# NITRIC OXIDE IN NEURODEGENERATIVE DISEASES

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## 1. ABSTRACT

Nitric oxide (NO) free radical as a major signaling molecule in nervous systems has been shown to have close relationship with neurodegenerative diseases. The results about the relation of NO and neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and stroke in recent years are reviewed. Particularly, the results of antioxidants and NO in neurodegenerative disorders from author's own laboratory are included in this paper.

## 2. INTRODUCTION

Nitric oxide (NO) is a simple gas with free radical properties. It appears as a major signaling molecule in cardiovascular, immune and nervous systems (1, 2, 3, 4, 5). NO is generated by three isoforms of NO synthase (NOS), endothelial NO synthase (eNOS), neuronal NO synthase (nNOS) and inducible NO synthase (iNOS) in different cells (6, 7) and it is known to be an important physiological molecule in the regulation of blood pressure, vascular tone, however, excessive NO produced by iNOS results in inhibition of cardiac contractility, impairment of mitochondrial respiration and apoptosis (8, 9). NO has been shown to influence neurotransmitter release and synaptogenetic processes, modulate the synaptic plasticity (10, 5), indicating that NO plays an important role in the development, maintenance and regulation of brain circuits. Increasing evidences indicate that if there is excessive NO, it may damage neurons and even cause neurodegenerative diseases, such as AD, PD and stroke (11). In this paper, I reviewed the results about this project in my laboratory and other publications in recent year.

## 3. EFFECT OF NO ON NEURONS

The effects of NO on neuron cells show a typical "sword with two edges". In the central nervous system (CNS), NO is produced enzymatically in postsynaptic structures in response to the activation of the excitatory amino acid receptor (12). It plays multiple roles in the developing and mature brain (13, 14), including the synaptic modulation, the learning and memory, the neurotoxicity and the neuron death (13, 15, 16).

We studied the developmental expression and activity variation of NOS in the brain of golden hamster. The expression of NOS during the postnatal development of the visual cortex was investigated by both electron spin resonance (ESR) and Western blot methods. A typical NO signal was found in the visual cortex of different age golden hamsters by ESR technique. The signal intensity increased after birth, peaked at postnatal day 14 (PD14) and then gradually decreased. An analysis of variance (ANOVA) implied that the NOS expression significantly correlated with the developmental processes. Results of Western blot further confirmed the developmental relating expression pattern of NOS shown by ESR technique (5). Apoptosis is now recognized as a normal feature in the development of the nervous system and also plays a role in neurodegenerative diseases and aging. Then we studied the role of NO in the developing visual cortex of the golden hamster. We used an inhibitor of NOS, N-nitro-L-arginine (L-NNA) to block the NOS activity of newborn golden hamster and it was found that L-NNA treatment caused an increasing of mortality and suppression on both body weight gain and nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity in the early phase of treatment (before postnatal day 14, PD14). The growth of NADPH-d positive neurons was also suppressed by the treatment. Massive apoptotic neurons were detected on PD 14 and apoptosis mainly affected cells in layer II-III. NOS inhibition largely rescued neurons from going apoptosis, indicating that NO may serve as a signal triggering apoptosis and play a role in the maturation of the visual cortex.

Exposure of neuronal cells to NO donor, S-nitrosoglutathione (GSNO, 250 M) or sodium nitroprusside (SNP, 500 M) induced apoptosis in immature cultures of cerebellar granule cell, which was characterized by chromatin condensation, nuclear fragmentation and DNA laddering. Exposure of neuronal

cells to NO donor let to a decrease in the mitochondrial transmembrane potential and intracellular ATP content, which suggested that NO treatment caused mitochondrial dysfunction (9). While pretreatment with free radical scavengers L-ascorbic acid 2-[3,4-dihydro- 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecy)-2H-1- benzopyran-6yl-hydrogen phosphate] potassium salt (EPC-K1), a combination of vitamin E and vitamin C, attenuated NO induced mitochondrial dysfunction and oxidative stress and prevented the cells from apoptosis effectively. This result suggests that a peroxynitrite mediated oxidative stress may be an important pathway leading to NO-associated neuronal damage and antioxidant EPC-K1 attenuated NO induced neurotoxicity by scavenging the ROS and its breakdown products (17). EPC-K1 was a moderate scavenger on hydroxyl radicals and alkyl radicals, a potent scavenger on lipid radicals, and an effective inhibitor on lipid peroxidation. EPC-K1 could react with hydroxyl radicals with a rate constant of $7.1 \times 10^8$ dm$^3$mol$^{-1}$ s$^{-1}$ and react with linoleic acid radicals with a rate constant of $2.8 \times 10^6$dm$^3$mol$^{-1}$s$^{-1}$. After administration of EPC-K1, the ability of rat brain to scavenge superoxide radicals was significantly increased. The potent scavenging effects of EPC-K1 on both hydrophilic and hydrophobic radicals were relevant with its molecular structure, which consisted of both hydrophilic and hydrophobic groups (18).

