New Insights into S-nitrosylation in Multiple Sclerosis

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Abstract

S-nitrosylation is a biologically relevant post-translational protein modification with signaling consequence. In eukaryotes, a large number of proteins have been identified as S-nitrosylation targets. Derangement in protein S-nitrosylation has been implicated in the pathogenesis of a number of different disease entities including Multiple Sclerosis (MS). A growing body of evidence has shown that Nitric oxide (NO) plays a critical role in MS. NO and other reactive nitrogen species (RNS) are involved in neuroinflammation and neurodegeneration in MS. Signaling by RNS is carried out mainly by S-nitrosylation of critical cysteine residues in targeted proteins. In recent years, newer roles in MS have been attributed to RNS. These roles relate to S-nitrosylation of cysteines in proteins which has emerged as a potential new paradigm in signal transduction and regulation of protein function. In the present review we discuss the evidence for the diverse roles of S-nitrosylation in MS, including nitrosative stress-induced gene expression in MS, and S-nitrosylation of transcription factors in MS. In addition, S-nitrosylation can be therapeutically used in MS. Recent studies providing evidence for SNO-based therapy strategy in the treatment of MS will also be discussed. Undoubtedly, new exciting results will contribute to the expanding area of MS research.

Keywords: Multiple sclerosis; S-nitrosylation; Nitric oxide; iNOS; Transcription factor; Biomarkers; Therapy; Posttranslational modification; Autoimmune disease

Introduction

Nitric Oxide (NO) has long been recognized as a modulator of gene expression both in prokaryotic and eukaryotic cells and is an import molecule involved in many physiological and pathological processes [1,2]. NO is synthesized by Nitric Oxide Synthase (NOS) which oxidizes a guanidine nitrogen of L-arginine releasing nitric oxide in the form of a free radical and citrulline. Three isoforms of the NOS have been identified, including neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2), and endothelial NOS (eNOS or NOS-3). S-nitrosylation is one of the key mechanisms by which NO regulates the function of various target proteins is through the coupling a nitroso moiety from NO-derived metabolites to a reactive cysteine leading to the formation of a S-nitrosothiol (SNO) [3]. SNOs are stable, bioactive forms of NO and are known to regulate the immune response [4]. Classic NO signaling delineates a pathway by which NOS-derived NO diffuses to and then binds to the heme moiety of guanylate cyclase inducing a conformational change that results in enzyme activation and increased formation of cyclic GMP (cGMP) [5]. Nitrosative stress has been implicated in the pathophysiology of MS and its animal model experimental autoimmune encephalomyelitis (EAE) [6-8]. It was reported that protein SNOs accumulate in the brain of MS patients and SNO levels are also increased in EAE [6,9].

MS is a chronic inflammatory demyelinating disease of the central nervous system (CNS), which is the most frequent disabling neurological disease in young adults. MS afflicts over 2 million people worldwide. According to the temporal course of disease, MS can be subdivided into three clinical groups: relapsing remitting MS (RR-MS), secondary progressive MS (SP-MS) and primary progressive MS (PP-MS). Most evidence supports that the activation of autoreactive T-cells is a central event in the development of autoimmune response in MS and the pathogenesis of MS in most patients is likely to result from autoreactive, activated CD4+ T cells moving from the periphery across the blood brain barrier (BBB) into the CNS [10]. There are numerous symptoms associated with the neurologic damage in MS patients, including fatigue, spasticity, depression, bowel and bladder dysfunction, pain, and impaired mobility. Several therapies (eg. modafinil, dalfampridine, baclofen, diazepam, gabapentin, and opioids) are used for symptomatic treatment of disability and symptoms, but these do not improve disease outcome [11]. This chronic immune-mediated disease potentially requires more definitive symptomatic and disease-modifying therapies.

Nitrosative stress induces the generation of protein and non-protein nitrosothiols, resulting in alterations in tissue function [12,13]. Accumulating evidences points to an important role for NO in the pathogenesis of MS and to its contribution to the various facets of the disorder: inflammation, oligodendrocyte injury, changes in synaptic transmission, axonal degeneration, and neuronal death [14]. Boullenne et al. found that S-nitrosothiols was detected in MS patients and EAE animals [15,16]. Calabrese et al. also reported that the concentration of both nitric oxide metabolites and unidentified low molecular weight nitrosothiols were increased in serum and cerebrospinal fluid (CSF) from patients with active MS [17]. Recent studies have reported that SNOs accumulate in brain white matter of MS patients, indicating that the occurrence of protein S-nitrosylation correlates with the inflammatory demyelinating disorders in MS patients [8]. This review paper provides insights into the role of protein S-nitrosylation in the pathophysiology of MS and summarizes the SNO-based therapy strategy in the treatment of MS.

