Substance P and Antagonists of the Neurokinin-1 Receptor in Neuroinflammation Associated with Infectious and Neurodegenerative Diseases of the Central Nervous System

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ABSTRACT
This review addresses the role that substance P (SP) and its preferred receptor neurokinin-1 (NK1R) play in neuroinflammation associated with select bacterial, viral, parasitic, and neurodegenerative diseases of the central nervous system. The SP/NK1R complex is a key player in the interaction between the immune and nervous systems. A common effect of this interaction is inflammation. For this reason and because of the predominance in the human brain of the NK1R, its antagonists are attractive potential therapeutic agents. Preventing the deleterious effects of SP through the use of NK1R antagonists has been shown to be a promising therapeutic strategy, as these antagonists are selective, potent, and safe. Here we evaluate their utility in the treatment of different neuroinfectious and neuroinflammatory diseases, as a novel approach to clinical management of CNS inflammation.
Introduction

The SP/NK1R system plays an important role in neuroinflammation. SP is encoded by the TAC1 gene and belongs to a large family of structurally related peptides, the tachykinins. These are small peptides broadly distributed in the central (CNS) and peripheral nervous systems (PNS). The major mammalian tachykinin peptides include SP, neurokinin A (NKA), and neurokinin B (NKB), together with NH₂-terminally extended forms of NKA, neuropeptide K (NPK), and neuropeptide γ (NKγ). The primary structures of SP, NKA and NKB are very similar in all mammalian species.

SP has a high affinity for the NK1 receptor, to which it binds preferentially. It also binds to other tachykinin receptors, NK2R and NK3R, with lower affinity. SP and its cognate receptors are present in neurons, as well as in microglia, endothelial cells, and peripheral immune cells. Due in part to its diverse anatomical distribution, the SP/NK1R system is found to be involved in a variety of complex physiological responses including pain, emotion, neuroinflammation, and microvascular permeability. Preventing the actions of SP through the use of NK1 receptor antagonists is emerging as a promising therapeutic approach for treatment of neuroinflammatory conditions. Thus, we reviewed preclinical and clinical studies on the effectiveness of NK1R antagonist treatment of CNS inflammation due to bacterial, viral, parasitic or neurodegenerative diseases.

Structure and Processing of SP and the NK1R

SP, an undecapeptide, is derived from the preprotachykinin-A gene, which is differentially spliced to form different mRNAs. It is synthesized, mostly by neurons, as a large protein, and is transported to the neuronal terminal endings, where it is enzymatically converted into the active form and stored in vesicles ready for release. Its preferred endogenous receptor, NK1R, is a G protein-coupled receptor with seven transmembrane domains and diverse downstream pathways, which vary depending upon the cell type where the receptor is located. There are two NK1R isoforms: a truncated isoform (311 aa) that lacks most of the intracellular C-tail and a full-length form (407 aa). The full-length form is expressed in several brain regions, with the exception of the cerebellum. The truncated form is predominately found in peripheral tissues, elicits a diminished calcium response and has a 10-fold lower binding affinity to SP than the full-length form. Upon SP stimulation, NK1R is internalized by the clathrin-dependent mechanism to the acidified endosomes, where the complex disassociates. The fate of the internalized receptor depends on the stimulation conditions. Thus, low concentrations of SP lead to its degradation and recycling of NK1R to the cell surface. Conversely, after sustained incubation with high SP concentrations, as may occur during inflammation, NK1R is ubiquitinated and degraded.
the presence and absence of an NK1R antagonist indicated that activation of the NK1R by SP may contribute to the pathophysiology of Lyme neuroborreliosis\textsuperscript{23}. Also, SP has been shown to enhance inflammatory glial responses to \textit{Streptococcus pneumonia}, the major Gram-positive causative agent of bacterial meningitis\textsuperscript{22}.

SP has also been studied in the context of neuroparasitic infections. It has been linked to the induction of seizures in a rodent model of neurocysticercosis (NCC), caused by the helminth \textit{Taenia crassiceps}\textsuperscript{24}. SP was also implicated in the development of post-treatment inflammatory encephalopathy (PTRE) in a murine model of \textit{Trypanosoma brucei} infection. This model shows remarkable similarities to the pathology seen in the CNS of individuals with African trypanosomiasis, also known as sleeping sickness\textsuperscript{25,26}.

