Minireview

Nitric oxide modulation in neuroinflammation and the role of mesenchymal stem cells

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Impact statement

Neurodegenerative diseases remain an unresolved health burden. Pharmacotherapeutics targeting distinct disease hallmarks have failed at phase III clinical trials, leaving only symptomatic treatments. A new angle for therapy is required and nitric oxide is a target worth paying attention to as it is a major player of neurotoxicity in these diseases. This work details crucial insights on NO modulation and draws a hypothetical map for experimental explorations of nitric oxide inhibition and mesenchymal stem cell modulations of NO for the development of novel therapeutic targets for neurodegenerative diseases. We predict that nitric oxide inhibition in combination with other immunomodulatory effects of mesenchymal stem cells on microglia has beneficial effects that can be exploited for producing more effective therapeutics with fewer side effects and are therefore safer and more tolerable to alleviate the burden of the disease.

Abstract

Nitric oxide is a versatile mediator formed by enzymes called nitric oxide synthases. It has numerous homeostatic functions and important roles in inflammation. Within the inflamed brain, microglia and astrocytes produce large amounts of nitric oxide during inflammation. Excessive nitric oxide causes neuronal toxicity and death and mesenchymal stem cells can be used as an approach to limit the neuronal damage caused by neuroinflammation. Mesenchymal stem cell therapy ameliorates inflammation and neuronal damage in disease models of Alzheimer's disease, Parkinson's disease, and other neuroinflammatory disorders. Interestingly, we have reported that *in vitro*, mesenchymal stem cells themselves contribute to a rise in nitric oxide levels through microglial cues. This may be an undesirable effect and highlights a possible need to explore acellular approaches for mesenchymal stem cell therapy in the central nervous system.

Keywords: Nitric oxide, neuroinflammation, neurodegenerative diseases, mesenchymal stem cells, microglia

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Introduction

Nitric oxide (NO) is a diatomic, short-lived gas that regulates a wide range of homeostatic functions, mainly in the cardiovascular system and nervous system.[1] It also has cardinal functions in inflammation, causing vasodilation, increased leukocyte adhesion, and vascular permeability.[2] Most immune cells and various non-immune cells such as endothelial cells, fibroblasts, hepatocytes, and keratinocytes produce NO. NO is generated by nitric oxide synthase (NOS) during the conversion of L-arginine to L-citrulline.[3] Within the brain, glial cells generate NO in response to inflammation and excessive levels of NO can exacerbate neuroinflammation, causing neuronal death and tissue damage.[4] The resulting neuronal death acts as a further stimulus for NOS expression, generating more NO and thus perpetuating a cycle of tissue damage. NO therefore is a plausible target for therapeutics in managing neuroinflammatory conditions. There are, however, numerous factors that can influence the outcome of NO-targeted therapy, including NOS isoforms, NO concentration, the kinetics of NO expression, and the way therapeutic interventions are administered.

Microglia are the tissue-specific macrophages of the brain and spinal cord. They constantly sample the central

nervous system (CNS) parenchyma for changes in homeostasis, upon which they assume a reactive phenotype that initiates inflammation.[5] This reactive phenotype is characterized by the production of proinflammatory cytokines, reactive oxygen and nitrogen species, and their proliferation and migration.[6] Although inflammation is an important and necessary defence mechanism, prolonged or excessive inflammatory responses of microglia can cause neuronal damage and are implicated in a wide range of CNS conditions.[7]

MSCs are multipotent stem cells with immunomodulatory effects. Numerous reports have described direct and indirect modulatory effects on microglial inflammatory responses. We have demonstrated MSCs to decrease the production of inflammatory mediators and markers by microglia, and reduce microglial proliferation *via* a slowdown of their cell cycle.[8,9] Interestingly, these effects were also coupled with an increase in NO levels, a possible undesirable effect. In this review, we discuss the complexities of NO's roles in inflammation, leading-in to a discussion of mesenchymal stem cells as a form of therapy for neuroinflammation, specifically in modulating NO levels.

