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Review article
Pregledni članak

NITRIC OXIDE – MEDIATED SIGNALIZATION AND NITROSATIVE STRESS IN NEUROPATHOLOGY

AZOT OKSID – POSREDOVANA SIGNALIZACIJA I NITROZATIVNI STRES U NEUROPATOLOGIJI

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Summary: Nitric oxide (NO) is an important signalling molecule in a variety of physiological pr ocesses. NO, a gas, is produced from L-arginine by differ ent isoforms of the nitric oxide synthase and ser ves as mediator in important physiological functions, such as pr omoting vasodilation of blood vessels and mediating communication between ner vous system cells. Contradictor y to its physiologic actions, fr ee radical activity of NO can cause cellular damage by the induction of nitr osative stress with significant implications on nervous system diseases. Although the mechanism of NO - mediated neurodegeneration still r emains unclear, numerous studies suggest its cr ucial role in modification of pr otein functions by nitr osylation and nitr o-tyrosination. NO contributes to glutamate excitotoxicity, participates in or ganelle fragmentation, inhibits mitochondrial r espiratory complexes and mobilizes zinc from the internal stores. Recently, NO has been emerged as a mediator of epigenetic gene expr ession and chromatin changes. Besides, NO is a key mediator in the regulation of inflammator y and immune r esponse of the central nervous system. It is involved in down r egulation of several aspects of CNS inflammation, but also has a dual r ole in that it is r equired for inflammation in some situations.

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

Kratak sadr`aj: Azot monoksid je važan signalni molekul u brojnim fiziološkim procesima. Ovaj gas se stvara iz L-arginina dejstvom tri izofor me azot monoksid sintaze i medijator je važnih fizioloških funkcija, kao što su posr edovanje u r egulaciji tonusa kr vnih sudova i komunikaciji ćelija ner vnog sistema. Nasuprot ovim ulogama, kao slobodni radikal, NO može izazvati ćelijsko oštećenje indukcijom nitr ozativnog stresa, što ima značajne implikacije u bolestima ner vnog sistema. Mada je mehanizam neur odegeneracije posredovane azot monoksidom još uvek nerazjašnjen, br ojne studije ukazuju na njegovu ključnu ulogu u modifikaciji funkcije pr oteina kroz procese S-nitrozilacije i nitrovanja tirozina. On doprinosi glutamatnoj ekscitotoksičnosti, učestvuje u fragmentaciji organela, inhibiše mitohondrijalne r espiratorne komplekse i mobiliše cink iz unutrašnjih depoa. Nedavno je ukazano da može biti medijator epigenetske genske ekspr esije i promena hromatina. Pored toga, NO je ključni posr ednik u r egulaciji inflamatornog i imunog odgovora CNS. On učestvuje u nishodnoj regulaciji nekoliko aspekata inflamacije CNS, ali takođe ispoljava dualističke efekte u uslovima inflamacije.

Klju~ne re~i: azot monoksid, nitrozativni stres, S nitrozilacija proteina, neuroinflamacija, neurodegeneracija

Introduction

Nitric oxide (NO), a gaseous signalling molecule, regulates a diverse range of physiological processes, including the r egulation 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 (1).

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However, NO has been also viewed as a major agent of neuropathology when, escaping the controlled production, it may dir ectly or indir ectly promote oxidative and nitrosative stress. The exact bor derline between physiological, and ther efore neuroprotective, and pathological, and therefore neurodegenerative, actions of NO is a matter of contr oversy 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-nitr osylation, NO may switch apoptosis to necrosis. Due to its multifaceted functions in CNS, NO attracts the attention of investigators as a neur omodulator, 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 ar e known to r egulate different functions in CNS. NO is able to r eact with many intracellular targets to trigger a variety of signal transduction pathways, r esulting in stimulator y or inhibitor y signals. If it was pr oduced in excess, NO would un dergo oxidative-reductive reactions, forming the reactive oxygen species, which can cause cellular damage (2). Reactive nitrogen species (RNS) ar e a gr oup of chemically reactive molecules derived fr om the nitric oxide, the main members of which ar e peroxynitrite ($ONOO^-$), S-nitrosothiols (RSNOs) and nitrogen dioxide (NO2). NO2 is a potent oxidizing and nitrating agent, while peroxynitrite is highly r eactive NO derivative, capable of r eacting 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 r esponsible for cellular damage in some neur ological diseases. The in creased superoxide production can cause NO consumption, thus promoting the per oxynitrite formation and inducing the cell death. P eroxynitrite can ir reversibly inactivate superoxide dismutase (SOD), incr easing the availability of super oxide anion for the r eaction with NO. Beside, NO acutely stimulates super oxide production in mitochondria, inhibiting the r espiratory chain.