The effect of SP on viruses that cause neurocognitive impairment, such as human immunodeficiency virus (HIV) has also been investigated. \textit{An in vitro} study using human fetal brain cell cultures expressing full-length NK1R showed that SP enhances HIV-1 infection\textsuperscript{27}. Likewise, \textit{in vivo} studies demonstrated that SP drives macrophage polarization and inflammation in HIV patients with neurocognitive impairment\textsuperscript{28-30}. Together, these studies confirm that higher levels of SP are associated with exacerbation of the neuroinflammatory process.

Traditionally defined neuroinflammatory diseases (such as multiple sclerosis [MS] or encephalitides) are often distinguished from neurodegenerative diseases, such as Alzheimer’s disease (AD) or Parkinson’s disease (PD), based on what kind of inflammation they evoke. Thus, tissue invasion of blood-derived leukocytes of the adaptive immune system (T and B lymphocytes) is prominent in MS and widely accepted to be disease-promoting\textsuperscript{31}. In contrast, the pathogeneses of PD and AD are thought to be naturally occurring processes that involve innate components such as microglia, astrocytes, the complement system, and cytokines\textsuperscript{32}. The immunomodulatory properties of SP and its role in autoimmune neuroinflammation have been investigated in more detail using the experimental autoimmune encephalomyelitis (EAE) model, as it resembles MS\textsuperscript{33,34}. This EAE model features inflammation of complex pathogenesis, demyelination, which is the hallmark of MS, axonal loss or damage, and gliosis\textsuperscript{34}. Studies confirmed that SP-mediated signaling contributes to the maintenance of inflammation in the CNS during the chronic phase of EAE\textsuperscript{35} and likely stimulates Th1 and Th17 autoreactive cells. These migrate to the CNS, enhance BBB crossing, and perpetuate inflammation\textsuperscript{19}. Additionally, genome-wide linkage studies provided data to suggest that the SP precursor protein-encoding TAC1 gene is a possible susceptibility gene for MS\textsuperscript{36,37}.

PD is the most common motor neurodegenerative disorder affecting approximately 4 million people worldwide. It is characterized by loss of dopaminergic neurons in the SN, an integral part of the basal ganglia\textsuperscript{38,39}. High levels of SP are present in the SN, where it binds to NK1Rs expressed on dopaminergic neurons. Subsequent internalization of the SP/NK1R complex activates a cascade of events that lead to the release of dopamine into the striatum\textsuperscript{40}. SP and dopamine regulation work in a positive feedback mechanism as dopamine can potentiate the release of SP\textsuperscript{41}. Therefore, SP decline is seen in post-mortem PD brains and in models that replicate the late stages of the disease\textsuperscript{42-44}, as a secondary effect of dopaminergic degeneration. The functional role of SP in the regulation of neuroinflammation and dopaminergic neuron survival remains elusive. One study showed that SP helps to restore dopamine deficit in the brain in an animal model of PD\textsuperscript{45}. Others have shown that exacerbation of SP levels accelerates PD progression\textsuperscript{13,39}. These discrepancies might be the result of different study designs, where the suggested beneficial role of SP is based on a short-term analysis of early disease stage that only contemplated dopamine deficit restoration by SP. The downstream effects of early SP exacerbation in PD include nigral BBB breakdown through neurogenic inflammation, as well as local inflammatory response, and are therefore detrimental. A noteworthy recent finding showed that the most effective drug in the treatment of PD, levodopa (L-3,4-dihydroxyphenylalanine or L-DOPA), increases nigral SP levels\textsuperscript{46}.