A review of the literature was performed with the following keywords: nitric oxide, inducible nitric oxide synthase, endothelial nitric oxide synthase, nitrosative stress, reactive nitrogen species, neuroinflammation, neurotoxicity, neurodegenerative disease, microglia, neuronal excitotoxicity, glutamate, mitochondrial respiration, vascular permeability, endothelial leukocyte adhesion, NOS inhibitor, iNOS/eNOS knockout, mesenchymal stem cells, immunomodulation, immunosuppression, NO modulation, MSC secretome. The databases on which the literature search was performed were PubMed, ScienceDirect, Scopus and Google Scholar. The literature search strategy included a focus on reviewing studies using NOS inhibitors and NOS knockouts that allowed a more direct implication for NO in neurological disease. We also focused on rat and mice models, occasionally citing human studies. Importantly, to implicate roles for NO in neuroinflammation, we focused our literature search on iNOS. The key studies and their findings obtained from the literature search are summarized in Table 1.

NO has a complex and multifaceted role in inflammation

NO is produced by two constitutive forms of NOS, neuronal (NOS-1/nNOS) and endothelial (NOS-3/eNOS), and one inducible form (NOS-2/iNOS).[38] Typically, the

Table 1. Summary of the literature reviewed.

Headings	Main findings	References
NO has a complex and multifaceted role in inflammation	– iNOS is expressed during inflammation, producing high volumes of NO for longer periods compared to other NOS isoforms.	10,11
	– The effect of NO on leukocyte adhesion and vascular permeability is dependent on the concentration of NO produced and the NOS isoform.	12, 13, 14, 15
NO is implicated in the pathophysiology of neuroinflammatory diseases	– iNOS deficiency in APP/PS1 mouse models of AD reduces beta-amyloid plaques and phosphorylated tau protein and increases survival.	16, 17
	– On the contrary, iNOS deletion in the APPSwDI mouse model of AD worsens AD-like pathology.	18
	– iNOS inhibition improves outcome in PD mouse models by preventing loss of dopaminergic neurons and improving motor impairment.	19, 20, 21
	– Inhibiting NO prevents clinical progression in the EAE model of MS.	22
	– Conversely, EAE mice lacking the iNOS gene have worse clinical symptoms and increased mortality rates.	23
MSC therapy for NO modulation in neuroinflammation	– In AD mouse models, human placental-derived MSCs restore cognition, reduce Aβ plaques, gamma-secretase activity, beta-secretase 1 and iNOS expression and increase anti-inflammatory cytokine expression.	24, 25
	– MSCs inhibit iNOS expression and peroxynitrite formation, improving neurological recovery in rats with intracerebral hemorrhage.	26, 27
	– Implantation of adipose-derived MSCs in a rat model of cerebral ischemia/reperfusion injury downregulates iNOS expression and reduced neuronal apoptosis.	28
	– BM-MSCs produce NO in response to soluble factors of activated microglia, and not to LPS alone.	8, 29
	– NO does inhibit microglia proliferation.	9
	– BM-MSCs express NO upon exposure to IFNγ and TNFα, but not to LPS alone. The NO produced by MSCs suppresses T cell proliferation.	30, 31
Acellular approaches for MSC therapy in NO modulation	– MSC-conditioned media reduces LPS-induced expression of TNFα, IL-6 and iNOS mRNA in astrocytes.	32
	– Conditioned media from human BM-MSCs protect rodent cerebellar neurons from NO-induced death.	33
	– Exosomes from adipose-derived stem cells improve neurogenesis and reduce microglia activation in a rat model of stroke.	34
	– In rat models of traumatic brain injury, MSC-derived exosomes improve functional recovery and spatial learning, accompanied by reduced neuroinflammation.	35, 36
	– MSC-derived exosomes reduce cortical damage in a model of traumatic brain injury, which is associated with microglia shifting to an anti-inflammatory phenotype.	37

