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·Review·

Nitric oxide in neurodegeneration: potential benefits of non-steroidal anti-inflammatories

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Abstract: The cellular messenger nitric oxide (NO) has been linked to neurodegenerative disorders due to the increased expression of the enzymes that catalyze its synthesis in postmortem tissues derived from sufferers of these diseases. Nitrated proteins have also been detected in these samples, revealing that NO is biologically active in regions damaged during neurodegeneration. Modulation of NO levels has been reported not only in the neurons of the central nervous system, but also in the glial cells (microglia and astroglia) activated during the neuroinflammatory response. Neuroinflammation has been found in some neurodegenerative conditions, and inhibition of these neuroinflammatory signals has been shown to delay the progress of such disorders. Thus NO and the pathways triggering its release are emerging as an important research focus in the search for strategies to prevent, halt or cure neurodegenerative diseases.

Keywords: Alzheimer's disease; neurodegenerative disease; nitric oxide; neuroinflammation; Parkinson's disease

1 Introduction and physiological actions of nitric oxide (NO)

The cellular messenger NO is a small, highly-diffusible molecule that, although it has a short half-life of a few seconds, mediates a large number of physiological processes. This highly reactive regulatory molecule acts as a messenger, a neurotransmitter or a signalling molecule and thus triggers responses in a wide range of cell types from neurons and glia of the nervous system to fibroblasts, myocytes and blood cells in the periphery. NO is a rapidly diffusing hydrophobic molecule, which allows it to move swiftly through tissues to reach its site of action[1]. Within seconds of diffusing through tissue, NO can enter red blood cells where it is rapidly destroyed by oxyhaemoglobin[2], which serves as one of the mechanisms of NO level control.

Generation of NO from *L*-arginine is regulated by cytoplasmic nitric oxide synthases (NOSs). There are three NOS isoforms, which differ in their localization and signalling properties. Inducible NOS (iNOS) rapidly produces large quantities of NO[3], while the other two NOS isoforms, neuronal NOS (nNOS) and endothelial NOS (eNOS), release sustained low levels of this molecule[4,5]. The names of the three NOS isoforms reflect the circumstances of their discovery. nNOS was first identified in the nervous system, although it is now known to be expressed also in other tissues such as fast-twitch muscle fibres[6], the mammary gland[7] and the kidney[8]. eNOS was initially identified in the endothelium but is also known to be expressed in the bladder[9], the olfactory epithelium[10], and the brain[11]. iNOS, as suggested by its name, is activated in response to a given stimulus. Thus it is constitutively expressed at low levels in both the central nervous sys-

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tem and the peripheral tissues[11]. It has been established that astroglia upregulate iNOS in response to proteins and ribonucleic acids of pathogenic origin, and that iNOS expression is triggered in these cells in response to pro-inflammatory cytokines[12]. The activity of iNOS is known to be calcium-independent whilst that of eNOS and nNOS is generally thought to be triggered by a rise in intracellular calcium[13]. However, it should be noted that eNOS can also be activated in a calcium-independent pathway following arterial sheer-stress and that this pathway requires tyrosine phosphorylation[13].

Within the nervous system, NOS-derived NO plays a plethora of physiological roles. The low levels of NO expressed following activation of eNOS or nNOS can activate soluble guanylate cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP) from guanosine-5'-triphosphate (GTP). It is well-established that NO/sGC signalling is required for long-term depression in cerebellar Purkinje cells[14], that it can enhance synaptic transmission[15], and that it may be involved in some forms of long-term potentiation[16]. To specifically consider the ion channels that underpin many of its neurophysiological functions, a previous study determined that NO uses both sGC signalling and direct S-nitrosylation of target proteins to modulate voltage-gated calcium channels in rat hippocampal neurons[17]. Furthermore, NO can bind to protein residues and S-nitrosylate them, thus directly modifying protein functions in a sGC-independent manner[18,19]. It has also been shown that NO regulates several types of potassium channels, including adenosine triphosphate (ATP)-dependent and calcium-activated potassium channels in the central nervous system[20]. Furthermore, it has been shown that NO triggers the activity of hyperpolarization-activated cyclic nucleotide-modulated cation channels which bind cGMP, and that activation of these channels is important for the mediation of NO signalling in deep cerebellar nuclei neurons[21]. Within neurons, a number of important signalling pathways can be triggered by NO/sGC, including activation of an Akt/glycogen synthase kinase 3 pathway to prevent neuronal death[22], direct targeting of the mitochondrial permeability transition pore to mediate apoptosis[23] and activation of the cGMP-stimulated phosphodiesterase which is involved in the regulation of cellular cyclic adenosine monophosphate levels by NO[24]. Thus NO can activate a number of signalling intermediaries that regulate neurophysiology, leading to a wide range of neuronal functions that can be modulated by NO (Fig. 1).