The convergence between work devoted to nitric oxide, apoptosis and exitotoxicity shed light on our understanding of the pathophysiology of brain ischemia, neurodegeneration and acute or chronic inflammation. Excessive and uncontrolled production of nitric oxide through a Ca$^{2+}$-independent and iNOS by astrocytes or microglia is associated with these disorders (19, 20). In addition, NO and its metabolites were found to induce apoptotic cell death in primary neurons, as well as in established neuronal cell lines (21, 22). The execution mechanisms of NO induced apoptosis are multifactor, among which oxidative stress is considered one mediator (23, 17, 9). It may serve as an inducer and also participate in the apoptotic signal pathway. According to the high oxygen consumption and low glutathione content, the central nervous system is particularly vulnerable to oxidative stress (24, 25, 26). Another mediator of the cytotoxic effects of NO is inhibition of mitochondria respiration. NO can cause inhibition of cytochrome oxidase (27, 28), a sensitive enzyme of electron transport chain (ETC), and lead to an increased formation of mitochondrial superoxide, exacerbation of cellular oxidative stress and potential mitochondrial damage (29). The ETC damage may lead to a loss of membrane potential, ATP deficiency, opening of the permeability transition pore and the release of factors capable of initiating apoptosis (30). The redox status of the cell is one of the key factors modulating the apoptotic pathway in which glutathione (GSH) plays a critical role. GSH can protect cells against damage elicited by NO due to its reactive ability with NO and its metabolites (29). The proteins of Bcl-2 family also involve in intracellular apoptotic signal transduction and oxidative damage/antioxidant protection (31, 32, 33). Bcl-2 and related proteins are known to regulate levels of ROS or their intermediates in cells, which is one possible

mechanism of anti-apoptosis. Overexpression of Bcl-2 can exert an anti-oxidative effect by raising SOD activity and GSH levels, diminishing ONOO$^-$ formation, and increasing proteasome function (34). It also rescues neurons from apoptosis and hypertrophys the nervous system (35, 36). On the other hand, it was reported that Bcl-2 was down regulated in NO-induced apoptosis (37).

The neurode-ROSs and RNSs are generated under normal cellular functioning, mainly during mitochondrial respiration, and are deactivated by endogenous antioxidants and scavengers. Various substances in high concentration can enhance the production of toxic free radicals, such as intracellular Ca$^{2+}$ and NOS (38, 39). Activation of NOS results in the increased formation of NO, which can then react with superoxide anion to form peroxynitrite. Peroxynitrite can activate the enzyme poly ADP ribosyl synthase (PARS), which goes on to polyribosylate proteins with ADP which can lead to the depletion of ATP (40, 41). The oxidation of membrane lipids (lipid peroxidation) by free radicals produces a cytotoxic byproduct, 4-hydroxynonenal (HNE), which has recently been identified as a mediator of oxidative stress-induced neuronal cell death (42, 43, 44).

## 4. NO AND AD

AD is a neurodegenerative disorder characterized by the presence of senile plaques and neurofibrillary tangles in the brain accompanied by progressive loss of synapses, neurons, and cognitive function (45, 46). An increasing amount of evidence has shown that oxidative reactions occur in AD and that β-amyloid (Aβ) may be one molecular link between oxidative stress and AD-associated brain dysfunction (47, 48, 49, 50). It has been demonstrated that aggregation of Aβ can attract inflammatory mediators (51) which, in turn, generate NO radicals (52). Together with inflammatory mediators, Aβ also stimulates NO production and expression of the high-output isoform, iNOS, in mouse microglia or in rat astrocytes. This Aβ-induced iNOS expression can result in an overproduction of NO, which may react with superoxide free radical to yield highly reactive peroxynitrite and may, therefore, increase the overall radical burden in Aβ-loaded brain regions (53). The widespread occurrence of peroxynitrite can lead to damage of neuronal cells in AD and nitration of synaptic proteins (54), thus affecting signal transduction pathways of cellular regulation.