Nitric oxide in the experimental autoimmune encephalitis and multiple sclerosis

In CNS, elevated levels of nitric oxide ar e associated with a number of inflammator y and neur odegenerative diseases, including multiple sclerosis (MS). Proinflammatory cytokines, chemokines and enzymes generated by a number of cells, including microgliae, produce NO and r eactive oxygen species that pr omote damage of oligodendr ocytes 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 ar e elevated in CNS in both multiple scler osis (4) and experimental model of multiple scler osis, namely experimental allergic encephalomyelitis (EAE) (5). It has been reported that high level of NO metabolites is the evidence in blood and cer ebrospinal 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 chr onic inflammatory disease wher e NO pr oduction played an important role, not only in disease onset, but also in MS aggravation. R eactive NO species pr omote myelin and oligodendr ocytes 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 inflammator y process, a lot of studies have investigated its participation in the mechanisms of MS onset and development (9). New literatur e data suggest the incr ease of NO pr oducts in CSF of MS patients (6, 7). NO r eactive species (nitrate, ni trite, peroxynitrite), produced as a consequence of activated glia cells, pr omote nitrosative stress disorders – myelin and ner ve tissue destruction. It is supposed that NO provokes block in the action potential transmission, since NO donors occur as a r eversible blocking agents in nor mal and demyelinating conditions, not only in the central, but also in the peripheral nerve system (10). This could be the r esult of direct NO effects on glutamatergic transmission, since it has been reported to induce N-methyl-D -aspartate (NMDA) up regulation due to nitr osylation processes (11). The results, discussed here, suggest the increased NO plasma levels even in the r emission of disease, which is in agreement with those of Roghani et al. (12).

In normal CNS, ther e are two constitutively ex pressed forms of the nitric oxide synthases (eNOS and nNOS), which produce low (nanomolar) concentrations of NO , contributing to r egulation 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 scler osis patients are associated with the reactive astrocytes expressing iNOS (4). The sour ces of NO , reactive microglia and astrocytes have been implicated in the pathogenesis of the white matter disor ders, 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

mechanism involving both glial cell types. Oxidative damage, mediated particularly by peroxynitrite, is associated with the formation of reactive intermediates capable of modifying the proteins post-translationally and targeting 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 i 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 profusely 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 neurons and astrocytes, Stewart et al. (19) reported that INF 1b pretreatment prevented neurons from mitochondria damages that occurred 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 INFβ1b 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 INFβ1b treatment, pointing out that 3-NT can be measured in biological materials as new biomarker of MS disease intensity, and, on the other hand, plasma NT concentration has been shown to correlate with INFβ1b therapy efficacy.

Nitric oxide in neurodegenerative diseases

A convergent feature of most neurodegenerative disorders is an excessive generation of the reactive nitrogen and oxygen species, which can contribute to neuronal cell injury and death (24). Since many intra- and extracellular molecules may participate in neuronal injury, accumulation of nitrosative stress due to excessive generation of NO appears to be a potential factor, contributing to neuronal cell damage and death (25). On one hand, an excessive activation of NMDA receptors and the consequent Ca^{++} 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 irreversible protein tyrosine nitration in target proteins. The protein modifications, caused by high NO concentrations, are mostly irreversible. 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 thiol, 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. Through 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 secondar y 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 disor ders 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 r ole in pr oducing energy. In the ner ve system, the importance of bioenergetics of mitochondria for highly consuming A TP is that neur ons can meet the high ener gy demands of pr oper neuronal function. There are two bioener getic 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 pr oduction, which results in bioener getic impairment, synaptic damage and neuronal loss (32).

Nitric oxide also binds to cytochr ome c oxidase, and is able to inhibit cell r espiration in a process that is reversible and in competition with the oxygen. This action can also lead to the r elease of super oxide anion from the mitochondrial r espiratory chain, resulting in peroxynitrite formation. It has been shown that, in brain cells, per oxynitrite persistently inhibits cytochrome c oxidase activity (33). It is consider ed that this mitochondrial interaction r epresents a molecular switch for cell signalling pathways involved in the contr ol 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 cor related with neuronal dysfunction. In addition, the brain tissue of these patients expr esses NOS2 (35), and *in vitro* incubation of cortical astr ocytes with β-amyloid potently promotes NOS2 induction thr ough a mechanism involving the nuclear factor κB (36). Thus, there is a str ong correlation between regional-specific NO for mation and specific persistent damage to cytochrome c oxidase in this disor der (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 r esults suggest that per oxynitrite formation in Alzheimer's disease may be r esponsible for the obser ved cytochrome c oxidase deficiency , and it may be r elevant for understanding the pathogenesis of this disor der, in which it is known to be a profound disruption of energy metabolism (39).