constitutive forms of NOS produce tonic, pulsatile volumes of NO, while iNOS produces the high volumes that are synonymous with inflammation.[3] Namely, iNOS produces more (micromolar) NO for longer (hours) compared to eNOS and nNOS that produce less NO (nanomolar amounts) for shorter periods (seconds to minutes).[10,11] In inflammation, NO acts as a vasodilator and increases leukocyte adherence to the endothelium of blood vessels.[12,39] NO also increases the permeability of blood vessel walls,[13–15] allowing leukocyte transmigration into extravascular spaces.[40,41] The levels of NO and the mode of its secretion (tonic/low and continuous or acute/high and transient) influence the downstream functions of NO. For instance, in physiological conditions, the low and constant levels of NO produced by eNOS allow white blood cells to remain suspended in blood as this basal level of NO inhibits leukocyte adhesion.[12] However, during inflammation, eNOS contributes to an acute increase in NO, removing the inhibition on leukocyte adhesion and increasing diapedesis.[12,39] Therefore, NO has a dual role in leukocyte adhesion that is dependent on the levels that are produced.

Recent studies on the effect of NO on vascular permeability have shown that it is not just the concentration of NO that has differential effects, but also the isotype of NOS that catalyses the NO formation. eNOS is more functional in mediating vascular endothelial growth factor (VEGF)-induced and platelet-activating factor (PAF)-induced vascular permeability during inflammation than NO derived from iNOS. Studies using eNOS and iNOS knockout mice showed that VEGF-induced vascular permeability was unaffected in iNOS knockout and wild-type mice but markedly reduced in $eNOS^{-/-}$ mice.[13] Hatakeyama *et al.* arrived at the same conclusion with PAF; PAF increased vascular permeability in cremaster muscles and the mesentery of wild type and $iNOS^{-/-}$ mice but not in $eNOS^{-/-}$ mice.[14] Deletion of the eNOS gene results in a dramatic drop in NO, implicating eNOS as the main contributor to VEGF-induced NO production.[13] It is important to note that in human endothelial cells, VEGF induced only eNOS and not iNOS, indicating different functional importance of NOS isoforms between species.[42] The contribution of each NOS isozyme in inflammatory conditions is therefore highly context-dependent, and selective modulation of NOS isoforms is important to consider in therapeutics.[13] If NOS inhibitors are to be used in therapy, the pathophysiology of NO-mediated effects must be accurately determined, including the forms of NOS involved and concentrations of NO produced, to prevent tipping the balance towards deleterious inflammatory consequences.

Although NO is critical for the various biological process during inflammation, high and persistent concentrations of NO can be damaging to cells. There are several ways in which NO causes cellular damage including (i) S-nitrosylation of proteins that causes mitochondrial damage and protein misfolding,[43] (ii) formation of peroxynitrite ($ONOO^-$), a reactive nitrogen species (RNS) that increases oxidative stress and leads to apoptosis,[44] and (iii) inhibition of oxidative phosphorylation and glycolysis that affects mitochondrial respiration.[45] Within the CNS, neurons are highly susceptible to NO-induced cell damage, which contributes to the pathophysiology of various diseases. iNOS is expressed in microglia and astrocytes and is the only form of NOS reported in microglia.[46] Therefore, modulating NO (and broadly iNOS) in microglia is perceived to derive favorable outcomes in chronic inflammatory diseases of the CNS. In fact, studies have shown that inhibiting iNOS in microglia-neuron co-cultures,[47] and glia-neuron co-cultures ameliorated neurotoxicity.[48]

While reducing NO in an inflammatory milieu remains prospective, experimental evidence gathered in the last couple of decades indicates that it is not an all or none beneficial phenomenon. The outcome is influenced by the extent of modulation, phase of inflammation, and method and modulation route.[49] For instance, the route of NOS inhibitor administration is decisive in the outcome of pleurisy. Systemic administration of an NO inhibitor was beneficial in improving pleurisy, although local (intrapleural) administration exacerbated the inflammation.[49] Additionally, NO's crucial roles in inflammation make regulating NO concentrations to a level that derives only favorable outcome a daunting task. A detailed account of *in situ* and experimental regulation of NO synthesis has been elegantly presented by Cinelli *et al.*[50] The next section will review the impact of regulating NO in neuroinflammatory environments, focusing on experimental evidence from iNOS modulation.

