NITRIC OXIDE – MEDIATED SIGNALIZATION AND NITROSATIVE STRESS IN NEUROPATHOLOGY

AZOT OKSID – POSREDOVANA SIGNALIZACIJA I NITROZATIVNI STRES U NEUROPATOLOGIJI

Ivana Stojanović1, Srđan Ljubisavljević1, Ivana Stevanović2, Radmila Pavlović1, Tatjana Cvetković1, Vidosava B. Đorđević1, Dušica Pavlović1, Slobodan Vojinović1, Jelena Bašić1

1Faculty of Medicine, University of Niš
2Institute for Medical Research, Military Medical Academy, Belgrade

Summary: Nitric oxide (NO) is an important signalling molecule in a variety of physiological processes. NO, a gas, is produced from L-arginine by different isoforms of the nitric oxide synthase and serves as mediator in important physiological functions, such as promoting vasodilation of blood vessels and mediating communication between nervous system cells. Contradictory to its physiologic actions, free radical activity of NO can cause cellular damage by the induction of nitric oxidative stress with significant implications on nervous system diseases. Although the mechanism of NO-mediated neurodegeneration still remains unclear, numerous studies suggest its crucial role in modification of protein functions by nitrosylation and nitro-tyrosination. NO contributes to glutamate excitotoxicity, participates in organelle fragmentation, inhibits mitochondrial respiratory complexes and mobilizes zinc from the internal stores. Recently, NO has been emerged as a mediator of epigenetic gene expression and chromatin changes. Besides, NO is a key mediator in the regulation of inflammatory and immune functions. It is involved in down regulation of several aspects of CNS inflammation, but also has a dual role in that it is required for inflammation in some situations.

Keywords: nitric oxide, nitrosative stress, protein S-nitrosylation, neuroinflammation, neurodegeneration

Introduction

Nitric oxide (NO), a gaseous signalling molecule, regulates a diverse range of physiological processes, including the regulation of vascular tone, neuronal function and immune function. Nitric oxide (NO) has been established as an important messenger molecule in various steps of brain physiology, from development to neur onal survival, differentiation, neurotransmitter release regulation, synaptic plasticity, learning and memory.
However, NO has been also viewed as a major agent of neuropathology when, escaping the controlled production, it may directly or indirectly promote oxidative and nitrosative stress. The exact borderline between physiological, and therefore neuroprotective, and pathological, and therefore neurodegenerative, actions of NO is a matter of controversy among researchers in this field. In high concentrations (>1 μmol/L), NO can induce cell death mainly either by oxidative/nitrosative stress-induced apoptosis or by energy depletion-induced necrosis. By caspase inhibition due to S-nitrosylation, NO may switch apoptosis to necrosis. Due to its multifaceted functions in CNS, NO attracts the attention of investigators as a neurotransmitter, a neuroprotector and a neurotoxic agent.

Nitric oxide is synthesized from L-arginine in the reaction catalyzed by the nitric oxide synthases (NOS) family: neuronal (nNOS, NOS1), endothelial (eNOS, NOS3) and inducible (iNOS, NOS2). Different members of NOS family are known to regulate different functions in CNS. NO is able to react with many intracellular targets to trigger a variety of signal transduction pathways, resulting in stimulatory or inhibitory signals. If it was produced in excess, NO would undergo oxidative-reductive reactions, forming the reactive oxygen species, which can cause cellular damage (1). Reactive nitrogen species (RNS) are a group of chemically reactive molecules derived from the nitric oxide, the main members of which are peroxynitrite (ONOO−), S-nitrosothiols (RSNOs) and nitrogen dioxide (NO2). NO2 is a potent oxidizing and nitrating agent, while peroxynitrite is highly reactive NO derivative, capable of reacting with various cell compounds, and directly causing DNA damage.

The initial studies had led to the hypothesis that peroxynitrite, formed in the reaction between NO and superoxide anion, might be responsible for cellular damage in some neurological diseases. The increased superoxide production can cause NO consumption, thus promoting the peroxynitrite formation and inducing the cell death. Peroxynitrite can irreversibly inactivate superoxide dismutase (SOD), increasing the availability of superoxide anion for the reaction with NO. Beside, NO acutely stimulates superoxide production in mitochondria, inhibiting the respiratory chain.

