Anti-Inflammatory Drugs in the Treatment of Neurodegenerative Diseases: Current State

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Abstract: Increasing evidence indicates that inflammation is involved in the pathogenesis of many neurological, particularly neurodegenerative diseases. Even if inflammation is not a primary causative process, its presence may contribute to the continued loss of CNS neurons. Therefore, it seems reasonable to propose that use of anti-inflammatory drugs might diminish the cumulative effects of inflammation in the brain. Indeed, some epidemiological studies performed to date, especially in Alzheimer's disease, suggests that sustained use of anti-inflammatory drugs (AIDs) may prevent or slow down the progression of neurodegenerative diseases. However, small number of clinical trials carried out so far using AIDs, were minimal and equivocal in their outcome. Potential reasons for these mixed results include timing of AIDs administration, nonselective inhibition of cyclooxygenase (COX), inappropriate use of particular anti-inflammatory drugs for a given disease or disease progression/ severity, sub-optimal dose in target site, or limited penetration to the brain through the blood-brain barrier (BBB). Therefore, design of AIDs for the treatment of neurodegenerative diseases based upon better BBB penetration, and with minimal adverse events, would be appropriate. In addition, relevant genetic differences among patients should be considered planning new AIDs, for improved efficacy. Furthermore, due to the possible co-involvement of oxidative stress and excitotoxicity in the pathogenesis of these diseases, combination therapy with antioxidants or glutamate antagonists or a multi-potent drug might be much more effective in successfully treating neurodegenerative diseases.

Key Words: Inflammation, Microglia, Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Anti-inflammatory drugs (AIDs), Steroids, Non-steroid anti-inflammatory drugs (NSAIDs), Blood brain barrier (BBB).

I. INTRODUCTION

A. Microglia in Inflammation-Induced Neurodegeneration

Inflammation plays a key role in the pathogenesis of age-related neurological disorders and especially chronic neuro-degenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) [1]. These diseases are accompanied by a spectrum of degenerative changes, which affect all body tissues and especially the brain, since neurons are post mitotic cells and their degeneration results in irretrievable loss of function. In addition, the pathogenesis of these diseases also involves oxidative stress (OS) and excitotoxicity [for review see 2]. Inflammation in the brain is caused by either exposure to an infectious agent or as a result of neuronal injuries induced by environmental, genetic, or age-related factors. The accumulation of amyloid and extra cellular tangles in AD, or Lewy bodies in PD apparently act as irritants, causing activation of the complement system, and the initiation of reactive changes in microglia, releasing neurotoxic products, such as reactive oxygen species (ROS) and glutamate [1]. The exact origin of microglia cells of the mononuclear phagocyte lineage remains a subject of debate. However, numerous studies over the last three decades, have generally supported the view that microglia derive from mesodermal precursor cells of possible hematopoietic lineage that enter the brain during the embryonic and early postnatal phases of development [for reviews, see 3, 4]. In the mature brain and under certain physiological conditions, resting microglia cells adopt the characteristic ramified morphological appearance and serve as immune surveillance and host defense. However, microglia are particularly sensitive to changes in their microenvironment and readily become activated in response to infection or injury. Activated microglia up-regulate a variety of surface receptors, including the major histocompatibility complex (MHC), especially class II, complement receptors and other plasma membrane molecules. They also undergo dramatic morphological changes from being resting ramified cells to become activated amoeboid microglia [5]. The majority of factors produced by activated microglia are pro-inflammatory and neurotoxic. These include the cytokines' tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), free radicals such as nitric oxide (NO) and superoxide, and fatty acid metabolites such as eicosanoids, and quinolinic acid. Studies using both cell culture and animal models of degeneration, have demonstrated that excessive quantities of individual factors, such as nitric oxide, produced by activated microglia can be deleterious to neurons [6-8]. Furthermore, microglia-derived factors are often associated with the induction of neurodegeneration (Fig. **1**). For example, Chao *et al.* [9] reported that the combination of IL-1 and TNF-α, but not either cytokine alone, induced the degeneration of human fetal brain cell cultures composed of neurons and glial cells while Jeohn and colleagues [10] have shown that the combination of IL-1,