NO regulation as a therapeutic approach for CNS diseases

NO produced in the CNS during inflammation causes neuronal death via glutamate release and subsequent excitotoxic cell death. Inhibition of NO rescues neurons from death, shown both *in vitro* and in animal models. We elaborate on these studies further in the following section.

NO is implicated in the pathophysiology of neuroinflammatory diseases

Excessive NO production in neuroinflammation is now recognized as an important pathological component of diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) and modulating NO in these diseases would be beneficial. Accumulation of beta-amyloid (Aβ) plaques and neurofibrillary tangles are characteristic of AD pathology.[51] Kummer *et al*. showed that products of iNOS activation such as NO and $ONOO^-$ cause nitrotyrosination of $A\beta_{42,}$ accelerating their aggregation into amyloid plaques.[16] In their study, iNOS knockout mice ($iNOS^{-/-}$) expressing a chimeric mouse/human amyloid precursor protein and mutant human presenilin 1 (APP/PS1) showed a pronounced (74%) reduction of nitrated Aβ (3NTyr10-Aβ) and lesser memory deficits than controls. The $iNOS^{-/-}$ mice also exhibited reduced Aβ, fewer plaques, lesser phosphorylated tau protein, and better survival than mice expressing iNOS.[16] In a similar AD model of human APP and PS1-expressing mice, Nathan *et al*. showed that concurrent with overall reduced disease pathology, the mice also exhibited fewer reactive microglia.[17] However, whether fewer microglia in $iNOS^{-/-}$ mice reflect the reduction in

pathology or the absence of NO affecting microglia recruitment is not clear. Notably, NO have both disease-inducing and disease-perpetuating roles in AD. In a lipopolysaccharide (LPS)-induced learning impairment rat model, NO inhibition using aminoguanidine reduced sera levels of inflammatory mediators, reduced oxidative stress and importantly, improved cognitive deficits, exemplifying the pathological significance of NO.[52] However, there are outcomes contradictory to those described above. For example, Wilcock *et al.* demonstrated that deletion of the iNOS gene worsens AD-like pathology in mice bred to produce Aβ plaques (APPSwDI/NOS2$^{-/-}$), resulting in extensive tau pathology, amyloid deposition, and significant neuron loss in the hippocampus.[18]

For PD, the prodrug 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) models the dopaminergic damage in the substantia nigra and striatum. Inhibiting iNOS with S-methylisothiourea (SMT) increased substantia nigral dopaminergic neuron number, decreased nitrate/nitrite levels, decreased lipid peroxidation, and reduced caspase-3 activity in MPTP-treated mice. Importantly, inhibiting iNOS reduced signs of bradykinesia.[19] Another common PD model is the 6-hydroxydopamine (6OHDA) mouse model. In this model, motor impairment can be assessed by the amphetamine rotation test. Studies have shown that administration of NG-nitro-L-arginine methyl ester (L-NAME), a non-specific NOS inhibitor, in 6OHDA mice inhibited amphetamine-induced rotation,[20,21] alongside improved levels of dopamine and its metabolites.[20] The iNOS inhibitor GW274150 also improved outcome in 6OHDA mice, with reduced tyrosine hydroxylase (TH)-positive neuron loss in the substantia nigra.[53]

