopamine in the striatum. After approx. 50% of the dopaminergic neurons and 75-80% of striatal dopamine are lost, patients start to exhibit the classical symptoms of PD including bradykinesia, postural reflex impairment, resting tremor, and rigidity.

Despite many years of focused research, the causes of the degeneration of dopaminergic neurons remains to be elucidated. Understanding the cause of PD is critical as that knowledge could lead to directed research that will develop new and potent therapies. The relative contributions of environmental versus genetic factors regarding the cause of PD have been hotly debated: Epidemiological studies have for a long time suggested that pesticide exposure is associated with an increased risk of developing PD [1-3]. Compelling evidence in favour of an environmental agent came from the discovery that inadvertent injections of the protoxin *n*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) cause a parkinsonian syndrome in humans that is virtually indistinguishable from PD [4]. Researchers have capitalised on this discovery to develop an animal model of PD. After administration MPTP crosses the blood-brain barrier and is converted in astrocytes by monoamine oxidases to its active metabolite 1-methyl-4-pyridinium (MPP+). MPP+ was found to be a mitochondria poison that inhibits mitochondrial respiration at complex I of the electron transport system [5]. The selectivity of MPP+ for dopaminergic neurons is due to the fact that it is an excellent substrate for the dopaminergic transporter and is thereby accumulated preferentially in dopaminergic neurons [6]. Following recognition of MPTP´s neurotoxicity and its mechanism of action, several laboratories reported a selective defect in complex I of the electron transport chain in PD. Other agricultural chemicals have been shown to induce a parkinsonian state as well: One of them is rotenone, a common pesticide and naturally occurring compound derived from the roots of certain plant species [7]. Rotenone is commonly used as an insecticide in vegetable gardens, and is also used to kill or sample fish populations in lake and reservoirs. Exposure to the herbicide 1, 1´dimethyl-4´-bipyridinium, or paraquat has emerged as another putative risk factor for PD on the basis of its structural similarity to MPP+. Systemic injection of paraquat into mice causes a dose-dependant decrease in dopaminergic nigral neurons and striatal

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dopaminergic neurons followed by reduced ambulatory movements [8]. Manganese, which is used in overlapping geographical areas with paraquat, has been shown to decrease locomotor activity and potentate MPTP effects, suggesting that exposure to mixtures of chemicals may also be relevant etiologically. These results highlight the possibility that environmental toxins, including pesticides that inhibit mitochondrial function, may contribute to degeneration of dopaminergic neurons in PD.

While different mechanisms including environmental toxins and genetic factors initiate neuronal damage in the substantia nigra and striatum, there is now unequivocal evidence that activation of neuroinflammatory cells, especially microglia, with subsequent production of pro-inflammatory cytokines and molecules aggravates this neurodegenerative process: It was shown that following an acute exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), manganese and other toxins the degenerative process continues for years in absence of the toxin [9]. Large numbers of reactive microglia, the resident macrophages of the CNS have been observed in the substantia nigra pars compacta in human post-mortem tissue from patients with PD [10] as well as patients suffering from MPTP-induced parkinsonism [9], indicating that this inflammatory process might aggravate neurodegeneration. By releasing various kinds of noxious factors such as cytokines or proinflammatory molecules (e.g. proteolytic enzymes, reactive oxygen intermediates or nitric oxide) microglia may damage CNS cells [11-14] Nitric oxide (NO) is neurotoxic due to inhibition of complex 1 and 2 of the respiratory chain. Moreover it reacts with superoxide anion to generate peroxynitrite, a highly reactive molecule capable of oxidizing proteins, lipids and DNA, which causes striatal neurodegeneration in a mouse model of PD *in vivo* [15]. An increased nitrotyrosine immunostaining is found in Lewy bodies of PD patients. The density of glial cells expressing the inducible form of nitric oxide synthase (iNOS) is markedly increased in the SN of PD patients as compared to controls as well [16], increased concentrations of the NO metabolite nitrite were found in the cerebrospinal fluid (CSF) of PD patients as compared to unaffected subjects as well as in MPTP-intoxicated mice [17, 18]. The cytokine tumor necrosis factor-α (TNF-α) is an important factor in the regulation of inflammatory processes and apoptotic cell death: In brains of PD patients, TNF-α producing glial cells have been detected in the substantia nigra and immunoreactivity for TNF-α receptors was found in cell bodies and processes of most dopaminergic neurons [19]. The stimuli triggering microgliosis in Parkinsonian syndromes are unknown so far: However, analysis of neuronal loss in the mesencephalon of PD patients shows that it is not uniform but that neurons containing neuromelanin (NM) are predominantly involved [20, 21]. Moreover, NM was found in patients suffering from juvenile [22], idiopathic and (MPTP)-induced parkinsonism [9]. In an autopsy study of a patient with (MPTP)-induced parkinsonism extraneuronal NM was found in close vicinity to activated microglial cells even 12 years after exposure to the neurotoxin [9]. We have hypothesized that extraneuronal melanin might trigger microgliosis, microglial chemotaxis and microglial activation in PD with subsequent release of proinflammatory and possible neurotoxic mediators like NO and TNF-α. The addition of human NM to microglial cell cultures induced positive chemotactic effects, activated the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) *via* phosphorylation and degradation of the inhibitor protein κB (IκB), and led to an upregulation of TNF-α and NO. These findings demonstrate a crucial role of NM in the pathogenesis of Parkinson´s disease by augmentation of microglial activation, leading to a vicious cycle of neuronal death, exposure of additional neuromelanin and chronification of inflammation [23]. The impairment of NF-κB function by the IκB-Kinase inhibitor sulfasalazine was paralleled by a decline in neurotoxic mediators. NM also activated p38 mitogen-activated protein kinase, the inhibition of this pathway by SB203580 diminished phosphorylation of the transactivation domain of the p65 subunit of NF-κB. Further insight into the involvement of inflammation-mediated neurodegeneration in PD has come from other experimental models of the disease: Indeed, lipopolysaccharide (LPS), an element of bacterial cell walls, which activates microglial cells and induces the expression of proinflammatory cytokines, has been shown to kill dopaminergic neurons in mixed neuron-glial but not pure neuronal cultures. Furthermore, the toxicity of agents capable of inducing the death of dopaminergic neurons, such as MPTP or 6-hydroxydopamine, was enhanced when the dopaminergic neurons were cultured with LPS-activated astrocytes [24]. *In vivo* models of PD also support the notion that activated glial cells may participate in the degeneration of dopaminergic neurons: Herrera and co-workers reported that a single intranigral injection of LPS induces damage to dopaminergic neurons in the substantia nigra with preservation of GABAergic and serotinergic neurons [25]. More recently it has been shown that dexamethasone, a potent anti-inflammatory drug that interferes with many of the features characterizing proinflammatory reaction, prevented the loss of catecholaminergic content, tyrosine hydroxylase activity, and tyrosine-hydroxylase immunostaining induced by LPS injection and also the bulk activation of microglia [26].

