Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Experimental Neurology 205 (2007) 295–312
Experimental Neurology
www.elsevier.com/locate/yexnr

Review

Non-steroidal anti-inflammatory drugs in Parkinson's disease

Ennio Esposito [a], Vincenzo Di Matteo [a], Arcangelo Benigno [b], Massimo Pierucci [a], Giuseppe Crescimanno [b], Giuseppe Di Giovanni [b,*]

[a] *Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, Santa Maria Imbaro (Chieti), Italy*
[b] *Dipartimento di Medicina Sperimentale, Sezione di Fisiologia Umana, "G. Pagano", Università degli Studi di Palermo, Corso Tuköry 129, 90134 Palermo, Italy*

Received 21 November 2006; revised 5 February 2007; accepted 13 February 2007
Available online 23 February 2007

Abstract

Parkinson's disease (PD) is known to be a chronic and progressive neurodegenerative disease caused by a selective degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc). A large body of experimental evidence indicates that the factors involved in the pathogenesis of this disease are several, occurring inside and outside the DAergic neuron. Recently, the role of the neuron–glia interaction and the inflammatory process, in particular, has been the object of intense study by the research community. It seems to represent a new therapeutic approach opportunity for this neurological disorder. Indeed, it has been demonstrated that the cyclooxygenase type 2 (COX-2) is up-regulated in SNc DAergic neurons in both PD patients and animal models of PD and, furthermore, non-steroidal anti-inflammatory drugs (NSAIDs) pre-treatment protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6 hydroxydopamine (6-OHDA)-induced nigro-striatal dopamine degeneration. Moreover, recent epidemiological studies have revealed that the risk of developing PD is reduced in humans who make therapeutical use of NSAIDs. Consequently, it is hypothesized that they might delay or prevent the onset of PD. However, whether or not these common drugs may also be of benefit to those individuals who already have Parkinson's disease has not as yet been shown.

In this paper, evidence relating to the protective effects of aspirin or other NSAIDs on DAergic neurons in animal models of Parkinson's disease will be discussed. In addition, the pharmacological mechanisms by which these molecules can exert their neuroprotective effects will be reviewed. Finally, epidemiological data exploring the effectiveness of NSAIDs in the prevention of PD and their possible use as adjuvants in the therapy of this neurodegenerative disease will also be examined.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Parkinson's disease; Aspirin; Cyclooxygenase inhibitors; Neuroprotection; Neurodegenerative disease; Inflammation; Hydroxyl radicals

Contents

Introduction . . . 296
Inflammation in Parkinson's disease . . . 296
Old and new mechanisms of action for NSAIDs . . . 298
Neuroprotective effects of NSAIDs in Parkinson's disease: experimental evidence . . . 301
Neuroprotective effects of NSAIDs in Parkinson's disease: epidemiological evidence . . . 305
Conclusions and implications . . . 307
Acknowledgments . . . 307
References . . . 307

* Corresponding author. Fax: +39 0916555823.
E-mail address: g.digiovanni@unipa.it (G. Di Giovanni).

0014-4886/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2007.02.008

Introduction

Parkinson's disease (PD) is the most prevalent neurological disorder of the basal ganglia, and it is characterized by a progressive loss of dopaminergic (DAergic) neurons in the caudate nucleus, putamen and substantia nigra (SN) (Ehringer and Hornykiewicz, 1960; Riederer and Wuketich, 1976). The loss of DAergic neurons in the substantia nigra pars compacta (SNc) is the principal feature of PD (Bernheimer et al., 1973) and results in cardinal motor symptoms such as tremor at rest, bradykinesia, muscular rigidity, stooped posture and instability (Sian et al., 1999). Hitherto, despite the recent progress in understanding the etiopathogenesis of PD, the modalities whereby the neurodegenerative process begins and progresses are still unclear. The situation is further complicated by the large number of factors that seem to be involved in the onset of this disease, such as aging, genetic vulnerability, exogenous or endogenous toxins, hydroxyl radicals production, neuronal metabolic disturbances and inflammation (Hirsch et al., 1998; Sian et al., 1999; Jellinger, 2000; Gebicke-Haerter, 2001; Jenner and Warren, 2006). The consequent cumulative neuronal insults attributable to these metabolic stress factors may promote premature SNc DAergic degeneration through the activation of apoptotic programs (Hartmann and Hirsch, 2001; Novikova et al., 2006; Nair et al., 2006). However, the specifics and sequential neuroapoptotic events associated with premature, progressive SNc neuronal atrophy remain undefined.

Thus far, among the various accepted experimental models of PD, neurotoxins still represent the most popular tools to produce selective death of DAergic neurons both in *in vitro* and *in vivo* systems. Even though recent genetic discoveries have lead to a number of different genetic models of PD, none of these shows the typical degeneration of DAergic neurons (Beal, 2001; Fleming et al., 2005). Among the neurotoxins, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a product of synthetic meperidine derivative, and 6-hydroxydopamine (6-OHDA), hydroxylated dopamine derivatives are the most used for inducing parkinsonian features in cells and animal species. MPTP is metabolized to the 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase-B (MAO-B) (Langston et al., 1984). This highly toxic metabolite is selectively taken up into DAergic neurons, via the dopamine (DA) transporter (Snyder and D'Amato, 1986), where it provokes an intracellular accumulation of Ca^{2+}, interfering with the function of nerve terminals in the striatum (Sun et al., 1988) and inhibiting complex 1 (NADH-ubiquinone oxidoreductase) of the respiratory chain causing progressive cell death (Cleeter et al., 1992). On the other hand, the neurotoxic effects of 6-OHDA are mediated by the generation of hydroxyl radicals, pro-inflammatory mediators or pro-apoptotic agents (Cohen, 1984; Jeon et al., 1995; Bové et al., 2005). The results of the administration of each neurotoxin, albeit by different mechanisms, is DA depletion in the nigro-striatal pathway of laboratory animals and molecular alterations comparable to those seen in PD's patients (Blum et al., 2001). Recently, it has been shown that 6-OHDA and MPTP like the bacterial lipopolysaccharide (LPS) induce the death of DA cells activating an immune response (Wang et al., 2005; Vijitruth et al., 2006; de Meira Santos Lima et al., 2006). These animal models have been crucial in the study of PD and have allowed the formulation of different hypotheses about its etiopathogenesis, and recently, they have been utilized to determine the role of inflammation in DA neuronal death. Moreover, toxin-based models have been useful in developing neuroprotective and neurorestorative strategies and in testing new drugs for the treatment of this disorder. In this review, experimental data regarding the role of neuroinflammation in the aetiology of PD, the effect of non-steroidal anti-inflammatory drugs (NSAIDs) and the possibility for their use as a new therapeutic approach for this neurodegenerative disease will be reviewed.

Inflammation in Parkinson's disease

Decades of research on the aetiology of Parkinson's disease have resulted in much information, but little has been gained in establishing the events causing the initiation and progression of the disease. Recently, the involvement of neuroinflammation and microglial activation in the pathogenesis of PD (Table 1) has been emphasized (Mogi et al., 1994a,b; Mogi et al., 1995; Blum-Degen et al., 1995; Rowe et al., 1998; Langston et al., 1999; Mirza et al., 2000; Knott et al., 2000, 2002; McGeer et al., 2001; McGeer et al., 2002; Imamura et al., 2003; Ouchi et al., 2005; Ishida et al., 2006; Kim and Joh, 2006). Results of neurotoxin models of PD, corroborating findings obtained in transgenic animal models and epidemiological studies, strongly

Table 1
Evidence of inflammation in PD-Human data

Study	Evidence from PD patients
McGeer et al., 1988	Up-regulation of MHC molecules in brains
Mogi et al., 1994a,b	Increased level TNF-α, in the striatum and CSF
Mogi et al., 1995	Increased levels of β2-microglobulin, the light chain of MHC, in striata
Blum-Degen et al., 1995	Increased levels of IL-1β and IL-6 in the CSF
Rowe et al., 1998	Presence of antibody reactivity to quinone-modified proteins
Langston et al., 1999	Presence of gliosis and clustering of microglia around nerve cells in MPTP-induced parkinsonism in humans
Mirza et al., 2000	Absence of reactive astrocytosis in the inflammatory process in PD autopsies.
Knott et al., 2000	Up-regulation of nitric oxide synthase- and cyclo-oxygenase-1- and -2-containing amoeboid microglia
Knott et al., 2002	Up-regulation of glial neurotrophins (BDNF, NT-3) in response to signals released from failing nigral neurons.
McGeer et al., 2002	Association of interleukin-1 beta polymorphisms with idiopathic PD
Imamura et al., 2003	The number of activated microglia is higher not only in the SN and putamen but also in the hippocampus, transentorhinal cortex, cingulate cortex and temporal cortex in PD.
Ouchi et al., 2005	Parallel changes in microglial activation and corresponding dopaminergic terminal loss in the affected nigrostriatal pathway in early PD.
Ishida et al., 2006	Increased expression of PAR-1 in astrocytes in SNpc of PD brain.

support the hypothesis that this neurodegenerative disease is not purely neuronal, as it has been previously considered (McGeer and McGeer, 2004; Hald and Lotharius, 2005). DAergic neuronal degeneration is the likely result of multiple pathogenic factors occurring both within and outside the cell. The cross-talk between neurons and glia is becoming more and more important for the understanding of brain pathophysiology. This new finding, unfortunately, does not allow us to diagnose the disease any earlier because the neuroinflammatory process is silent and unnoticed due to the absence of pain fibres in the brain, but it at least gives a glint of hope for new potential therapeutic targets for the slowing of neuronal degeneration.

Neuroinflammation is not a distinctive characteristic of PD but it has been clearly revealed in a broad spectrum of neurodegenerative diseases that share with it a common pathological process, such as Alzheimer's disease (AD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) (Minghetti, 2005; Kim and Joh, 2006; Pavese et al., 2006). The scenario is still obscure, but inflammation in PD is not any longer considered a non-specific consequence of neuronal degeneration as it was originally thought to be. Indeed, neuroinflammation may aggravate the course of the disease and, as has recently been suggested, may be a primary factor in some cases of PD (Hald and Lotharius, 2005; Marchetti and Abbracchio, 2005; Kim and Joh, 2006; Jenner and Warren, 2006). In fact, postmortem examinations have shown that neuronal degeneration in PD is associated with massive gliosis due to a population of activated glial cells, the microglia (McGeer et al., 1988; Członkowska et al., 2002; Teismann et al., 2003), evidence that has been confirmed in MPTP-induced parkisonism in monkeys (McGeer et al., 2003) and humans (Langston et al., 1999). Interestingly, the SN, usually prone to the deleterious effects of oxidant stress, containing DA neurons high in iron and low in glutathione (Jenner and Olanow, 1998, 2006), is also one of the brain regions more sensitive to inflammation. Indeed, the healthy SN exhibits the highest concentration of microglia in the brain especially in the ventral tier of the pars compacta (Lawson et al., 1990; Kim et al., 2000). Normally, very few microglial cells are detected in the vicinity of DAergic neurons, and when present, they appear to be resting with fine, long processes. Neuronal damage, aggregated proteins with abnormal conformations present in Lewy bodies and other unknown factors increase the number and change the shape of glial cells, to such an extent that they can be found in proximity to DA cells with short cellular processes (Zhang et al., 2005). Activated microglia are recruited to the SNc from various structures and finally stuck to DA neurons. It has been shown that glial cells once activated become phagocytes that ingest degenerating DA neurons piece-by-piece. This occurs early in neuronal degeneration, starting at the extending fibres, such as the dendrite which extends into the SN reticulata (Sugama et al., 2003). Hence, activated glial cells release detrimental compounds such as, interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α) and interferon γ (IFN- γ), which may act by stimulating inducible nitric oxide synthase (iNOS), or which may exert a more direct deleterious effect on DAergic neurons by activating receptors that contain intracytoplasmic death domains involved in apoptosis (Kataoka et al., 1997; Mogi et al., 1998; Kaku et al., 1999; Kurkowska-Jastrzębska et al., 1999a,b; Knott et al., 2000; Iravani et al., 2002; Teismann et al., 2003; Przybyłkowski et al., 2004; de Meira Santos Lima et al., 2006). Microglia can also induce neuritic beading (Takeuchi et al., 2005) or synaptic stripping along dendrites (Schiefer et al., 1999) leading to synaptic disconnection and loss of trophic support and cell death (Isacson et al., 1985; Jiang et al., 2006). Given that glial cells are potent activators in lymphocyte invasion, animal studies using MPTP have shown that the immune reaction might evolve, ultimately leading to the infiltration of lymphocytic $CD4^+$ and $CD8^+$ T cells into the injured SN and striatum (Kurkowska-Jastrzębska et al., 1999a,b). Moreover, activated lymphocytes have been shown in the SN of patients with PD and they could be responsible for the immune reaction-associated inflammatory process seen in the PD brain (McGeer et al., 2001; Baba et al., 2005).

Such activation of microglia is, nevertheless, not only disadvantageous to neurons. Indeed, some investigations indicate that activated microglial cells and macrophages tend to synthesise and produce neurotrophic factors (brain-derived neurotrophic factor, BDNF and glia-derived neurotrophic factor, GDNF) through certain compensatory mechanisms following neuronal injury and induce sprouting surrounding the wound in the striatal DA terminals (Batchelor et al., 1999; Minghetti et al., 2005). Moreover, activated glia play a role in gradually removing the dead DA neurons as a defence mechanism, although some healthy DA neurons might also be phagocytosed during the process (Novikova et al., 2006; Cho et al., 2006). As a consequence, inflammation has been rightly defined as a double-edged sword. It normally starts as a defence reaction but, for the failure of its control mechanism, can lead to an uncontrolled and continuous extremely damaging immune response. Furthermore, brief pathogenic insult, can induce an ongoing inflammatory response and the toxic substances released by the glial cells may be involved in the propagation and perpetuation of neuronal degeneration. This theory is plausible, corroborated by the evidence that several years after exposure to MPTP, increased levels of factors such as, IL-1β, IL-6 and TNF-α have been found in the basal ganglia and cerebral spinal fluid (CSF) of patients with toxin-induced PD (McGeer et al., 2001).