Literature data point out the role of NO in the pathogenesis of MS (3). There is evidence that NO and the inducible form of its synthesizing enzyme are elevated in CNS in both multiple sclerosis (4) and experimental model of multiple sclerosis, namely experimental allergic encephalomyelitis (EAE) (5). It has been reported that high level of NO metabolites is the evidence in blood and cerebrospinal fluid (CSF) of patients with MS, even in the absence of neurological signs of disease (6). Danilov et al. (7) also suggested that high levels of NO and its derivatives, even in disease remission, pinpointed that MS was a chronic inflammatory disease where NO production played an important role, not only in disease onset, but also in MS aggravation. Reactive NO species promote myelin and oligodendrocyte destruction due to its cytotoxic effects on nerve and glial cells (8). Glia cells excrete NO metabolites which interact with the superoxide anions forming the peroxynitrite anion (ONOO−), promoting the nerve cell destruction.

At the same time with the cognition of NO critical role in the inflammatory process, a lot of studies have investigated its participation in the mechanisms of MS onset and development (9). New literature data suggest the increase of NO products in CSF of MS patients (6, 7). NO reactive species (nitrate, nitrite, peroxynitrite), produced as a consequence of activated glia cells, promote nitrosative stress disorders – myelin and nerve tissue destruction. It is supposed that NO provokes block in the action potential transmission, since NO donors increase the emission of disease, which is in agreement with those of Roghani et al. (12).

In normal CNS, there are two constitutively expressed forms of the nitric oxide synthases (eNOS and nNOS), which produce low (nanomolar) concentrations of NO, contributing to the regulation of blood flow and participating in synaptic transmission. During inflammation, owing to appearance of the inducible form of NOS, NO is produced continuously which results in much higher NO concentrations. Demyelinating lesions in the brain of multiple sclerosis patients are associated with the reactive astrocytes expressing iNOS (4). The source of NO, reactive microglia and astrocytes have been implicated in the pathogenesis of the white matter disorders, such as multiple sclerosis through NO-dependent mechanisms. Microglial phagocytosis of myelin debris has been known to activate the secretion of both reactive oxygen species and astrocyte stimulator IL-1β, resulting in peroxynitrite production through a cooperative

Nitric oxide in the experimental autoimmune encephalitis and multiple sclerosis

In CNS, elevated levels of nitric oxide are associated with a number of inflammatory and neurodegenerative diseases, including multiple sclerosis (MS). Proinflammatory cytokines, chemokines and enzymes generated by a number of cells, including microglia, produce NO and reactive oxygen species that promote damage of oligodendrocytes and axons. Literature data point out the role of NO in the pathogenesis of MS (3). There is evidence that NO and the inducible form of its synthesizing enzyme are elevated in CNS in both multiple sclerosis (4) and experimental model of multiple sclerosis, namely experimental allergic encephalomyelitis (EAE) (5). It has been reported that high level of NO metabolites is the evidence in blood and cerebrospinal fluid (CSF) of patients with MS, even in the absence of neurological signs of disease (6). Danilov et al. (7) also suggested that high levels of NO and its derivatives, even in disease remission, pinpointed that MS was a chronic inflammatory disease where NO production played an important role, not only in disease onset, but also in MS aggravation. Reactive NO species promote myelin and oligodendrocyte destruction due to its cytotoxic effects on nerve and glial cells (8). Glia cells excrete NO metabolites which interact with the superoxide anions forming the peroxynitrite anion (ONOO−), promoting the nerve cell destruction.

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mechanism involving both glial cell types. Oxidative damage, mediated particularly by peroxynitrite, is associated with the for mation of reactive intermediates capable of modifying the proteins post-translationally and targeted to the unsaturated membrane lipids, promoting free-radical cascade reactions.

It is well known that inflammatory process in CNS is characterized by the activation of proinflammatory cytokines, i.e. TNF-α and INF-γ which induce iNOS (13). Animal and cellular models of the disease have confirmed this hypothesis. Thus, in the multiple sclerosis mice experimental model of allergic encephalomyelitis, NOS2 is preferentially expressed in either infiltrating macrophages (14) or in microglia of the white matter (15). The increased NO synthesis is demonstrated by powerful iNOS expression within the CNS at the onset and development of EAE clinical signs, followed by decreased expression in remission (16). Thus, the increase of NO production may be expected in disease aggravation, characterized by the intensive inflammation.

NO has been detected by several indirect methods, such as nitrite and nitrate in cerebrospinal fluid (17), immunodetection of nitrosoamino acids in sera (18) or S-nitrosothiols in several proteins of the white matter (8) in patients suffering from this disorder. In coculture of neur ons and astrocytes, Stewart et al. (19) reported that INF-1b pretreatment prevented neurons from mitochondria damages that occur red as a consequence of excessive NO production. So, the limitation of NO and its derivatives production, with the consecutive prevention of neighbour neuron damages, could be the possible mechanism of INF-1b effects in MS. The results of our investigation show that INF-1b therapy decreases plasma level of NO metabolites in treated MS patients (20), which correlates with better clinical findings.