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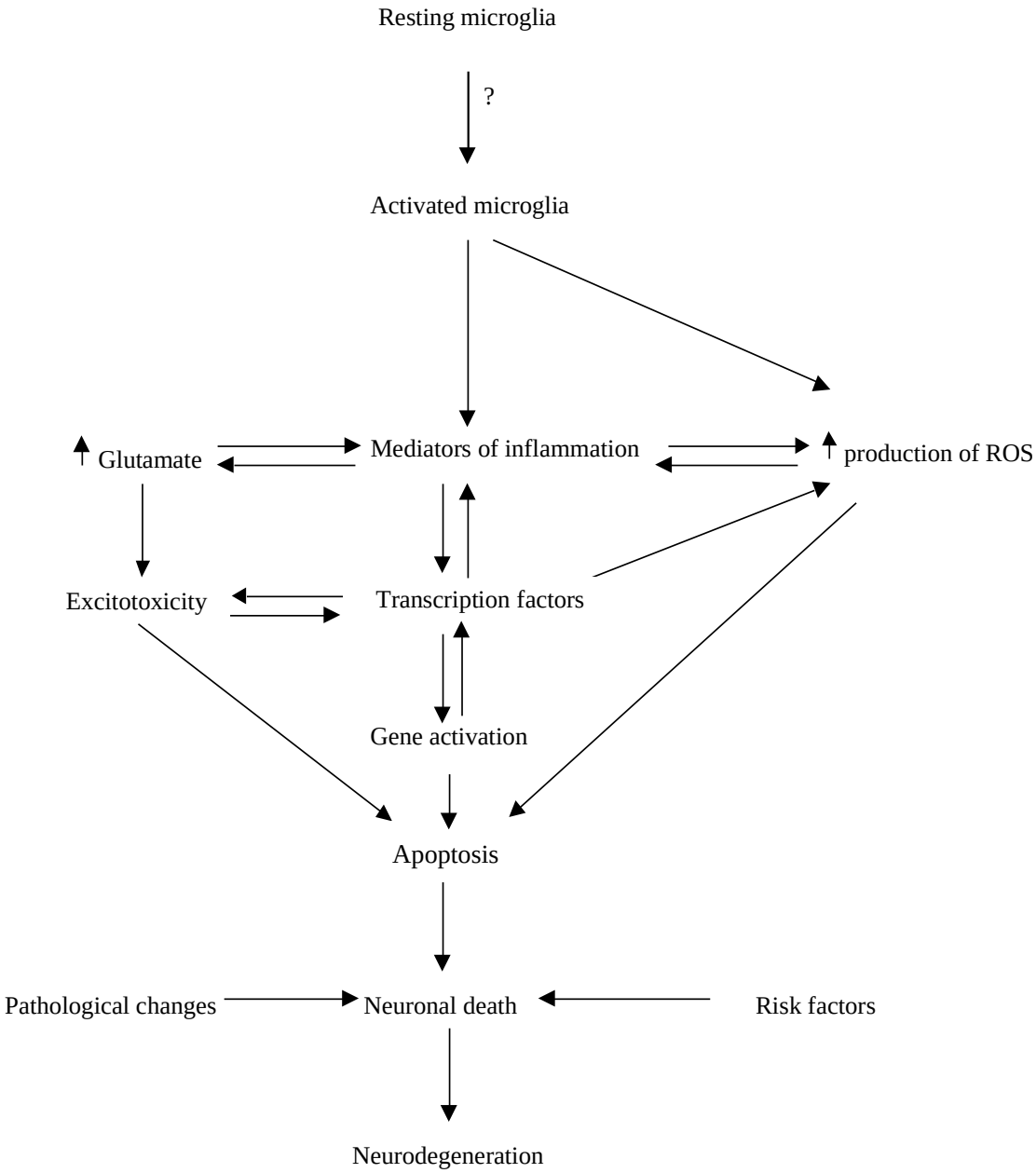

Fig. (1). Suggested mechanisms involved in inflammation-induced neurodegenerative diseases.

TNF, and interferon-γ work in synergy to induce degeneration of murine primary mixed neuron/glia cultures. Recently, Xie *et al*. [11] demonstrated that peroxynitrite, possibly a product of superoxide and NO, is a major mediator of neurotoxicity induced by lipopolysaccharide (LPS) or amyloid peptide (1-42).

B. Inflammation and Aging

Aging in mammalian species appears to be the result of normal developmental and metabolic processes responsible for physiological changes. Aging is also the most dominant risk factor for neurodegenerative diseases. Interestingly, the brain appears to have an enhanced inflammatory response with aging. For example, astrocyte activation, as evidenced by glial fibrillary acidic protein (GFAP) expression, increases with age in rodents as well as humans [12] and the number and distribution of activated macrophage/microglial cells increases with age in rat brain [13]. Other cited reasons for aging include accumulation of oxidative damage, mitochondrial mutations, loss of plasticity, and vascular changes [14, 15].

