Novel Anti-Inflammatory and Neuroprotective Agents for Parkinson’s Disease

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Abstract: Parkinson’s disease (PD) is a type of motor system disorder that results from the progressive loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) of the midbrain. It is one of the most common neurodegenerative disorders, with an incidence that is second only to Alzheimer’s disease (AD). Although replacement of dopamine can temporarily alleviate the symptoms of PD patients, it cannot prevent the progression of the disease. Increasing evidence has suggested that neuroinflammation significantly contributes to the progress of PD. Therefore, anti-inflammatory therapy could represent a promising neuroprotective intervention with the potential to delay or prevent onset of the disease. This review summarizes several novel potential agents/candidates that might open new avenues for the treatment of PD. In addition to possessing demonstrated anti-inflammatory activities that operate through different molecular mechanisms, these agents exert neuroprotective effects by enhancing the production of neurotrophic factors or interfering with the apoptosis of neurons.

Keywords: Triptolide, minocycline, glatiramer acetate, Parkinson’s disease, anti-inflammation.

INTRODUCTION

Parkinson’s disease (PD) is one of the major neurodegenerative disorders, with an incidence that is second only to AD in aged people. The clinical symptoms of PD include tremor, rigidity and bradykinesia, and the pathological hallmark of the disease is the progressive loss of dopamine (DA)-containing neurons that project from the substantia nigra (SN) to the caudate-putamen (striatum). In the past three decades, the most widely used medication for PD treatment is the DA precursor levodopa. Unfortunately, although levodopa can alleviate the symptoms of the disease, it cannot prevent the progression of the neurodegenerative process. Furthermore, its long-term utility is limited by side-effects that include motor complications. Moreover, approximately 15-20% of PD patients do not respond to levodopa. Therefore, tremendous efforts have been made to seek more effective therapeutic strategies to arrest the progression of PD. Recently, various studies that used human [1-3] or animal models [4-7] have implicated neuroinflammation in the pathogenesis of PD. Inhibition of the inflammatory reaction was found to attenuate the degeneration of nigrostriatal DAergic neurons in several PD models [8-12].

THE DUAL ROLES OF NEUROINFLAMMATION IN PD

Inflammation is somewhat of a two-edged sword in the human body. Though it represents the first line of defense against injury and infection, an excessive or sustained inflammatory response can also cause serious damage to the host’s intact cells.

In physiological conditions, the brain has a relatively low adaptive immune response due to the protection of the blood-brain barrier (BBB). As a result of their inability to divide and limited capacity to recover from injury, neurons are extremely vulnerable to auto-destructive immune and inflammatory processes [13]. In most situations, inflammation in the brain is driven by the activation of resident glial cells, especially microglia. With the exception of oligodendrocytes, both astrocytes and microglia have so far been implicated in PD. By removing cellular debris, destroying invaded pathogens and releasing neurotrophic factors, some aspects of inflammation are beneficial for combating the disease and promoting tissue repair. However, the inflammatory actions become destructive and do harm to neurons when there is a loss of proper control.

Microglia, commonly described as the surveillance cells of the CNS (Central Nervous System), are the key mediators of neuroinflammatory responses. In the normal brain, resting microglia function as local sentinels that detect any changes/threats in their environment and stand ready to support endangered neurons. Minor deviations from normal neuronal activity are sufficient to alert them. Recently, it has been established that microglia do not constitute a single, uniform cell population, but rather comprise a family of cells with diverse phenotypes. Whereas the actions of some types of microglia are beneficial, the CNS can barely tolerate the actions of certain other types, and these are therefore destructive. Whether inflammation is good or bad mostly depends on the phenotype of the triggered microglia and the mechanism by which they are regulated [14].

GLIAL REACTION IN PD PATIENTS AND EXPERIMENTAL MODELS

The presence of activated microglia has been observed in many kinds of neurodegenerative diseases, including AD, PD, multiple sclerosis (MS), AIDS dementia, trauma and stroke [15]. Under neuropathological conditions, activated microglia are thought to contribute to neuronal damage via the release of pro-inflammatory and neurotoxic factors. These factors include pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL)-1, reactive nitrogen species, proteases, reactive oxygen species (ROS), eicosanoids and excitatory amino acids [15, 16].