Current findings on the neuroinflammatory process in AD have suggested a neuroprotective role of SP. This contrasts with the detrimental effect observed in MS. AD is the most common neurodegenerative disorder. It is characterized by the formation of neurofibrillary tangles in the cortex, which are mainly constituted by altered phosphorylated and truncated portions of tau protein and by abnormal extracellular deposition of neurotoxic beta amyloid (Aβ) peptides\textsuperscript{46}. Reduced SP levels were found in cortical regions of post-mortem brain tissues of patients suffering from AD\textsuperscript{47,48} but conflicting results were obtained regarding SP levels in the CSF, where they were elevated or reduced depending on the study\textsuperscript{49-51}. Transgenic mouse models of AD based on the known genetic origins of familial AD have significantly contributed to the understanding of the molecular mechanisms involved in the onset and progression of the disease\textsuperscript{52}. Studies using animal models showed that administration of SP provides protection from the cognitive impairment induced by Aβ infusion\textsuperscript{53}, prevents Aβ induced neuronal loss\textsuperscript{54} and improves memory functions\textsuperscript{55}. Despite the observed benefits, it is unlikely that administration of SP will be used as a treatment of AD as it induces multiple effects including risk of emesis and nausea.

**NK1R Antagonists in Neuroinflammation**

Specific NK1R antagonists block binding of SP to its receptor. Several NK1R antagonists have been investigated and show promise for neuroinfectious and neuroinflammatory disease treatment. Different compounds have been developed by various
pharmaceutical companies but the only NK1R antagonist that is currently approved for clinical use is aprepitant (Emend)\textsuperscript{56}. Fosaprepitant (Emend for injection), a water-soluble phosphoryl prodrug for intravenous use, is also available and is marketed as Ivmend\textsuperscript{57}. These drugs are well tolerated and used as antiemetics to combat chemotherapy-induced nausea in cancer patients\textsuperscript{11}. Investigators have focused on the use of NK1R antagonists as an alternative to classical anti-inflammatory drugs. The antagonists have the advantage of targeting chronic inflammation without impairing acute immune responses. In addition, nonpeptide NK1R antagonists can cross the BBB and exert CNS effects\textsuperscript{15}.

**NK1R Antagonists and CNS-Invasive Bacteria**

Studies have shown that systemic administration of the NK1R antagonist L703,606 oxalate significantly inhibits \textit{N. meningitidis} and \textit{B. burgdorferi} induced CNS gliosis, demyelination, and associated inflammatory cytokine elevations in a murine model, while attenuating concomitant decreases in IL-10 levels\textsuperscript{58}. The therapeutic potential of L703,606 oxalate was also tested in a murine model of pneumococcal meningitis\textsuperscript{59}. Results showed that pharmacological targeting of the NK1R prevented the development of damaging inflammation when administered prophylactically, and limited neuroinflammation associated with \textit{S. pneumonia} infection. In addition, it was demonstrated that this therapeutic intervention was capable of reversing infection-associated gliosis and demyelination\textsuperscript{22}. In line with these findings, primary explant cultures from nonhuman primate brain and dorsal root ganglia infected with \textit{B. burgdorferi} and treated with L703,606 oxalate showed significantly decreased levels of proinflammatory cytokines (IL-6, CXCL8, CCL2), as well as decreased oligodendrocyte and neuronal apoptosis, compared to untreated explant cultures\textsuperscript{23}. CCL2/CCR2 and MAPK, chiefly MEK/ERK, signaling play a major role in neuroinflammation\textsuperscript{59,60} and SP is known to selectively enhance inflammatory chemokine production via ERK/p38 MAPK-mediated NF-kB activation\textsuperscript{23,61}. Hence, this result is consistent with the possibility that blockage of NK1R may act by limiting these signaling cascades and extends prior work in murine models, which showed that SP/NK1R interactions are required \textit{in vivo} for inflammation after challenge with bacteria.

**NK1R Antagonists and CNS-Invasive Parasites**

As mentioned, SP is present in the brain of NCC patients and co-localizes with areas of inflammation adjacent to degenerating worms\textsuperscript{24}. Studies using \textit{T. crassiceps} infected mice showed that, similar to \textit{T. solium} infection in humans, cysts cause little or no inflammation, while dead or dying parasites initiate a granulomatous reaction. SP was shown to be required to generate epileptogenic granuloma extracts, and the NK1 receptor was required to respond to these extracts with seizure activity\textsuperscript{62}. This notion led to the investigation of NK1R antagonists as potential treatment to prevent seizures. Therefore, in a mouse model of NCC, pretreatment with aprepitant resulted in complete abrogation of seizure activity. These findings suggest that NK1R antagonists may be used to prevent and/or treat seizures in NCC in humans and, perhaps, function as an adjuvant or replacement for the treatment of these seizures with antiepileptic drugs\textsuperscript{24}.