C. Inflammation and Oxidative Stress

It is well known that inflammation might raise reactive oxygen species (ROS) levels leading to oxidative stress (OS). OS is one of the major factors contributing to the

pathology of neurodegenerative diseases, and acute central nervous system (CNS) injury [for reviews see 2, 16]. One of the most abundant sources of ROS, except from the electron-transport chain of mitochondria, is the respiratory burst system of activated microglia. This system operates intermittently: when it is turned on, large quantities of ROS, especially superoxide ions ($O_2^{\cdot-}$) are generated on the microglial external membrane, from which they are released into the surroundings. Other ROS includes hydroxyl radical (OH·), hydrogen peroxide (H_2O_2), peroxynitrite (ONOO) and nitric oxide ($NO^{\cdot}$), which was shown to be produced by reactive astrocytes via cytokine-mediated induction of nitric oxide synthase [17]. All these agents can oxidize cardinal cellular macromolecules, such as DNA, lipids, and proteins, leading to cell damage [18, 2].

D. Inflammation and Excitotoxicity

Inflammation itself is insufficient to produce acute neurodegeneration. Several recent studies using LPS provide evidence that inflammation and excitotoxicity are interrelated either directly or by an indirect mechanism involving OS [19, 20]. Glutamate and related excitatory amino acids account for most of the excitatory synaptic activity in the mammalian CNS and are released by an estimated 40% of all synapses, by activating ionotropic receptors, including the N-methyl-D-aspartate (NMDA), a-amino-3-hydroxy-5-methyl-4-isoxasole-proprionic acid (AMPA) and kainic acid (KA) receptors. The calcium-mediated effect of glutamate receptor over-activation leads to excitotoxicity [2]. Bal-Price *et al*. [19] showed that glia cells, activated *in-vitro* either by LPS or interferon-γ (IFN-γ), kill neurons via NO which inhibits neuronal respiration resulting in glutamate release and subsequent excitotoxicity. An *in-vivo* study performed by Morimoto *et al.* [20] showed that LPS injection into the rat hippocampus increased microglial infiltration and exacerbated excitotoxicity in rat hippocampus, leading to neurodegeneration.

E. Inflammation in Neurodegenerative Diseases

1. The Inflammatory Process in Alzheimer's Disease

Alzheimer disease (AD) is the most common progressive neurodegenerative disorder prevalent in the elderly. It is characterized by progressive memory loss, decline in language skills, and dementia [for review see 21]. Many studies examined the association of cellular elements and inflammation mediators with AD. These studies found a lot of inflammatory changes in the AD brain, including increased concentrations of pro-inflammatory cytokines and complement proteins, together with the presence of large numbers of activated microglia [for review see 22].

A strong candidate for the induction of inflammation in AD is Aβ, a neurotoxic peptide derived from the amyloid precursor protein (APP), the principal component of senile plaque. Studies have hypothesized that Aβ is a factor in glial activation, since reactive glial cells were found close to the Aβ deposits [23, 24]. *In vitro* studies in astrocytes have convincingly demonstrated that Aβ and APP activate glia in a dose- and time-dependent manner as measured by morphological response and expression of the potent pro-inflammatory cytokines such as IL-1 and TNF-α [25-27]. Moreover, the release of proteins from activated microglia enhances Aβ-induced glial activation creating a continuous cycle of inflammatory stimuli [27].

The mechanism by which Aβ stimulates microglia is still unclear. However, it was shown that the peptide induces the activation of nuclear-factor B (NF-κB) a transcription factor implicated in the induction of numerous genes, including cytokines, acute phase proteins and immunoreceptors [26, 28, 29]. Experiments with primary rodent glial cell cultures demonstrated an Aβ-induced increase in IL-1 and IL-6 mRNAs, while pretreatment with anti-sense NF-κB oligo-nucleotides inhibited its expression [29]. It was suggested that activation of NF-κB may be through Aβ's chemokine-like activity at formyl chemotactic receptors [30], or through Aβ's binding to the receptor for advanced glycation end products (RAGE) [31, 32]. Moreover, antagonists to both these receptors have prevented Aβ-stimulated IL-1 release [31, 32]. As mentioned previously, one of the hallmarks of inflammation in AD is the presence of large numbers of activated microglia. These cells can be found within and immediately surrounding maturing amyloid plaques that contain Aβ fibrils, and are reasonable candidates for early cellular respondents in the Aβ-mediated pathogenic cascade.

As Aβ assemblies are apparently never observed during brain development, the immature nervous system will probably treat it as foreign material. Based on this assumption, microglia and astrocytes will perceive Aβ oligomers and fibrils will respond. Indeed, an initial component of the complement cascade, C1q, has been shown to bind to Aβ, *in vitro*. Moreover, it triggers one of the complement complexes, the membrane C5b-9. Microglia could also be the source of interleukin 1β and a variety of other pro-inflammatory cytokines detected in AD brain sections. In addition, astrocytes also play an important role in the pathogenic process of neurodegeneration in AD since reactive astrogliosis frequently observed in the lesioned regions of brains in AD patients [33]. Such astrocytes might be the source of a variety of pro-inflammatory and neurotoxic factors, such as NO and IL-1 [21, 33].