The contributing factor is also decr eased oxidative defense. Some authors suggest that, even S-nitrosoglutathione (GSNO) is pr esent in micr omolar concentrations in rat brain, it is several fold mor e potent than GSH against oxidative str ess caused by peroxynitrite (40). The most of RSNO pr oduction is mediated by S-nitrosoglutathione, thus, GSNO as the most potent modulator of NO bioavailability , regulates CNS NO storage due to r everse decomposition to NO and GSSG in spontaneous or thiols-mediated catalyzed reaction (41). NO r eacts with reduced glutathione (GSH), forming GSNO, thus decreasing CNS protein thiol content and changing the r edox state (42). That is why cell r edox status is fateful for CNS signaling, growth, survival and cell death.

S-nitrosylation in Parkinson's disease. Parkinson's disease is the second most pr evalent disease characterized by the pr ogressive loss of dopaminer gic neurons accompanied with appearance of Lewis bodies, containing misfolded and ubiquitinated pr oteins, the hallmark of this disease. It has been pr oved that nitrosative/oxidative stress can pr omote protein misfolding, leading to aggr egation of these misfolded or aberrant proteins (43). Parkin – an E3 ubiquitin ligase that ubiquitinates pr oteins important for the sur vival of dopaminergic neurons is S-nitr osylated in the *in vivo* mouse model of P arkinson'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 r eactive 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-tr eated rats (46). A dditional support for this notion is pr ovided by obser vations showing that MPTP and NOS2 induction syner gistically promote degeneration of dopaminer gic neurons in cultur e (47). Thus, S-nitr osylation 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 odegeneration (48, 49).

S-nitrosylation in Alzheimer's disease. In addition to PD, S-nitr osylation is likely to affect critical thiol groups on other chaper ons, 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 wer e 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-nitr osylation 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 energy deletion-induced necrosis. NO/RNS/ROS can also switch apoptosis to necrosis by inhibiting the caspases due to S-nitrosylation 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.