Nitrosative Stress-induced Protein S-nitrosylation in MS

NO has been linked to numerous physiological and pathophysiological events. It is very important to identify the protein targets of S-nitrosylation which include metabolic, structural and signalling proteins. Previous studies indicated that protein S-nitrosylation acts as a physiological signalling mechanism in MS [18].

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Cytoskeletal proteins

Jaffrey et al. reported that the proteins which were S-nitrosylated comprise neurofilament heavy chain (NFH), α/β-tubulin, and β/α-actin when the rat cerebellum homogenates were incubated with NO donors in vitro experiments [18]. In vivo, S-nitrosylation of both α-tubulin and β-tubulin was increased only during acute EAE [19]. The S-nitrosylation of the major microfilament protein β-actin was also detected only in animals with acute EAE [20], but β-actin were not modified by S-nitrosylation in either control or EAE tissues as well as dynein, ankyrin, and tropomyosin. It is likely that the abnormal S-nitrosylation of several structural proteins such as NFPs, tubulin, and β-actin in EAE may contribute to the pathophysiology of MS.

Proteolipid protein (PLP)

Exposure to NO donors causes myelin decompaction, accompanied by S-nitrosylation of a cysteine-rich proteolipid protein (PLP) [7,14]. Indeed, incubation of rat spinal cord slices with GSNO resulted in the S-nitrosation of a number of proteins [6,21]. In myelin, one of the major S-nitrosated substrates was identified as PLP, an abundant cysteine-rich protein that is responsible for the intraperiod line (IPL) stabilization [6]. It is proposed that NO-mediated nitrosation of sulfhydryl groups is likely to interfere with the normal function of PLP and other important CNS myelin proteins leading to the structural demise of this membrane. These findings are relevant to multiple sclerosis and other inflammatory demyelinating disorders where both excessive NO production and myelin instability are known to occur [7]. S-nitrosylation of PLP has been linked to decompaction of CNS myelin at the level of the intraperiod line, where this protein plays an adhesive role.

Metabolic enzymes

In vitro experiment, incubation of rat spinal cord slices with GSNO leads to S-nitrosylation of four metabolic enzymes including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), creatine kinase (CK), hexokinase 1 (HK), and glycogen phosphorylase (GP) [18]. Among the four metabolic enzymes that are S-nitrosylated in vitro, only GAPDH was S-nitrosylated to a higher level in EAE in vivo experiment. The S-nitrosylation of GADH Cys-149 at the active site significantly attenuates the activity of this glycolytic enzyme [22,23]. It was also found that GSNO inhibited GAPDH activity in both purified enzyme preparations and endothelial cells [24]. In addition, recent studies indicated that the S-nitrosylation of GAPDH induces its binding to the E3 ubiquitin ligase Siah1 to cause nuclear translocation and to promote apoptosis [25]. GSNO induced S-nitrosylation of HK causes enzyme inactivation, but the effect apparently is caused by S-nitration of several tyrosine residues instead of by S-nitrosylation of cysteine thiols [26]. GSNO also inhibits the activity of Triosephosphate isomerase (TPI), Phosphofructokinase (PFK), Neuron-specific enolase (NSE), GP and Creatine kinase (CK) through S-nitrosylation [27–29]. In vivo experiment, TPI, PFK, and GP were S-nitrosylated to the same extent in control and EAE tissues. Bizzozero and Zheng reported that NSE is heavily modified in acute EAE and is minimally S-nitrosylated in control spinal cords [6]. CK was barely modified in control and EAE spinal cord [29,30]. These finding suggest that S-nitrosylation of important metabolic enzymes such as GAPDH and NSE could lead to neuronal death later in the disease process in MS.

Ion-channels-related proteins

The NO donors induced the S-nitrosylation of 3 ion channel-related proteins including N-methyl-d-aspartate (NMDA)-glutamate receptors, hyperpolarization activated cation channel (HCN3), and Na1/K1 ATPase α-2 subunit [18]. In vivo, HCN3 was not S-nitrosylated either in control or in EAE tissues. Na/K ATPase α-2 subunit was modified equally in control and EAE spinal cords. In contrast, the proportion of S-nitroso-NR2A increased in both acute and chronic EAE [6]. Choi et al. reported that S-nitrosylation of a single cysteine residue in NR2A modulates its channel activity [5]. Site-directed mutagenesis identified a critical cysteine residue (Cys 399) on the NR2A subunit whose S-nitrosylation under physiological conditions underlies this modulation. Bizzozero et al. found that the proportion of S-nitrosylated NMDA receptors increased in EAE [6]. They also discovered that neuronal specific enolase is the major S-nitrosylated protein in acute EAE. Given that S-nitrosylation affects protein function, it is likely that the observed changes are significant to the pathophysiology of inflammatory demyelination in MS [6]. The NMDA receptor (NMDAR)-associated ion channel was modulated not only by exogenous NO but also by endogenous NO. In cell systems expressing NMDARs with mutant NR2A subunits in which single cysteine was replaced by an alanine, the effect of endogenous NO was lost [5]. Thus endogenous S-nitrosylation can regulate ion channel activity.