2. The Inflammatory Process in Parkinson's Disease

Parkinson's disease (PD) is characterized by progressive and selective destruction of the nigrostriatal dopaminergic system that is important in regulating of body movements. Apart from early onset and familial PD (5% of PD cases), the clinical symptoms of the majority, as well as of sporadic cases of PD, occur late in life and progress over decades [34]. PD may prove to be the consequence of interplay of multiple factors that include genetic predisposition, exposure to environmental toxins and the unique vulnerability of substantia nigra (SN). Microglial activation certainly assumes an important role in the pathogenic, and perhaps even the initiation stage of PD. The detection of elevated levels of pro-inflammatory cytokines and evidence of oxidative stress-mediated damage in postmortem PD brains has lent strong support to the notion of microglial involvement in the degenerative process. More compelling, epidemiological studies and case reports seem to indicate a positive correlation between early-life brain injuries and later development of PD [35]. Indeed, inflammation in the SN, and specifically HLA-DR-positive reactive microglia

activation were suggested to play a critical role in the early stage(s) of the pathogenesis of this disorder [36, 37]. Moreover, it has long been speculated that populations of people exposed to certain viruses or other infectious drugs have an increased probability of developing post-encephalitic Parkinsonism, sometimes several decades later [38]. In addition, intrauterine fetal brain inflammation following exposure to viruses or endotoxins has also suggested playing a role in the late development of PD [39]. Dopaminergic neurons in the SN are known to have a reduced antioxidant capacity, evidenced by a decreased level of the intracellular glutathione, This fact, together with the finding that the SN is particularly rich in microglia [40, 41], rendering dopaminergic cells uniquely vulnerable to a variety of insults, including oxidative stress [42]. Thus, it is logical to infer that activation of microglia, as a consequence of neuronal injury or infection, represents a risk factor that may trigger the onset of a cascade of events leading to the progressive degeneration of dopaminergic neurons.

Interestingly, as demonstrated in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced degeneration of nigral dopaminergic neurons in mice, astrogliosis is also evident, usually lagging behind the occurrence of microgliosis [43].

3. The Inflammatory Process in Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease, typically resulting in death within 5 years of onset. Most ALS cases are sporadic, but 5-10% are familial, being associated with several genes or genetic loci [44]. ALS tissue is characterized by inflammatory changes, observed in both sporadic and familial ALS and in a transgenic mouse model bearing the human superoxide dismutase 1 (SOD1) mutant. Accumulation of reactive microglia can be found in degenerative areas such as spinal cord and motor cortex [45-47]. In addition, numerous inflammation-induced biochemical changes such as prostaglandins and inflammatory cytokines, as seen in other neurodegenerative diseases, were observed in ALS [48].

II. ANTI INFLAMMATORY DRUGS IN THE TREATMENT OF NEURODEGENERATION

A. Anti-inflammatory Drugs

The main anti-inflammatory drugs (AIDs) available today include steroids, mainly glucocorticoids, and non-steroid anti-inflammatory drugs (NSAID's).

Steroids

All steroids, mainly glucocorticoids, have powerful anti-inflammatory and immunosuppressive effects. They inhibit the early manifestations of inflammation, such as initial redness, heat, pain, and swelling, and the late stages of wound healing, repair and proliferation reactions seen in chronic inflammation. Steroids, in general, bind to specific intracellular receptor generating a complex that interacts with DNA to modify gene transcription that induces the synthesis of some proteins and inhibits others. Glucocorticoids, for example, interact with a transcription factor activator protein termed "AP-1", a heterodimer of Fos and Jun proteins [49]. AP-1 inhibition decreases the activity of leukocyte, lymphocyte and mononuclear cells, and reduces the secretion of various pro-inflammatory cytokines (such as IL-2, and TNF-α). In addition, steroids inhibit the expression of cyclooxygenase-2 (COX-2), which is normally induced by inflammatory mediators and gives rise to inflammatory prostanoids. Furthermore, they block vitamin D_3 induced osteocalcin expression in osteoblasts. They are also known to modify the expression of collagenase and reduce the synthesis of lipocortin 1, an anti-inflammatory mediator protein, which inhibits phospholipase A_2 [49].

Adverse Events

The potential effect of steroids is limited due to adverse events, which mainly include the suppression of response to infection and of endogenous glucocorticoid synthesis, osteoporosis and iatrogenic Cushing's syndrome. These adverse events (AE's) are seen mainly after high dose and long-term systemic use [49].

NSAIDs

NSAIDs are group of pharmacologically related compounds with analgesic, antipyretic (usually in low to moderate doses) and anti-inflammatory properties (usually in high doses). Their shared pharmacological action is due to their inhibitory effect on arachidonic acid cyclooxygenase (COX). This inhibition causes decrease in IL-1β and possibly IL-6 synthesis via pathways that rely on vasodilator prostaglandin synthesis (PGE_2, PGI_2), which causes mainly edema [50]. The COX inhibition by NSAIDs might repress its oxidation, which associates with calcium-dependent glutamate release. In this way, NSAIDs indirectly reduce the glutamate-induced neurodegeneration [51].