References

1. Guix FX, Uribesaigo I, Coma M, Munoz FJ. The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 2005; 76: 126–52.
2. Patcher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007; 87: 315–24.
3. Ibragić S, Sofić E, Suljić E, Avdagić N, Bajraktarević A, Tahirović I. Serum nitric oxide concentrations in patients with multiple sclerosis and patients with epilepsy. J Neural Transm 2011; 119(1): 7–11.
4. Bagasra O, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Fu ZF et al. Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. Proc Natl Acad Sci USA 1995; 92(26): 12041–5.
5. Cross AH, Keeling RM, Goorha S, San M, Rodi C, Wyatt PS, et al. Inducible nitric oxide synthase gene expression and enzyme activity correlate with disease activity in murine experimental autoimmune encephalomyelitis. J Neuroimmunol 1996; 71(1–2): 145–53.
6. Rejdak K, Petzold A, Stelmasiak Z, Giovanoni G. Cerebrospinal fluid proteins in relation to nitric oxide metabolites during relapse of multiple sclerosis. Mult Scler 2008; 14(1): 59–66.
7. Danilov AI, Andersson M, Bavand N, Wiklund NP, Olsson T, Brundin L. Nitric oxide metabolite determinations reveal continuous inflammation in multiple sclerosis. J Neuroimmunol 2003; 136: 112–8.
8. Bizzozero O, DeJesus G, Bixler HA, Pastuszyn A. Evidence of nitrosative damage in the brain white matter of patients with multiple sclerosis. Neurochem Res 2005; 30(1): 139–49.
9. Encinas JM, Manganas L, Enikolopov G. Nitric oxide and multiple sclerosis. Curr Neurol Neurosci Rep 2005; 5(3): 232–8.
10. Redford EJ, Kapoor R, Smith KJ. Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. Brain 1997; 120: 2149–57.
11. Broillet MC. S-nitrosylation of proteins. Cell Mol Life 1996; 55: 1036–42.
12. Roghani M, Mahboudi F, Saharian MA, Etemadifar M, Esfahani AN, Nahrevanian H et al. Concentrations of nitric oxide metabolites in the serum of Iranian multiple sclerosis patients. J Neurol Sci 2010; 294(1–2): 92–4.
13. Stewart WC, Giovannoni G, Land JM, McDonald I, Clark JB, Heales JR. Pretreatment of astrocytes with interferon-β impairs interferon–γ induction of nitric oxide synthase. J Neurochem 1998; 68: 2547–51.
14. Okuda Y, Nakatsuji Y, Fujimura H, Esumi H, Ogura T, Yanagihara T et al. Expression of the inducible isoform of nitric oxide synthase in the central nervous system of mice correlates with the severity of actively induced experimental allergic encephalomyelitis. J Neuroimmunol 1995; 62(1): 103–12.
15. Garthwaite G, Batchelor AM, Goodwin DA, Hewson AK, Leeming K, Ahmed Z et al. Pathological implications of iNOS expression in central white matter: an ex vivo study of optic nerves from rats with experimental allergic encephalomyelitis. Eur J Neurosci 2005; 21(8): 2127–35.
16. Ljubisavljević S, Stojanović I, Pavlović R, Stojnev S, Stevanović I, Sokolović D, Pavlović D. The reduced glutathione and S-nitrosothiols levels in acute phase of experimental demyelination – pathophysiological approach and possible clinical relevancy. Neuroscience 2012; doi.10.1016/j.neuroscience.2012.05.062
17. Johnson AW, Land JM, Thompson EJ, Bolaños JP, Clark JB, Heales SJ. Evidence for increased nitric oxide production in multiple sclerosis. J Neurol Neurosurg Psychiatry. 1995; 58(1): 107.
18. Boullerne AI, Rodrıguez JJ, Touil T, Brochet B, Schmidt S, Abrous NR, Le Moal M, Pua JR, Jensen MA, Mayo W, Arnason BGW, Petry KG (2002), Anti-S-Nitrosocysteine antibodies are a predictive marker for demyelination in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neurosci 22 (1): 123–132.
19. Stewart WC, Land JM, Clark JB, Heales JR. Pretreatment of astrocytes with interferon-β prevents neuronal mitochondrial respiratory chain damage. J Neurochem 1997; 70: 432–4.
20. Stojanović I, Vojinović S, Ljubisavljević S, Pavlović R, Basić J, Pavlović D, Ilić A, Cvetković T, Stukalov M. IFN-β1b therapy modulates L-arginine and nitric oxide metabolism in patients with relapse remittens multiple sclerosis. (in press).
21. Miller E, Walczak A, Saluk J, Ponczek MB, Majsterek I. Oxidative modification of patient's plasma proteins and its role in pathogenesis of multiple sclerosis. Clin Biochem 2011; 45 (1–2): 26–30.

22. Foster MW, McMahon TJ, Stamler JS. S-nitr osylation in health and disease. Trends Mol Med 2003; 9: 160–68.

23. Drel VR, Lupachyk S, Shevalye H , Vareniuk I, Xu W , Zhang J et al. New Therapeutic and Biomarker Discovery for Peripheral Diabetic Neur opathy: PARP Inhibitor, Nitrotyrosine, and Tumor Necrosis Factor-α. Endocrinology 2010; 151(6): 2547–55.

24. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006; 443: 787–95.

25. Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat R ev Drug Discov 2006; 5: 160–70.

26. Cassina P, Peluffo H, Pehar M, Martinez-Palma L, Ressia A, Beckman J et al. P eroxynitrite triggers a phenotypic transformation in spinal cord astrocytes that induces motor neuron apoptosis. J Neurosci Res 2002; 67: 21–9.

27. Estevez AG, Spear N, Manuel SM, R adi R, Henderson CE, Barieto et al. Nitric oxide and super oxide contribute to motor neuron apoptosis induced by trophic factor deprivation. J Neurosci 1998; 18(3): 923–31.

28. Zhang K, Kaufman RJ. The unfolded protein response: a stress signaling pathway critical for health and disease. Neurology 2006; 66: S102–9.

29. Muchowski PJ, Wacker JL. Modulation of neur odegeneration by molecular chaperones. Nat Rev Neurosci 2005; 6:11–22.

30. Bence NF, Sampat RM, K opito RR. Impair ment of the ubiquitin-proteasome system by pr otein aggregation. Science 2001; 292: 1552–5.

31. Barsoum MJ, Yuan H, Gerencser AA, Liot G, Kushnareva Y, Graber S et al. Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. EMBO J 2006; 25: 3900–11.

32. Cho DH, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z et al. S-Nitr osylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. Science 2009; 324: 102–5.