Substantial evidence has been presented for the existence of a state of chronic inflammation in the brain of PD patients. For example, postmortem studies have revealed microglial activation in the vicinity of dying dopamine-containing neurons in the SN from human PD patients [2] and also in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-intoxicated patients [1]. Excessive production of pro-inflammatory cytokines, such as TNF-α, IL-1β and interferon γ (IFN-γ), in the SN of PD patients has also been demonstrated [16]. In addition, inflammation has been implicated in the neurodegenerative process in animal models of PD that were created using neurotoxic compounds such as MPTP, 6-hydroxydopamine (6-OHDA), or rotenone [17]. Furthermore, microglial activation induced by pro-inflammatory LPS (lipopolysaccharide) is capable of initiating the
1. Triptolide and its Analogues

**Tripterygium wilfordii** Hook.f. (TWHF) is a woody vine that is native to Eastern and Southern China, Korea, Japan and Taiwan. TWHF has a long history in traditional Chinese medicine (TCM) for the treatment of swelling, fever, chills, sores, joint pain, and inflammation [26, 27]. Since the 1960s, TWHF has also been prescribed as an allopathic medicine for the treatment of autoimmune disease rheumatoid arthritis (RA) [28] and other inflammatory disorders, cancer, chronic nephritis, hepatitis, systemic lupus erythematosus, ankylosing spondylitis, and a variety of skin conditions [29, 30].

Triptolide is the major active component of Tripterygium extracts, which possesses potent anti-inflammatory and immunosuppressive properties [31, 32]. As a type of triterpene diolignoid that has a low molecular weight (MW 360), triptolide (Fig. 1A) has a lipophilic character that can facilitate its penetration across the BBB. Triptolide and its analogue, trichlorolide (Fig. 1B, MW 397), have been demonstrated to exert both neuroprotective and neurotrophic activities in PD models [8, 9, 33-35]. Recently, several reviews have summarized the clinical and pharmacological effects of triptolide [36-38]. Here we want to draw attention to its potency as a candidate for the treatment of PD and other neurodegenerative diseases.

1.1. Neuroprotection in PD Animal Models

Triptolide has been reported to protect DAergic neurons from neurodegeneration in the LPS- and MPTP-induced rat models of Parkinsonism [8, 33]. After a single unilateral intranigral injection of LPS (10 μg), the percentage of DAergic neurons remaining in the SNpc (substantia nigra pars compacta) ipsilateral to the injection site was 29% of that in the contralateral SNpc, and the DA levels in the ipsilateral striatum were reduced to 37% of that in the contralateral striatum. Treatment with triptolide (5 μg/kg/d for 24 days) significantly increased the percentage of DAergic neurons and the DA levels to 79% and 68%, respectively. Moreover, triptolide was found to inhibit LPS-induced microglial activation and suppress the overproduction of TNF-α and IL-1β in the LPS-injected site. Trichlorolide (an analogue of triptolide that exerts its biological activity after being spontaneously converted to triptolide [39]) was also demonstrated to possess neuroprotective effects on DAergic neurons in rats in which PD was induced by MFB (Medial Forebrain Bundle) transection [9, 34] and in mice in which PD was induced by MPTP injection [35]; anti-inflammatory mechanisms were shown to be involved.

1.2. Pharmacological Mechanisms

1.2.1. Inhibiting Microglial Activation and Release of Pro-Inflammatory Cytokines

In general, the neuroprotective mechanisms of triptolide can largely be attributed to its anti-inflammatory activities. Several studies have shown that triptolide inhibits the proliferation of T cells and the production of many pro-inflammatory cytokines, such as TNF-α, IL-1, 2, 6, 8 [40, 41]. It also has similar effects on microglia that are the immune cells of the CNS. Triptolide was demonstrated to inhibit microglial activation and to depress the release of pro-inflammatory cytokines, such as TNF-α, IL-1β [42] that were induced by LPS in primary neuron-glia mixed cultures. The effect of triptolide on different inflammatory cytokines is varied. Whereas at concentrations as low as 0.1 nM, triptolide can inhibit TNF-α production, at 0.1 μM, it can almost completely block its release. However, triptolide showed no obvious effect on the release of IL-1β at low concentrations (0.1–1 nM). Indeed, it only exhibited potent inhibitory activity for IL-1β at higher concentrations (0.1–1 μM).