The main reservoir of iNOS in the central nervous

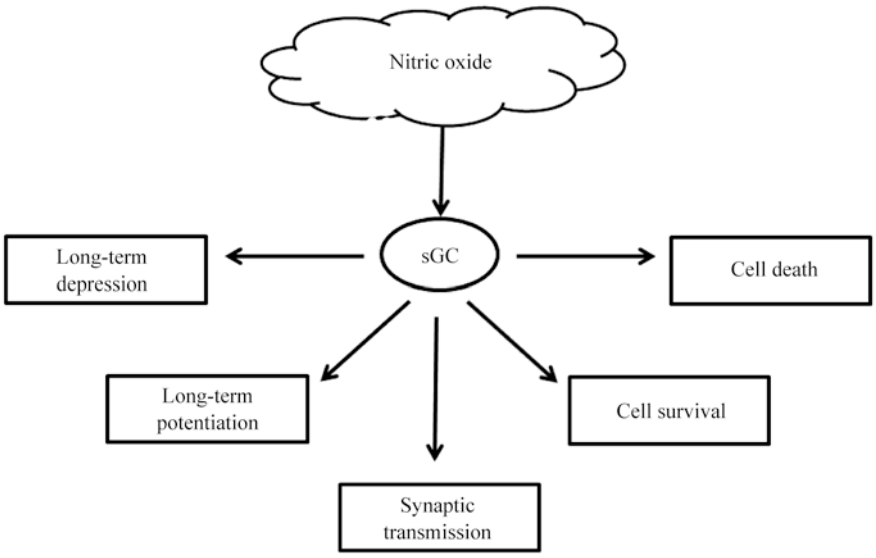

Fig. 1 Physiological roles of nitric oxide (NO) in the nervous system. Cellular NO has been linked to modulation of both neuronal survival and neuronal function. sGC: soluble guanylate cyclase.

system is in the microglia and astrocytes. These non-neuronal cells perform various functions including nutrient and biochemical support, repair processes, protection against oxidative stress, and immune defence within the nervous system. Thus exposure of microglia to the bacterial endotoxin lipopolysaccharide (LPS) induces iNOS activation and subsequent NO release via a protein kinase C (PKC)-dependent mechanism[25]. NO release, downstream of iNOS activation, is an integral part of the immune defence mechanism as exhibited by the inhibitory effects of iNOS on the spread of murine cytomegalovirus within the retina[26]. Details of some of the pro-inflammatory stimuli that induce iNOS activity will be further discussed later in this review. Importantly however, stimulation of glial cells can lead, via iNOS activation, to the generation of large quantities of NO, which can be released from the astrocytes and microglia and target the adjacent neurons. It is well-established that high concentrations of NO have deleterious effects on neurophysiology and neuroviability. Thus, evidence is accruing that the delayed neuronal damage after trauma is mediated via NO synthesized by iNOS in activated microglia[132]. As such, this delayed damage is not observed in iNOS-deficient animals[27]. In contrast, after transient focal cerebral ischemia, iNOS-mediated release of NO does not influence infarct size, revealing that the involvement of glial iNOS in neurodegeneration may be highly dependent upon the initial trigger of neurological damage[28].

It is becoming evident that the release of NO from activated astrocytes and microglia is likely to be an important source of NO in neurodegenerative disorders. NO has a number of maladaptive properties associated with such disorders. First, NO can mediate neuronal viability. Studies have demonstrated both neuroprotective[29-31] and neurotoxic[32-34] effects of NO. Furthermore, in addition to the direct effects of NO on cell survival, it has been established that within cells, NO interacts with superoxide to form peroxynitrite. As for high concentrations of NO *per se*, peroxynitrite has also been shown to be important in triggering neuronal death[35-37]. It is known that NO induces neuronal death by either necrotic or apoptotic pathways, both of which have been implicated in neurodegeneration[38]. To induce necrosis, NO depletes cell energy by damage to the mitochondria[39-42]. Necrosis can also be triggered via S-nitrosylation of GAPDH, leading to inhibition of glycolysis as well as acetylation and ubiquitination of nuclear targets[43-45]. NO-induced apoptosis can be triggered by endoplasmic reticulum (ER) stress[46] or activation of the mitochondrial pore transition, leading to cytochrome *c* release, caspase activity and thus apoptosis[47]. Generally, NO induces neuronal death when present at relatively high concentrations following iNOS activation, while lower concentrations of NO following nNOS or eNOS activity can be neuroprotective[48]. Thus, activation of a pro-survival phosphatidylinositol3-kinase/Akt pathway has been shown to be the downstream of nNOS activation[49] and similarly, increased expression of the anti-apoptotic protein Bcl-2 has been detected[50].

Nitrosative stress is the term that refers to the ability of reactive nitrogen species to damage components of the cellular environment. NO *per se* is a highly reactive molecule that can directly modify cellular targets and, as mentioned above, readily react with superoxide to form peroxynitrite. It is estimated that 15% of all superoxide produced by the mitochondria interacts with NO to form peroxynitrite[51]. Given that the glial cells within the nervous system can, upon stimulation, act as a source of NO, peroxynitrite formation downstream of NO release may be an important mediator of its maladaptive effects. It has been determined that within mitochondria, peroxynitrite competes with molecular oxygen to block the activity of the respiratory chain and also leads to the release of cytochrome *c*, both of which can cause cell death[52,53]. Moreover, peroxynitrite can be converted to other highly toxic molecules including nitrogen dioxide, a known pro-apoptotic molecule[54], with the concomitant release of carbonate and hydroxyl radicals[51].

An important mechanism whereby reactive nitrogen species may contribute to neuronal damage is through their ability to mediate excitotoxicity. This is the state wherein overstimulation of a given receptor leads to neuronal loss. Activation of the N-methyl-*D*-aspartic acid receptor

(NMDA-R) causes a rise in intracellular calcium, which, in turn, leads to excessive nNOS activity[55]. The release of NO as noted above can have deleterious effects on neurons, and it has been demonstrated that blockade of NOS activity or scavenging of NO prevents the excitotoxicity associated by overstimulation of NMDA-R by glutamate. Thus NMDA-R-associated excitotoxicity is triggered by over-activity of the receptor, leading to an excessive influx of calcium, which in turn leads to aberrant activity of nNOS and the resulting excessive production of NO. NO then acts as, or converts to, other damaging reactive nitrogen species leading to nitrosative stress.