There are two COX isoforms: COX-1, which is a constitutive enzyme expressed in most tissues and involved in cell-cell signaling and tissue homeostasis, and COX-2, which is highly inducible enzyme that is particularly related to inflammation, specifically in the brain since it is known to be produced in neurons, astrocytes, and endothelial cells. Recently a variant of COX-1 named COX-3 was identified, and found to be selectively inhibited by analgesic/antipyretic drugs such as acetaminophen, phenacetin, antipyrine, and dipyrone, and is potently inhibited by some NSAIDs. Thus, inhibition of COX-3 could represent a primary central mechanism by which these drugs decrease pain and possibly fever [52].

COX-1 and COX-2 enzymes are products of distinctly different genes, and their catalytic pockets that receive arachidonic acid are sufficiently different. Most conventional NSAIDs in current use are inhibitors of both isoenzymes, causing unwanted AE's, due to their inhibition of COX-1 (e.g., aspirin, ibuprofen, naproxen, diclofenac, nabumetone, sulindac, and oxaprozin). In contrast, the new compounds developed have selective action on COX-2, which are thought to be more potent. These novel drugs, including celecoxib (Celebrex®, Pharmacia) and rofecoxib (Vioxx®, Merck), are meant to target inflammatory sites, sparing noninflammed areas where COX-mediated prostaglandin synthesis may be beneficial (e.g., gastric mucosa). However, the dramatic withdrawal of rofecoxib (Vioxx), the first COX-2 selective non-steroidal anti-inflammatory drug (NSAID),

by Merck Sharp & Dohme on 30 September 2004, along with safety concerns about other cyclo-oxygenase (COX)-2 inhibitors (especially valdecoxib), raises important concerns. Significantly increased risk of acute myocardial infarction was shown in patients receiving either high daily dosage (>25 mg/day) or for a long period of time (> 18 months). As the precise mechanism responsible for this phenomenon remains unknown, the specific implications and future usage of COX-2 inhibitors is still undetermined.

Another property of NSAIDs is the activation of the proliferator-activated receptor-γ (PPAR-γ) [55-57], resulting in transcriptional regulatory actions causing the suppression of a wide range of pro-inflammatory molecules and microglial activity. Moreover, some NSAIDs show antioxidant activity [58-60], and blockade of NF-κB activation [61]. Therefore, as inflammation is involved in neurodegenerative diseases, and considering the other properties of NSAIDs, it is reasonable to suggest that COX inhibitors, especially COX-2 and maybe COX-3, are possible candidates for the treatment of neurodegenerative diseases.

Adverse Events

It is important to note that high dose and sustained use of NSAIDs is a leading cause of iatrogenic illnesses incurring significant financial burden owing to the medical intervention needed for the treatment of renal and gastro-intestinal complications [62-64]. COX-1 inhibition is generally implicated in gastrointestinal toxicity, while recent studies showed that the new COX-2 selective compounds exhibited a lower incidence of gastro-intestinal toxicity [65, 66]. However, additional studies over extended time periods are needed in order to prove this point, especially in older people.

Blood-Brain-Barrier Penetration of AIDs

The blood-brain-barrier (BBB) is a major obstacle that separates the brain microenvironment from the blood within the cerebrovascular tree to allow complex neural signaling without external interference. The endothelial cells found in BBB differ fundamentally in two ways from those in peripheral tissues. Firstly, they have very few endocytotic vesicles, limiting the amount of trans-cellular flux. Secondly, they are coupled by tight junctions or a zipper-like structure that seal the intercellular cleft and restrict par-cellular flux. Normally, the tight junctions of the BBB permit the diffusion of only very small amounts of water-soluble compounds (par-cellular aqueous pathway), while the large surface area of the lipid membranes of the endothelium offers an effective diffusive route for lipid-soluble agents [67].

Some studies of BBB membrane models have shown that steroids, due to their lipophilic properties, probably enter the brain through a passive diffusion mechanism [68]. However, the topic of NSAIDs' penetration into the brain has only been considered in three studies [69-71] asserting that NSAIDs, such as diclofenac and other NSAIDs-related drugs, have if any, an inadequate brain transfer capacity. This may be related, in part, to their high plasma binding percentages and apparently low distribution volumes. Therefore, to attain a therapeutic dose in the brain, this concern should be taken into consideration when evaluating novel NSAIDs, especially those of COX-2 and COX-3.