1.2.2. Inhibiting Expression of Pro-Inflammatory Enzymes by Interfering with both MAPKs and NF-κB Signal Pathways

In addition to the effect on cytokine release, triptolide was found to inhibit the expression of two important proinflammatory enzymes – iNOS and COX-2. Triptolide (100 nM) caused a marked suppression of LPS-stimulated iNOS mRNA levels in primary microglial cultures, and significantly inhibited LPS-induced accumulation of nitrite (reduced by 54% and 91% compared to the control, at 10 and 100 nM, respectively) [42]. Gong et al. reported that at 10-50 nM, triptolide could inhibit the LPS-stimulated expression of COX-2 and production of PGE2 in microglia in a dose-dependent manner. It was suggested that the mechanisms underlying this suppression were associated with two parallel signaling pathways: 1. JNK→PGE2; Triptolide suppressed PGE2 expression by inhibiting the phosphorylation of c-jun NH2-terminal kinase (JNK); 2. p38 MAPK→NF-κB→COX-2→PGE2; Triptolide inhibited p38 MAPK, but not JNK or extracellular signal-regulated kinase (ERK), to reduce the transcriptional activity of NF-κB; this resulted in a down-regulation in the expression of COX-2 and PGE2 [43].
1.2.3. Inhibiting Oxidative Stress Activity, Excitotoxicity and Ca\textsuperscript{2+}

Overload

Triptolide can also exert neuroprotection through anti-oxidative stress activity and the suppression of glutamate toxicity and Ca\textsuperscript{2+} overload. Triptolide (at 0.1 or 1 nM) could protect PC12 cells from necrosis and apoptosis that was induced by glutamate; the underlying mechanisms may be involved in the inhibition of ROS formation and the decrease of mitochondrial membrane potential [44]. Pre-treatment with triptolide (0.01 nM) for 48 h significantly inhibited the apoptosis of PC12 cells and the incremental increases in intracellular Ca\textsuperscript{2+} concentration that were induced by β Amyloid [45].

1.2.4. Increasing the Production of Neurotrophic Factors

In addition, triptolide could provide neuroprotection by increasing the production of neurotrophic factors. An in situ hybridization study revealed that treatment with triprolidide (10\textsuperscript{-12} to 10\textsuperscript{-9} M) for 48 h induced significant up-regulation of brain-derived neurotrophic factor (BDNF) mRNA expression in the primary cultured mesencephalic neurons and promoted the axon growth of DAergic neurons [9]. Xue et al. [46] found that although triptolide could increase both mRNA and protein levels of nerve growth factor (NGF), it did not affect the synthesis and release of either BDNF or glial cell line-derived neurotrophic factor (GDNF) in a rat astrocyte culturing system. These data may suggest its potential application for other neurodegenerative diseases, such as AD.

1.3. Pharmacokinetics

The majority of triptolide given by oral administration is absorbed in the intestine. It is mainly distributed to organs with high blood flow, such as the liver, spleen, lung, kidney, heart and brain. It is predominantly excreted from urine and feces in the original form or as the metabolite, but it can also be excreted from bile [47]. The elimination rate is relatively slow, and \( t_{1/2b} \) (elimination half-life) is 58.6 h and 59.5 h in mouse and rat, respectively. It is worth noting that triptolide possesses nonlinear pharmacokinetics; the area under significant (AUC) increased, the clearance (CL) decreased and \( t_{1/2b} \) was prolonged in higher dosage groups [48]. Cumulative intoxication would probably occur if a high dose was administered.

1.4. Toxicology and Strategy to Reduce Toxicity

Thus far, there is insufficient data regarding the toxicology of triptolide. However, the observed side-effects of Tripterygium extracts include: (1) Hepatic injury (mildly fatty degeneration of hepatocytes), non-specific gastrointestinal injury (anorexia, vomiting, abdominal pain, diarrhea, esophageal burning), renal injury (acute renal function failure) and cardiac injury (myocardial damage); (2) Productive system injury (long-term utility may cause male infertility and amenorrhea); (3) Hematological system injury (leukopenia and thrombocytopenia, which could recover after drug withdrawal); (4) Immunological organ injury (lymphatic organ atrophy and lymphocytes necrosis); (5) Neurological injury (mainly manifested as dizziness, anergy, drowsiness, muscular soreness, limbs anesthesia, convulsion and agitation). The incidence of the adverse effects and their severity depend on the dosage [49].