A prominent factor in neuroinflammatory reactions in PD seems to be the activation of the complement system (Mirza et al., 2000; Loeffler et al., 2006; Bonifati and Kishore, 2007) a major mediator of immune/inflammation reactions. Indeed, increased mRNA levels of complement components have been found in affected brain regions (McGeer and McGeer, 2004). The presence of complement components, including all constituents of the membrane attack complex (MAC), has been shown intracellularly on Lewy bodies and on oligodendroglia in the SN of PD patients (Yamada et al., 1991, 1992, 1993). Accumulation of Lewy bodies can apparently cause the activation of complement, the initiation of reactive changes in microglia, and the release of potentially neurotoxic products such as the MAC, hydroxyl radicals, and excess glutamate (GLU) (McGeer and McGeer, 1998).

So far, among the plethora of toxic factors released by the reactive glia it is not clear which one of them is responsible for

the DAergic neuronal death. Reactive oxygen species (ROS), hydroxyl radicals , NO and its peroxinitrite ($ONOO^-$), are the likely candidates (Di Matteo et al., 2006a,b). From this evidence it appears clear that the inflammatory process and oxidative stress derived from DA metabolism, constitute a vicious cycle that lead to the final demise of nigral DA cells (Fig. 1) (Hald and Lotharius, 2005).

Furthermore, experimental evidence has also shown that inflammatory loss of DA nigro-striatal neurons might be mediated by apoptosis (Cassarino et al., 2000; Lee et al., 2001; Furuya et al., 2004; Arimoto et al., in press; Ruano et al., 2006). Indeed, inflammation induced by intranigral injection of LPS could be mediated, at least in part, by the mitogen-activated protein kinase p38 (MAPK p38) signal pathway leading to activation of inducible nitric oxide synthase (iNOS) and cysteine protease caspase-11 (Ruano et al., 2006). Consistent with this evidence, it has been recently shown that LPS-induced inflammation causes apoptosis in the SNc due to increased pro-inflammatory cytokine levels of mRNA for TNF-α, IL-1α, IL-1β and IL-6, and the apoptosis-related genes Fas and Bax and caspase-3 immunoreactivity (Arimoto et al., in press). These data have also been confirmed in a MPTP mouse model, neurotoxic effect seems to be mediated via activation of the caspase-11 cascade and inflammatory cascade, as well as the mitochondrial apoptotic cascade (Furuya et al., 2004). The link between apoptotic signalling cascade and inflammation could follow other pathways. In fact, in a chronic MPTP model of PD, activation of the nuclear transcription factor NF-κB, that is well known for its role in preventing apoptotic cell death, has been revealed (Dehmer et al., 2004). NF-κB, among other effects, promotes the synthesis of cyclooxygenase types 2 (COX-2) (Baeuerle and Baichwal, 1997). COX-2 induction, in turn increases inflammatory response with the formation of different types of free radicals, a tyrosyl one and two different carbon-centred free radicals as well (Fig. 1), capable of causing phospholipid peroxidation (Marnett, 2000; Jiang et al., 2004).

The release of arachidonic acid (AA) also inhibits GLU uptake contributing to the neurodegenerative processes seen in PD (Dugan and Choi, 1999). COX-2 could also be induced by pro-inflammatory cytokines such as TNF-α via the c-Jun N-terminal kinase (JNK) pathway (Westwick et al., 1994; Adams et al., 1996; Teismann et al., 2003).

Nonetheless, it is with underlining that the interactions between apoptotic neurons and microglia don't always have detrimental effects but they can lead microglia to acquire an anti-inflammatory phenotype. Indeed, recent studies from Minghetti's group have provided evidence that under chronic stimulation the interaction with apoptotic cells contributes to a progressive down-regulation of glial pro-inflammatory molecule expression and a sustained release of immunoregulatory substances, such as PGE_2 and TGF-β_1, while promoting the synthesis of other products with potential immunoregulatory and protective activities (De Simone et al., 2004; Minghetti et al., 2005; Minghetti, 2005).

Old and new mechanisms of action for NSAIDs

The above discussion makes it plausible that drugs with the capacity to rescue DA neurons from microglia toxicity and inflammatory processes, may result in an amelioration of parkinsonian symptoms by delaying the onset and slowing the progression of the disease (Gao et al., 2003; Marchetti and Abbracchio, 2005; McCarty, 2006). Several agents have been shown to inhibit microglial or monocytic cell neurotoxicity (Klegeris and McGeer, 2005; McCarty, 2006). Among them much attention has been devoted to NSAIDs since it has been shown by experimental and clinical observation that they may represent a possible new therapeutic approach for treating PD.

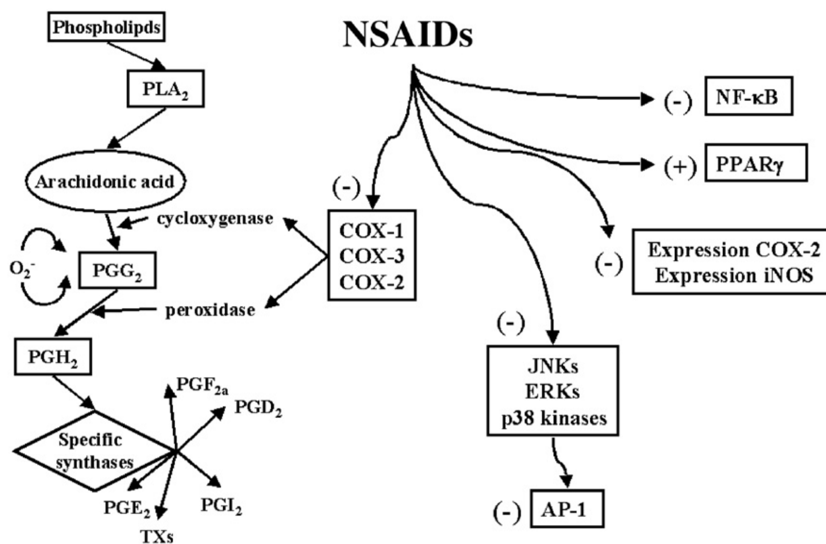

Fig. 1. Classical versus non-classical effects of NSAIDs. Abbreviations: COX cyclooxygenase; PLA_2, phospholipase A_2; PGG_2, Prostaglandin G_2; PGH_2, Prostaglandin H_2; Prostaglandin F_{2alha}, $PGF_{2\alpha}$; Prostaglandin D_2, PGD_2; Prostaglandin I_2, PGI_2; Prostaglandin E_2, PGE_2; Thromboxanes, TXs; Nuclear Factor kappa B, NF-κB; Peroxisome proliferator-activated receptor gamma, PPARγ; Inducible nitric oxide synthase, iNOS; c-Jun N-terminal kinases, JNKs; Extracellular signal-regulated kinases, ERKs; P38 mitogen-activated protein kinases, p38 kinases; factor activator protein 1, AP-1.

NSAIDs are an heterogeneous group of compounds which share many pharmacological properties (and side effects) and are the main drugs used as analgesics and antipyretics to reduce the untoward consequences of inflammation. NSAIDs together with steroidal anti-inflammatory drugs (SAIDs) are capable of halting eicosanoids synthesis and suspending inflammatory process progression. SAIDs, which include both cortisone and its derivatives, inhibit phospholipase A_2 (PLA_2) blocking both the production of leukotrienes (LTs) and PGs via the discontinuance of AA synthesis. Differently, NSAIDs only inhibit COX activity inducing a diminution of PGs levels, accompanied by a compensatory increase of LTs levels (Fig. 2). The NSAIDs can be classified into three groups based on their COX inhibition ratios (affinity of inhibition for COX-1 or COX-2) chemical structures or inhibitory kinetics (Taketo, 1998) (Table 2). Recently, a new class of NSAIDs has been synthesized named nitric oxide-donating nonsteroidal anti-inflammatory drugs (NO-NSAIDs), consisting of a traditional NSAID to which a NO releasing moiety is covalently attached, these drugs may have an important role in colon cancer prevention and/or treatment (Rigas and Kashfi, 2004). The main pharmacological action of these compounds is on the metabolism of AA, through the inhibition of the enzymes possessing COX activity (Vane, 1971) but also suppressing the COX-2 gene expression as well (Shishodia et al., 2004; Wu, 2006) (Fig. 2). Whenever an inflammatory process occurs, there is a consequent activation of specific enzymes in the cell wall. Among them, one of the first activated is PLA_2 phospholipase A_2 (PLA_2) that deacylates fatty acids from the 2nd carbon atom of the triglyceride backbone of membrane phospholipids, producing AAs and lyso-phospholipids (Furst, 1999; Vane and Botting, 1996, 2003). AA is subjected to the action of two families of enzymes: lipoxygenases (LOX) and COX. These enzymes are able to insert oxygen into the molecule of AA in a specific way. The 5-lipoxygenase (5-LO) subtype catalyzes oxidation of AA producing a variety of bioregulators such as LTs, that play important roles in the maintenance of the homeostasis of the neuronal cells (Christie et al., 1999). COX enzymes produce five prostanoids: PGE_2, PGF_2, PGD_2, PGI_2 (prostacyclin), and thromboxane A_2 (TxA_2) through the intermediate PGH_2. These prostanoids bind to specific G-protein-coupled receptors designated EP (for E-prostanoid), FP, DP, IP, and TP receptors, respectively (Narumiya, 2003; Bos et al., 2004). LTs are commonly known as vasoconstrictor and bronchospastic agents whereas PGs play a pivotal role in all the biochemical mechanisms inducing pain, hyperpyrexia and classical signs of inflammation, cytoprotective and cytotoxicity processes.Bergstrom and colleagues (1964) first described PGs in the brain more than 40 years ago. Since then, numerous studies have shown that PGs are formed in certain regions of the brain and in the spinal cord as a response to a variety of stimuli and in 1976 the enzyme which is key in the synthesis of PGs from AA, COX, was purified (Hemler et al., 1976). Subsequent to the cloning of the COX-1 gene, Dan Simmons and colleagues identified a second gene with COX activity (COX-2) (Xie et al., 1991). Recently, a third variant of COX has been described, initially called COX-3 (Diaz et al., 1992; Chandrasekharan et al., 2002), that might be the target of acetaminophen (paracetamol) (Chandrasekharan et al., 2002). COX-3 has been more appropriately renamed COX-1b being a splice variant of COX-1 which has retained intron-1 during translation (Chandrasekharan et al., 2002). It has a completely different amino acid sequence than the known cyclooxygenases and it does not seem to show cyclooxygenase activity in mice (Kis et al., 2006) and rats (Snipes et al., 2005), thus it may well be that COX-1b is not relevant to humans.

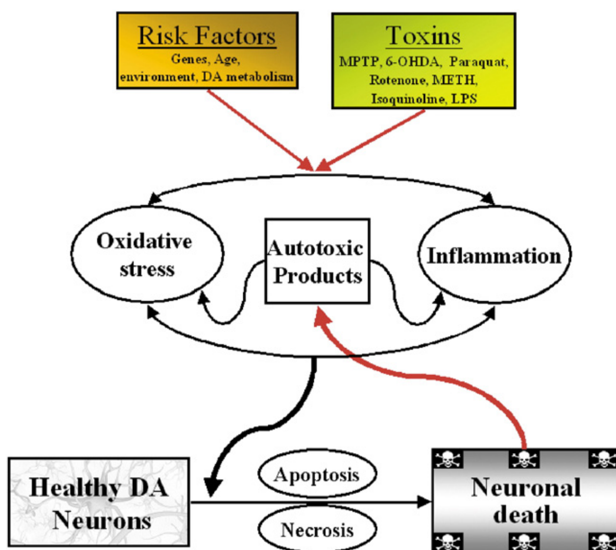

Fig. 2. Interacting synergistic mechanisms involved in dopaminergic death in Parkinson's disease. The role of the positive feed back (vicious) circle between neuronal death, neuroinflammation and/or oxidative stress is depicted. Abbreviations: Dopamine, DA; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP; 6 hydroxydopamine, 6-OHDA; Methamphetamine, METH; lipopolysaccharide, LPS.

The properties of COX-1 are different to those of COX-2. It was originally thought that the function of constitutive COX-1 was involved in physiological phenomena, such as cytoprotection of the stomach, platelet aggregation, and kidney functions, whereas that of COX-2 was involved in various pathologies. However, recent studies suggest that the inducible isoform COX-2 also plays an important role in development and homeostasis (Hinz and Brune, 2002). In the central nervous system (CNS), COX-2 plays an important role in membrane excitability, synaptic transmission and participates in memory consolidation during REM sleep (Cole-Edwards and Bazan, 2005; Chen and Bazan, 2005).

COX-1 and COX-2 are widely and both constitutively expressed under normal physiological conditions in human organs (Yasojima et al., 1999), even though only COX-2 is dramatically up-regulated during inflammatory processes. Similarly to the other tissues, the two isoforms are distributed heterogeneously among the brain cells, COX-1 and COX-1b are detected in microglial cells, while COX-2 is found in neuronal and glial cells, astrocytes do not express significant COX levels (Hoozemans et al., 2001; Kis et al., 2003). Normally, COX-2 is expressed in low levels in nigral DA neurons, but it becomes up-regulated in both patients and experimental PD models (Feng et al., 2002, 2003; Teismann et al., 2003; Wang et al.,

Table 2
Biological, pharmacokinetic and chemical subdivision of NSAIDs

Cox-2/Cox-1 ratio	Inhibition kinetics	Chemical structure
Nonselective COX inhibitors (e.g. ketorolac or piroxicam, with ratio ≈1);	simple, competitive	Carboxylic acids
	(e.g. ibuprofen and Naproxen)	(e.g. Aspirin and Ibuprofen)
Selective COX-1 inhibitors (e.g. Dexketoprofene and SC 560 with ratio <0.01)	competitive, time-dependent, reversible	Pyrazoles
	(e.g. Indomethacin and DuP 697)	(e.g. Phenilbutazone and Kebuzone)
Preferential COX-2 inhibitors (e.g. ibuprofen and indomethacin, with ratio 15–60)	competitive, time-dependent, irreversible	Oxicams
	(e.g. Aspirin and Valeryl salicylate)	(e.g. Piroxicam and Isoxicam)
Selective COX-2 inhibitors (e.g. coxibs, selective COX-2, with ratio >1000)		Sulphonamides
		(e.g. Valdecoxib and Celecoxib)
		Methylsulphones (e.g. Rofecoxib and Etoricoxib)
		Arylacetic acid (e.g. Lumiracoxib)

2005; Vijitruth et al., 2006; de Meira Santos Lima et al., 2006; Tyurina et al., 2006).