While epidemiological data suggest that use of nonsteroidal anti-inflammatory drugs (NSAID) may delay or prevent the onset of PD [27], the use of anti-inflammatory drugs to prevent dopaminergic degeneration in PD has not been formally tested in patients. However, various drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) with their targets COX NFkB and others, including steroids, immunophilins, thalidomide, and phosphodiesterase IV inhibitors have been studied with variable results in animal models: While aspirin acting *via* inhibition of COX 1 / 2 and NFkB inhibited neurodegeneration in the MPTP mouse model [28], other inhibitors of COX 1/ 2 like paracetamol, diclofenac or ibuprofen [28] failed to exert a neuroprotective effect. Acting upon the steroid receptor dexamethasone or hydrocortisone showed either just a partial [29] or no neuroprotective effect [30]**.** In a different approach it has been demonstrated that activation of peroxisome proliferator-activated receptor- χ (PPAR-χ), a member of the nuclear receptor superfamily, inhibits inflammatory properties in monocytes [31] and microglia *in vitro* [32] and *in vivo* [33]. Orally administered pioglitazone, a selective PPAR-χ agonist attenuated the MPTP-induced microglial activation and prevented the dopaminergic cell loss in the substantia nigra [34]**.** Moreover, minocycline a tetracycline derivative that inhibits microglial activation as well was recently shown to reduce microglial activation and protects dopaminergic structures in the entire

striatonigral system in the MPTP mouse [35] or 6-OH-DOPA model [36]. These findings demonstrate the potentially deleterious role of activated glia for the pathogenesis of PD.

Increased binding of a ligand for the peripheral benzodiazepine binding receptor (PBR) is currently used in PET-studies as an in-vivo measurement of inflammation in diseases like Multiple Sclerosis [37, 38], Alzheimer´s disease [39] and progressive suprannuclear palsy [40]. Previous reports suggest that elevated PBR levels in brain tissue are specific to activated microglia. Since its functional role is unknown, we determined if this receptor is expressed on microglia *in vitro* and, moreover if benzodiazepines modulate proliferation of microglial cells and release of the inflammatory molecules nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) in cell culture supernatants of primary rat microglia. Compared to lipopolysaccharide-activated microglia the release of NO was markedly decreased in benzodiazepine (Clonazepam, Midazolam, Diazepam) treated cultures. Moreover, release of TNF-α and proliferation was significantly inhibited in the benzodiazepine treated groups [41].

In conclusion the data reviewed here suggest an involvement of the glial reaction and inflammatory processes in the progression of neuronal degeneration in parkinsonian syndromes. Pharmacological manipulation of these pathways affords a relative degree of neuroprotection. Yet it remains to be determined if these molecules will be neuroprotective in PD.

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