Acute toxicity study showed LD\textsubscript{50} of triptolide in mice is approximately 0.86 mg/kg (i.p.) [50]. In subacute toxicity study in dogs, when the dosage of triptolide was 20 \( \mu \)g/kg/d (i.v., for 7 days), no significant changes were found in hemogram, bone marrow picture, ECG, liver function and kidney function. Meanwhile, no significant histological changes were observed in the parenchymatous organs (such as heart, kidney, liver and spleen). When the dosage increased to 40-80 \( \mu \)g/kg/d, reversible toxic reactions were found in heart, hemopoiesis, and gastrointestinal tract. Dogs in lethal dosage group (160 \( \mu \)g/kg/d) showed bone marrow depression, cardiac and hepatic injury [51].

1.4.1. Strategy to Reduce Toxicity

The clinical utility of triptolide is limited to some extent by its poor water solubility and toxicity. To solve these problems, it is important to validate both the crucial bioactive and toxic domains of triptolide [49]. Using structure-activity relationship (SAR) analysis, it has been demonstrated that the C-14 beta-hydroxyl and gamma-butyrolactone moieties of the triptolide molecule are crucial for its anti-inflammatory/anti-proliferative properties and cytotoxicity [52].

Moreover, some triptolide analogs that are less toxic and/or more effective, have already been successfully synthesized. Triprolidide, which can either be extracted from tripterygium or obtained by modifying the structure of triptolide, is one such example; it boasts improved water solubility and immunosuppressive activity, but reduced toxicity [53]. (5R)-5-hydroxytriptolide (LLDT-8) (Fig. 1C, MW 376.4), is another analog that is formed by hydroxylation of C-5 of triptolide. Compared to its parent compound, the toxicity of LLDT-8 is significantly lower; LLDT-8 has a 122-fold lower cytotoxicity in vitro and a 10-fold lower acute toxicity in vivo [50]. It was also found that whereas the immunosuppressive activities of triptolide were largely correlated with its cytotoxicity, those of LLDT-8 were not. The therapeutic efficacy of LLDT-8 has been demonstrated in the experimental model of MS — experimental autoimmune encephalomyelitis (EAE) [54] (triprolide has a similar effect [55]). Its main effects, reductions in the incidence and severity of EAE, were associated with inhibition of the MOG (myelin/oligodendrocyte glycoprotein) 35-55 lymphocyte recall response, anti-MOG 35-55 T cell responses, and the production of IL-2 and IFN-γ. Whether LLDT-8 is effective for PD is still unknown.

Another approach to reducing the toxicity of triptolide is to develop a new drug carrier for administering the drug. Liu et al. reported that the use of poly (D, L-lactic acid) nanoparticles as a drug carrier could significantly decrease the hepatic and renal toxicity of orally-administered triprolidide in Wistar rats [56]. To reduce the toxicity of triptolide, drug delivery systems with controlled release, such as solid lipid nanoparticle (SLN) and microemulsion, have also been developed [57-59].

1.5. Clinical Study

Thus far, there are no clinical data available concerning the use of triptolide to treat PD. However, in TCM tripterygium is frequently used in a herbal compound recipe for PD treatment. Bao reported in a clinical study that 86 PD patients accepted the treatment with Pingpa decoction (a compound recipe containing tripterygium). A total of 76 of 86 patients showed significant improvements, with an average effective rate of 88.3% [60]. In another study of 30 PD patients, Zhenchan decoction (another compound recipe containing tripterygium) and Madopa were combined as the treatment. Marked improvements were found in 14 patients (46.7%), improvements were observed in 12 (40%), and the treatment was ineffective in 4 patients (13.3%). The total effective rate was 86.7% [61].

Currently, the application of triptolide for PD is still at the pre-clinical stage. Its clinical application warrants further investigations. Triptolide or its related compounds are being evaluated in several clinical trials for the treatment of other diseases, including psoriasis vulgaris, diabetic nephropathy, nephritic syndrome and organ transplantation [62-65].