A study of a mouse model of PTRE demonstrated that SP plays an important role in this phenomenon\textsuperscript{26}. This model reproduces the effects of human African trypanosomiasis in the CNS at a late stage of the disease that is characterized by fatal encephalopathy. The experimental design consisted in infecting mice with the protozoan parasite \textit{Trypanosoma brucei} and treating them subcutaneously with the trypanocidal drug diminazene aceturate, to induce an acute inflammatory meningoencephalitis. Treatment with RP67,580, a NK1R antagonist, showed reduction in the severity of the inflammatory response and the degree of astrocyte activation in the brain\textsuperscript{27}. These findings pointed to the development of novel anti-inflammatory therapies in human African trypanosomiasis by modulating NK1R-mediated responses.

**NK1R Antagonists and CNS-Invasive Viruses**

Patients with HIV infection, even if successfully treated, may have a high frequency of neurocognitive impairment that is associated with residual chronic inflammation. Studies have shown elevated levels of SP in the sera of HIV patients\textsuperscript{3} and simian immune deficiency (SIV)-infected rhesus macaques\textsuperscript{63}. SP facilitates HIV replication, and reciprocally, NK1R antagonists block HIV in part through the co-receptor CCR5, as well as through blocking SP-driven macrophage polarization and inflammation\textsuperscript{29,30}. Initial \textit{in vitro} studies using the NK1R antagonists CP-96,345 and aprepitant led to further investigation of NK1R antagonists as potential HIV therapeutic and immunomodulatory agents\textsuperscript{54,64}. A phase 1B randomized, placebo controlled, double-masked study to evaluate the safety, antiviral activity, pharmacokinetics, and immune-modulatory effects of aprepitant in HIV-infected adults revealed that aprepitant was safe but did not show significant antiviral or immunologic improvement at the doses used (125 or 250 mg for 14 days and follow-up study for 42 days)\textsuperscript{30}. A subsequent clinical trial increased the aprepitant dose to 375 mg per day in order to determine its \textit{in vivo} safety, antiviral activity and the effect on inflammatory markers\textsuperscript{28} in HIV patients not receiving antiretroviral therapy. Despite the higher dose used, aprepitant did not show a significant antiviral activity but the investigators believe that this dose is still too low based on the target concentrations required to elicit an antiviral effect in \textit{in vitro} and preclinical experiments. Aprepitant treatment was, however, associated with decreased programmed death-1 (PD-1) expression on CD4+ T cells, and decreased plasma levels of SP and soluble CD163 (an indicator of monocyte activation) [28], which
are markers associated with poor disease prognosis. In addition, when the results of both studies were combined (125–250–375 mg) a significant reduction in plasma levels of several pro-inflammatory cytokines including IL-6 and TNFa was observed. These results are encouraging and suggest that blockade of the NK1R pathway has a role in modulating monocyte activation in HIV infection. Hence, prospective studies to evaluate the immunomodulatory and anti-inflammatory effects of aprepitant with longer treatment (4 weeks) and co-administration with ritonavir to boost aprepitant plasma concentrations in virollogically suppressed patients on combination antiretroviral therapy are ongoing (Clinical Trials.gov # NCT02154360).

**NK1R Antagonists and Neurodegenerative Diseases**

MS is an inflammatory demyelinating disease and, at least in part, an immune-mediated disease where both innate and adaptive immune responses play a role. SP-reactive cells are found in MS lesions, where they enhance proinflammatory cytokine production by T cells. Activated T cells can then produce more SP, up-regulate NK1R, and proinflammatory cytokine production by T cells. Activated reactive cells are found in MS lesions, where they enhance SP stimulation. The up-regulation of IL-12 and IL-23 that was induced by the specific NK1R antagonist CP-96,345 suppressed IFN-γ/IL-17 in the context of MS. Treatment of PBMCs with the IL-12/IL-23 family of cytokines and the associated cells (PBMCs) evaluated the interactions of SP and NK1R, showing beneficial effects in the chronic stages of the disease. Consequently, the genetic absence of NK1R or its suppression using the synthetic antagonist SR140333 showed beneficial effects in the chronic stages of the disease, but not during the acute phase. Similarly, in a different study using the EAE model, the authors evaluated the effect of CP-96,345, a selective NK1R antagonist, and found marked suppression of clinical and histological signs of EAE after early treatment, although severity could not be modulated if treatment was started at the peak of disease. The beneficial anti-inflammatory effects of antagonist treatment consisted in the decreased expression of adhesion molecules in CNS endothelia, and reduced secretion of proinflammatory Th1 cytokines. Another study that used human peripheral blood mononuclear cells (PBMCs) evaluated the interactions of SP and NK1R with the IL-12/IL-23 family of cytokines and the associated IFN-γ/IL-17 in the context of MS. Treatment of PBMCs with the specific NK1R antagonist CP-96,345 suppressed the up-regulation of IL-12 and IL-23 that was induced by SP stimulation. Given the similarity of the in vitro results with those from the experiments in mice it is possible to suggest that NK1R antagonists are therapeutic candidates in MS.