Signal transduction proteins

Jaffrey et al. found that the retinoblastoma (Rb), heat-shock protein 72 (Hsp72), isoforms 2 of the collagen-responder mediator protein (CRMP2), and calbindin were S-nitrosylated when rat cerebellum homogenates were incubated with the NO donors GSNO [18]. In vivo, Rb protein was not detected in either the total homogenate of mouse Spinal cords (T1-L5). HSP-72, CRMP-2, and calbindin were detected in the total homogenates of mouse Spinal cords (T1-L5) [6]. These findings indicate that, although some proteins are susceptible to S-nitrosylation in vitro with various NO donors, they may not be modified in vivo to any appreciable extent even under severe nitrosative stress conditions.

S-nitrosylation of Transcription Factors in MS

NF-κB

NF-κB is a transcription factor activated by cell surface receptor signaling to meet stress and inflammatory responses, regulating key cellular processes such as inflammation, innate and adaptive immunity, and cell growth and survival [31]. Accumulating evidences indicate that NF-κB plays an important role in controlling expression of genes relevant to the pathogenesis of autoimmunity. Genetic factors related to NF-κB may also be determinants of MS susceptibility [32]. Within chronic active MS lesions and adjacent white matter, both NF-κB and c-jun/JNK reactivity was markedly up-regulated on glial cells and inflammatory elements [1]. NF-κB p50-deficient mice were significantly resistant to EAE induced by myelin oligodendrocyte glycoprotein. The resistance to EAE in NF-κB p50-deficient mice was associated with a deficiency of myelin oligodendrocyte glycoprotein-specific T cells to differentiate into either Th1- or Th2-type effector cells in vivo, suggesting that NF-κB plays crucial roles in the activation and differentiation of autoreactive T cells in vivo and that blocking NF-κB function can be an effective means to prevent autoimmune encephalomyelitis [2]. NO acts as second messenger molecular which through S-nitrosylation has shown to control important cellular processes by regulation of activity of NF-κB [33]. NF-κB activity is exquisitely sensitive to cellular NO levels with multiple steps in the signaling pathway targeted by S-nitrosylation. In addition, both p50 and p65 have been shown to be targeted by S-nitrosylation in cytokine-stimulated respiratory epithelial cells [34]. In addition to direct modification of NF-κB proteins, NO can also alters NF-κB activity through S-nitrosylation of proteins in other...
signal transduction pathways that cross-talk with NF-kB [34]. NO inhibits TLR-4 activation of NF-kB via S-nitrosylation of MyD88 [35]. Based on the role of NF-kB in MS, S-nitrosylation of NF-kB could be considered as a new therapeutic target in MS.

**HIF**

Hypoxia-inducible factor (HIF) is a transcription factor that regulates cellular hypoxic responses, and it has therapeutic potential in MS. An increased expression of HIF-1α in MS normal-appearing white matter (NAWM) in oligodendrocytes was detected by in situ hybridization analysis and quantitative RT-PCR [36]. HIF-1α, a key regulator of hypoxia-induced gene regulation, and its downstream genes were significantly unregulated in MS NAWM in the microarray study [37]. The upregulation of HIF-1α in oligodendrocytes supports the view of oligodendrocyte and/or neuronal dysfunction in the NAWM as a possible primary cause. These studies suggest an endogenous inflammatory reaction throughout the whole white matter of MS brain, in which oligodendrocytes actively participate. Recent studies also demonstrate that HIF stabilization and transcriptional activity is achieved through S-nitrosylation of HIF pathway components [38]. HIF-1 plays a critical role in the mammalian program by which cell respond to hypoxia in both physiological and pathological situations. HIF-1 transcriptional activity, protein stabilization, protein-protein interaction, and cellular localization are mainly modulated by post-translation modifications such as hydroxylation, acetylation, phosphorylation, S-nitrosylation, and SUMOylation [39]. Under normal oxygen tension, HIF-1 activity is usually suppressed due to the rapid, oxygen-dependent degradation of HIF-1α. Normoxic HIF-1 activity can be upregulated through NO-mediated S-nitrosylation and stabilization of HIF-1α [40].