2. Minocycline

Minocycline belongs to the group of semisynthetic second-generation tetracyclines. Compared to the first-generation product, it has certain molecular modifications that result in a significantly
improved ability to penetrate the BBB and a longer half-time (Fig. 2) [66]. In addition to its antimicrobial actions, minocycline also possesses beneficial anti-inflammatory and anti-apoptotic properties. Clinical studies have shown minocycline and related tetracyclines to be useful in treating both rheumatoid arthritis and osteoarthritis. Minocycline’s neuroprotective and anti-neuroinflammatory activities were first reported in 1998 in a gerbil model of global brain ischemia [67]. Thereafter, minocycline was also demonstrated to be effective for the treatment of spinal cord injury [68] and the following neurodegenerative diseases: PD, AD, Huntington’s disease, amyotrophic lateral sclerosis and MS [10, 69-72].

2. Minocycline

![Fig. (2). Structures of Tetracycline and Minocycline.](image)

### 2.1. Neuroprotection in PD Animal Models

Minocycline treatment (45 mg/kg, i.p. twice a day for 8 days) significantly decreased the loss of TH (Tyrosine hydroxylase)-positive neurons in LPS-induced PD rats at 7 days post-injection. After the injection of a single dose of LPS (3 μg) into the left SN, the number of TH-positive neurons decreased to 5257 ± 1278 cells from 10,506 ± 1632 cells (right SN). Minocycline recovered the number to 8736 ± 1048 cells, which was close to control levels (10,572 ± 442 cells). Immunostaining revealed that minocycline prevented activation of microglia and decreased the area that was devoid of astrocytes around the LPS injection site in SN. Minocycline also decreased the expression of IL-1β and TNF-α mRNAs and diminished the peroxynitrite-mediated nitration of proteins and disruption of the BBB that was induced by inflammation [73].

In the MPTP-induced mouse PD model, minocycline (90, 120 mg/kg/day for 9 days, oral administration) could significantly increase the percentage of viable TH-positive neurons in the SNpc; these ranged from 37% of control (no minocycline treatment) to 56% and 77% of control, respectively (P < 0.01 and P < 0.001, respectively) [10]. Post-treatment with minocycline (120 mg/kg, 4 h after MPTP administration) could also protect DAergic neurons from MPTP toxicity. Similar neuroprotection was detected by minocycline treatment in 6-OHDA injection-induced PD mice, in parkin null mice and mutant weaver mice [74-76]. Using Western Blotting analysis, minocycline was found to block the MPTP-induced expression of iNOS and caspase 1, both in vitro (in midbrain homogenates from MPTP-induced PD mice) and in vitro (in primary cultures of mouse astrocytes and the mouse microglial cell line BV2) [10].

### 2.2. Mechanisms of Bioactivities

#### 2.2.1. Anti-Inflammatory and Anti-Apoptotic Actions

The exact neuroprotective mechanism of minocycline is not fully understood. In animal models, after focal and global ischemia, the minocycline-mediated neuroprotection was associated with a marked reduction in microglial activation, and this was accompanied by reductions in the expression of IL-1β-converting enzyme (caspase 1), COX-2, and iNOS mRNA in the affected brain regions [10, 77]. In the R6/2 transgenic mouse model of Huntington’s disease, the neuroprotective effect of minocycline was demonstrated to be associated with the inhibition of caspases 1 and 3 [70]. Minocycline has also exhibited similar anti-inflammatory and anti-apoptotic actions in various different PD models. For example, minocycline was also found to protect TH-positive neurons from microglial activation-mediated cell death in 6-OHDA induced-PD mice, parkin null mice and mutant weaver mice [74-76]. Using Western Blotting analysis, minocycline was found to block the MPTP-induced expression of iNOS and caspase 1, both in vivo (in midbrain homogenates from MPTP-induced PD mice) and in vitro (in primary cultures of mouse astrocytes and the mouse microglial cell line BV2) [10].