The empirical evidence reviewed above simultaneously establishes a causative role for iNOS in the progression of neurodegenerative diseases and reveals therapeutic promise for NO inhibition. Non-pharmacological approaches for regulating iNOS expression, including short interfering RNAs (siRNAs), microRNAs (miRNAs) and genetic knockouts are excellent research tools; however, their clinical application remains very limited. Alternatively, regulation of NO in the CNS can be achieved pharmacologically using inhibitors of NOS or NO scavengers. Structural analogues of L-arginine such as L-NAME, NG-monomethyl-L-arginine (L-NMMA), and N-iminoethyl-L-ornithine (L-NIO) can be used as competitive NOS inhibitors[54,55] but their non-selective nature to other isoforms of NOS is a limitation for deciphering the actions of iNOS alone.[56] Selective iNOS inhibitors such as L-N-iminoethyl lysine (L-NIL), 1400 W, GW273629, GW274150, and aminoguanidine, overcome such limitations and are now being tested in multiple disease models.[52,57]

Although blocking NO production in disease has shown some success, in some cases it resulted in undesirable outcomes. For instance, inhibiting iNOS prevented clinical progression in the experimental allergic encephalomyelitis (EAE) model of multiple sclerosis (MS).[22] However, in iNOS$^{-/-}$ knockout mice, symptoms worsened and mortality rates increased compared to wild-type animals, suggesting that NO may have some protective effects in EAE.[23] These observations indicate that targeting iNOS in these diseases should be balanced to harness the beneficial effects of NO while blocking its harmful activities.[58] Many iNOS inhibitors have exhibited high selectivity and potency *in vitro* and in animal models; however, they have not been approved for clinical use in humans.

MSC therapy for NO modulation in neuroinflammation

MSCs have been pursued in therapy for their regenerative potential, although their potential to limit inflammation has comparatively appeared more fruitful.[59] Trophic factors from MSCs exert anti-inflammatory, immunomodulatory, and cytoprotective effects on a range of immune cells including T cells, B cells, and dendritic cells.[60] Here, we discuss the potential therapeutic effects of MSCs on neuroinflammation with a specific focus on the modulation of nitrosative stress.

Multiple studies have shown the effectiveness of MSC therapy in modulating NO in neurodegenerative disease models. A single intravenous injection of human placental-derived MSCs into the Aβ_{1-42} mouse model of AD restored cognition by decreasing Aβ plaques, gamma-secretase activity, beta-secretase 1 (BACE1) expression, and iNOS.[24] Similarly, IV injection of human placenta amniotic membrane-derived MSCs into the APPSwe transgenic mouse was showed to improve AD pathology by expressing high levels of Aβ-degrading enzymes (matrix metallopeptidase-9 and insulin-degrading enzyme), reducing levels of proinflammatory cytokines IL-1 and TNF-α, and increasing levels of anti-inflammatory cytokines TGF-β and IL-10.[25] Similarly, in SOD1G93A mice (a mouse model of amyotrophic lateral sclerosis (ALS)), soluble factors of MSCs significantly reduced TNF-α, interleukin 6 (IL-6), and iNOS expression in astrocytes.[32] An interesting effect was that MSCs upregulated fractalkine (CX3CL1) mRNA expression in astrocytes from mutant SOD1G93A transgenic mice. Fractalkine is a chemokine that serves as a calming signal for microglia. Correspondingly, MSCs increased expression of the fractalkine receptor, CX3CR1, in mutant SOD1G93A transgenic microglia.[32] The interaction of CD3CL1-CX3CR1 suppressed microglia activation and improved neuronal survival in the mice.[32] Similarly, we have found that MSCs attempt to restore CX3CL1 expression to constitutive levels in microglia stimulated with LPS, which seems to be an attempt to scale down the inflammatory phenotype of microglia.[9]