COX-2 expression in neurons has been proposed to increase the vulnerability of neurons to GLU mediated excitotoxicity. In the CNS, COX-2 expression increases in neurons following GLU receptor activation (Nogawa et al., 1997, Hewett et al., 2000; Carlson, 2003) and is thought to contribute to increased neuronal death. Genetic evidence also indicates that neuronal expression of COX-2 leads to excitotoxic cell death. Transgenic mice that overexpress neuronal COX-2 are more susceptible to excitotoxic cell death (Kelley et al., 1999) and age associated neuronal loss (Andreasson et al., 2001). This evidence has been confirmed in rat ischemic hippocampus, where COX-2 expression was substantially and significantly upregulated in vulnerable CA1 and not in resistant CA3 and dentate granule cells (Choi et al., 2006). In contrast, COX-2 null (knockout) mice exhibit less neuronal death following ischemia, challenge with NMDA (Iadecola et al., 2001), and MPTP (Feng et al., 2002, 2003; Miller and Hong, 2005; Vijitruth et al., 2006). Pharmacological and genetic inhibition of COX-2 is capable of sheltering DA neuronal bodies in the SNc as well as the striatal TH-stained fibres against toxin effects, suggesting a protection of the entire nigrostriatal pathway (Vijititruth et al., 2006). Potential downstream effectors of COX-2 neurotoxicity on SNc DA neurons are PGE_2 and free radicals generated through both the oxygenase and peroxidase part of the cycle (Marnett, 2000; Jiang et al., 2004), levels of which have been found to be enhanced in experimental models and in PD post-mortem samples (Teismann et al., 2003; Di Matteo et al., 2006a,b; Tyurina et al., 2006; Vijitruth et al., 2006). PGE_2 mediates COX-2 neurotoxicity essentially through the activation of EP_1/EP_3 receptors that disrupt Ca^{2+} homeostasis by increasing its cellular concentration thus causing excitotoxic neuronal death (Carlson, 2003; Kawano et al., 2006). Conversely, the activation of the prostanoid EP_2/EP_4 G-protein-coupled receptors seems normally to be associated with neuroprotection (Lee et al., 2004; Echeverria et al., 2005). PGE_2 is present in ventral midbrain neurons and derives primarily from COX-1 (Teismann et al., 2003). After a few days of neuronal insult, PGE_2 concentration almost doubles due to MPTP-induced COX-2 up-regulation, although more than half still depends on COX-1 activity (Teismann et al., 2003). Microglia-DAergic neurons interaction is necessary for MPP^+-induced COX-2 activation and PGE_2 production (Wang et al., 2005). The toxin first induces reactive microgliosis and secretion of its proinflammatory factors, among them PGE_2. These will enhance COX-2 DA neuronal activity and lead to a second wave of neuronal damage, which in turn, could reinforce the microgliosis process. The strong correlations found between COX-2 and PGE_2 levels, microglial activation and dopaminergic neurodegeneration suggest that COX-2 may mediate microglial activation and may play a key role in amplifying the inflammatory response and other toxic effects in a vicious circle, which ultimately exacerbates dopaminergic neuronal loss (Fig. 1) (Sugama et al., 2003; Teismann et al., 2003; Wang et al., 2005; Vijitruth et al., 2006).

The detrimental effects have been discussed above, however, within the brain, PGE_2 production, depending on its level, has also been associated with protective effects on neurons and glial cells behaving as an anti-inflammatory molecule (Zhang and Rivest, 2001; Minghetti, 2004). Indeed, in spite of its classic role as a pro-inflammatory molecule, several recent in vitro observations indicate that prostaglandin E2 can inhibit microglial activation. At lower (nanomolar) concentrations, PGE_2 protects hippocampal and cortical neuronal cultures against excitotoxic injury or LPS-induced cytotoxicity (Akaike et al., 1994; Thery et al., 1994; Kim et al., 2002; McCullough et al., 2004). The protective effect of EP_2 receptor activity has been confirmed in vivo, in a model of transient forebrain ischemia, in which the genetic deletion of this PGE_2 receptor exacerbates the extent of neuronal damage (McCullough et al., 2004). PGE_2 has also been shown to down-regulate microglial activation and expression of pro-inflammatory genes, including TNF-α, both *in vitro* and *in vivo* (Levi et al., 1998; Zhang and Rivest, 2001). Minghetti's group found that the interaction of microglial cells with apoptotic neurons promotes the synthesis of PGE_2 along with neuroprotective and immunoregulatory molecules such as TGF-β and NGF (De Simone et al., 2003, 2004). In addition, they have recently given further evidence for the anti-inflammatory PGE_2 effect, showing that it is involved in the brain cholinergic anti-inflammatory pathway. In fact, glial α7 nicotinic receptor stimulation reduces the LPS-induced release of TNF-α and enhances the expression of COX-2 and the synthesis of PGE_2 (De Simone et al., 2005).

COX-2 activation, moreover, might result in direct DAergic cell demise by producing the neurotoxic oxidant species DA-quinone (Teismann et al., 2003; Asanuma and Miyazaki, 2006), and by increasing DNA damage inducing the formation of

etheno-DNA adducts that arise as a consequence of COX-2-mediated lipid peroxidation (Lee et al., 2005; Williams et al., 2006).

Aspirin (acetylsalicylic acid, ASA) is the most frequently used drug in the world to treat inflammation and pain. Aspirin is the progenitor of the NSAIDs family, it is known to preferentially inhibit COX-1 rather than COX-2 in an irreversible way, by acetylating the active site of these enzymes, producing salicylic acid (SA) (Vane and Botting, 1987, 2003). Although many of ASA's and other NSAIDs' pharmacological actions are related to the ability to inhibit prostaglandin biosynthesis, some of their beneficial therapeutic effects are not completely understood. NSAIDs are able to inactivate the transcription factors NF-κB and factor activator protein-1 (AP-1) which is critical for the induction of neoplastic transformation and the induction of multiple genes involved in inflammation and infection (Vane and Botting, 1987; Weissmann, 1991; Kopp and Ghosh, 1994; Grilli et al., 1996; Dong et al., 1997; Mitchell et al., 1997; Amann et al., 2001). Diverse noxious cellular stimuli free NF-κB from any endogenous inhibitor, permitting the translocation of free NF-κB from the cytoplasm to the nucleus. Consequently, NF-κB binds to DNA and activates a number of genes involved in the inflammatory and immune responses. Some of these gene products, such as TNF could exert cytotoxic effects by switching on apoptotic self-destruct programs (Wright et al., 1992; Vaux and Strasser, 1996). ASA and COX-2 selective inhibitors exert antitumor effects partly through blocking AP-1 activation. AP-1, consisting of Jun/Fos dimers, is a downstream target of MAP kinase family members including extra-cellular signal regulated kinases (ERK-1 and -2; p42/p44 MAPK), Jun kinases (JNK), and p38 MAPK. NSAIDs suppress AP-1 activation through different mechanisms blocking the activation of ERK and JNK as well as P38 mitogen-activated protein kinase (p38 kinase) (Dong et al., 1997; Wong et al., 2004). Furthermore, ASA and salicylate at therapeutic concentrations inhibit COX-2 protein expression pointing towards a possible (cell-specific) target of NSAIDs upstream to COX-2 enzyme activity through interference with the binding of CCAAT/enhancer binding protein beta (C/EBPbeta) to its cognate site on COX-2 promoter/enhancer. Expression of other genes, such as iNOS and interleukin-4, may be inhibited by ASA and salicylate through a C/EBP-dependent mechanism or inhibiting NF-κB activation (Xu et al., 1999; Wu, 2003; Wu, 2006).

Among the COX independent actions, it has been shown that NSAIDs in neuronal cells, might directly and dose-dependently scavenge ROS and reactive nitrogen species (RNS) blocking their detrimental effects (Grilli et al., 1996; Asanuma et al., 2001). Besides this, the agonistic activity shown at high concentration by some NSAIDs such as ibuprofen and indomethacin toward the peroxisome proliferator-activated receptor-γ (PPARγ) seems relevant to neuroprotection (Lehmann et al., 1997). This receptor PPARγ is a ligand-activated inhibitory transcription factor that antagonizes the activity of NF-κB, AP-1, signal transducer and activator of transcription-1 (STAT-1) and nuclear factor of activated T cells (NFAT) (Lemberger et al., 1996; Jiang et al., 1998). Its cellular activation is associated with a reduction in the expression of several inflammatory genes (Ricote et al., 1998) and the production of inflammatory cytokines (i.e., IL-1, IL-6, TNF) (Jiang et al., 1998). In vitro studies have shown that the selective agonists pioglitazone, indomethacin and ibuprofen can activate PPARγ in microglia, reducing the Aβ-mediated secretion of inflammatory cytokines and neurotoxicity, decreasing the number of activated microglia and reactive astrocytes (Bishop-Bailey and Warner, 2003; Heneka et al., 2005). Drug treatment reduces the expression of the proinflammatory enzymes COX-2, iNOS and beta-secretase-1 (also called β-site of APP cleaving enzyme, BACE1) mRNA and protein levels (Heneka et al., 2005). In addition, PPARγ depletion potentiates beta-secretase mRNA levels by increasing BACE1 gene promoter activity. Conversely, overexpression of PPARγ, as well as NSAIDs and PPARγ activators, reduced BACE1 gene promoter activity. These recent results suggest that PPARγ could be a repressor of BACE1 binding to a peroxisome proliferator responsive element (PPRE) located in the BACE1 gene promoter. These effects may explain the overexpression of BACE1 in the brain under inflammatory conditions and emphasize the hypothesis that neuroinflammatory mechanisms significantly contribute to the pathogenesis of AD. This could be a potential mechanism by which NSAIDs have a protective effect against the development of AD (Sastre et al., 2006).

Currently, selective COX-2 inhibitors are used more frequently than the other NSAIDs because they produce the same pharmacological effects as non-selective COX inhibitors without the attendant COX-1 inhibition related toxic effects on stomach lining. Unfortunately, this class of drugs has recently been shown to increase the risk of cardiovascular events, resulting in the withdrawal of some of these drugs from the market and the halting of some clinical trials. Among those halted, a trial with celecoxib in AD (Couzin, 2004).

Neuroprotective effects of NSAIDs in Parkinson's disease: experimental evidence

Since the end of the 1980s, when McGeer and colleagues (McGeer et al., 1988) in their seminal study reported a large number of reactive leukocyte antigen-DR (HLA-DR)-positive microglial cells in the SNc and striatum of patients with PD, several experimental investigations have provided further plausible evidence for the activation of a proinflammatory response in this disease (Mogi et al., 1994a,b; Mogi et al., 1995; Blum-Degen et al., 1995; Rowe et al., 1998; Langston et al., 1999; Mirza et al., 2000; Knott et al., 2000, 2002; McGeer et al., 2002; Imamura et al., 2003; Ouchi et al., 2005; Ishida et al., 2006). The importance of the subject, has engaged several groups in the emerging and promising theme of NSAIDs and neurodegeneration. As far as we are aware, nineteen studies have been carried out in which the effects of NSAIDs have been tested on animal (mouse and rat) models of PD and cell cultures (Table 3). This evidence supports the use of NSAIDs in reducing the pathological burden of the disease; ASA was the most tested drug (8 studies), followed by its metabolite SA (5 studies), only 1 studied the effect of COX-1 but 10 focused on

Table 3
Experimental studies with NSAIDs

Study	Experimental model	NSAIDs	Outcome
Grilli et al., 1996	Primary cultures of rat cerebellar granule cells and hippocampal slices	ASA (1, 3 mM) SA (3,10 mM) indomethacin (1–20 μM)	Protection ↓NF-κB Protection ↓NF-κB NO protection
Aubin et al., 1998	MPTP mouse model of PD, MPTP (15 mg/kg, s.c.)	ASA (100 mg/kg) Aspegic (200 mg/kg) SA (100 mg/kg) Paracetamol (100 mg/kg) Diclofenac (100 mg/kg) Ibuprofen (20 mg/kg) Indomethacin (100 mg/kg)	Protection ROS scavenging Protection ROS scavenging Protection ROS scavenging NO protection NO protection NO protection NO protection
Ferger et al., 1999	MPTP mouse (C57BL/6) model of PD, MPTP 30 mg/kg or 40 mg/kg s.c.	SA (50 mg/kg or 100 mg/kg i.p.)	Protection ROS scavenging
Mohanakumar et al., 2000	MPTP mouse model of PD, MPTP (30 mg/kg i.p. twice, 16 h apart).	SA (25–100 mg/kg, i.p.)	Protection ROS scavenging ↓akinesia or catalepsy
Casper et al., 2000	Cultured primary rat embryonic neurons from mesencephalon GLU-toxicity	ASA (1 mM) Paracetamol (1 mM) Ibuprofen (0.1 mM)	Protection mechanism?? Protection mechanism?? Protection mechanism??
Teismann and Ferger, 2001	MPTP mouse (C57BL/6) model of PD, MPTP (30 mg/kg s.c.)	ASA (10, 50, 100 mg/kg i.p.) Meloxicam (2, 7.5, 50 mg/kg i.p.)	Protection ↓COX-1/COX-2 ↓akinesia or catalepsy Protection ↓COX-2 ↓akinesia or catalepsy
Carrasco and Werner, 2002	Cultured primary rat embryonic neurons from mesencephalon 6-OHDA (1.25–25 μM) MPP+ (0.625–20 μM)	 ASA (1 mM) ASA (1 mM)	 Protection mechanism?? Protection mechanism??
Kurkowska-Jastrzębska et al., 2002	MPTP mouse model of PD	Indomethacin (1 mg/kg, i.p.)	Protection ↓ inflammation
Sairam et al., 2003	MPP+ rat model of PD, intrastriatal 100 nmol (in 4 μl/animal)	SA (50 and 100 mg/kg, i.p.), diclofenac (5–100 mg/kg, i.p.) celecoxib (2.5–50 mg/kg, i.p.)	Protection ROS scavenging NO protection NO protection
Teismann et al., 2003	MPTP mouse (C57/BL/6) model of PD, MPTP (20 mg/kg i.p. four injections)	Rofecoxib (12.5–50 mg/kg, i.p. for 5 days before and after	Protection ↓COX-2
Klivenyi et al., 2003	MPTP mouse (C57/BL/6) model of PD, MPTP (20 mg/kg i.p)	Rofecoxib	Protection ↓COX-2
Przybyłkowski et al., 2004	MPTP mouse (C57/BL/6) model of PD, MPTP (60 mg/kg i.p)	Rofecoxib (10 mg/kg, i.p., for 21 days 1 day after the injury MPTP)	NO protection
Maharaj et al., 2004	MPP+ rat model of PD, intrastriatal (32 nmol in 1 μl)	ASA (100 mg/kg, i.p. four injections, after MPP+ infusion) Paracetamol (100 mg/kg, , i.p. four injections, after MPP+ infusion)	Protection ROS scavenging Partial protection ROS scavenging
Carrasco et al., 2005	Rat mesencephalic neuronal cultures 6-OHDA (2.5–10 μM) MPP+ (2.5–10 μM)	Ibuprofen (25, 100, 250 μM) SC-560 (6.5 μM) NS-398 (5–50 μM) and Cayman 10404 (0.1–10 nM)	Protection for both toxins ↓COX-2 NO protection Protection against only 6-OHDA-toxicity ↓COX-2
Sánchez-Pernaute et al., 2004	6-OHDA rat model of PD, intrastriatal (22.5 μg)	Celocoxib (20 mg/kg i.p., −1 up to +12 or 21 days)	Protection ↓COX-2
Morioka et al., 2004	PC12 cells MPP+ (30 μM)	Indomethacin (100 μM) Ibuprofen (100 μM) Ketoprofen (100 μM) Diclofenac (100 μM) ASA (100 μM)	Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP Potentiation of neurotoxicity ↓MRP No effect
Wang et al., 2005	Primary mesencephalic mixed neuron-microglia cultures MPP+ (0.5 μM)	DuP697 (10 nM)	Protection ↓COX-2
Di Matteo et al., 2006a,b	Rat model of PD, intrastriatal MPP+ or 6-OHDA 1 mM (10 min 1 μl/min)	ASA (100 mg/kg i.p.) Meloxicam (50 mg/kg i.p.)	Protection for both toxins ROS scavenging NO protection both toxins
Maharaj et al., 2006	Rat model of PD, intranigral 1 mM (32 nmol in 1 μl)	ASA (100 mg/kg i.p.) Paracetamol (100 mg/kg i.p.)	Protection ROS scavenging and ↓superoxide anion generation