#### 2.2.2. Interference in p38 MAPKs Signal Pathways

So far, the direct molecular target of minocycline that is involved in signaling pathways has been reported to be p38 MAPK [78]. MAPKs are serine threonine kinases that are critically involved in both the activation of microglia and in the apoptosis of neurons. Correspondingly, minocycline was found to regulate p38 MAPKs in both microglia and neurons. In mixed spinal cord cultures that were treated with glutamate, kainite, or N-methyl-D-aspartate, minocycline was shown to reduce microglia activation through p38 MAPK-dependent mechanisms and increase the survival of neurons [79]. In cerebellar granule neurons (CGN), it could inhibit the NO-induced phosphorylation of p38 MAPK [10]. In vitro, a very low concentration of minocycline was demonstrated to be effective at blocking NO toxicity in the culturing system of both CGN and rostral mesencephalic neurons (RMN). Therefore, in addition to the indirect neuroprotective effect that comprises an inhibition of microglia, minocycline may also exert direct beneficial effects on neurons.

#### 2.3. Recent Clinical Studies

In addition to assessing its efficacy on treating the symptoms of early PD, the safety and tolerability of minocycline have been tested in a randomized, double-blind, Phase II clinical trial [80]. Patients that had received a diagnosis of PD within the previous 5 years received minocycline (200 mg per day) for 12 months. Based on the Deprenyl And Tocopherol antioxidative Therapy Of Parkinsonism (DATATOP) futility threshold, minocycline could not be rejected as futile (p = 0.66). Consequently, to determine whether it alters the long-term progression of PD, it should be considered for definitive Phase III trials. Tolerability in the minocycline group was 77%, and common adverse events included upper respiratory symptoms (26%), joint pain (19%), and nausea (17%).

### 3. Glatiramer Acetate

Glatiramer acetate (GA; formerly known as Copaxone or Copolymer 1) is an artificial copolymer of a pool of peptides (45 ~ 100 amino acids, MW 4.7 ~ 11 kDa) [81]. It is composed of random sequences of the following four amino acids in a defined molar ratio: L-alanine: L-lysine: L-glutamine: L-tyrosine = 4.2: 3.4: 1.4: 1.0. It was initially developed in the 1970s at the Weizmann Institute of Science in Israel [82]. Distinct from the two aforementioned agents, GA exerts anti-inflammatory through adaptive immunomodulation. Thus far, it has been approved by several national health authorities for the treatment of relapsing–remitting MS (RRMS) [82]. Recent preclinical data demonstrated that it was also effective in CNS injury and neurodegenerative diseases, including PD, AD and major depressive disorder [83-86].

#### 3.1. Immunomodulation of GA in MS

MS is an idiopathic disease in which the body’s immune response attacks the CNS (brain and spinal cord), resulting in demyelination. Although its exact etiology and pathogenesis are
still unclear, there is much evidence to suggest a type of T cell-triggered inflammation. In MS, peripheral T cells gain entry into the brain via the BBB, recognize myelin as foreign and attack it as if it were an invading virus. This triggers inflammatory processes and stimulates other immune cells and soluble factors such as cytokines and antibodies.

GA was originally synthesized to mimic the activity of myelin basic protein (MBP) and expected to induce EAE in laboratory animals. However, it was found to be non-encephalitogenic and even discovered to suppress MBP-induced EAE [81]. Due to the similarity of pathologic features between GA and MS, the potential of GA for the treatment of MS was investigated. In clinical trials, GA has been shown to significantly reduce the relapse rate and progression of disability in patients with RRMS; it is characterized by long-term efficacy, considerable safety, and tolerability.

The precise mechanisms underlying the effectiveness of GA on MS are not yet fully understood. Nevertheless, some important immunological properties of GA have been discovered. GA shows partial cross-reactivity with MBP, and it can bind to the relevant MHC proteins and activate suppressor T cells that are triggered by determinants common to GA and MBP. Therefore, the autoimmune response to several myelin antigens that is mediated by T-cells (which is the core pathogenetic process of MS), was inhibited. In addition, the protective role of inflammatory cells induced by GA may also involve secretion of neurotrophic factors that exert neuroprotection to the injured CNS neurons [82].