Pharmacogenomics of AIDs

Due to population heterogeneity, a specific genotype may be important in determining the effects of a medication for a particular population or disease. Indeed, a recent study with thirty-eight healthy participants, demonstrated that a single nucleotide polymorphism (SNP) in the COX-1 gene, markedly increased the efficacy of acetylsalicylic acid [72]. Thus, the individual's genetic profile must be validated and tailored for each AIDs-therapeutic indication.

B. IN NEURODEGENERATIVE DISEASE

1. Alzheimer's Disease

1.1. Animal Models

Studies of several different animal model systems indicate that neuro-inflammation can be attenuated by NSAIDs. For example, indomethacin partially suppressed microglial activation in rats infused i.v. with Aβ [73]. In another AD model, chronic infusion of LPS into the basal forebrain of rats was associated with robust microglial activation and expression of inflammatory mediators, degeneration of basal forebrain cholinergic neurons, and behavioral deficits in spatial memory tasks [74, 75]. In addition, the use of CI987, a combined COX-2/lipooxygenase inhibitor, attenuated microglial activation and cortical acetylcholine content loss [76]. In a different model of basal forebrain degeneration, injection of quisqualic acid into the nucleus basalis led to cholinergic degeneration, microglial activation, and increased production of IL-1 and PGE_2 [77]. Treatment with nimesulide, an NSAID that inhibits COX-2, resulted in reduced numbers of activated microglia and reduced production of IL-1 and PGE_2.

A number of reports have demonstrated glial activation and expression of inflammatory mediators in transgenic mice over-expressing mutant human APP genes [78-81]. In mice over-expressing the Swedish mutation of APP (Tg2576), 6 months' treatment with ibuprofen, starting at 10 months of age when plaques first appear, led to a decrease in IL-1 and glia fibrillary acidic protein (GFAP) levels, number of plaques, and ubiquitin-positive neurites [82]. Although these results suggest that ibuprofen, an inhibitor of both COX-1 and COX-2, reduces the inflammatory reaction to Aβ deposition, the possibility that it does so indirectly, by influencing the deposition process itself, has not been excluded.

1.2. Epidemiological Findings

There have been over 20 epidemiological studies in the last twenty-five years of anti-inflammatory therapies, particularly the use of NSAIDs, to examine their beneficial effect in AD. A critical meta-analysis, published by McGeer and its colleagues [83] grouped the previous studies. Seven retrospective case-control studies examined arthritis as a risk factor for AD [84-90]. For example, Breitner and colleagues used twins [89] and individuals genotyped for apolipoprotein E4, an established risk factor for AD [91], and concluded that the use of an NSAID could delay the onset of AD by 5-7 years. Two other case-controlled studies examining the prevalence of AD in populations with and without arthritis were inconsistent, maybe due to their small samples [92, 93].

Arthritis was also examined as a risk factor in three population-based studies. Two showed much lower occurrence of AD in subjects with arthritis, compared to the general population-based [94, 95], while the third reported a substantially higher occurrence of AD than the other studies among individuals with arthritis [96]. Three other cross-sectional studies reached the conclusion that NSAIDs' use was associated with a reduced risk of AD, but they did not include McGeer's meta-analysis, due to their different methods of analysis [97-99]. Following McGeer's meta-analysis, six major population-based studies of NSAIDs and AD have been published. Two were retrospective case-control studies that found no evidence that NSAIDs reduced the risk of AD [100, 101], while two recent studies observed a significant inverse association between NSAIDs and prevalent AD [102, 103].

As compared to many earlier investigations, which were case-controlled design, the prospective Baltimore Longitudinal Study of Aging with 1686 participants confirmed that NSAID is beneficial in preventing AD [104]. The conclusions of this study were that the standard NSAIDs such as ibuprofen had beneficial effects increasing with the duration of drug use, and that administration of aspirin was associated with a smaller, but statistically insignificant effect. However, the Rotterdam study, the first prospective analysis, failed to confirm Stewart's findings (of 74 incident AD cases and 232 age- and sex-matched population controls) [105].

Another study indicated that AIDs, such as the steroid prednisone and NSAIDs, including ibuprofen and indomethacin, are associated with a decreased risk of AD [106]. Support for the disease-modifying effect of NSAIDs derives from data showing that, in addition to reducing the formation of Aβ-42 [106], these compounds limit plaque production in mice that over-express the mutant genes causing AD and initiate widespread microglial activation [107]. However, although the anti-inflammatory action of NSAIDs is attributable to COX inhibition, blockade of Aβ-42 formation appears to be *via* a direct (i.e. COX-independent) mechanism, possibly by the inhibition of secretase [108]. A recent prospective study performed by In't Veld and his colleagues [109] reported that long-term use of NSAIDs (2 or more years of treatment) might protect against AD, but not against vascular dementia. These results were inconsistent with many previous observational studies regarding to the association between NSAIDs and AD risk [e.g., 110-113].