The increased 3-nitrotyrosine (3-NT) concentration, a stable footprint of peroxynitrite injury, has been also documented (21) in plasma of MS patients, showing the correlation with the disease development and intensity, as well as reflecting the patients’ therapy response (14). 3-NT has direct detrimental effects on nerve tissue followed by nerve dysfunction (22). Thus, Drel et al. (23) reported the importance of nitrosative stress in neurological disorders, suggested 3-NT accumulation, not only in nerve cells, but also in all cell types in the peripheral nervous system (endothelial and Schwann cells, astrocytes and oligodendrocytes of the spinal cord, and glial cells of dorsal root ganglia). Our study finding supports the results from the others, showing that the increased 3-NT concentrations are evident in MS patients, but become reduced upon INF-1b treatment, pointing out that 3-NT can be measured in biological materials as a new biomarker of MS disease intensity, and, on the other hand, plasma NT concentration has been shown to correlate with INF-1b therapy efficacy.

Nitric oxide in neurodegenerative diseases

A convergent feature of most neurodegenerative disorders is an excessive generation of reactive nitrogen and oxygen species, which can contribute to neuronal cell injury and death (24). Since many intra- and extracellular molecules may participate in neuron injury, accumulation of nitrosative stress due to excessive generation of NO appears to be a potential factor, contributing to neuron cell damage and death (25). On one hand, an excessive activation of NMDA receptors and the consequent Ca2+ influx activate neuronal NOS, leading to NO generation together with the increased ROS formation due to NADPH oxidase activation and mitochondrial respiration. On the other, in neurodegenerative diseases, as in neural injury, there is an activation of microglia and astrocytes with subsequent release of high concentrations of NO, independent of NMDA receptor activation (26). In sporadic ALS, NO-mediated damage is seen. In ALS dementia, neurons that normally generate and utilize NO suddenly become NO-sensitive and die (27).

NO damages lipids and proteins. Reactive nitrogen species can elicit reversible S-nitrosylation of thiol groups and irreversibly protein tyrosine nitration in target proteins. The protein modifications, caused by high NO concentrations, are mostly reversible. In addition, one of the key cellular roles of NO is reversible protein modification, such as S-nitrosylation, a covalent addition of NO group to cysteine thiols, resulting in the formation of S-nitrosothiol derivative (RSNO). This influences protein activity, protein-protein interactions, protein location, serving as the prototypical redox-based signal. Though RSNO, NO modifies multiple proteins in CNS, alters their structures and functions, either allosterically or by direct modification of an active site cysteine, and promotes dual role in pathophysiological ongoings.

S-nitrosylation and subsequent further oxidation of critical cysteine residues can lead to protein misfolding and mitochondrial dysfunction, the characteristic features of neurodegenerative diseases.

S-nitrosylation and protein misfolding

The relationship between RNS/ROS and protein misfolding is thought to be a pathogenic trigger of neurodegenerative diseases. Under degenerative conditions, RNS may significantly participate in the process of protein misfolding through protein S-nitrosylation, and, possibly, protein nitration. Increased nitrosative/oxidative stress is associated with chaperone and proteasomal dysfunction, resulting in accumulation of misfolded proteins which have tendency to aggregate (28). This affects neuronal connectivity and plasticity and trigger cell death signaling pathways (29). These aggregates may consist of oligomer-
ic complexes of non-native secondary structures and demonstrate poor water solubility. Degenerating brain contains aberrant accumulations of misfolded, aggregated proteins, such as α-synuclein and synphilin-1 in Parkinson’s disease (PD), and β-amyloid (Aβ) and tau in Alzheimer’s disease (AD). Other disorders manifesting protein aggregation include Huntington’s disease, amyotrophic lateral sclerosis and prion disease (30).

S-nitrosylation and mitochondrial dysfunction

Mitochondria are powerhouse in cells because of their vital role in producing energy. In the nerve system, the importance of bioenergetics of mitochondria for highly consuming ATP is that neurons can meet the high energy demands of proper neuronal function. There are two bioenergetic processes in mitochondria – fission and fusion, important for morphology and number regulation. Increased nitrosative stress can elicit dysfunction of mitochondrial bioenergetics, resulting in generation of the excessive mitochondrial fragmentation (31). In AD, it is triggered by oligomeric Aβ via increased NO production, which results in bioenergetic impairment, synaptic damage and neuronal loss (32).