3.2. Potential for PD Treatment

Distinct from MS, the inflammation that occurs in PD is mostly related to the activation of resident microglia, rather than driven by peripheral T-cells or macrophages. Previously, this type of inflammation was considered to be only a local immune response. However, a different idea has emerged from studies that indicated that it is also mediated by the adaptive arm of the immune system. The peripheral immune cells (in particular autoimmune T cells) were found to exert a beneficial effect in protecting the injured CNS from the ongoing spread of damage [14]. They could provide the activated microglia with optimal conditions, while minimizing the risk of inducing neurodegenerative disease. In addition, T cell-mediated therapeutic immunization has been demonstrated to protect dopaminergic neurons in PD animal models [83-87], which provides support for the use of immunomodulatory strategies for Parkinson’s disease.

3.2.1. GA-Specific T Cell Accumulation in CNS

When induced in the periphery either by injection or by oral treatment, GA-specific regulatory cells have been demonstrated to cross the BBB, accumulate in the brain, and increase the expression of anti-inflammatory cytokines. This finding was manifested by their isolation from brains of actively sensitized GA-treated mice and by the localization of GA-specific cells in the brain after their passive transfer to the periphery [88-90].

3.2.2. Neuroprotection in PD Models

Benner et al. first reported that a vaccine for GA could confer neuroprotection on DAergic neurons [83]. After the adoptive transfer of copolymer-1 immune cells (5x10^7 donor splenocytes, i.v.) to MPTP recipient mice, T cells were found to accumulate within the SNpc. This effect not only mitigated the deleterious action of MPTP on DAergic nerve fibers in the striatum and on the cell bodies in the SNpc, but it also protected neuronal dopamine metabolism. The effects involved in this effect were the CD4+ T cell population, whereas CD8+ T cells showed no significant neuroprotective activities [91]. GA could induce the conversion of peripheral CD4+CD25+ to CD4+CD25+ regulatory T cells (Tregs, which are known to suppress immune activation and maintain immune homeostasis and tolerance) through the activation of transcription factor Foxp3 [92]. The adoptive transfer of CD3-activated Tregs has been shown to provide greater than 90% protection of the nigrostriatal system in MPTP-intoxicated mice. The response was dose-dependent and paralleled modulation of microglial responses and up-regulation of GDNF and TGF-β [87]. Whether a vaccine for GA is effective in LPS-induced PD or in other kinds of PD animal models still requires further investigation.

3.2.3. Increasing Expression of Anti-Inflammatory Cytokines

As an immunomodulator in case of inflammation, GA could induce a T-helper (Th1 to Th2) cytokine shift in the GA-reactive lymphocyte population [93, 94]. In untreated MS patients, as well as in healthy subjects, the majority of GA-reactive lymphocyte clones that were obtained by *in vitro* stimulation belong to the Th1 subset; these cells produce proinflammatory cytokines, such as IL-2 and IFN-γ. After a period of GA treatment as long as 1 month, the majority of GA-reactive lymphocyte clones display a Th2 phenotype and release IL-4, -5, -10, and -13, which usually exert an anti-inflammatory effect. According to these findings, GA could increase central anti-inflammatory cytokine expression and hence help decrease inflammatory processes that are implicated in PD.

In MPTP/GA mice (MPTP-intoxicated mice receiving splenocytes from GA-immunized mice), the mRNA level of CD11b (a cell surface receptor upregulated by activated microglia) is lower than in either MPTP (MPTP-intoxicated mice receiving no splenocytes) or MPTP/OVA (MPTP-intoxicated mice receiving splenocytes from ovalbumin-immunized mice) mice. CD3+ T cells were readily seen in close association with activated microglial cells [83]. Thus, T cells may affect their function by secreting anti-inflammatory cytokines.

3.2.4. Increasing Production of BDNF and GDNF

It is postulated that in PD, the brain tissue is unable to generate sufficient levels of various growth factors for sustaining the viability of dopamine-producing neurons in the presence of toxic factors. Neurotrophins, such as BDNF and GDNF, have been shown to exert trophic effects on DAergic neurons in rat models of PD [95, 96].