Although extensive genetic data implicate Aβ in the neurodegenerative cascade of AD, the molecular mechanisms underlying its effects on neurons and glia and the relationship between glial activation and neuronal death are not well defined. It is therefore crucial to identify specific Aβ-induced molecular pathways mediating these responses in activated glia. Akama *et al.* report that Aβ stimulates the activation of the transcription factor NFκB in rat astrocytes, that NFκB activation occurs selectively from p65 transactivation domain 2, and that Aβ-induced NOS expression and NO production occur through an NFκB-dependent mechanism. This demonstration of how Aβ couples an intracellular signal transduction pathway

involving NFκB to a potentially neurotoxic response provides a key mechanistic link between Aβ and the generation of oxidative damage. These results also suggest possible molecular targets upon which to focus future drug discovery efforts for AD (52).

In AD, Aβ plaques are surrounded by activated astrocytes and microglia. A growing body of evidence suggests that this activated glia contribute to neurotoxicity through the induction of inflammatory cytokines such as interleukin (IL)-1b and tumor necrosis factor-α (TNFα) and the production of neurotoxic free radicals, mediated in part by the expression of iNOS. Akama and Eldik address the possibility that Aβ-stimulated iNOS expression might result from an initial induction of IL-1b and TNFα. They found that in Aβ-stimulated astrocyte cultures, IL-1b and TNFα production occurred before iNOS production, new protein synthesis was required for increased iNOS mRNA levels, and the IL-1 receptor antagonist IL-1ra could inhibit nitrite accumulation. Likewise, dominant-negative mutants of tumor necrosis factor-a receptor-associated factor (TRAF) 6, TRAF2, and NFκB inducing kinase (NIK), intracellular proteins involved in IL-1 and TNFα receptor signaling cascades, inhibit Aβ-stimulated iNOS promoter activity. These data suggest that Aβ stimulation of astrocyte iNOS is mediated in part by IL-1β and TNFα, and involves a TRAF6-, TRAF2-, and NIK-dependent signaling mechanism (53).

## 5. NO AND PD

PD is a progressive neurodegenerative disorder and the hallmark of this disease is selective loss of dopaminergic neurons in the substantia nigra pars compacta (55). Recently, the death of dopaminergic neurons has been reported to occur by apoptosis (56). Some researchers found that the elevated expression of iNOS in PD brain (57) and the involvement of NO in the pathogenesis of PD, and both iNOS and nNOS were included (58, 59).

MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can induce Parkinson's disease in animals (60, 61, 62). It was found that in MPTP treated mice, dopamine nerve terminals were destroyed almost completely in the substantia nigra pars compacta and TH-immunostained cells were diminished compared with those control animals. In both water-treated control and MPTP groups, nNOS proteins in the substantia nigra were increased compared with that of nothing-treated intact groups. In mice (60, 61) and baboons (63), inhibition of neuronal NO synthase (nNOS) prevents PD induced by MPTP. In addition, mutant mice lacking iNOS gene are significantly more resistant to MPTP than their wild type littermates. NOS was inhibited by 7-nitroindozole (7-NI) in a dose and time-dependent fashion when MPTP was injected into mice. 7-NI dramatically protected MPTP-injected mice against indices of severe injury to the nigrostriatal dopaminergic pathway, including reduction in striatal dopamine contents, decreases in numbers of nigral tyrosine hydroxylase-positive neurons, and numerous silver-stained degenerating nigral neurons. The resistance of 7-NI injected mice to MPTP is not due to alterations in striatal pharmacokinetics or content of MPP$^+$, the active metabolite of MPTP. To study specifically the role of neuronal NOS, MPTP was administered to mutant mice lacking the nNOS gene. Mutant mice are significantly, more resistant to MPTP-induced neurotoxicity compared with wild-type littermates (59).