The ability of NO to modulate both neuronal survival and physiology raises the question of whether it is an important molecule in neurodegenerative diseases. These conditions are characterised by the large-scale death of neurons in specific regions of the nervous system coupled with loss of neuronal function. They remain currently incurable despite the on-going research strategies aiming to replace lost neurons, support the survival of the remaining cells and boost their functions. The causes of neurodegeneration are multiple and complex, with a myriad of identified genetic and lifestyle factors. A vast array of pharmacological research tools is available to target NO, either enhancing or repressing its functions. Due to the role of NO in both neurodegeneration and neuroinflammation, the following discusses the potential of anti-inflammatory substances as an approach to prevent or halt progressive neural damage in two common neurodegenerative diseases, Alzheimer's disease (AD) and Parkinson's disease (PD).

2 The relationship between NO, neuroinflammation and neurodegeneration—the pathological consequences of NO activity

2.1 Evidence for the involvement of NO and neuroinflammation in AD A plethora of studies links NO to neurodegenerative diseases, and one of the most prevalent neurodegenerative conditions is AD. This disorder was first described by German doctor Alois Alzheimer in 1907, with a fuller histopathological picture published in 1911[56-58]. AD is a progressive dementia characterised by neuronal loss in brain regions associated with cognition such as the hippocampus and cerebral cortex. At the cellular level, AD is diagnosed histopathologically by the presence of intracellular neurofibrillary tangles, extracellular senile plaques, dystrophic neurites, degenerating neurons and neuroinflammation[59]. The involvement of NO in the pathogenesis of AD has been indicated by many studies. It is known that NO binds to protein residues and S-nitrosylates them. A major intraprotein target for this reaction is tyrosine. Nitrotyrosine residues within the neurofibrillary tangles are detected immunohistochemically in the postmortem brains of AD patients, while no similar staining is observed in age-matched control brains[60]. This implies that the proteins within these tangles have been modified by NO. It has been revealed that high levels of nitrosative stress facilitates protein misfolding and aggregation, both of which are linked to AD and other neurodegenerative conditions[61]. Moreover, protein disulphide isomerase, which under conditions of non-critical endoplasmic reticulum stress protects against neurotoxicity within cells, can however, be S-nitrosylated by NO, leading to a decrease in its neuroprotective function and therefore to neuronal death[62]. Protein disulphide isomerase immunoreactivity has recently been detected within the neurofibrillary tangles in postmortem AD brains[63]. Its inclusion within the tangles, where we know that proteins are S-nitrosylated due to the detection of nitrotyrosine[60], suggests that this protein may be dysregulated in AD, potentially via NO, enhancing neuronal death triggered by ER stress.

There are some potential sources of NO in AD (Fig. 2). It has long been known that amyloid beta (Aβ) peptide is overexpressed in the brains of AD sufferers. In addition, *in vitro*, elevated Aβ expression has been shown to increase NO release[64], revealing one potential source for the NO that targets the proteins in the neurofibrillary tangles of AD patients. Furthermore, in postmortem brain tissues from AD patients, Aβ plaques are strongly associated with reactive microglia. As stated above, glial cells are an important source of NO that can rapidly pass into neurons[65]. Given that microglia and monocytes are stimulated by Aβ to upregulate the expression of iNOS[66], they serve as another

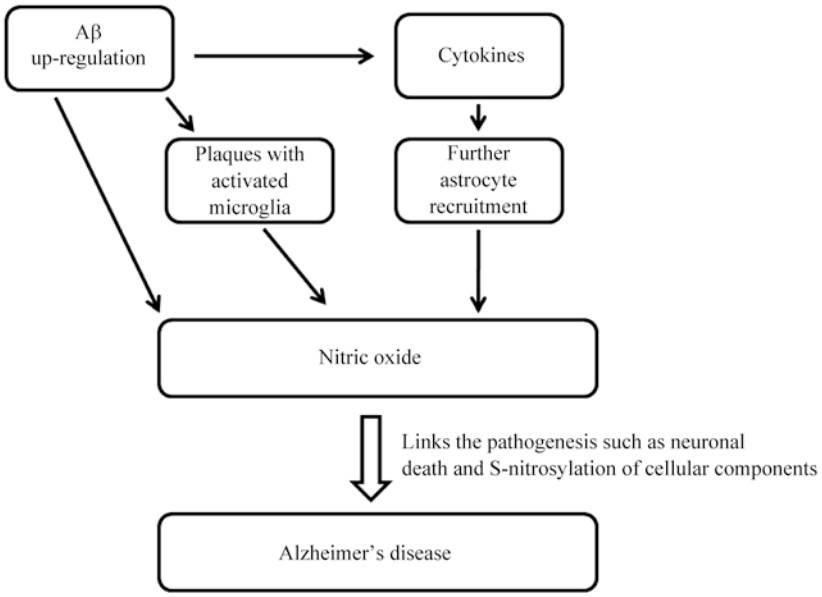

Fig. 2 Potential sources of nitric oxide downstream of Aβ up-regulation, one of the events linked to pathogenesis of Alzheimer's disease.

source of NO. From these data, it can be suggested that glial cells contribute to the pathogenesis of AD via NO release, leading to neurotoxicity. Besides, a previous study indicated that Aβ activates microglia by binding to the receptor for advanced glycation end products[67]. In addition to NO release, Aβ triggers the release of cytokines from microglia, which in turn recruit astrocytes that further enhance the localised immune response[68]. Amongst the cytokines released by activated glia is tumor necrosis factor-α (TNF-α), which is potentially neurotoxic[69]. Some drugs targeting TNF signalling have been used successfully in the periphery, such as the anti-TNF monoclonal antibody adalimumab, which has been successfully used to treat chronic inflammatory disorders such as psoriasis[70]. This raises a question of whether inhibition of TNF signalling could benefit AD sufferers. Accordingly, in a small-scale study, patients with mild-to-severe AD were administered with etanercept, an antagonist of human TNF receptor II, into the spinal fluid. After 6 months of drug administration, cognitive improvement was observed in a subset of these patients, suggesting that inhibiting TNF-α signalling can give symptomatic improvement in some AD patients[71]. However, the method of drug delivery was invasive and the beneficial results were restricted to a subset of patients, implying that the current TNF-targeting therapeutic tools may be of limited benefit to AD sufferers.