GA can increase the production of BDNF. The effect of GA in producing BDNF was first reported by Ziessen et al.; they discovered that GA-specific T-helper (Th) cell lines could be stimulated to produce BDNF [97]. This finding is further supported by the observation that GA-specific T cells in the CNS strongly express BDNF [98]. Furthermore, in humans, it has been demonstrated that the decreased BDNF levels in the serum and CSF of RRMS patients can be corrected by GA therapy [99]. GA could also increased mRNA expression of GDNF in astrocytes of the ventral midbrain in MPTP/GA mice [83]. From these findings, GA may increase central BDNF/GDNF production, which in turn may facilitate neuroprotection and restoration of nigrostriatal dopamine neurons in PD. However, because neurotrophins can also act as proinflammatory factors under certain circumstances (such as oxidative stress), their actions should be carefully evaluated and managed [100,101].

4. Other Candidates

Dexamethasone, an anti-inflammatory steroid, has been reported to attenuate the degeneration of dopamine-containing neurons that is induced by LPS [102]. However, whether it can also provide neuroprotection in the MPTP-induced PD model is still controversial. Although one paper reported that it was effective [103], another paper reported failure [104]. Moreover, its side-effects render its long-term clinical use almost impossible.

NSAIDs, inhibitors of COX-2, have also been suggested to block the degeneration of DAergic neurons induced by MPTP [105-107] or LPS [108]. Epidemiological studies have also suggested that NSAIDs can reduce the incidence of PD. However, to the best of our knowledge, there is no available clinical evidence to support its neuroprotective effect. In addition, not all of those effects are...
associated with the mitigation of inflammation. Therefore, its potential for PD treatment remains to be established [13].

Naloxone, an opioid peptide receptor antagonist, has been shown to protect DAergic neurons against LPS-induced inflammatory damage by inhibiting microglial activation and superoxide production. This property is separate from its antagonistic effect on opioid receptors. Dextromethorphan, a dextrorotatory morphanin that is widely used as non-opioid antitussive agent, also reduces LPS-induced degeneration of rat dopamine-containing neurons [13].

Vasoactive intestinal peptide (VIP), a neuropeptide with a potent anti-inflammatory effect, has been found to significantly decrease MPTP-induced dopaminergic neuronal loss in SNpc and nigrostriatal nerve-fiber loss in vivo [109]. Silymarin, a polyphenolic flavonoid derived from milk thistle, could protect neurons against LPS-induced neurotoxicity in mesencephalic mixed neuron-glia cultures by inhibition of microglia activation and the production of iNOS. Both of these agents emerge as potentially valuable neuroprotective agents for PD treatment [110].

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ABBREVIATIONS

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<th>Agent</th>
<th>Mechanisms of Action</th>
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<td>Triptolide</td>
<td>Inhibit microglial activation and pro-inflammatory cytokines release (TNF-α, IL-1β)</td>
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<td>Inhibit expression of pro-inflammatory enzymes (iNOS, COX-2) by interfering both MAPKs and NF-κB signal pathways</td>
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<td>Increase expression of anti-inflammatory cytokines</td>
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<tr>
<td></td>
<td>Increase the production of BDNF</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Anti-inflammatory steroid</td>
</tr>
<tr>
<td>COX-2 inhibitors</td>
<td>Inhibit COX-2</td>
</tr>
<tr>
<td>Naloxone</td>
<td>Inhibit microglial activation</td>
</tr>
<tr>
<td></td>
<td>Reduce superoxide production</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>Inhibit microglial activation</td>
</tr>
<tr>
<td>Vasoactive intestinal peptide</td>
<td>Block microglial activation</td>
</tr>
<tr>
<td></td>
<td>Suppress the expression of iNOS, TNF-α, IL-1β</td>
</tr>
<tr>
<td>Silymarin</td>
<td>Block microglial activation</td>
</tr>
<tr>
<td></td>
<td>Inhibit iNOS</td>
</tr>
<tr>
<td></td>
<td>Reduce superoxide and TNF-α production</td>
</tr>
</tbody>
</table>

Table 1. Anti-Inflammatory Agents for PD Treatment

CONCLUSION

Despite the above optimistic data, further investigations are still required to develop therapeutic strategies that concern the targeting of microglia in neurodegenerative disorders. It is also important to uncover the neuroprotective mechanisms of these anti-inflammation agents/candidates. Because most of the findings are based on experimental models, their effects must be rigorously corroborated in human studies. Their therapeutic effects, safety and tolerability must be carefully evaluated before they are administered to PD patients.
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