the role of COX-2 using selective inhibitors for this isoform of the enzyme. The results of 10 years of research will be reported chronologically.

The first piece of experimental evidence in the field was published by Grilli and co-workers a few years after the McGeer study (Grilli et al., 1996). These authors showed that ASA and its metabolite SA, at concentrations compatible with amounts in plasma during treatment of chronic inflammatory states, were protective against neurotoxicity elicited by GLU in primary cultures of rat cerebellar granule cells and hippocampal slices. Indomethacin, however, was unable to prevent GLU-induced cell death. The common molecular target for ASA and SA but not for indomethacin was identified as COX-independent and involved specific inhibition of GLU-mediated induction of NF-κB, suggesting, for the first time, a link between neuroprotection and the nuclear event (Grilli et al., 1996).

Moreover, Aubin and colleagues (1998) confirmed these ASA neuroprotective effects in a low dosage MPTP mouse model of PD. In accordance with the previous study, using an ex vivo and in vitro approach they found that the protective effect of ASA, its soluble lysine salt (Aspegic) and SA, is probably not due to COX inhibition. Their assertion was just a speculation based on the fact that other COX inhibitors such as paracetamol, diclofenac and indomethacin were ineffective. Likewise, the involvement of NF-κB was ruled out based on the lack of effect of dexamethasone, a glucocorticoid known to powerfully repress this nuclear factor function. ROS scavenging activity as a possible mechanism for explaining SA and ASA's neuroprotection was instead proposed by these authors (Aubin et al., 1998).

In addition, SA was found to be neuroprotective, even if not completely, in a higher dosage MPTP mouse model of PD (Ferger et al., 1999). SA acted on both the terminal and cell body area of the nigrostriatal system as might be deduced by the pronounced effect against both MPTP-induced striatal DA depletion and loss of tyrosine hydroxylase (TH) immunoreactive on nigral cell bodies. Ferger and colleagues in their paper pointed out that SA neuroprotective properties are based on its effective hydroxyl radicals scavenger rather than on its COX-inhibitory action (Ferger et al., 1999).

This piece of evidence has been further confirmed by Mohanakumar et al. (2000), in a MPTP mouse model of PD, where SA demonstrated a clear antioxidant action blocking toxin-induced glutathione (GSH) and DA depletion acting as a hydroxyl radicals scavenger in the brain and indicates its strength as a valuable neuroprotectant. SA did not inhibit MAO-B as has been previously shown by Aubin et al. (1998), overruling the possibility that its observed neuroprotective effects were caused by the possible blockade on the production of MPP^+ from MPTP due to the presence of this enzyme in the brain. It is worth noting that these authors showed for the first time that SA pre-treatment also significantly improved motor activity, blocking akinesia or catalepsy caused by MPTP administration (Mohanakumar et al., 2000).

An in vitro study evaluated the effects of some NSAIDs on cultured primary rat embryonic neurons from rat embryos mesencephalon also containing glial cells, an experimental preparation that reflects the cellular composition of the brain well, and is therefore useful in the study of neuroinflammation (Casper et al., 2000). Incubation with ASA, paracetamol or ibuprofen protected DAergic neurons against GLU toxicity, considering as indices the reduction of the decrease in DA uptake caused by GLU, and the attenuation of the TH-positive cells loss. Among the NSAIDs tested, ibuprofen was the most effective and surprisingly increased the number of DA cells in basal condition most likely protecting them from the excitoxicity associated with culture medium change (Casper et al., 2000).

So far experimental evidence has suggested that NSAIDs act as neuroprotectants essentially through a nonclassical mechanism. Against the general trend, the role of COX-1 and COX-2 enzymes was reassessed by Teismann and Ferger (2001), who proposed the use of COX-2 inhibitors as a new non-DAergic therapy for PD. Their assumption was based on the effects of ASA and meloxicam, the latter a preferential antagonist for the COX-2 isoform, in a MPTP mouse model of PD. Both drugs, at higher dosages, showed an almost complete protection against MPTP toxicity. ASA and meloxicam antagonized MPTP-induced striatal DA depletion, attenuated the reduction of TH immunereactivity of the SNc and the MPTP-induced decrease in locomotor activity (Teismann and Ferger, 2001).

Carrasco and Werner (2002), using a neuronally enriched mesencephalic culture system, showed that ASA was also able to increase the survival of DA neurons exposed to low doses of 6-OHDA and MPP^+ but not to counteract the morphological changes induced by the toxins. However, the authors did not investigate the possible protective mechanism of ASA (Carrasco and Werner, 2002).

The role of COX-activity in the pathogenesis of PD was studied for the first time by Kurkowska-Jastrzębska et al. (2002) using indomethacin in a MPTP mouse model of PD. This drug protected SNc DA neurons against the toxin effect and it was associated with diminished microglial activation and lymphocytic infiltration in the damaged areas. Thus, reduced inflammation by indomethacin might result in less damage to DAergic neurons. However, in this study, microglial and lymphocytes accumulation was decreased only in association with less neuronal impairment, when indomethacin was given before MPTP. Indomethacin in higher dose or given 24 h after intoxication did not decrease inflammatory reaction. Therefore, the anti-inflammatory effect of indomethacin might be secondary to the diminished neural injury which probably results from a direct interaction of indomethacin maybe on neurons scavenging ROS. However, indomethacin appeared to be toxic in high doses indicating that doses of NSAIDs should be considered carefully in clinical trials (Kurkowska-Jastrzłbska et al., 2002).

In view of conflicting reports so far on the role of the NSAIDs as neuroprotectants and the involvement of COX isoenzymes in their effects, Sairam et al. (2003), used SA, diclofenac and celecoxib in a model of PD induced infusing MPP^+ directly into the striata of rats. These three anti-inflammatory agents have different mechanisms of action. SA is well known to have an effect independent of the COX activity, diclofenac is a non-selective reversible COX-inhibitor

and celecoxib is instead a specific COX-2 inhibitor. The failure of celecoxib and diclofenac to protect animals against MPP^+-induced DA depletion, together with a significant attenuation of severe DA depletion (>65%) induced by SA indicate the absence of the involvement of prostaglandins (PGs) in MPP^+ action. The authors conclude that the difference in neuroprotection among the NSAIDs used in the study is mostly dependent on their antioxidant activity (Sairam et al., 2003).

Further insights into the field have been provided by Teismann and colleagues (2003) in a very elegant in vitro study from MPTP-treated mice and post-mortem PD samples. These researchers showed that COX-2 isoenzyme is up-regulated in the SNc DAergic neurons in both animal and human samples, COX-2-mediated neurodegeneration might be correlated to its catalytic activity through the production of prostaglandins and maybe also to the oxidation of catechols such as DA (Asanuma and Miyazaki, 2006). Treatment with rofecoxib, before and after MPTP-injection, blocked the increase of PGE_2 in the midbrain, doubled the number of the surviving TH-positive neurons, and prevented the rise in protein cysteinyldopamine, an index of DA quinones production. Surprisingly, neither pharmacological nor genetic abrogation of COX-2 activity mitigate inflammatory processes (Teismann et al., 2003).

The neuroprotective effects of rofecoxib have also been shown by Klivenyi and colleagues (2003) in MPTP model of PD in mice. They showed that the selective COX-2 inhibitor either alone or in combination with creatine, that facilitates metabolic channelling and shows antiapoptotic properties (Wyss and Kaddurah-Daouk, 2000) protected against striatal DA depletions and loss of SN TH-immunoreactive neurons. Administration of rofecoxib with creatine produced significant additive neuroprotective effects against DA depletions. These results suggest that a combination of a COX-2 inhibitor with creatine might be a useful neuroprotective strategy for PD (Klivenyi et al., 2003).

The work of Przybyłkowski and colleagues (2004) is also noteworthy, they have shown, in the MPTP model of PD in mice, that rofecoxib has no neuroprotective effect when it is given after MPTP intoxication, even for a long period, revealing that the time of COX-2 inhibition is critical to achieve a protective effect. Consequently, COX-2 activity, prostaglandins production and oxygen species formation might not play a detrimental role in neuronal cells death, at least when the injury process has started already. The inhibition of COX-2 activity could, nevertheless, be harmful to neurons injured by MPTP. Indeed, the authors showed that, in later stages of injury, COX-2, through the formation of cyclopentenone prostaglandins derived from PGD_2, may participate in the resolution of inflammation and even in the regeneration process (Przybyłkowski et al., 2004).

Maharaj et al. (2004) on the other hand, showed that ASA given after MPP^+ administration, completely blocked MPP^+-induced striatal DA depletion. Similar treatment with paracetamol resulted instead only in a partial protection. In both experimental conditions, rat brain homogenates and rats intranigrally treated with MPP^+, ASA and paracetamol acted mainly as antioxidants. They were also capable of blocking hydroxyl radicals production and lipid peroxidation in vitro, but in this ASA was the weaker compared to paracetamol. In conclusion, ASA appears to offer itself as a prophylactic as well as an adjuvant therapy for PD and its neuroprotective effect is only partially mediated by ROS scavenging properties (Maharaj et al., 2004).

Sánchez-Pernaute and colleagues (2004) in a 6-OHDA rat model of PD, showed that selective inhibition of COX-2 by treatment (pre and post lesion) with celocoxib is protective against the neurotoxin effect. The authors evaluated celocoxib effects using micro PET and immunohistochemical techniques, and observed a decrease in microglial activation in the striatum and ventral midbrain associated with a prevention of the progressive degeneration seen in the intrastriatal 6-OHDA retrograde lesioned rats treated with the vehicle. The benefit of COX-2 activity inhibition might be attributed to a selective decrease of the harmful glial cells and to the no effect on the protective astroglia. Celocoxib's rescue of DA toxin-insulted neurons from death could be mediated by both neuronal and glial COX-2, but in any case the effect obtained by this drug is to create favourable conditions for the prevention of progressive neurodegenerative cascades during and after neuronal injury similar to that seen in PD (Sánchez-Pernaute et al., 2004).

Furthermore, results obtained in cultures of embryonic rat mesencephalic neurons treated with 6-OHDA and MPP^+ showed that these two neurotoxins act differently in the killing of DA neurons, neuronal COX-2 activity and PG production is involved only in the 6-OHDA-neurotoxic effect whereas MPP^+ toxicity does not require COX involvement (Carrasco et al., 2005). This evidence comes from experiments carried out with ibuprofen, a non-selective COX inhibitor, SC-560 a COX-1 selective inhibitor and two selective COX-2 inhibitors, NS-398 and Cayman 10404, showing that COX-2, but not COX-1, is involved in 6-OHDA toxicity. Since ibuprofen attenuated both 6-OHDA and MPP^+-neurotoxicity, the authors proposed that this drug has additional COX-independent effects as yet not well identified (Carrasco et al., 2005). Some discrepancies with the previous study have been reported by Wang et al. (2005). These authors found that MPP^+ induces DAergic degeneration enhancing COX-2 expression in both glial and DA cells in primary mesencephalic mixed neuron-microglia cultures. Its toxicity is undoubtedly mediated through PGE_2, the levels of which almost doubled. They observed that the COX-2 specific inhibitor DuP697, attenuates microgliosis by decreasing PGE_2 production, and leads DA neurons to the rescue from a secondary lethal neurotoxicity attack (Wang et al., 2005). Valdecoxib, another selective COX-2 inhibitor, acted similarly in a mouse model of PD abating microglia activation and the consequential MPTP-induced toxicity, confirming that COX-2 and activated microglia play an important role in secondary injury of DA neurons. Moreover, these cellular protective effects of valdecoxib pretreatment were confirmed in the behavioural counterpart of the experimentation in which it also alleviated locomotor deficits induced by the toxin, assessed in open field and vertical activity (Wang et al., 2005).