In addition to TNF-α, a number of other cytokines such as interleukin-1 (IL-1) have been shown to be up-regulated in AD[72]. One mechanism underlying IL-1 action is through increasing the synthesis of amyloid precursor protein (APP), which is cleaved to form Aβ[73]. Furthermore, postmortem analysis of AD tissue has demonstrated an elevated number of activated microglia overexpressing IL-1 compared with age-matched controls. Associated with these cells is an increase in hyperphosphorylated tau-positive neurons[74]. Furthermore, it has been shown that IL-1β increases the level of phosphorylated tau in rat cortical neurons *in vitro*[75]. Thus, it can be postulated that if pro-inflammatory cytokines such as TNF-α and IL-1 have detrimental effects on the neurons that degenerate in AD, elevated expression of anti-inflammatory cytokines, such as interleukin-10 (IL-10) may be of benefit. It has been elucidated that AD patients have a higher incidence of the -1082A polymorphism in the IL-10 gene, which is associated with a reduced production of IL-10 as compared to other genotypes, suggesting an association between a low

level of IL-10 and AD in this subset of patients[76]. Taken together, it can be concluded that activated glia contribute to AD in several ways, producing potentially neurotoxic cytokines and also releasing NO.

It is therefore not surprising that research has been carried out into the potentially beneficial effects of anti-inflammatory drugs in the prevention or treatment of neurodegeneration. Findings from *in vitro* studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) decrease both NO release from, and expression of iNOS mRNA within, a macrophage cell line[77]. In addition, it has been demonstrated that NSAIDs can exert their neuroprotective actions by direct scavenging of NO[78]. Thus, as will be discussed later, the prospect of targeting NO signalling with these drugs to interfere with AD progression may be promising.

2.2 NO and neuroinflammation in PD PD is a progressive neurological disorder that involves the loss of dopaminergic neurons from the substantia nigra with consequent dopamine depletion from the striatum leading to motor control problems. The cellular hallmarks of PD include dopaminergic neuronal death, intracytoplasmic inclusions (Lewy bodies), dystrophic neurites, mitochondrial dysfunction and neuroinflammation[79]. In many ways, the roles of both NO and neuroinflammation in PD have been far more rigorously investigated than in AD, at least in part due to the larger number of *in vitro* and *in vivo* models of PD. This has led to a number of lines of evidence linking NO to PD being uncovered (Fig. 3).

There are several methods of recreating the dopaminergic neuronal loss observed in PD in laboratory animals, including administration of neurotoxins, all of which show some relevance to aspects of the disorder but fail to fully recreate PD and therefore have certain disadvantages. Administration of 6-hydroxydopamine (6-OHDA) induces loss of dopaminergic neurons of the substantia nigra coupled with reduced dopamine release in the striatum. However, like most laboratory methods of inducing PD-like degeneration, this neuronal loss is acute, occurring over days and weeks as compared to the period of years of neuronal degeneration in PD. Furthermore, the neuronal loss is limited to the substantia nigra, the site of 6-OHDA administration, and other brain regions, such as the locus coeruleus, which is affected in PD, are spared in this

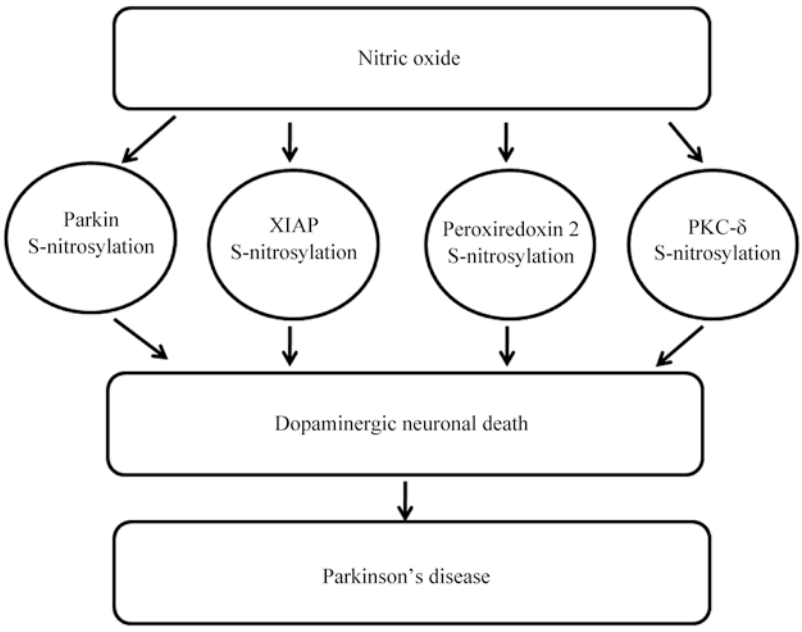

Fig. 3 Cellular targets of nitric oxide (NO) in Parkinson's disease (PD). Research data have determined a number of ways in which NO could lead to the pathological changes associated with PD. XIAP: X-linked inhibitor of apoptosis protein. PKC-δ: protein kinase C-delta.