Nitric oxide also binds to cytochrome c oxidase, and is able to inhibit cell respiration in a process that is reversible and in competition with the oxygen. This action can also lead to the release of super oxide anion from the mitochondrial respiratory chain, resulting in peroxynitrite formation. It has been shown that, in brain cells, peroxynitrite persistently inhibits cytochrome c oxidase activity (33). It is considered that this mitochondrial interaction represents a molecular switch for cell signalling pathways involved in the control of physiological functions in brain. These include super oxide- or oxygen-dependent modulation of gene transcription, calcium-dependent cell signalling r esponses, changes in mitochondrial membrane potential or AMP-activated protein kinase-dependent control of glycolysis. The brain cortex (34) of autopsied patients with Alzheimer’s disease displays a loss of cytochrome c activity that is correlated with neuronal dysfunction. In addition, the brain tissue of these patients expresses NOS2 (35), and in vitro incubation of cortical astrocytes with β-amyloid potently promotes NOS2 induction through a mechanism involving the nuclear factor κB (36). Thus, there is a strong correlation between regional-specific NO formation and specific persistent damage to cytochrome c oxidase in this disorder (37). Finally, a correlation has been demonstrated between β-amyloid deposition and protein nitration in the hippocampus of the brain samples of patients with Alzheimer’s disease (38). These results suggest that peroxynitrite formation in Alzheimer’s disease may be responsible for the observed cytochrome c oxidase deficiency, and it may be relevant for understanding the pathogenesis of this disorder, in which it is known to be a profound disruption of energy metabolism (39).

The contributing factor is also decreased oxidative defense. Some authors suggest that, even S-nitrosoglutathione (GSNO) is present in micromolar concentrations in rat brain, it is several fold more potent than GSH against oxidative stress caused by peroxynitrite (40). The most of RSNO production is mediated by S-nitrosoglutathione, thus, GSNO as the most potent modulator of NO bioavailability, regulates CNS NO storage due to reverse decomposition to NO and GSSG in spontaneous or thiols-mediated catalyzed reaction (41). NO reacts with reduced glutathione (GSH), forming GSNO, thus decreasing CNS protein thiol content and changing the redox state (42). That is why cell redox status is fateful for CNS signaling, growth, survival and cell death.

S-nitrosylation in Parkinson’s disease. Parkinson’s disease is the second most prevalent disease characterized by the progressive loss of dopaminergic neurons accompanied with appearance of Lewy bodies, containing misfolded and ubiquitinated proteins, the hallmark of this disease. It has been proved that nitrosative/oxidative stress can promote protein misfolding, leading to aggregation of these misfolded or aberrant proteins (43). Parkin – an E3 ubiquitin ligase that ubiquitinates proteins important for the survival of dopaminergic neurons is S-nitrosylated in the in vivo mouse model of Parkinson’s disease and in the brain of patients with this disorder (44). These results are compatible with reported overproduction of NO in this disorder by NOS2 in the reactive glia, as seen in in vivo substantia nigra of the MPTP (1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine) mice model (45) and in LPS-treated rats (46). An additional support for this notion is provided by observations showing that MPTP and NOS2 induction synergistically promote degeneration of dopaminergic neurons in culture (47). Thus, S-nitrosylation and inactivation of parkin by NO may contribute to pathogenesis of Parkinson’s disease by preventing the degradation of parkin substrates, which accumulation triggers neur degeneration (48, 49).

S-nitrosylation in Alzheimer’s disease. In addition to PD, S-nitrosylation is likely to affect critical thiol groups on other chaperons, such as HSP90 (heat shock protein 90), which normally stabilize misfolded proteins and modulates the activity of cell signalling proteins, including NOS and calcineurin (50). In the Alzheimer’s disease brains, HSP90 levels were increased in both cytosolic and membranous fractions, where HSP90 was thought to maintain tau and Aβ in a soluble conformation, thereby averting their aggregation (51). The study of Martinez-Ruiz (52) provides an explanation of the mechanism by which S-nitrosylation of HSP90 in neur ons of AD brains may contribute to accumulation of tau and Aβ aggregation.
Conclusion

Excessive production of ROS and RNS is thought to be a contributing factor of CNS inflammation and neurodegenerative diseases. Nitric oxide can induce CNS cell death mainly by oxidative/nitrosative stress-induced apoptosis or by ery did deletion-induced necrosis. NO/RNS/ROS can also switch apoptosis to necrosis by inhibiting the caspases due to S-nitrosoylation or oxidation of reactive thiols. In degenerative conditions, by the process of S-nitrosylation, NO induces protein misfolding and mitochondrial dysfunction and fragmentation, leading to synaptic damage and death of neurons.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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Attila Bonert, Aron O. S. Schumacher, and Jürgen Weyermann

300 Stojanović et al.: Nitrite oxide – mediated signalization and nitrosative stress in neuropathology}