1.3. Clinical Trials

Evidence for inflammation-related changes in AD brain and transgenic models accompanied with the suggested benefits of NSAIDs in disease treatment and prevention, encouraged the implementation of several clinical trials of AIDs in AD.

1.3.1. Nonsteroidal Anti-Inflammatory Drugs

There are several reports on randomized double blind, placebo-controlled trials in which treatment of AD has been attempted with AID. Rogers *et al*. [114] conducted a small clinical trial showing that patients with early AD that took indometachin maintained constant mini-mental state examination (MMSE) scores over the 6-month period of the trial, while controls (taking placebo) exhibited the expected 10-12% deterioration [114]. The interpretation of this trial is problematic due to its short duration, the dropout rate and adverse events in the indomethacin group. Therefore, on the basis of this trial, indomethacin cannot be recommended for the treatment of mild to moderate AD.

Scharf *et al*. [115] assayed the effect of diclofenac, a mixed COX-1/COX-2 inhibitor, in a small placebo-controlled trial. In this study, diclofenac was administered in combination with misoprostol, a prostaglandin E_2 analog that confers some protection to the gastric mucosa. Despite this strategy, 12 of 27 AD patients dropped out of the study while only two of 17 in the placebo group withdrew. Ultimately, only 12 patients completed the full 25 weeks of therapy. A modest beneficial trend was observed in several outcome measures denoting reduced deterioration in the diclofenac group, but results were not statistically significant, probably due to the small sample.

A large multi-center trial of the COX-2 selective inhibitor, celecoxib versus placebo was completed in 1999. This study enrolled over 400 AD patients at sites in Europe and the United States. Duration of treatment was 6 months and the primary outcomes were measures of cognitive capacity. Despite the large number of AD patients, no difference in the rate of cognitive decline was found between the celecoxib-treated and placebo groups [116].

It will be interesting to see the results of the Alzheimer's Diseases Collaborative Studies, a multi-center, double blind, placebo control trial scheduled to be completed in 2003. This study examines whether the COX-2 selective NSAID, rofecoxib, or naproxen, a nonselective NSAID, will slow the rate of cognitive decline in AD. The sample group consists of 320 patients with mild to moderate AD who will be treated for 1 year. It is hoped that this study will provide an answer to the question of whether NSAIDs are effective in slowing the progression of AD.

1.3.2. Steroids

Results of low-dose prednisone for the treatment of Alzheimer's disease have been published [117]. Despite known problems with long-term therapy, this trial was undertaken because of the potential benefit afforded by the broad anti-inflammatory and immunosuppressive actions of glucocorticoids. The treatment regimen consisted of 20 mg prednisone QD for 4 weeks, 10 mg prednisone for 1 year, followed by a slow tapering dose over 16 additional weeks. Of 138 randomly sampled patients, 50 subjects in the prednisone and 58 in the control group completed the course of treatment. No difference in cognitive decline was found between the two groups. However, the prednisone group had greater increases in Blessed Dementia Rating Scale subscores reflecting agitation and hostility/suspicion [117].

In summary, the majority of studies focused on the use of NSAIDs and only a minority on the use of glucocorticoids and related anti-inflammatory therapies. This is due, in part, to the widespread use of such compounds in the general population. In addition, although subclasses of NSAIDs may have divergent levels of efficacy in AD treatment, it is worthwhile to note that epidemiological evidence is of patients prior to clinical manifestations of AD. In contrast, therapeutic studies are conducted on patients with illnesses

severe enough to exceed the clinical detection threshold, presenting a significantly different pathophysiological environment than early onset patients.

A major challenge in designing AIDs trials for AD is the selection of a patient population with some degree of risk for the disease such as those with mild cognitive impairment (MCI), which is a transitional stage between normal aging and dementia, characterized by memory deficit without clinically meaningful functional impairments. MCI is associated with up to 50% probability of progressing to symptomatic AD within a 4-year period. Thus, the rate of progression from MCI to AD is approximately 12% per year, supporting the concept that MCI partially represents the preceding stage of AD [118, 119]. A trial of rofecoxib for prevention of AD is currently in progress and approaches this issue by selecting subjects with mild MCI. The outcome will be people progressing to a clinical diagnosis of AD.

2. Parkinson's Disease

2.1. Animal Models

There are no epidemiological or clinical evidence existing with regard to NSAIDs use in PD. However, AIDs have been shown to be beneficial in animal models of Parkinson's disease. Matsuura and his colleagues [120, 121] showed that cyclosporin A, but not glucocorticoid treatment, attenuates degeneration of dopaminergic neurons induced by 6-hydroxydopamine in the mouse brain.