model. Histologically, there is no evidence of the development of Lewy bodies following 6-OHDA insult, further highlighting the differences between this animal model and human PD[80]. Another frequently-used method of inducing a parkinsonian phenotype in laboratory animals is through administration of the dopaminergic neurotoxin 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP). This compound is metabolised by astrocytes to its active form, 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPP^+), which mediates its neurotoxicity to dopaminergic neurons. Following administration, there is specific loss of dopaminergic neurons of the substantia nigra coupled with dopamine depletion from the striatum. Again, however, the neuronal death is acute and therefore does not reflect the progressive nature of this process in human PD. Also, because MPP^+ acts by inhibiting complex I of the mitochondrial respiratory chain solely in dopaminergic neurons, the effects of mitochondrial dysfunction in other cells cannot be ascertained from this model[80]. In addition to these and other *in vivo* methods, midbrain dopaminergic neurons derived from embryonic rodents can be readily maintained in culture[81]. The disadvantages of such cultures include their derivation from embryos rather than from mature animals and the mixed nature of the cultures such that they contain a number of cell types and are not purely dopaminergic neurons. Whilst these cultures, in many ways, more accurately depict the situation *in vivo*, where a population of neurons does not exist as a purified cohort of a single cell type, they do not lend themselves well to biochemical analysis. To this end, some dopaminergic cell lines have been established, which, although less 'true to life' than primary neuronal cultures, are more readily manipulated in the laboratory. Thus, a large number of research models are available to study PD, and this has helped to drive research forward.

Studies on the *in vivo* models for inducing a PD-like phenotype have shown that following MPTP administration, poly(ADP-ribose) polymerase is activated, DNA is damaged and, crucially for this review, NO is released[82]. In addition, the neurodegeneration induced in laboratory rodents by MPTP is critically dependent upon iNOS activity[83], underpinning the role of NO in this model system. It has also been determined that co-treatment with 6-OHDA and an NO donor worsens the phenotype of experimental rats leading to greater neuronal loss, which highlights the vulnerability of these neurons that degenerate in PD to NO. Conversely, co-administration of 6-OHDA and an NO inhibitor lessens the damage as compared to application of 6-OHDA alone, revealing that NO release contributes, at least partially, to the neuronal death observed in the 6-OHDA model[84]. Taken together, these data imply that NO plays a role in the neurodegeneration in the nigrostriatal pathway of experimental rodents treated with either MPTP or 6-OHDA. The presence of NO downstream of both neurotoxins suggests that it is a common denominator for neurodegeneration of dopaminergic neurons and therefore a potential molecule of interest in PD.

Research has revealed direct effects of NO on key cellular components linked to PD. For instance, parkin, a component of the E3 ubiquitin ligase complex that targets proteins for degradation by polyubiquitination, protects dopaminergic neurons from damage and loss. Failure of this system leads to the build-up of faulty or superfluous proteins, which in turn have deleterious consequences for cell signalling and survival. It has been demonstrated that parkin is S-nitrosylated *in vitro* and in a murine model of PD *in vivo* as well as in the postmortem brains of PD patients, but not in age-matched control tissues. S-nitrosylation of parkin inhibits its E3 ligase activity and therefore its protective function. This enhances the vulnerability of dopaminergic neurons to cell death[85]. In addition, NO alters the solubility of parkin, leading to its intracellular aggregation, and loss of neuroprotective function[86]. In addition to parkin, S-nitrosylation of the antioxidant enzyme peroxiredoxin-2 has been linked to PD. NO-linked modification of peroxiredoxin-2 leads to loss of its antioxidant and thus neuroprotective functions, thus exacerbating oxidative stress and dopaminergic neuronal death[87]. Similarly, the pro-survival X-linked inhibitor of apoptosis protein (XIAP) is S-nitrosylated *in vitro*, in a PD model *in vivo* and in postmortem tissue derived from PD patients. This protein acts to protect cells in two ways. First, like parkin,

it has E3 ubiquitin ligase activity and thus can target proteins for degradation. Second, it prevents neuronal death via apoptosis by binding to and inhibiting caspases. NO-induced modification of XIAP does not compromise its E3 ligase activity as S-nitrosylation does to parkin, but it does compromise its anti-caspase 3 activity, which in turn promotes cell death[88]. Since modification of proteins by NO need not lead to the loss of their function but can instead enhance their activity, this again can have negative consequences for cell viability and function. It has been demonstrated that NO nitrates and activates protein kinase C-delta (PKC-δ). This in turn phosphorylates p53, leading to dopaminergic neuronal cell death[89]. Thus S-nitrosylation of key intracellular components can enhance the vulnerability of dopaminergic neurons to cell death either by activating them, as is the case with PKC-δ, or by inactivating their protective functions, as seen with parkin, XIAP and peroxiredoxin-2.

As in AD, the focus in the search for a source for NO in the PD brain has turned to the glial cells. Activated microglia have been reported in postmortem brain tissues from PD patients[90]. In addition, microglial activation has been found in some PD models *in vivo*, suggesting neuroinflammation as a common feature of both PD patients and laboratory models[91,133-135]. Moreover, enhanced iNOS expression has been detected in the microglia of MPTP model animals, and mutant mice that lack iNOS exhibit a reduced susceptibility to this agent[92]. This implies that the microglia, by upregulating iNOS, can produce a toxic mediator NO that contributes to MPTP-induced neuropathology. In addition, the activated microglia also release neurotoxic cytokines, including TNF-α. The potential toxicity of this to midbrain dopaminergic neurons has been established[69], and accordingly, mice that possess a null mutation in the TNF-α gene or in which TNF-α biosynthesis has been pharmacologically blocked exhibit less nigrostriatal degeneration following MPTP administration[93]. In addition to their neurotoxicity *per se*, pro-inflammatory cytokines can also induce iNOS activity in microglia, creating further toxic stimuli for the neurons in the region of neuroinflammation[94]. Thus activated glia can simultaneously produce NO, other ROS and cytokines, which together form the basis for the neuroinflammatory response observed in neurodegenerative conditions including PD[95].