**IRF**

Interferon regulatory factor (IRF) family is a group of transcription factors that are induced following treatment with type I interferon (IFN) [41]. Following the initial identification of two structurally related members, IRF-1 and IRF-2, seven additional members have now been reported [42]. IRF-1 is an interferon-induced transcription factor with pro-inflammatory and pro-injurious functions. New evidences emerged over past decade indicated that IRF-1 gene is associated with progressive MS and the elevated expression of IRF-1 was detected in active and chronic-active MS lesions [43-45]. IRF-1 was detected in the areas of CNS inflammation and co-localized with the perivascular active and chronic-active MS lesions [43-45]. IRF-1, a key regulator of hypoxia-induced gene regulation, and its downstream genes were significantly unregulated in MS NAWM in the microarray study [37]. The upregulation of HIF-1α in oligodendrocytes supports the view of oligodendrocyte and/or neuronal dysfunction in the NAWM as a possible primary cause. These studies suggest an endogenous inflammatory reaction throughout the whole white matter of MS brain, in which oligodendrocytes actively participate. Recent studies also demonstrate that HIF stabilization and transcriptional activity is achieved through S-nitrosylation of HIF pathway components [38]. HIF-1 plays a critical role in the mammalian program by which cell respond to hypoxia in both physiological and pathological situations. HIF-1 transcriptional activity, protein stabilization, protein-protein interaction, and cellular localization are mainly modulated by post-translation modifications such as hydroxylation, acetylation, phosphorylation, S-nitrosylation, and SUMOylation [39]. Under normal oxygen tension, HIF-1 activity is usually suppressed due to the rapid, oxygen-dependent degradation of HIF-1α. Normoxic HIF-1 activity can be upregulated through NO-mediated S-nitrosylation and stabilization of HIF-1α [40].

**SNO-based Therapy Strategy in the Treatment of MS**

Multiple sclerosis is the most frequent chronic inflammatory, demyelinating and neurodegenerative disease in young adults, but has no definitive pharmacological treatment. Most therapeutic agents used in MS including immunosuppressive and immunomodulatory drugs and cell cycle interruption drugs are only used for the treatment of RR-MS. These therapeutic agents can lessen the relapse rate in RR-MS and time to progression, but cannot cure MS. Therefore, there is a need for new efficient treatments for all types of MS. A more definitive therapy for MS should reduce relapse rate, prolong remission, limit the onset of new MS lesions, and postpone the development of long-term disability. There is a growing interest in developing a treatment strategy focused on protein posttranslational modification in MS, including S-nitrosylation. Herein, we are drawing attention to S-nitrosylation as a potential therapeutic strategy in MS (Figure 1).

**The S-nitrosylated protein biomarkers for detection of MS**

The symptoms of MS include independent processes of inflammation, demyelination, neurodegeneration, glosis and repair. The progress made in the search for new biomarkers in MS is helpful for the early diagnosis, prognosis, evaluation of the development of the disability caused by the disease and the response to therapy [55]. Biomarkers are very helpful to make decision in clinical diagnostics and important for guiding therapeutic treatment. MS is a class of disorders that need early diagnosis and steady monitoring. Now it was confirmed that S-nitrosylation affects the immunogenicity of self-protein antigens, and triggering an autoimmune response. In this context, S-nitrosylated peptides provided a more valuable tool with respect to isolated or recombinant proteins to selectively detect autoantibodies as disease biomarkers. It is now well established that some posttranslational modifications can generate new self-antigens or even mask antigens normally recognized by the immune system in physiological conditions. The most extensively studied putative self-antigens are components of normal myelin of the central nervous system, or of their post-translational modified forms [55]. Peptides can

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Figure 1: The role of S-nitrosylation in Multiple Sclerosis (MS).

Neuroprotective activity of S-nitrosylation in MS through the regulation of NO

S-nitrosylation can be therapeutically used in MS. The main focus of study on the role of NO in MS has been the iNOS isoform because it is the high-output form of NO and can produce several orders of magnitude more NO than eNOS or nNOS [33]. iNOS has been found to be a major contributor to initiation/exacerbation of CNS inflammatory conditions through the production of excessive NO which generates RNSs. iNOS expression is mainly controlled at the level of transcription and can be induced by an appropriate combination of cytokines in almost every cell type [33]. Recent studies have reported that MS and EAE are resulting from an increase in iNOS [6,9]. Activation of iNOS and NO generation were identified as a marker and therapeutic target in neuroinflammatory conditions in MS. The positive modulators of iNOS and NO generation were identified as a marker and therapeutic target in neuroinflammatory conditions in MS.

References