It is worth noting that in the MPTP-induced animal model, inflammatory reactions are reported to contribute to neuronal damage [122]. Supporting these findings, Kitamura *et al*. [123] showed that pretreatment with FK-506, a novel immunosuppressant, and cyclosporin A, significantly protected MPTP-induced DA depletion in the striatum of mice. In addition, thalidomide [124], aspirin, and salicylate [125] were shown to protect dopaminergic neurons from MPTP toxicity in mice. However, in the study of Aubin and his colleagues, other COX activity inhibitors such as paracetamol, indomethacin, diclofenac or the COX expression inhibitor, dexamethasone, were found to be ineffective in protecting neurons against MPTP neurotoxicity, suggesting that the effects were due to actions other than COX inhibition [125]. In addition, the non-selective high dose aspirin, as well as the COX-2 inhibitor meloxicam, have been reported to confer neuroprotection in MPTP-induced dopamine (DA) depletion in mice [126, 127].

Two studies showed that Minocycline, a second-generation tetracycline that effectively crosses the BBB [128] inhibited microglial activation and prevented dopaminergic neurodegeneration in the MPTP model of PD [129, 130].

Recently it has been demonstrated that sodium salicylate (50/100 mg/kg, i.p.), but not diclofenac or celecoxib, protected against MPTP-induced neurotoxicity in rats [131]. The study's data indicate the absence of prostaglandin involvement in MPP^+ action, and that the neuroprotective ability of sodium salicylate is rendered by inactivation of $OH\cdot$ and is independent of prostaglandin mediation.

Interestingly, it has been found that the steroid dexamethasone decreases the production of pro-inflammatory cytokines and protects nigral dopaminergic neurons against LPS-induced degeneration in cell cultures and animal models [132, 133].

Offen *et al*. [134] have shown that vasoactive intestinal peptide (VIP), a neuropeptide with anti-inflammatory effects, was efficacious against PD's relevance neurotoxins in neuronal cultures. In addition, VIP was found to protect the MPTP mouse model for PD [135]. VIP treatment significantly decreases MPTP-induced dopaminergic neuronal loss in SN and nigrostriatal nerve-fiber loss. VIP prevents MPTP-induced activation of microglia in SN and striatum and the expression of the cytotoxic mediators, iNOS, IL-1β, and TNF-α [135]. The authors concluded that VIP might be a valuable neuroprotective agent treating pathologic conditions of the CNS, such as PD, where inflammation-induced neurodegeneration occurs.

3. Amyotrophic Lateral Sclerosis

3.1. Animal Models

Although there are no clinical studies regarding AIDs for ALS treatment, there are some showings that AIDs might be effective in elevating motor performance and/or survival of mice bearing the human SOD mutation. Two early studies showed no efficacy of steroids in the mouse model [136, 137]. However, encouraging preliminary results have been obtained in the G93A transgenic mouse model with the COX-2 inhibitor SC236, in which a 20% prolongation of survival was reported [138]. A very mild improvement in this same mouse model was described with lysine acetylsalicylic acid, a soluble form of aspirin, in which the COX-1 inhibitor was delayed from appearing at 12-14 weeks of age, although survival was not prolonged [139].

Other investigators have observed increased COX-2 expression and PGE_2 levels in ALS patients as well as in a mouse model of the disease [140, 141]. Studies in a spinal cord organotypic model of ALS suggest that COX-2 inhibition protects motor neurons [142]. Whether such findings results from direct neuroprotection versus anti-inflammatory roles of the COX-2 inhibiting drugs, remains to be determined.

Two other recent studies showed that Minocycline, with or without creatine, inhibited cytochrome C release from the mitochondria and delayed progression of ALS in mice [143, 144]. The results of AIDs in animal models of ALS should be confirmed in clinical studies with large samples.

III. CONCLUSIONS AND FUTURE APPROACHES

There is growing evidence that inflammation is involved in the pathogenesis of many neurological, particularly neurodegenerative, diseases. Therefore, it seems reasonable to propose that use of anti-inflammatory drugs might diminish the cumulative damaging effects of inflammation in the brain. However, in practice and so far, clinical trials using anti-inflammatory drugs are equivocal in their outcome. The reasons for these mixed results might include timing or AIDs administration, nonselective inhibition of COX, inappropriate use of particular anti-inflammatory drugs for a given disease or disease progression/severity, sub-optimal dose in target site, or limited penetration to the brain through the

BBB. Consequently the design of new anti-inflammatory drugs for treating neurodegenerative diseases, based upon better BBB transport, and improved safety profile might result in effective therapy. In addition, the design of new AID should take population genetics' heterogeneity and pathology severity into consideration. More research is needed to understand the mechanism and the chemistry of AIDs delivery into the brain. Furthermore, combination of AID therapy with other potentially effective drugs, such as antioxidants or glutamate antagonists, might offer more effective therapies for patients with neurodegenerative diseases.

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