A recent study threw some light on this question by confirming that ASA has a protective effect against neuronal damage induced by intrastriatal infusion of MPP^+ and 6-OHDA using a microdialysis approach in conscious rats (Di Matteo et

al., 2006a,b). What makes this study noteworthy, is that the ASA neuroprotective effect was evidenced in vivo, indeed, this has been observed only under in vitro and ex vivo conditions to date. Pre-treatment of rats with ASA, protected DA neurons in both animal models (MPP^+ and 6-OHDA-lesioned) as indicated by electrochemical and TH immunostaining evidence, whereas meloxicam, a selective COX-2 inhibitor, was devoid of any activity. The authors have confirmed these findings also in vitro, in a human neuroblastoma cell culture line. In fact, ASA, but not meloxicam, inhibited cell death induced by treatment with MPP^+, in a dose-dependent manner (unpublished observation). The mechanism of action of ASA seemed to be different in each model since it was associated with ROS scavenging activity in the 6-OHDA model, but not in the MPP^+ model that surprisingly did not induce any hydroxyl radicals formation at the concentration used in this study. Therefore, it is likely that the protective effect exerted by ASA, in vivo, may be due to inhibition of MPP^+ toxicity at the cell level, possibly by blocking NF-κB or caspase activation, providing further evidence that the neuroprotective effect of NSAIDs might be independent from COX-2 inhibition. Other mechanisms, such as hydroxyl radicals scavenging activity, as in the model of 6-OHDA-induced damage, cannot be ruled out however. (Di Matteo et al., 2006a).

Finally, Maharaj et al. (2006) have provided novel information by highlighting the role of NSAID agents on a different molecular target, the mitochondrion. These authors studied the effect of MPP^+ on striatal mitochondrial function and the ability of MPP^+ to generate superoxide hydroxyl radicals and the effect of ASA and paracetamol. These NSAIDs prevented MPP^+-induced inhibition of the mitochondrial electron transport chain and complex I activity. Also, ASA and paracetamol significantly attenuated MPP^+-induced superoxide anion generation. The results of this study suggest that these NSAIDs not only serve as hydroxyl radicals scavengers but also prevent mitochondrial dysfunction and subsequent superoxide anion generation (Maharaj et al., 2006).

Conversely, Morioka et al. (2004), have shown that treatment with some NSAIDs might instead aggravate the neurodegenerative processes. They reported that coincubation of PC12 cells with indomethacin, ibuprofen, ketoprofen, or diclofenac, markedly enhanced MPP^+-induced cell death. This additive detrimental effect was not observed after treatment with ASA and NS-398, a COX-2 selective inhibitor, that had no effects on the toxin action. The authors showed that the potentiating effect of some NSAIDs on MPP^+-induced cell death was not associated with any of the classical and non, actions attributed to them so far (i.e., inhibition of COX enzymes, ROS scavenging, antagonism at PPARγ, caspase-3-apoptotic cell death pathway). The possible mechanism whereby NSAIDs potentate MPP^+-induced cell death might be the increase of intracellular accumulation of MPP^+. These drugs actually suppressed the cellular efflux of MPP^+ by the blockade of multidrug resistance proteins (MRP) (Morioka et al., 2004).

The use of different experimental conditions (i.e., in vivo versus in vitro, ex vivo, species or strain of animal used, cell types) drugs (NSAIDs are a heterogeneous chemical group also with different potency in crossing the blood–brain barrier), therapy duration (i.e., pre-treatment versus post-treatment or combination of both), time of observation, dose and type of neurotoxins used, may explain the differences among the studies here reviewed and, sometimes, the conflicting results. Overall, there is no doubt that ASA, SA, ibuprofen and especially COX-2 selective inhibitors exert neuroprotective effects, although the mechanism through which they act still remains controversial. Hitherto, the involvement of classical versus non-classical mechanisms in the beneficial effects of NSAIDs remains to be fully unravelled. However, their broad sites of action and pharmacological effects (from anticancer to antipyretic) might be the basis on which their efficacy in neurodegenerative disease is founded.

Neuroprotective effects of NSAIDs in Parkinson's disease: epidemiological evidence

Despite the evidence of inflammation in the brains of patients with PD, confirmed successively in animal models of PD, since the mid 1990s, NSAIDs have not yet been formally tested in PD. To date, only five epidemiological studies have been carried out analyzing the association between regular use of NSAIDs and the risk of PD with conflicting results (Table 4).

The first piece of evidence was provided by Chen et al. (2003) from the Harvard School of Public Health. They published the first study investigating prospectively the potential benefit in humans of the use of NSAIDs in reducing the risk of PD. These researchers found that regular users of these drugs had a lower risk of PD than non-users. The study was conducted among participants in the Health Professionals Follow-Up Study and the Brigham and Women's Hospital based Nurses Health Study who were free of PD, stroke or cancer at the start of the research. More than 44,000 men and nearly 99,000 women were followed for 14 years and 18 years, respectively. Use of ASA and non-ASA NSAIDs (such as, diflunisal, ibuprofen, indomethacin, naproxen) was assessed via biennial questionnaires. A total of 236 men and 179 women developed PD during the course of the study. The risk of developing PD was 45% lower among regular users of non-ASA NSAIDs compared to non-users (pooled multivariate relative risk (RR), 0.55; 95% confidence interval (CI), 0.32–0.96). Regular use of non-ASA NSAIDs was reported by 6.1% of the men at the beginning of the study and 3.7% of the women. A similar decrease in risk was also found among participants who took two or more tablets of ASA per day compared to non-

Table 4
Epidemiological studies of NSAIDs and PD

Study	Duration NSAIDs use	Overall cohort	PD cases	Relative risk	95% CI
Chen et al., 2003	>14 years	142,902	415	0.55	0.32–0.96
Chen et al., 2005	8 years	146,565	413	0.65	0.48–0.88
Case control	>3 years	7896	1258	0.93	0.80–1.08
Hernán et al., 2006		women	493	1.21	0.95–1.54
		men	765	0.79	0.65–0.96
Bower et al., 2006	20 years	404	202	0.50	0.20–1.5
Ton et al., 2006	20 years	589	206	0.90	0.59–1.35

users (RR, 0.56; 95% CI, 0.26–1.21). No benefit was found among those who took smaller amounts of ASA per day or paracetamol. Additionally, increasing benefits were observed with longer duration of use of non-ASA NSAIDs (Chen et al., 2003). It is worth noting, that the Chen study may underestimate the protective effect of NSAIDs, since PD is much more common in people over 75 years old, an age group not included in the Chen team's data. Therefore, benefits of even greater magnitude might be demonstrable if this intervention were applied to the same population as it aged beyond 75 years. These data, however, provide little support for the routine use of NSAIDs as disease-modifying agents in PD, since to prevent one additional case of PD 98 individuals would have to be treated with them (Schiess, 2003).

A subsequent prospective study conducted by the same group has provided further insights (Chen et al., 2005). Chen and co investigators continued examining the relationship between NSAIDs use and risk of PD this time, utilizing another large cohort, the American Cancer Society's Cancer Prevention Study II Nutrition Cohort of 146,565 people. Between 1992 and 2000 they recorded 413 new cases of PD in the cohort. Ibuprofen was associated with 35% lower risk of PD (RR, 0.65; CI 95% 0.48–0.88), with similar risk reductions for men and women regardless of age or smoking status. There was a significant trend for lower risk with increasing consumption of ibuprofen (from RR 0.73 with fewer than 2 tablets per week to RR 0.61 for daily use) but duration of use made little difference. In contrast to the previous study, no significant associations were found for ASA, other NSAIDs or paracetamol (Chen et al., 2005). These discrepancies might be simply explained by the fact that considerably more people in the cohort used ibuprofen than other medications. However, the authors also did not exclude that there may be an ibuprofen-specific effect against PD, related to its unique molecule. Another limitation of this study is that because ibuprofen is an over-the-counter medication, the so-called nonusers could have taken ibuprofen years ago, consequently, a short-term clinical study might not give complete data. Long-term data would be necessary to more fully discern users and nonusers.

Recently, another group from the Harvard School of Public Health has conducted a case-control study on subjects with no history of PD or parkinsonism-related drug use at baseline (Hernán et al., 2006). Their study was nested within a cohort of the world's largest computerised database, the British General Practice Research Database (GPRD). The authors analyzed 1,258 PD cases and 6,638 controls, and reported a surprising finding: nonASA NSAIDs use reduces PD risk only in men but not in women. Use of nonASA NSAIDs was associated with a 20% reduction in the incidence of PD among men (odds ratio (OR), 0.79; CI 95% 0.65–0.96), and a 20% increase in the incidence of PD among women (OR, 1.21; CI 95% 0.95–1.54) (Hernán et al., 2006). Although sex differences in PD risk have been previously reported for caffeine consumption (Ascherio et al., 2001) and alcoholism (Hernán et al., 2004), this was an unexpected finding that warrants further research.

Less promising insights have been provided by Bower and colleagues (2006) from the Mayo Clinic College of Medicine in Rochester, Minnesota. They explored the association of PD with the use of NSAIDs in a population-based, case-control study for a total of 392 individuals. The investigators used the medical records linkage system of the Rochester Epidemiology Project to identify 196 subjects who developed PD from 1976 to 1995. Consistent with the previous epidemiological studies (Chen et al., 2003, 2005; Hernán et al., 2006), Bower and colleagues found that cases of PD used NSAIDs (excluding ASA) less frequently than controls (OR 0.5; CI 95% 0.2–1.5); however, the difference did not reach significance. This trend finding was similar for both NSAIDs and steroidal agents considered separately. The use of ASA was not significantly associated with PD as shown previously (Chen et al., 2005; Hernán et al., 2006). These investigators also showed a significant association between pre-existing immune-mediated diseases and the later development of PD (OR 1.8; 95% CI: 1.1–3.1). The association was stronger for women and for earlier onset of PD cases, but neither of these differences reached significance. These results support the hypothesis that there is an inflammatory component in the pathogenesis of PD and provide a rationale for the use of NSAIDs as neuroprotectants capable of delaying onset or slowing progression of the disease (Bower et al., 2006). Since patients with diseases of immediate-type hypersensitivity are genetically predisposed to initiate a humoral response to low levels of antigens, they might also be predisposed to initiate neuroinflammatory responses as well and play a role in the aetiology of PD (McGeer and McGeer, 1997).

The latest available data on the subject come from Ton and colleagues (2006) from the University of Washington and, unfortunately, they have continued in dampening the initial enthusiasm. In an American population-based case-control study these investigators did not observe a significant association between PD and NSAIDs. Subjects among enrollees of a health maintenance organization included 206 cases between ages 35 and 89 with a new diagnosis of idiopathic PD between 1992 and 2002, and 383 randomly selected controls. Exposure to NSAIDs was ascertained from an automated pharmacy database. After adjusting for age, sex, smoking, duration of enrolment, and clinic, the risk of PD among individuals who received non-ASA NSAIDs between 1977 and 1992 was 0.90 (95% CI: 0.59–1.35) and 1.67 (95% CI: 0.60–4.60) between 1993 and 2002. Use of ibuprofen was not associated with PD (OR: 0.89; 95% CI: 0.60–1.32). The risk of PD associated with ASA or ASA-containing medications was 0.74 (95% CI: 0.49–1.12). These results provide only limited support for the hypothesis that use of ASA may reduce the risk of this disease, but this association was statistically imprecise and no clear trend according to number of ASA prescription was observed. In addition, no indication at all of protection from other NSAIDs was revealed (Ton et al., 2006).

Differences in the methods of ascertaining medication exposures, in the extent or timing of exposure to NSAIDs, as well as chance, may account for the discrepant findings from this and the earlier studies. These findings offer, at most, a limited support for the hypothesis of neuroprotection from ASA, and no indication of protection from other NSAIDs.

Larger studies that include medication records and over-the-counter medication use will clarify these associations. Indeed, these unclear indications must be clarified and corroborated by clinical trial before any firm conclusions can be drawn. In addition, the role of selective COX-2 inhibitors might be investigated since only the effect of traditional NSAIDs has been analyzed by epidemiological studies. Selective COX-2 inhibitors have not been in use long enough for epidemiological data to be collected. The side effects of NSAIDs therapy, such as gastrointestinal lesions and cardiovascular risks should also be carefully evaluated.

Conclusions and implications

From the large amount of literature here reviewed it appears evident that inflammatory processes are involved in the pathophysiology of PD. Neuroinflammation, a processes orchestrated by activated resident microglia cells and sustained by them and other immune cells, might be contributing to the demise of nigral DA cells, perpetuating the neurodegenerative phenomenon. A large body of information on the molecular and cellular mechanisms whereby inflammation might induce neuronal death has been generated in the past few years by researchers in the neuroscience community. Nevertheless, further clarification of the role of inflammation in the pathophysiology of basal ganglia disorders is required, since the overall picture is still confusing. Complicating the situation is the fact that inflammation is a *double-edged sword* and probably starts as a beneficial defence mechanism that at some point evolves into a destructive and uncontrollable chronic reaction. Thus, the ideal approach would be to inhibit the deleterious effects associated with neuroinflammation while preserving the inflammatory pathways that lead to neuroprotection. From the above discussion it seems clear that drugs inhibiting inflammation and microglial activation might be an important feature of the treatment of PD and also the dementia, often associated with the disease (Gao et al., 2003; McGeer and McGeer, 2004; Marchetti and Abbracchio, 2005; McCarty, 2006; Prodan et al., 2006). Consequently, a rational use of NSAIDs could be useful as a therapeutic intervention in PD and in other major neurological diseases with similar etiopathology, such as AD, ASL and MS. Despite the fact that experimental and epidemiological evidence has been provided for future use of anti-inflammation agents, they have not been rigorously corroborated in trial studies for the treatment of motor disorders as yet and most of the data have yielded contradictory results. This may be a result of the peculiar characteristics of these drugs, so different both at the chemical and action level. In fact, NSAIDs might exert their neuroprotective actions not only inhibiting COX enzymes but also by acting on NF-κB, iNOS, PPARγ, suppressing the formation of DA quinones, scavenging ROS and RNS activity and probably by other unknown mechanisms. Indeed, recently it has also been proposed that anti-inflammatory compounds might act inhibiting microglial proliferation, modulating the cell cycle progression and apoptosis (Elsisi et al., 2005).