3 Pharmacological approaches to deal with neuroinflammation—NSAIDs

The presence of an inflammatory response at the site of neurological damage in both AD and PD reveals that neuroinflammation is an integral part of the cellular processes in these disorders. Research has revealed that the activated glia release a variety of factors including neurotoxic cytokines and NO. There are numerous ways to target an inflammatory response, from targeting specific pro-inflammatory cytokines such as TNF-α[70,71,93] to initiating strategies to prevent microglial activation such as targeting with cannabinoid-derived therapeutics[96]. Given the multitude of mechanisms outlined above by which NO causes damage to neurons, compounds that target activated microglia as a potential source of NO and that also inhibit NO *per se* would have great potential in treating neuroinflammation. In this context, the dual abilities of NSAIDs as both anti-inflammatory agents and as compounds that directly scavenge NO could prove very interesting (Fig. 4).

One main target of NSAIDs is the intracellular enzymes known as cyclooxygenase (COX). COX-1 is constitutively expressed in most cell-types including microglia[97], while COX-2 is expressed mainly during inflammatory responses but is also found in discrete neuronal populations in the hippocampus and cortex[98]. Traditional NSAIDs such as aspirin, ibuprofen, and naproxen target both COX-1 and COX-2, and compounds such as celecoxib, rofecoxib and nimesulide have been developed to solely target COX-2. Particular interest in COX-2 has stemmed from the discoveries that COX-2 is upregulated in the early stages of AD[97] and that mice overexpressing COX-2 exhibit cognitive deficits coupled with neuronal apoptosis[99]. In addition to targeting the COX enzymes, some NSAIDs such as ibuprofen, indomethacin and naproxen, act as agonists of the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ)[100]. PPARγ transcription factors inhibit the expression of a wide range of pro-inflammatory

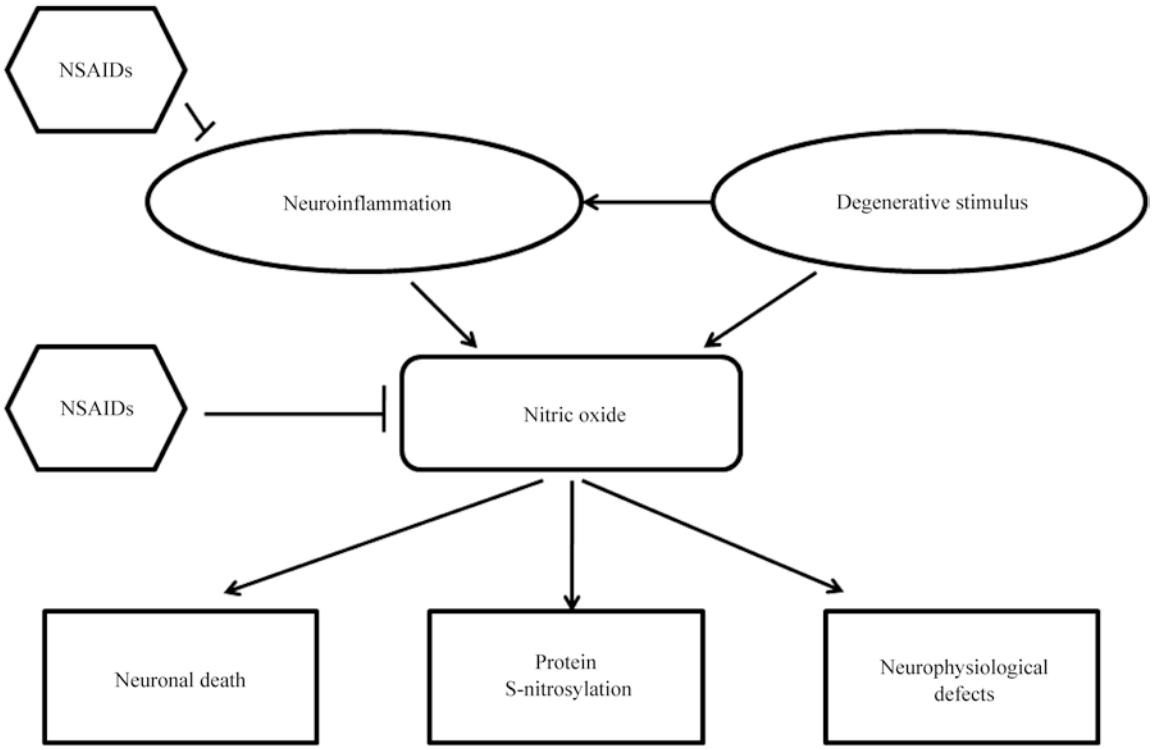

Fig. 4 Non-steroidal anti-inflammatory drugs (NSAIDs) can block the nitric oxide (NO)-mediated neurodegenerative pathway by reducing neuroinflammation and by direct scavenging of NO. Other abilities of NSAIDs that may be relevant to their apparent beneficial pharmacological effects in neurodegeneration include agonism of peroxisome proliferator-activated receptor-γ, a transcription factor that inhibits inducible NO sythase expression and downregulation of Aβ expression, for which a number of different pathways have been suggested.

genes[101]. Interestingly, compounds that activate PPARγ are also known to inhibit iNOS expression and thus NO production by glial cells[102]. Although the full mechanisms by which NSAIDs defend against neurodegeneration have yet to be elucidated, individual compounds have demonstrated properties that would be of benefit in the fight against neurodegeneration.