MSCs also improve outcome in brain injury. In a radiation-induced brain injury model, the intranasal administration of MSCs promoted repair by limiting the activation of damage-induced c-AMP response element-binding signalling (CREB). Notably, iNOS expression and oxidative stress biomarkers were decreased, both of which were related to better cognitive performance and neuronal survival.[61] In rat models of intracerebral hemorrhage, MSCs decreased peroxynitirite levels in lesioned brain tissues, reduced blood–brain barrier leakage, and improved neurological recovery.[26,27] Regulation of NO after MSCs transplantation was also observed in cerebral ischemia

and reperfusion injury.[62] Implantation of adipose-derived MSCs into the middle cerebral artery occlusion rat model downregulated iNOS expression and reduced neuronal apoptosis.[28] Taken together, these reports indicate that MSCs-mediated reduction of iNOS/NO, although not tested directly for its causative role, may have contributed to the disease improvement in these experiments. While the reduction of nitrosative stress was a common phenomenon, the transfer of MSCs also led to modulation of other inflammatory mediators (cytokines and oxidative radicals), thus defining the causative significance of NO in MSCs-mediated immunomodulation *in vivo* remains elusive.

Since NO is deleterious to neurons,[63] it raises the question of whether MSCs can reduce NO production by microglia as a means to reduce neurotoxicity? Conversely, we show that mouse BM-MSCs increase NO levels in LPS-stimulated BV2 microglial co-cultures.[8,29] The increased NO was observed irrespective of cell-to-cell contact, indicating a role for soluble factors in increasing the NO levels. In co-culture experiments, we are unable to identify the cell (s) responsible for this increase; however, both MSCs and microglia can produce large amounts of NO. A direct inflammatory stimulus (in this instance, LPS) does not induce NO expression in MSCs, and nor do soluble factors from inactivated microglia.[8] Only soluble factors from LPS-activated microglia initiated MSCs to produce substantial amounts of NO. The ability of MSCs to produce NO was further enhanced in MSCs pre-treated with LPS.[8] Generation of nitrosative free radicals by MSCs from different sources has been reported by others, including human bone marrow, human skin, human umbilical cord, and rat bone marrow.[64–67] Ren *et al*. have also demonstrated that mouse BM-MSCs exposed to IFN-γ and TNF-α (but not to LPS) induces NO production, [30] demonstrating again that the biologic cue for MSCs to produce NO appears to be from the cellular/tissue reaction of inflammation.

In an attempt to understand the reasons for MSCs/BV2 co-cultures causing an NO increase, we explored what others had shown to be a role for NO in the immunosuppression of T cells by MSCs. Ren *et al.* and Sato *et al.* showed that immunosuppression of T cells by MSCs, specifically the suppression of T cell proliferation was mediated by NO, through the inhibition of signal transducer and activator of transcription 5 (Stat5).[30,31] These were determined through the following observations: (i) MSCs from iNOS knockout mice were less able to suppress proliferation of T cells; (ii) Inhibiting NO in cocultures of MSCs and T cells *in vitro* restored T cell proliferation and phosphorylation of Stat5.[30,31] As our work had demonstrated that MSCs secrete considerable amounts of NO in the presence of microglia, and MSCs decrease microglia proliferation, we determined whether NO was mediating this effect. However, inhibiting NO production in co-cultures with L-NAME did not affect microglial proliferation.[9] The role of MSCs-produced NO in our cocultures, therefore, remains unknown, and the additional NO produced may promote neuronal cell death. These factors need to be further elucidated to improve MSC therapy for NO regulation.

Acellular approaches for MSC therapy in NO modulation

MSCs integration into injured tissue is not essential for them to exert their immunomodulatory effects, indicating that acellular aspects of MSCs are also protective. MSC secretomes containing soluble mediators and exosomes may be the magic bullet that harnesses the immunomodulatory and neuroprotective effects of MSCs while limiting the risk of an increase in NO.[68,69] The immunomodulatory effects of secreted factors from MSCs have been demonstrated in multiple *in vitro* experiments. The addition of conditioned media from mouse BM-derived MSCs reduced LPS-induced transcriptional increase of TNF-α, IL-6, and iNOS in astrocytes.[32] Interestingly, Kemp *et al*. demonstrated the addition of MSCs-conditioned media protects cerebellar neurons from NO-induced death.[33] MSC-derived secretomes and exosomes regulate microglia activation in a variety of CNS disease models. In rat models of traumatic brain injury, systemic administration of MSC-derived exosomes improved spatial learning and promoted functional recovery. These improvements were accompanied by reduced inflammation.[35,36] The exosomes' effects on reducing cortical damage in traumatic brain injury are associated with microglia shifting to an anti-inflammatory phenotype characterized by the reduced release of cytokines.[37] These immunomodulatory and neuroprotective effects were driven by miRNAs in the exosomes derived from MSCs.[37] In a middle cerebral artery occlusion model of stroke, exosomes from adipose-derived stem cells improved neurogenesis and reduced microglia activation.[34]