NSAIDs are *sui generis*, and the further anti-inflammatory agents research progresses, the greater the number of indications that are discovered. NSAIDs have carved out a unique career in such diverse fields as the treatment of pain, migraine, prevention of cardiovascular disorders, and the chemoprophylaxis of various types of cancer. Probably, we are on the threshold of a new promising career for NSAIDs especially in prevention of neurodegenerative disease rather than for their treatment. Indeed, it is quite possible that NSAIDs are ineffective once the pathological process has started, the pharmacological intervention should start very early in the pre-symptomatic period, according to some experimental a epidemiological evidence (Chen et al., 2003; Przyby3kowski et al., 2004; Chen et al., 2005; Hernán et al., 2006). This need is also corroborated by the recent failure of some promising clinical trials in AD (Salpeter et al., 2006; Firuzi and Pratico, 2006) shedding some doubts on the inflammation hypothesis of AD. Thus, the attractive thesis that NSAIDs might protect the remaining surviving DA neurons from the degeneration process and thus slow the ratio of progression of the illness sounds less promising. Due to the complexity of the disease, it is possible that combination therapy, concomitant use of agents with nonoverlapping or even synergistic mechanisms of action, may represent the best means available to enhance treatment effectiveness. Some results could be achieved, therefore, by combining NSAIDs with other rescue agents, such as MAO inhibitors (rasagiline, safinamide); mitochondrial function enhancers (coenzyme Q10, creatine); antiapoptotic agents; protein aggregation inhibitors and neurotrophic factors (Bonuccelli and Del Dotto, 2006). Although this hypothesis is worthy of consideration, it remains largely undocumented and certainly deserves further discussion. Furthermore, NSAIDs might be a beneficial adjuvant to L-DOPA therapy counteracting the toxicity induced by its long-term use, through anti-inflammatory action and the reduction of DA quinones generated by L-DOPA therapy itself (Asanuma and Miyazaki, 2006).

There are also many avenues that remain unexplored, so there are undoubtedly further advances to be made especially on the non-classical mechanisms of action of NSAIDs that might lead us towards a better knowledge of the neuropathological process. In the next few years, we believe that novel approaches (Di Giovanni et al., 2006; Schapira et al., 2006) will support the current dopamine-replacement therapy for PD. Furthermore, early diagnosis, early symptomatic treatment and particularly the introduction of neuroprotective therapies will improve PD pharmacological management, as disease modification remains the most important goal in PD. Compounds inhibiting neuroinflammation such as NSAIDs represent an important starting point that could, for the first time, lead us to the identification of disease-modifying agents for this devastating disease.

Acknowledgments

This work was supported in part by the Italian MIUR (Ministero Istruzione Università Ricerca). We want to thank Ms. Samantha Austen for the English revision of the manuscript.

References

Adams, J., Collaco-Moraes, Y., De Belleroche, J., 1996. Cyclooxygenase-2 induction in cerebral cortex: an intracellular response to synaptic excitation. J. Neurochem. 66, 6–13.

Akaike, A., Kaneko, S., Tamura, Y., Nakata, N., Shiomi, H., Ushikubi, F., Narumiya, S., 1994. Prostaglandin E2 protects cultured cortical neurons against *N*-methyl-D-aspartate receptor-mediated glutamate cytotoxicity. Brain Res. 663, 237–243.

Amann, R., Egger, T., Schuligoi, R., Heinemann, A., Peskar, B.A., 2001. Sodium salicylate enhances the expression of cyclooxygenase-2 in endotoxin-stimulated human mononuclear cells. Eur. J. Pharmacol. 433, 129–134.

Andreasson, K.I., Savonenko, A., Vidensky, S., Goellner, J.J., Zhang, Y., Shaffer, A., Kaufmann, W.E., Worley, P.F., Isakson, P., Markowska, A.L., 2001. Age dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. J. Neurosci. 21, 8198–8209.

Arimoto, T., Choi, D.Y., Lu, X., Liu, M., Nguyen, X.V., Zheng, N., Stewart, C.A., Kim, H.C., Bing, G., in press. Interleukin-10 protects against inflammation-mediated degeneration of dopaminergic neurons in substantia nigra. Neurobiol Aging.

Asanuma, M., Miyazaki, I., 2006. Nonsteroidal anti-inflammatory drugs in Parkinson's disease: possible involvement of quinone formation. Expert Rev. Neurotherapeutics 6, 1313–1325.

Asanuma, M., Nishibayashi-Asanuma, S., Miyazaki, I., Kohno, M., Ogawa, N., 2001. Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. J. Neurochem. 76, 1895–1904.

Ascherio, A., Zhang, S.M., Hernan, M.A., Kawachi, I., Colditz, G.A., Speizer, F.E., Willett, W.C., 2001. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 50, 56–63.

Aubin, N., Curet, O., Deffois, A., Carter, C., 1998. Aspirin and salicylate protect against MPTP-induced dopamine depletion in mice. J. Neurochem. 71, 1635–1642.

Baba, Y., Kuroiwa, A., Uitti, R.J., Wszolek, Z.K., Yamada, T., 2005. Alterations of T-lymphocyte populations in Parkinson disease. Parkinsonism Relat. Disord. 11, 493–498.

Baeuerle, P.A., Baichwal, V.R., 1997. NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. Adv. Immunol. 65, 111–137.

Batchelor, P.E., Liberatore, G.T., Wong, J.Y., Porritt, M.J., Frerichs, F., Donnan, G.A., Howells, D.W., 1999. Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor. J. Neurosci. 19, 1708–1716.

Beal, M.F., 2001. Experimental models of Parkinson's disease. Nat. Rev., Neurosci. 2, 325–334.

Bergstrom, S., Danielsson, H., Samuelsson, B., 1964. The enzymatic formation of prostaglandin E2 from arachidonic acid. Prostaglandins and related factors 32. Biochim. Biophys. Acta 90, 207–210.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., Seitelberger, F., 1973. Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. J. Neurol. Sci. 20, 415–455.

Bishop-Bailey, D., Warner, T.D., 2003. PPARgamma ligands induce prostaglandin production in vascular smooth muscle cells: indomethacin acts as a peroxisome proliferator-activated receptor-gamma antagonist. FASEB J. 17, 1925–1927.

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A.L., Sadoul, R., Verna, J.M., 2001. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog. Neurobiol. 65, 135–172.

Blum-Degen, D., Muller, T., Kuhn, W., Gerlach, M., Przuntek, H., Riederer, P., 1995. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. Neurosci. Lett. 202, 17–20.

Bonifati, D.M., Kishore, U., 2007. Role of complement in neurodegeneration and neuroinflammation. Mol. Immunol. 44, 999–1010.

Bonuccelli, U., Del Dotto, P., 2006. New pharmacologic horizons in the treatment of Parkinson disease. Neurology 67, S30–S38.

Bos, C.L., Richel, D.J., Ritsema, T., Peppelenbosch, M.P., Versteeg, H.H., 2004. Prostanoids and prostanoid receptors in signal transduction. Int. J. Biochem. Cell Biol. 36, 1187–1205.

Bové, J., Prou, D., Perier, C., Przedborski, S., 2005. Toxin-induced models of Parkinson's disease. NeuroRx 2, 484–494.

Bower, J.H., Maraganore, D.M., Peterson, B.J., Ahlskog, J.E., Rocca, W.A., 2006. Immunologic diseases, anti-inflammatory drugs, and Parkinson disease: a case-control study. Neurology 67, 494–496.

Carlson, N.G., 2003. Neuroprotection of cultured cortical neurons mediated by the cyclooxygenase-2 inhibitor APHS can be reversed by a prostanoid. J. Neurosci. Res. 71, 79–88.

Carrasco, E., Werner, P., 2002. Selective destruction of dopaminergic neurons by low concentrations of 6-OHDA and MPP+: protection by acetylsalicylic acid aspirin. Parkinsonism. Relat. Disord. 8, 407–411.

Carrasco, E., Camper, D., Werner, P., 2005. Dopaminergic neurotoxicity by 6-OHDA and MPP+: differential requirement for neuronal cyclooxygenase activity. J. Nurosci. Res. 81, 121–131.

Casper, D., Yaparpalvi, U., Rempel, N., Werner, P., 2000. Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro. Neurosci. Lett. 289, 201–204.

Cassarino, D.S., Halvorsen, E.M., Swerdlow, R.H., Abramova, N.N., Parker Jr., W.D., Sturgill, T.W., Bennett Jr., J.P., 2000. Interaction among mitochondria, mitogen-activated protein kinases, and nuclear factor-kappaB in cellular models of Parkinson's disease. J. Neurochem. 74, 1384–1392.

Chandrasekharan, N.V., Dai, H., Roos, K.L., Evanson, N.K., Tomsik, J., Elton, T.S., Simmons, D.L., 2002. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure and expression. Proc. Natl. Acad. Sci. U. S. A. 99, 3926–13931.

Chen, C., Bazan, N.G., 2005. Lipid signaling: sleep, synaptic plasticity, and neuroprotection. Prostaglandins Other Lipid Mediat. 77, 65–76.

Chen, H., Zhang, S.M., Hernan, M.A., Schwarzschild, M.A., Willett, W.C., Colditz, G.A., Speizer, F.E., Ascherio, A., 2003. Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. Arch. Neurol. 60, 1059–1064.

Chen, H., Jacobs, E., Schwarzschild, M.A., McCullough, M.L., Calle, E.E., Thun, M.J., Ascherio, A., 2005. Nonsteroidal antiinflammatory drug use and the risk for Parkinson's disease. Ann. Neurol. 58, 963–967.

Cho, B.P., Song, D.Y., Sugama, S., Shin, D.H., Shimizu, Y., Kim, S.S., Kim, Y.S., Joh, T.H., 2006. Pathological dynamics of activated microglia following medial forebrain bundle transection. Glia 53, 92–102.

Choi, J.S., Kim, H.Y., Chun, M.H., Chung, J.W., Lee, M.Y., 2006. Differential regulation of cyclooxygenase-2 in the rat hippocampus after cerebral ischemia and ischemic tolerance. Neurosci. Lett. 393, 231–236.

Christie, M.J., Vaughan, C.W., Ingram, S.L., 1999. Opioids, NSAIDs and 5-lipoxygenase inhibitors act synergistically in brain via arachidonic acid metabolism. Inflamm. Res. 48, 1–4.

Cleeter, M.W.J., Cooper, J.M., Schapira, A.H.V., 1992. Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement. J. Neurochem. 58, 786–789.

Cohen, G., 1984. Oxy-radical toxicity in catecholamine neurons. Neurotoxicology 5, 77–82.

Cole-Edwards, K.K., Bazan, N.G., 2005. Lipid signaling in experimental epilepsy. Neurochem. Res. 30, 847–853.

Couzin, J., 2004. Clinical trials. Halt of Celebrex study threatens drug's future, other trials. Science 306, 2170.

Członkowska, I., Kurkowska-Jastrzębska, A., Członkowski, A., Peter, D., Stefano, G.B., 2002. Immune processes in the pathogenesis of Parkinson's disease—A potential role for microglia and nitric oxide. Med. Sci. Monit. 8, RA165–RA177.

Dehmer, T., Heneka, M.T., Sastre, M., Dichgans, J., Schulz, J.B., 2004. Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. J. Neurochem. 88, 494–501.

de Meira Santos Lima, M., Braga Reksidler, A., Marques Zanata, S., Bueno Machado, H., Tufik, S., Vital, M.A., 2006. Different parkinsonism models produce a time-dependent induction of COX-2 in the substantia nigra of rats. Brain Res. 1101, 117–125.

De Simone, R., Ajmone-Cat, M.A., Tirassa, P., Minghetti, L., 2003. Apoptotic PC12 cells exposing phosphatidylserine promote the production of anti-inflammatory and neuroprotective molecules by microglial cells. J. Neuropathol. Exp. Neurol. 62, 208–216.

De Simone, R., Ajmone-Cat, M.A., Minghetti, L., 2004. Atypical antiinflammatory activation of microglia induced by apoptotic neurons: possible

role of phosphatidylserine–phosphatidylserine receptor interaction. Mol. Neurobiol. 29, 197–212.

De Simone, R., Ajmone-Cat, M.A., Carnevale, D., Minghetti, L., 2005. Activation of alpha7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. J. Neuroinflammation 2, 4.

Diaz, A., Reginato, A.M., Jimenez, S.A., 1992. Alternative splicing of human prostaglandin G/H synthase mRNA and evidence of differential regulation of the resulting transcripts by transforming growth factor beta 1, interleukin 1 beta, and tumor necrosis factor alpha. J. Biol. Chem. 267, 10816–10822.

Di Giovanni, G., Di Matteo, V., Pierucci, M., Benigno, A., Esposito, E., 2006. Serotonin involvement in the basal ganglia pathophysiology: could the 5-HT2C receptor be a new target for therapeutic strategies? Curr. Med. Chem. 13, 3069–3081.

Di Matteo, V., Pierucci, M., Di Giovanni, G., Di Santo, A., Poggi, A., Benigno, A., Esposito, E., 2006a. Aspirin protects striatal dopaminergic neurons from neurotoxin-induced degeneration: an in vivo microdialysis study. Brain Res. 1095, 167–177.

Di Matteo, V., Benigno, A., Pierucci, M., Giuliano, D.A., Crescimanno, G., Esposito, E., Di Giovanni, G., 2006b. 7-nitroindazole protects striatal dopaminergic neurons against MPP+-induced degeneration: an in vivo microdialysis study. Ann. N. Y. Acad. Sci. 1089, 462–471.

Dong, Z., Huang, C., Brown, R.E., Ma, W.Y., 1997. Inhibition of activator protein 1 activity and neoplastic transformation by aspirin. J. Biol. Chem. 272, 9962–9970.

Dugan, L.L., Choi, D.W., 1999. Hypoxic–ischemic brain injury and oxidative stress. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M. D. (Eds.), Basic Neurochemistry. Raven Press, New York, pp. 711–730.

Echeverria, V., Clerman, A., Dore, S., 2005. Stimulation of PGE receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following beta-amyloid exposure. Eur. J. Neurosci. 22, 2199–2206.

Ehringer, H., Hornykiewicz, O., 1960. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Klin. Wochenschr. 38, 1236–1239.

Elsisi, N.S., Darling-Reed, S., Lee, E.Y., Oriaku, E.T., Soliman, K.F., 2005. Ibuprofen and apigenin induce apoptosis and cell cycle arrest in activated microglia. Neurosci. Lett. 375, 91–96.

Feng, Z.H., Wang, T.G., Li, D.D., Fung, P., Wilson, B.C., Liu, B., Ali, S.F., Langenbach, R., Hong, J.S., 2002. Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra. Neurosci. Lett. 329, 354–358.

Feng, Z., Li, D., Fung, P.C., Pei, Z., Ramsden, D.B., Ho, S.L., 2003. COX-2-deficient mice are less prone to MPTP-neurotoxicity than wild-type mice. NeuroReport 14, 1927–1929.

Ferger, B., Teismann, P., Earl, C.D., Kuschinsky, K., Oertel, W.H., 1999. Salicylate protects against MPTP-induced impairments in dopaminergic neurotransmission at the striatal and nigral level in mice. Naunyn-Schmiedebergs Arch. Pharmacol. 360, 256–261.