Aspirin decreases the phosphorylation of tau, one of the cellular pathways that lead to the formation of neurofibrillary tangles in AD[103]. Multiple pathways have also been implicated in the NSAID-induced decrease of expression of AD-related Aβ. These include the ability of NSAIDs to inhibit Rho, the small GTPase known to modulate Aβ secretion[104], inhibition of nuclear factor-kappa B leading to decreased expression of beta secretase 1, a secretase responsible for the cleavage of APP[105], and direct targeting of presenilin-1, a protein regulating APP processing[106].

Population-based studies have demonstrated positive effects of NSAIDs in the prevention or delay of AD onset. In the Rotterdam study, analysis of NSAID use with respect to the development of AD in 6 989 participants revealed that cumulative use of NSAIDs for two or more years markedly reduced the risk of AD development as compared to individuals with no uptake of anti-inflammatories[107]. The Baltimore longitudinal study reported a similar decrease in AD incidence in NSAID users[108]. In addition, early treatment with the NSAID indomethacin appeared to slow disease progression in a small number of AD patients[109]. Importantly, epidemiological studies have also revealed that not all NSAIDs modify the AD disease process, with naproxen and celecoxib having no effects on AD modification[110]. Indeed, a weak detrimental effect of naproxen has been observed in AD

patients[111]. Similarly, epidemiological data suggest that NSAIDs may have a protective effect on PD[112], but not all NSAIDs have equal efficacy in this protection, because the protective effects were observed for non-aspirin NSAIDs but not for aspirin. Indeed, a negative effect of aspirin was found although researchers could not rule out confounding factors[112].

4 Conclusion and discussion

It is clear that NO plays a role in the pathogenesis of neurodegenerative conditions AD and PD, and that activated microglia, triggered as part of the neuroinflammatory response, are a key source of NO in these conditions. Furthermore, NO has been linked to a number of other neurodegenerative diseases that are beyond the scope of this review. Thus, inhibition of all three NOS isoforms potentiates the activity of pharmacological compounds known to counteract the effects of the neurotoxin 3-nitropropionic acid (3-NP) that is used to induce neuronal damage to model Huntington's disease[113,114]. As such, reducing NO helps prevent neuronal death in these circumstances, again linking elevated NO to neurodegeneration. In models of amyotrophic lateral sclerosis, nitrosative stress increases the likelihood of protein aggregation for key proteins[115], implying that NO could again mediate neurotoxicity in another degenerative disorder. The generality of the finding that high levels of NO may, at least in part, mediate neuronal damage and loss in a number of neurodegenerative conditions implies that NO modulation would be an important target for pharmacological strategies to prevent, cure or halt the progress of neurodegeneration. There are distinctions between these three therapeutic targets. Treatment to cure would involve replacement of lost neurons, correct reestablishment of axonal and dendritic pathways and regaining function within the system that has degenerated. However, if an individual is identified to be at risk, prevention is a much less complex prospect, as is halting the disease progression. It is within the context of preventing and halting disease progression that neuroprotective strategies have the most relevance. Strategies that aim to reduce the levels of NO may be effective in preventing neuronal death. In fact, empirical evidence has shown that NO inhibition can be neuroprotective, for instance, in ameliorating neuronal loss in the 6-OHDA-lesioned rat model of PD[84]. However, in the field of neurodegenerative research, protective effects are usually seen when a proposed treatment (e.g. NO inhibition) is applied at or close to the time of neurotoxic insult (e.g. 6-OHDA administration). This is clearly not easily implemented in the clinical setting. In contrast to the rapid occurrence of large-scale degeneration in laboratory models in response to a selected and often selective neurotoxin, cell loss and degenerative changes in the human patient occur over years and decades[116]. Furthermore, a large number of neurons may already be dead by the time the patient reports his or her symptoms to a doctor. Thus it is estimated that up to 80% of the neurons of the substantia nigra have already been lost at the time of diagnosis of PD[117]. Therefore, whilst strategies that reduce NO levels could prevent further neuronal loss and thus further degenerative changes, they may not prove as effective as they do in laboratory models due to the differences in time of application relative to the point of disease progression.

It is well-established in the scientific literature that NO protects against neuronal loss in certain circumstances[29-31]. The general consensus that is emerging is that low levels of NO can be neuroprotective while high levels are neurotoxic. This implies that any modulation of NO levels in a therapeutic setting would have to be tightly regulated, such that NO levels do not drop too low. It is important that sufficient NO remains to carry out its pro-survival functions in the neurons in which this is a critical pathway for their viability. Furthermore, it should be noted that NO is a widely used messenger both within and outside the nervous system, so care would be needed to have highly-specific targeting of those cells lost in a degenerative disorder while avoiding widespread NO depletion with associated side-effects.

This makes NO *per se*, in many ways, a poor choice as a therapeutic target. However, targeting the pathways that lead to excessive release of NO may be an easier and more realistic strategy for preventing neurodegeneration