The neurotoxic 6-hydroxydopamine (6-OHDA) lesion model is the classic and frequently utilized animal model of PD, as it produces oxidative stress and causes cell death in dopamine neuronal populations. SP expression was shown to be elevated following 6-OHDA treatment of dopaminergic neurons in vitro and, comparably, increased SP levels were found in the SN of the rat model. This early increase of SP level was associated with augmented BBB permeability, microglial and astrocyte activation, increased dopaminergic cell death and profound motor deficit. Moreover, intrastratial 6-OHDA injections combined with endogenous SP treatment accelerated disease progression, while blocking SP effects with NK1R antagonists (N-acetyl-L-tryptophan [NAT] or L-733,060) preserved barrier integrity, reduced inflammatory processes, attenuated 6-OHDA-induced cell death and resulted in a significant improvement in motor function. In another study, SP was able to modulate microglial function via both NK1R dependent and independent mechanisms, depending on its concentration. These findings suggest that SP could use different signaling pathways to produce proinflammatory and neurotoxic effects and, therefore, the use of NK1R antagonists would not be as effective.

The role of SP in the onset of L-DOPA induced dyskinesia (LID) or involuntary abnormal movements was also investigated. L-DOPA, the precursor to dopamine, combined with a peripheral L-DOPA decarboxylase inhibitor such as beneseride, is the gold-standard treatment for PD and results in maladaptive changes to brain signaling pathways and LID. Confirmation that SP is involved in the onset of LID was reported recently in an in vivo study where the combined administration of the NK1R antagonist N-acetyl-L-tryptophan with L-DOPA prevented the onset of mild to moderate LID. To corroborate this finding another group showed that treatment with a different NK1R antagonist (LY303870) was also capable of reducing LID in a rodent model by preventing dopamine efflux and the subsequent increase in secondary messenger systems. Importantly, both studies confirmed that NK1R antagonists do not interfere with L-DOPA-induced improvement in motor function. Taken together, these results suggest the use of NK1R antagonists as a novel adjunct therapy to L-DOPA in the treatment of PD.

To our knowledge there are no published studies involving AD and NK1R antagonists. However, several reports have indicated that SP has a beneficial rather than a detrimental role in AD. Very recently, a study in humans showed that patients with early AD had elevated CSF SP levels, which correlated with biomarkers of AD. This can possibly represent a compensatory mechanism to protect the AD brain from uncontrolled Aβ exposure. Future experiments are still needed to further explore the interaction between beta-amyloid deposits and SP.

**Conclusion**

The use of classical anti-inflammatory drugs for the treatment of acute and chronic CNS diseases is often limited by detrimental side effects. NK1 receptor antagonist treatment is an appealing alternative. However, there are still many challenges in developing NK1R antagonists to treat neuroinflammation, as many neuroinflammatory
diseases present complex pathologies. In addition, the extrapolation from animal to human studies is complicated by the differences in the potency with which certain antagonists interact with NK1Rs of different species. For this reason, interpretation of the effects of NK1R receptor antagonists in preclinical assays requires great caution. Also, when one receptor is antagonized, the other two (NK2R and NK3R) may compensate for this effect. Therefore, antagonists that target more than one NK receptor could be needed. Overall, however, the use of NK1R antagonists appears to be a promising therapeutic approach for neuroinflammatory diseases of diverse etiologies.

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