Firuzi, O., Pratico, D., 2006. Coxibs and Alzheimer's disease: should they stay or should they go? Ann. Neurol. 59, 219–228.

Fleming, S.M., Fernagut, P.O., Chesselet, M.F., 2005. Genetic mouse models of parkinsonism: strengths and limitations. NeuroRx 2, 495–503.

Furst, D., 1999. Pharmacology and efficacy of cyclooxygenase (COX) inhibitors. Am. J. Med. 107, 18S–26S.

Furuya, T., Hayakawa, H., Yamada, M., Yoshimi, K., Hisahara, S., Miura, M., Mizuno, Y., Mochizuki, H., 2004. Caspase-11 mediates inflammatory dopaminergic cell death in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. J. Neurosci. 24, 1865–1872.

Gao, H.M., Liu, B., Zhang, W., Hong, J.S., 2003. Novel anti-inflammatory therapy for Parkinson's disease. Trends Pharmacol. Sci. 24, 395–401.

Gebicke-Haerter, P.J., 2001. Microglia in neurodegeneration: molecular aspects. Microsc. Res. Tech. 54, 47–58.

Grilli, M., Pizzi, M., Memo, M., Spano, P., 1996. Neuroprotection by aspirin and sodium salicylate through blockade of NF-kappaB activation. Science 274, 1383–1385.

Hald, A., Lotharius, J., 2005. Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp. Neurol. 193, 279–290.

Hartmann, A., Hirsch, E.C., 2001. Parkinson's disease. The apoptosis hypothesis revisited. Adv. Neurol. 86, 143–153.

Hemler, M., Lands, W.E.M., Smith, W.L., 1976. Purification of the cyclooxygenase that forms prostaglandins: demonstration of two forms of iron in the holoenzyme. J. Biol. Chem. 251, 5575–5579.

Heneka, M.T., Sastre, M., Dumitrescu-Ozimek, L., Hanke, A., Dewachter, I., Kuiperi, C., O'Banion, K., Klockgether, T., Van Leuven, F., Landreth, G.E., 2005. Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. Brain 128, 1442–1453.

Hernán, M.A., Logroscino, G., Garcıa Rodrıguez, L.A., 2004. A prospective study of alcoholism and the risk of Parkinson's disease. J. Neurol. 251, VII14–VII17.

Hernán, M.A., Logroscino, G., García Rodríguez, L.A., 2006. Nonsteroidal anti-inflammatory drugs and the incidence of Parkinson disease. Neurology 66, 1097–1099.

Hewett, S.J., Uliasz, T.F., Vidwans, A.S., Hewett, J.A., 2000. Cyclooxygenase-2 contributes to *N*-methyl-D-aspartate-mediated neuronal cell death in primary cortical cell culture. J. Pharmacol. Exp. Ther. 293, 417–425.

Hinz, B., Brune, K., 2002. Cyclooxygenase—2–10 years later. J. Pharmacol. Exp. Ther. 2, 367–375.

Hirsch, E.C., Hunot, S., Damier, P., Faucheux, B., 1998. Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration? Ann. Neurol. 44, S115–S120.

Hoozemans, J.J., Rozemuller, A.J., Janssen, I., De Groot, C.J., Veerhuis, R., Eikelenboom, P., 2001. Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. Acta Neuropathol. 101, 2–8.

Iadecola, C., Niwa, K., Nogawa, S., Zhao, X., Nagayama, M., Araki, E., Morham, S., Ross, M.E., 2001. Reduced susceptibility to ischemic brain injury and *N*-methyl-D-aspartate-mediated neurotoxicity in cyclooxygenase-2-deficient mice. Proc. Natl. Acad. Sci. U. S. A. 98, 1294–1299.

Imamura, K., Hishikawa, N., Sawada, M., Nagatsu, T., Yoshida, M., Hashizume, Y., 2003. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. Acta Neuropathol. 106, 518–526.

Iravani, M.M., Kashefi, K., Mander, P., Rose, S., Jenner, P., 2002. Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 110, 49–58.

Isacson, O., Brundin, P., Gage, F.H., Bjorklund, A., 1985. Neural grafting in a rat model of Huntington's disease: progressive neurochemical changes after neostriatal ibotenate lesions and striatal tissue grafting. Neuroscience 16, 799–817.

Ishida, Y., Nagai, A., Kobayashi, S., Kim, S.U., 2006. Upregulation of protease-activated receptor-1 in astrocytes in Parkinson disease: astrocyte-mediated neuroprotection through increased levels of glutathione peroxidase. J. Neuropathol. Exp. Neurol. 65, 66–77.

Jellinger, K.A., 2000. Cell death mechanisms in Parkinson's disease. J. Neural Transm. 107, 1–29.

Jenner, P., Olanow, C.W., 1998. Understanding cell death in Parkinson's disease. Ann. Neurol. 44, S72–S84.

Jenner, P., Olanow, C.W., 2006. The pathogenesis of cell death in Parkinson's disease. Neurology 66, S24–S36.

Jeon, B.S., Jackson-Lewis, V., Burke, R.E., 1995. 6-Hydroxydopamine lesion of the rat substantia nigra: time course and morphology of cell death. Neurodegeneration 4, 131–137.

Jiang, C., Ting, A.T., Seed, B., 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 391, 82–86.

Jiang, J., Borisenko, G.G., Osipov, A., Martin, I., Chen, R., Shvedova, A.A., Sorokin, A., Tyurina, Y.Y., Potapovich, A., Tyurin, V.A., Graham, S.H., Kagan, V.E., 2004. Arachidonic acid-induced carbon-centered radicals and phospholipid peroxidation in cyclo-oxygenase-2-transfected PC12 cells. J. Neurochem. 90, 1036–1049.

Jiang, Q., Yan, Z., Feng, J., 2006. Neurotrophic factors stabilize microtubules and protect against rotenone toxicity on dopaminergic neurons. J. Biol. Chem. 281, 9391–9400.

Kaku, K., Shikimi, T., Kamisaki, Y., Shinozuka, K., Ishino, H., Okunishi, H., Takaori, S., 1999. Elevation of striatal interleukin-6 and serum

corticosterone contents in MPTP-treated mice. Clin. Exp. Pharmacol. Physiol. 26, 680–683.

Kataoka, M., Tonooka, K., Ando, T., Imai, K., Aimoto, T., 1997. Hydroxyl radical scavenging activity of nonsteroidal anti-inflammatory drugs. Free Radical Res. 27, 419–427.

Kawano, T., Anrather, J., Zhou, P., Park, L., Wang, G., Frys, K.A., Kunz, A., Cho, S., Orio, M., Iadecola, C., 2006. Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. Nat. Med. 12, 225–229.

Kelley, K.A., Ho, L., Winger, D., Freire-Moar, J., Borelli, C.B., Aisen, P.S., Pasinetti, G.M., 1999. Potentiation of excitotoxicity in transgenic mice overexpressing neuronal cyclooxygenase-2. Am. J. Pathol. 155, 995–1004.

Kim, Y.S., Joh, T.H., 2006. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp. Mol. Med. 38, 333–347.

Kim, W.G., Mohney, R.P., Wilson, B., Jeohn, G.H., Liu, B., Hong, J.S., 2000. Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. J. Neurosci. 20, 6309–6316.

Kim, E.J., Kwon, K.J., Park, J.Y., Lee, S.H., Moon, C.H., Baik, E.J., 2002. Neuroprotective effects of prostaglandin E2 or cAMP against microglial and neuronal free radical mediated toxicity associated with inflammation. J. Neurosci. Res. 70, 97–107.

Kis, B., Snipes, J.A., Isse, T., Nagy, K., Busija, D.W., 2003. Putative cyclooxygenase-3 expression in rat brain cells. J. Cereb. Blood Flow Metab. 23, 1287–1292.

Kis, B., Snipes, J.A., Gaspar, T., Lenzser, G., Tulbert, C.D., Busija, D.W., 2006. Cloning of cyclooxygenase-1b (putative COX-3) in mouse. Inflamm. Res. 55, 274–278.

Klegeris, A., McGeer, P.L., 2005. Non-steroidal anti-inflammatory drugs (NSAIDs) and other anti-inflammatory agents in the treatment of neurodegenerative disease. Curr. Alzheimer Res. 2, 355–365.

Klivenyi, P., Gardian, G., Calingasan, N.Y., Yang, L., Beal, M.F., 2003. Additive neuroprotective effects of creatine and a cyclooxygenase 2 inhibitor against dopamine depletion in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease. J. Mol. Neurosci. 21, 191–198.

Knott, C., Stern, G., Wilkin, G.P., 2000. Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol. Cell. Neurosci. 16, 724–739.

Knott, C., Stern, G., Kingsbury, A., Welcher, A.A., Wilkin, G.P., 2002. Elevated glial brain-derived neurotrophic factor in Parkinson's diseased nigra. Parkinsonism Relat. Disord. 8, 329–341.

Kopp, E., Ghosh, S., 1994. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 265, 956–959.

Kurkowska-Jastrzębska, A., Wronska, M., Kohutnicka, A., Członkowski, A., Członkowska, A., 1999a. The inflammatory reaction following 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine intoxication in mouse. Exp. Neurol. 156, 50–61.

Kurkowska-Jastrzębska, I., Wronska, A., Kohutnicka, M., Członkowski, A., Członkowska, A., 1999b. MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease. Acta Neurobiol. Exp. 59, 1–8.

Kurkowska-Jastrzębska, I., Babiuch, M., Joniec, I., Przybyłkowski, A., Członkowski, A., Członkowska, A., 2002. Indomethacin protects against neurodegeneration caused by MPTP intoxication in mice. Int. Immunopharmacol. 2, 1213–1218.

Langston, J.W., Irwin, I., Langston, E.B., Forno, L.S., 1984. Pargyline prevents MPTP-induced parkinsonism in primates. Science 225, 1480–1482.

Langston, J.W., Forno, L.S., Tetrud, J., Reeves, A.G., Kaplan, J.A., Karluk, D., 1999. Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenil-1,2,3,6-tetrahydropyridine exposure. Ann. Neurol. 46, 598–605.

Lawson, L.J., Perry, V.H., Dri, P., Gordon, S., 1990. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 39, 151–170.

Lee, H.J., Kim, S.H., Kim, K.W., Um, J.H., Lee, H.W., Chung, B.S., Kang, C.D., 2001. Antiapoptotic role of NF-kappaB in the auto-oxidized dopamine-induced apoptosis of PC12 cells. J. Neurochem. 76, 602–609.

Lee, E.O., Shin, Y.J., Chong, Y.H., 2004. Mechanisms involved in prostaglandin E2-mediated neuroprotection against TNF-alpha: possible involvement of multiple signal transduction and beta-catenin/T-cell factor. J. Neuroimmunol. 155, 21–31.

Lee, S.H., Williams, M.V., Dubois, R.N., Blair, I.A., 2005. Cyclooxygenase-2-mediated DNA damage. J. Biol. Chem. 280, 28337–28346.

Lehmann, J.M., Lenhard, J.M., Oliver, B.B., Ringold, G.M., Kliewer, S.A., 1997. Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J. Biol. Chem. 272, 3406–3410.

Lemberger, T., Desvergne, B., Wahli, W., 1996. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu. Rev. Cell Dev. Biol. 12, 335–363.

Levi, G., Minghetti, L., Aloisi, F., 1998. Regulation of prostanoid synthesis in microglial cells and effects of prostaglandin E2 on microglial functions. Biochimie 80, 889–904.

Loeffler, D.A., Camp, D.M., Conant, S.B., 2006. Complement activation in the Parkinson's disease substantia nigra: an immunocytochemical study. J. Neuroinflammation 3, 29.

Maharaj, D.S., Saravanan, K.S., Maharaj, H., Mohanakumar, K.P., Daya, S., 2004. Acetaminophen and aspirin inhibit superoxide anion generation and lipid peroxidation, and protect against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats. Neurochem. Int. 44, 355–360.

Maharaj, H., Maharaj, D.S., Daya, S., 2006. Acetylsalicylic acid and acetaminophen protect against MPP+-induced mitochondrial damage and superoxide anion generation. Life Sci. 78, 2438–2443.

Marchetti, B., Abbracchio, M.P., 2005. To be or not to be (inflamed)–is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? Trends Pharmacol. Sci. 26, 517–525.

Marnett, L.J., 2000. Cyclo-oxygenase mechanisms. Curr. Opin. Chem. Biol. 4, 545–552.

McCarty, M.F., 2006. Down-regulation of microglial activation may represent a practical strategy for combating neurodegenerative disorders. Med. Hypotheses 67, 251–269.

McCullough, L., Wu, L., Haughey, N., Liang, X., Hand, T., Wang, Q., Breyer, R.M., Andreasson, K., 2004. Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. J. Neurosci. 24, 257–268.

McGeer, E.G., McGeer, P.L., 1997. The role of the immune system in neurodegenerative disorders. Mov. Disord. 12, 855–858.

McGeer, P.L., McGeer, E.G., 1998. Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions. Alzheimer Dis. Assoc. Disord. 12, S1–S6.

McGeer, P.L., McGeer, E.G., 2004. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat. Disord. 10, S3–S7.

McGeer, P.L., Itagaki, S., Boyes, B.E., McGeer, E.G., 1988. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 38, 1285–1291.

McGeer, P.L., Yasojima, K., McGeer, E.G., 2001. Inflammation in Parkinson's disease. Adv. Neurol. 86, 83–89.

McGeer, P.L., Yasojima, K., McGeer, E.G., 2002. Association of interleukin-1 beta polymorphisms with idiopathic Parkinson's disease. Neurosci. Lett. 326, 67–69.

McGeer, P.L., Schwab, C., Parent, A., Doudet, D., 2003. Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenil-1,2,3,6-tetrahydropyridine administration. Ann. Neurol. 54, 599–604.

Miller, D.S., Hong, J.S., 2005. MPP+-induced COX-2 activation and subsequent dopaminergic neurodegeneration. FASEB J. 19, 1134–1136.

Minghetti, L., 2004. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J. Neuropathol. Exp. Neurol. 63, 901–910.

Minghetti, L., 2005. Role of inflammation in neurodegenerative diseases. Curr. Opin. Neurol. 18, 315–321.