33. Bolaños JP, Heales SJR, Land JM, Clark JB. Effect of peroxynitrite on the mitochondrial r espiratory chain: differential susceptibility of neurones and astrocytes in primary cultures. J Neurochem 1995; 64: 1965–72.

34. Davis RE, Miller S, Herrnstadt C, Ghosh SS, Fahy E, Shinobu LA, Galasko D et al. Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc. Natl A cad. Sci. USA 1997; 94: 4526–31.

35. Haas J, Storch-Hagenlocher B, Biessmann A, Wildemann B. Inducible nitric oxide synthase and ar gininosuccinate synthetase: co-induction in brain tissue of patients with Alzheimer's dementia and following stimulation with b-amyloid 1–42 in vitro. Neurosci Lett 2002; 322: 121–5.

36. Akama KT, Van Eldik LJ. β-Amyloid stimulation of inducible nitric-oxide synthase in astrocytes is interleukin-1β and tumor necrosis factor-α (TNFα)-dependent, and involves a TNF-α receptor-associated factor- and NF-κB-inducing kinase-dependent signaling mechanism. J Biol Chem 2000; 275: 7918–24.

37. Keil U, Bonert A, Marques CA, Scherping I, Weyermann J, Strosznajder JB et al. Amyloid b-induced changes in nitric oxide production and mitochondrial activity lead to apoptosis. J Biol Chem 2004; 279: 310–20.

38. Sultana R, Perluigi M, Butter field DA. Protein oxidation and lipid per oxidation in brain of subjects with Alz - heimer's disease: insights into mechanism of neur odegeneration from redox proteomics. Antioxid Redox Signal 2006; 8(11–12): 2021–37.

39. Mattson MP. Pathways towards and away fr om Alzheimer's disease. Nature 2004;430: 631–9.

40. Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a cur rent perspective. Trends Mol Med 2009; 15: 391–404.

41. Liu L, Yan Y, Zeng M, Zhang J, Hanes MA, Ahear n G et al. Essential roles of S-nitrosothiols in vascular homeostasis and endotoxic shock. Cell 2004; 116: 617–28.

42. Ljubisavljević S, Stojanović I, P avlović D, Sokolović D, Stevanović I. Aminoguanidine and N- Acetyl-Cysteine suppresses oxidative and nitr osative stress in EAE rat brains. Redox Rep 2011; 16 (4): 166–72.

43. Gu Z, Nakamura T, Lipton SA. Redox reactions induced by nitrosative stress mediate protein misfolding and mitochondrial dysfunction in neur odegenerative diseases. Mol Neurobiol 2010; 41: 55–72.

44. Chung KK, Thomas B, Li X, Pletnikova O , Troncoso JC, Marsh L, Dawson VL, Dawson TM. S-nitr osylation of parkin regulates ubiquitination and compromises parkin's protective function. Science 2004; 304, 1328–31.

45. Wu DC, Jackson-Lewis V , Vila M, Tieu K, T eismann P, Vadseth C et al. Blockade of micr oglial activation is neuroprotective in the L -methyl-4-phe- nyl-1,2,3,6-tetrahydropyridine mouse model of P arkinson disease. J. Neu - rosci 2002; 22: 1763–71.

46. Iravani MM, K ashefi K, Mander P , Rose S, Jenner P . Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 2002; 110, 49– 58.

47. Gao HM, Liu B, Zhang W, Hong JS. Synergistic dopaminergic neurotoxicity of MPTP and inflammagen lipo - polysaccharide: relevance to the etiology of P arkinson's disease. FASEB J. 2003; 17, 1957–59.

48. Goldberg D, Soleas G. Wine and health: a paradigm for alcohol and antioxidants, Jour nal of Medical Bio chemistry 2011; 30: 93–102.

49. Hübner-Woźniak E, Okecka-Szyman´ska J, Stupnicki R, Malara M, K ozdroń E. Age-Related blood antioxidant capacity in men and women. Jour nal of Medical Bio - chemistry 2011; 30: 103–8.

50. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci 2005; 6: 11–22.

51. Dou F, Netzer WJ, T anemura K. Chaper ones increase association of tau pr otein with micr otubules. Proc Natl Acad Sci USA 2003; 100: 721–6.

52. Martinez-Ruiz A, Villanueva L, Gonzalez de Orduna C. S-nitrosylation of Hsp90 pr omotes the inhibition of its ATPase and endothelial nitric oxide synthase r egulatory activities. Proc Natl Acad Sci USA 2005; 102: 8525–30.

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