from arising or progressing. It is within this context that the finding that the non-neuronal cells of the central nervous system may act as a source of damaging levels of NO is of great interest. The severity of the neuroinflammatory response has been shown to correlate with the severity of pathological changes in the AD brain[118]. It is also well-established that anti-inflammatory agents, such as NSAIDs, reduce neuroinflammation. In so doing we presume that both through the reduction in inflammatory cells, and by direct scavenging of NO, these drugs could demonstrate useful effects in modulating NO levels. Thus some promising observations have arisen with long-term NSAIDs protecting against PD and AD. However, it is clear that not all NSAIDs act to counteract neurodegeneration. Neither naproxen nor celecoxib has beneficial effects in AD patients[110], and aspirin has no beneficial effects on PD when investigated using meta-analysis of the published data[112]. The inability of celecoxib to protect against AD may at least in part be explained by the fact that it is a COX-2 inhibitor. The precise role of COX-2 in AD is somewhat controversial. Both up-regulation and down-regulation of COX-2 have been reported in postmortem brain samples from AD patients[119,120], with a high degree of variability in COX-2 levels between samples[119]. Similarly, findings for prostaglandin E_2 (PGE_2), a major product of COX-2 activity, in AD patients are inconsistent: whilst the CSF levels of PGE_2 are found to decrease in AD patients with the increase of the severity of the disease[121], elevated levels of PGE_2 are reported in other studies[122]. Thus it remains unclear what the role, if any, of COX-2 is in AD. The finding that aspirin does not have beneficial effects against PD[112] raises another point that must be mentioned when considering the role of NSAIDs in counteracting these conditions. The complexity of modelling neurodegeneration in the laboratory was briefly discussed in the section on PD earlier in this review. Thus aspirin has been found to protect midbrain dopaminergic neurons from degeneration in response to LPS in mixed neuron-glia cultures through its ability to reduce microglial activation and a reduction in NO release[123]. Furthermore, in rats with electrical ablation of the substantia nigra, aspirin administration can reduce the rigidity that is associated with this lesion[124], and it can also protect against neurotoxicity in an MPP^+-induced rat model of PD *in vivo*[125]. The conflicting data between the research models and the epidemiological evidence may be attributed to several factors, such as species difference, differences in the concentration and the relative timing of drug administration versus the time of neuronal loss, and the different nature of neuronal loss from the acute lesions and neuronal death seen in laboratory models versus the long period of progressive degeneration that occurs in human sufferers.

Although the epidemiological data revealing that long-term NSAID use can prevent or delay the onset of AD and PD seem convincing, there are also studies questioning the validity of these findings. For instance, a postmortem study has revealed that corticosteroids (which, interestingly, also have anti-inflammatory properties) but not NSAIDs decrease the incidence of the histopathological characteristics of AD, neuritic plaques and neurofibrillary tangles[126]. Indeed, an increased neuritic plaque load has been recorded in NSAID users, especially in those with high levels of use[127]. In addition to the reasons mentioned above, one of the possible explanations for the discord between the histopathological data and observations of clinical symptoms may relate to the beneficial effects of NSAIDs on neurophysiology. Thus in spite of the presence of histopathological change, the neural network continues to function due to the NSAID-induced reductions in inflammatory markers including cytokines and, of course, high concentrations of NO.

It may also be possible to modify the current generation of NSAIDs to obtain pharmacological compounds that have multiple beneficial effects in the nervous system, thus enhancing their efficacy. It has been demonstrated that hydrogen sulphide has several positive functions within the central nervous system, including protection of hippocampal neurons from ischemic damage[128], amelioration of parkinsonian symptoms in 6-OHDA-lesioned rats[129], and prevention of LPS-induced cognitive deficits[130]. These findings suggest that hydrogen sulphide-releasing drugs *per se* could be beneficial in the fight against neurodegen-

eration. Thus hybrid molecules that have both NSAID- and hydrogen sulphide-releasing properties have been generated and have demonstrated more significant anti-inflammatory properties than NSAIDs alone[131].

Obviously, the mechanism by which NSAID use appears to at least delay the onset of AD or PD in the population-based studies remains to be elucidated, and there is no definitive evidence that the beneficial effects of NSAIDs with respect to AD and PD are linked to their ability to modulate NO levels. Nonetheless, it is intriguing that a category of drugs that modulate NO can at least delay, if not prevent, the onset of AD, at least in the subset of individuals studied. It is also an important discovery that this category of drugs can be modified, for instance, to also release hydrogen sulphide. This implies that the possibility exists to modify these molecules further, perhaps even to enhance their NO-scavenging capacity.

In conclusion, given the wide range of evidence demonstrating the occurrence of S-nitrosylated proteins in animal models and tissues from patients with degenerative diseases, it is clear that NO is involved in the pathogenesis of these disorders at some level. The beneficial effects of blocking NO production and action in laboratory models of neurodegenerative disorders imply that NO is a valid target in the search for strategies to prevent, halt or treat these disorders. However, the localisation of NO messenger systems to multiple types of cells and tissues and the requirement of low levels of NO to promote neuronal viability imply that care needs to be taken in therapeutic manipulation of this molecule. To this end, it is evident that NSAIDs should be further investigated for their potential to modify NO release and thus neurodegeneration. The beneficial effects of the drugs identified to date give hope that their potential to manipulate NO signalling pathways may be a therapeutic hope to arrest these diseases.

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一氧化氮在神经退行性疾病中的作用：非类固醇性抗炎药的治疗前景

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摘要：一氧化氮(nitric oxide, NO)是一类胞内信使。研究表明，神经退行性病人脑组织中催化合成NO的酶的表达水平显著提高，提示NO与神经退行性疾病密切相关。此外，在这些组织中还检测到硝化的蛋白，提示NO在这些组织中具有生物活性。在神经免疫应答中，神经元和胶质细胞(包括小胶质细胞和星形胶质细胞)内都发生了NO水平的改变。很多神经退行性疾病都伴随有神经炎症，抑制神经炎症的信号通路能延迟这些疾病的发展。因此，NO及其释放通路已逐渐成为神经退行性疾病研究领域的热点，对它们的理解能帮助我们找到合适的方案来预防、减缓或者治愈这些疾病。

关键词：阿尔茨海默病；神经退行性疾病；一氧化氮；神经炎症；帕金森氏病