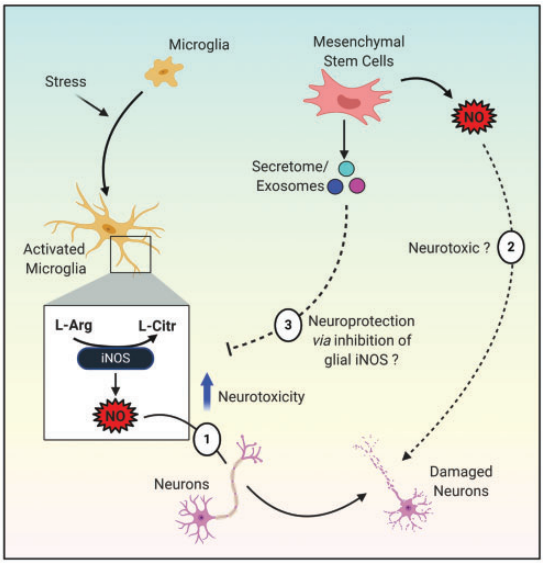

Figure 1. A graphical summary of the review. (1) In response to homeostatic changes in the brain, microglia are activated to express iNOS. NO is formed when iNOS converts L-arginine to L-citrulline. High levels of NO produced during inflammation increases neurotoxicity, causing neuronal damage. (2) MSCs also produce NO in the inflammatory milieu. Although MSCs are immunosuppressive and downregulate inflammation in the brain, their NO production may contribute to neurotoxicity. (3) Using acellular components from MSCs, can the immunosuppressive properties of MSCs be isolated from their NO production to confer neuroprotection via inhibition of glial iNOS? L-Arg: L-arginine; L-Cit: L-citrulline; NO: nitric oxide; iNOS: inducible nitric oxide synthase. (Graphics created with BioRender.com A color version of this figure is available in the online journal.)

Taken together, the secretome of MSCs appears to retain the beneficial effects of MSCs while potentially avoiding the risk associated with an increase in MSCs-derived inflammatory mediators such as NO. Furthermore, exosomes administered systemically are shown to cross the BBB and have low immunogenicity.[70,71]

It is important to note, however, that a secretome collected from MSCs cultured in an environment distinct from the injury site will not have the same physiological cues as MSCs. The effectiveness of acellular MSCs approaches therefore must be tested as they may even render undesirable outcomes. The exact molecular constituents of MSCs-derived exosomes and the paracrine mechanisms that protect neuroinflammation are not entirely understood and remain an avenue of active research.

Conclusion and future direction

The functions of NO in inflammation are diverse and complex. Harnessing the beneficial effects of NO while limiting their neurotoxicity is a balance that needs to be struck for therapeutics (see Figure 1 for a graphical summary of this review). Although MSCs downregulate microglial inflammatory responses, they seem to contribute to a surge in NO. Unlike for T cells, NO does not appear to be the mediator for MSCs-driven inhibition of microglial proliferation. Therefore, for the CNS, the outcome for this increase in NO levels has not been deciphered and conversely, may cause neurotoxicity. Acellular products of MSCs may be an interesting avenue to pursue for the management of neuroinflammatory disease, as MSCs exosomes and secretomes will potentially downregulate inflammation without contributing to NO levels.

AUTHORS' CONTRIBUTIONS

PML, NNAP, SV, and SJ wrote the manuscript; SV and SJ also planned and supervised the writing of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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