Minghetti, L., Ajmone-Cat, M.A., De Berardinis, M.A., De Simone, R., 2005. Microglial activation in chronic neurodegenerative diseases: roles of apoptotic neurons and chronic stimulation. Brain Res. Brain Res. Rev. 48, 251–256.

Mirza, B., Hadberg, H., Thomsen, P., Moos, T., 2000. The absence of reactive astrocytosis is indicative of a unique inflammatory process in Parkinson's disease. Neuroscience 95, 425–432.

Mitchell, J.A., Saunders, M., Barnes, P.J., Newton, R., Belvisi, M.G., 1997. Sodium salicylate inhibits cyclo-oxygenase-2 activity independently of transcription factor (nuclear factor kappaB) activation: role of arachidonic acid. Mol. Pharmacol. 51, 907–912.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Inagaki, H., Minami, M., Nagatsu, T., 1994a. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci. Lett. 80, 147–150.

Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., Nagatsu, T., 1994b. Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci. Lett. 165, 208–210.

Mogi, M., Harada, M., Kondo, T., Riederer, P., Nagatsu, T., 1995. Brain beta 2-microglobulin levels are elevated in the striatum in Parkinson's disease. J. Neural Transm., Parkinson's Dis. Dement. Sect. 9, 87–92.

Mogi, M., Togari, A., Ogawa, M., Ikeguchi, K., Shizuma, N., Fan, D., Nakano, I., Nagatsu, T., 1998. Effects of repeated systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to mice on interleukin-1beta and nerve growth factor in the striatum. Neurosci. Lett. 250, 25–28.

Mohanakumar, K.P., Muralikrishnan, D., Thomas, B., 2000. Neuroprotection by sodium salicylate against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. Brain Res. 864, 281–290.

Morioka, N., Kumagai, K., Morita, K., Kitayama, S., Dohi, T., 2004. Nonsteroidal anti-inflammatory drugs potentiate 1-methyl-4-phenylpyridinium (MPP+)-induced cell death by promoting the intracellular accumulation of MPP+ in PC12 cells. J. Pharmacol. Exp. Ther. 310, 800–807.

Nair, V.D., McNaught, K.S., Gonzalez-Maeso, J., Sealfon, S.C., Olanow, C.W., 2006. p53 mediates non-transcriptional cell death in dopaminergic cells in response to proteasome inhibition. J. Biol. Chem. 281, 39550–39560.

Narumiya, S., 2003. Prostanoids in immunity: roles revealed by mice deficient in their receptors. Life Sci. 74, 391–395.

Nogawa, S., Zhang, F., Ross, M.E., Iadecola, C., 1997. Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. J. Neurosci. 17, 2746–2755.

Novikova, L., Garris, B.L., Garris, D.R., Lau, Y.S., 2006. Early signs of neuronal apoptosis in the substantia nigra pars compacta of the progressive neurodegenerative mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid model of Parkinson's disease. Neuroscience 140, 67–76.

Ouchi, Y., Yoshikawa, E., Sekine, Y., Futatsubashi, M., Kanno, T., Ogusu, T., Torizuka, T., 2005. Microglial activation and dopamine terminal loss in early Parkinson's disease. Ann. Neurol. 57, 168–175.

Pavese, N., Gerhard, A., Tai, Y.F., Ho, A.K., Turkheimer, F., Barker, R.A., Brooks, D.J., Piccini, P., 2006. Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology 66, 1638–1643.

Prodan, C.I., Monnot, M., Ross, E.D., Coleman, A.E., 2006. Reversible dementia with parkinsonian features associated with budesonide use. Neurology 67, 723.

Przybyłkowski, A., Kurkowska-Jastrzębska, I., Joniec, I., Ciesielska, A., Członkowska, A., Członkowski, A., 2004. Cyclooxygenases mRNA and protein expression in striata in the experimental mouse model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration to mouse. Brain Res. 1019, 144–151.

Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J., Glass, C.K., 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 391, 79–82.

Riederer, P., Wuketich, S., 1976. Time course of nigrostriatal degeneration in Parkinson's disease: a detailed study of influential factors in human brain amine analysis. J. Neural Transm. 38, 277–301.

Rigas, B., Kashfi, K., 2004. Nitric-oxide-donating NSAIDs as agents for cancer prevention. Trends Mol. Med. 10, 324–330.

Rowe, D.B., Le, W., Smith, R.G., Appel, S.H., 1998. Antibodies from patients with Parkinson's disease react with protein modified by dopamine oxidation. J. Neurosci. Res. 53, 551–558.

Ruano, D., Revilla, E., Gavilan, M.P., Vizuete, M.L., Pintado, C., Vitorica, J., Castano, A., 2006. Role of p38 and inducible nitric oxide synthase in the in vivo dopaminergic cells' degeneration induced by inflammatory processes after lipopolysaccharide injection. Neuroscience 140, 1157–1168.

Sairam, K., Saravanan, K.S., Banerjee, R., Mohanakumar, K.P., 2003. Non-steroidal anti-inflammatory drug sodium salicylate, but not diclofenac or celecoxib, protects against 1-methyl-4-phenyl pyridinium-induced dopaminergic neurotoxicity in rats. Brain Res. 966, 245–252.

Salpeter, S.R., Gregor, P., Ormiston, T.M., Whitlock, R., Raina, P., Thabane, L., Topol, E.J., 2006. Meta-analysis: cardiovascular events associated with nonsteroidal anti-inflammatory drugs. Am. J. Med. 119, 552–559.

Sánchez-Pernaute, R., Ferree, A., Cooper, O., Yu, M., Brownell, A.L., Isacson, O., 2004. Selective COX-2 inhibition prevents progressive dopamine neuron degeneration in a rat model of Parkinson's disease. J. Neuroinflammation 1, 6.

Sastre, M., Dewachter, I., Rossner, S., Bogdanovic, N., Rosen, E., Borghgraef, P., Evert, B.O., Dumitrescu-Ozimek, L., Thal, D.R., Landreth, G., Walter, J., Klockgether, T., van Leuven, F., Heneka, M.T., 2006. Nonsteroidal anti-inflammatory drugs repress beta-secretase gene promoter activity by the activation of PPARgamma. Proc. Natl. Acad. Sci. U. S. A. 103, 443–448.

Schapira, A.H., Bezard, E., Brotchie, J., Calon, F., Collingridge, G.L., Ferger, B., Hengerer, B., Hirsch, E., Jenner, P., Le Novere, N., Obeso, J.A., Schwarzschild, M.A., Spampinato, U., Davidai, G., 2006. Novel pharmacological targets for the treatment of Parkinson's disease. Nat. Rev., Drug Discov. 5, 845–854.

Schiefer, J., Kampe, K., Dodt, H.U., Zieglgansberger, W., Kreutzberg, G.W., 1999. Microglial motility in the rat facial nucleus following peripheral axotomy. J. Neurocytol. 28, 439–453.

Schiess, M., 2003. Nonsteroidal anti-inflammatory drugs protect against Parkinson neurodegeneration: can an NSAID a day keep Parkinson disease away? Arch. Neurol. 60, 1043–1044.

Shishodia, S., Koul, D., Aggarwal, B.B., 2004. Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-kappa B activation through inhibition of activation of I kappa B alpha kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis. J. Immunol. 173, 2011–2022.

Sian, J., Gerlach, M., Youdim, M.B., Riederer, P., 1999. Parkinson's disease: a major hypokinetic basal ganglia disorder. J. Neural Transm. 106, 443–476.

Snipes, J.A., Kis, B., Shelness, G.S., Hewett, J.A., Busija, D.W., 2005. Cloning and characterization of cyclooxygenase-1b (putative cyclooxygenase-3) in rat. J. Pharmacol. Exp. Ther. 313, 668–676.

Snyder, S.H., D'Amato, R.J., 1986. MPTP: a neurotoxin relevant to the pathophysiology of Parkinson's disease. The 1985 George C. Cotzias lecture. Neurology 36, 250–258.

Sugama, S., Cho, B.P., Degiorgio, L.A., Shimizu, Y., Kim, S.S., Kim, Y.S., Shin, D.H., Volpe, B.T., Reis, D.J., Cho, S., Joh, T.H., 2003. Temporal and sequential analysis of microglia in the substantia nigra following medial forebrain bundle axotomy in rat. Neuroscience 116, 925–933.

Sun, C.J., Johannessen, J.N., Gessner, W., Namura, I., Singhaniyom, W., Brossi, A., Chiueh, C.C., 1988. Neurotoxic damage to the nigrostriatal system in rats following intranigral administration of MPDP+ and MPP+. J. Neural Transm. Gen. Sect. 74, 75–86.

Taketo, M.M., 1998. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J. Natl. Cancer Inst. 90, 1529–1536.

Takeuchi, H., Mizuno, T., Zhang, G., Wang, J., Kawanokuchi, J., Kuno, R., Suzumura, A., 2005. Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport. J. Biol. Chem. 280, 10444–10454.

Teismann, P., Ferger, B., 2001. Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease. Synapse 39, 167–174.

Teismann, P., Tieu, K., Choi, D.K., Wu, D.C., Naini, A., Hunot, S., Vila, M., Jackson-Lewis, V., Przedborski, S., 2003. Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. Proc. Natl. Acad. Sci. U. S. A. 100, 5473–5478.

Thery, C., Dobbertin, A., Mallat, M., 1994. Downregulation of in vitro neurotoxicity of brain macrophages b prostaglandin E2 and a beta-adrenergic agonist. Glia 11, 383–386.

Ton, T.G., Heckbert, S.R., Longstreth Jr., W.T., Rossing, M.A., Kukull, W.A., Franklin, G.M., Swanson, P.D., Smith-Weller, T., Checkoway, H., 2006.

Nonsteroidal anti-inflammatory drugs and risk of Parkinson's disease. Mov. Disord. 21, 964–969.

Tyurina, Y.Y., Kapralov, A.A., Jiang, J., Borisenko, G.G., Potapovich, A.I., Sorokin, A., Kochanek, P.M., Graham, S.H., Schor, N.F., Kagan, V.E., 2006. Oxidation and cytotoxicity of 6-OHDA are mediated by reactive intermediates of COX-2 overexpressed in PC12 cells. Brain Res. 1093, 71–82.

Vane, J.R., 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat., New Biol. 231, 232–235.

Vane, J.R., Botting, R.M., 1987. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J. 1, 89–96.

Vane, J.R., Botting, R.M., 1996. Mechanism of action of anti-inflammatory drugs. Scand. J. Rheumatol. 25, 9–21.

Vane, J.R., Botting, R.M., 2003. The mechanism of action of aspirin. Thromb. Res. 110, 255–258.

Vaux, D.L., Strasser, A., 1996. The molecular biology of apoptosis. Proc. Natl. Acad. Sci. U. S. A. 93, 2239–2244.

Vijitruth, R., Liu, M., Choi, D.Y., Nguyen, X.V., Hunter, R.L., Bing, G., 2006. Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J. Neuroinflammation 3, 6.

Wang, T., Pei, Z., Zhang, W., Liu, B., Langenbach, R., Lee, C., Wilson, B., Reece, J.M., Miller, D.S., Hong, J.S., 2005. MPP+-induced COX-2 activation and subsequent dopaminergic neurodegeneration. FASEB J. 19, 1134–1136.

Weissmann, G., 1991. Aspirin. Sci. Am. 264, 58–64.

Westwick, J.K., Weitzel, C., Minden, A., Karin, M., Brenner, D.A., 1994. Tumor necrosis factor alpha stimulates AP-1 activity through prolonged activation of the c-Jun kinase. J. Biol. Chem. 269, 26396–26401.

Williams, M.V., Lee, S.H., Pollack, M., Blair, I.A., 2006. Endogenous lipid hydroperoxide-mediated DNA-adduct formation in min mice. J. Biol. Chem. 281, 10127–10133.

Wong, B.C., Jiang, X.H., Lin, M.C., Tu, S.P., Cui, J.T., Jiang, S.H., Wong, W.M., Yuen, M.F., Lam, S.K., Kung, H.F., 2004. Cyclooxygenase-2 inhibitor (SC-236) suppresses activator protein-1 through c-Jun NH2-terminal kinase. Gastroenterology 126, 136–147.

Wright, S.C., Kumar, P., Tam, A.W., Shen, N., Varma, M., Larrick, J.W., 1992. Apoptosis and DNA fragmentation precede TNF-induced cytolysis in U937 cells. J. Cell. Biochem. 48, 344–355.

Wu, K.K., 2003. Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. Semin. Vasc. Med. 3, 107–112.

Wu, K.K., 2006. Transcription-based COX-2 inhibition: a therapeutic strategy. Thromb. Haemost. 96, 417–422.

Wyss, M., Kaddurah-Daouk, R., 2000. Creatine and creatinine metabolism. Physiol. Rev. 80, 1107–1213.

Xie, W., Chipman, J.G., Robertson, D.L., Erikson, R.L., Simmons, D.L., 1991. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc. Natl. Acad. Sci. U. S. A. 88, 2692–2696.

Xu, X.M., Sansores-Garcia, L., Chen, X.M., Matijevic-Aleksic, N., Du, M., Wu, K.K., 1999. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. Proc. Natl. Acad. Sci. U. S. A. 96, 5292–5297.

Yamada, T., McGeer, P.L., McGeer, E.G., 1991. Relationship of complement-activated oligodendrocytes to reactive microglia and neuronal pathology in neurodegenerative disease. Dementia 2, 71–77.

Yamada, T., McGeer, P.L., McGeer, E.G., 1992. Lewy bodies in Parkinson's disease are recognized by antibodies to complement proteins. Acta Neuropathol. 84, 100–104.

Yamada, T., McGeer, E.G., Schelper, R.L., Wszolek, Z.K., McGeer, P.L., Pfeiffer, R.F., Rodnitzky, R.L., 1993. Histological and biochemical pathology in a family with autosomal dominant parkinsonism and dementia. Neurol. Psychiatry Brain Res. 2, 26–35.

Yasojima, K., Schwab, C., McGeer, E.G., McGeer, P.L., 1999. Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. Brain Res. 830, 226–236.

Zhang, J., Rivest, S., 2001. Anti-inflammatory effects of prostaglandin E2 in the central nervous system in response to brain injury and circulating lipopolysaccharide. J. Neurochem. 76, 855–864.

Zhang, W., Wang, T., Pei, Z., Miller, D.S., Wu, X., Block, M.L., Wilson, B., Zhang, W., Zhou, Y., Hong, J.S., Zhang, J., 2005. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J. 19, 533–542.

View publication stats