Barthwal *et al.* investigated the NOS activity in the striatum following 6-OHDA induced neurodegeneration in rats. eNOS activity remained unaltered at 3,7 and 14 days after lesion, while a 43% and 45% decrease was observed at 30 and 50 days, respectively. iNOS activity was detected only on the 3$^{rd}$ days after lesion and not in subsequent days or the control striatum. The inhibitor of NOS, L-NAME pretreatment blocked the amphetamine-induced rotations and inhibited the iNOS activity at the 3$^{rd}$ day after the 6-OHDA injection. L-NAME pretreatment also significantly restored the striatal dopamine, dihydroxyphenyllacetic acid (DOPAC) and homovanillic acid (HVA) levels in 6-OHDA treated rats (64). Gomes and Del Bel investigated the degenerative changes in NO system of basal ganglia in animals with experimentally induced PD. In one procedure, rats were stereotaxically injected with 6-ODA in the right medial forebrain bundle; in another procedure, electrodes were implanted in the right substania nigra pare compacta (SNc). After 15 and 30 days animals were tested for rotational asymmetry induced by apomorphine. Apomorphine induced rotation in lesioned animals, towards the ipsilateral side after electrolytic lesion and towards contralateral side in 6-OHDA animals. Structural deficits in basal ganglia were quantified by NADPH-d histochemistry and by NOS immunoreactivity. 6-OHDA and electrolytic lesions induced a significant decrease in the number of NADPH-d/NOS positive cells in the lesion ipsilateral to SNc, in contrast with cell number increase in the ipsilateral dorsal striatum. By contrast, 6-OHDA-treated animals showed a decrease in the number of NOS immunoreactive cells in the contralateral nucleus accumbens. They conclude that populations of NO-synthesizing neurons are differentially regulated in PD induced by different experimental procedures (65).

We investigated the level of intracellular NO in SH-SY5Y cells after treated with 6-OHDA using fluorescence technique and it was found that treatment with 6-OHDA resulted in a 1.2-fold increase of NO in the cells. The expression of nNOS was detected by Western blot, using specific antibodies and it was found that 6-OHDA induced an up-regulation of nNOS. It was found that exposure of SY5Y cells to 100μM 6-OHDA for 24h led to a significant increase the level of $H_2O_2$ and resulted in a 2.4-fold elevation of $[Ca^{2+}]_i$ in the cells respectively.

Not all evidences demonstrate NO related neuron toxicity in 6-OHDA –induced cell apoptosis. Ha *et al.* reported about the protective effect of NO on 6-OHDA induced apoptosis of PC12 cells (66). They demonstrated that NO, produced either from the NO donor S-nitroso-N-acetyl-d,1-penicillamine(SNAP) or by transfection of neuronal NOS, suppressed 6-HODA induced apoptosis in PC12 cells by inhibiting mitochondrial cytochrome c release, caspase-3 and 9 activation, and DNA

fragmentation. This protection was significantly revised by the soluble guabyl cyclase inhibitor 1H-(1,2,4)-oxadiazole[4,3-a]quinoxalon-1-one, indicating that cGMP is a key mediator in NO-mediated anti-apoptosis. Moreover, the membrane-permeable cGMP analog 8-Br-cGMP inhibited 6-OHDA induced apoptosis. These anti-apoptotic effects of SNAP and 8-Br-cGMP were suppressed by cGMP-dependent protein kinase G (PKG) inhibitor KT5823, indicating that PKG is a downstream signal mediator in the suppression of apoptosis by NO and cGMP.

## 6. NO AND STROKE

Stroke is a major cause of death and disability in the world; it is the third leading cause of death and the primary cause of long-term disability in adult. Therefore, there is a great interest in the basic mechanisms by which ischemia/reperfusion cause damage and in the prevention of the disease. ROS generated from the respiratory chain in mitochondria, ischemia-activated xanthine/hypoxanthine oxidase and lipid fatty acid metabolism play an important role in the brain ischemia/reperfusion process (67, 68). Due to the high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids, relatively low antioxidant capacity, low repair activity and non-replicating nature of the neuronal cells, the brain is very susceptible to the damage caused by oxygen radicals (69). The burst in production of ROS results in damage to cellular proteins, lipid and DNA. In the cerebral circulation system, the burst in production of ROS damages the endothelium cell and smooth muscle cell, induces blood platelet aggregation and vascular permeability changes, and results in edema (70).

There two kinds of results about the effects of NO in stroke, one reported the potential destructive mechanisms of nitric oxide (54, 71, 72) but other reported the potential protective mechanisms (73, 74, 75). In first group, they suggest that NO promotes oxidative damage by avidly combining with superoxide anion to form peroxynitrite, a strong oxidant. Furthermore, NO induces loss of iron from the cell thereby making iron available for free radical generation via the Fenton reaction and facilitating lipid peroxidation. The purported mechanisms of the effect involve binding of NO to the Fe-S cluster of the iron response element binding protein (IRE-BP), the cytoplasmic homoLog of the mitochondrial enzyme aconitase. NO, or a NO oxidation product, binds to the cluster activating IRE-BP which, in turn leads to iron loss. NO can inhibit cellular energy production by different mechanisms. NO, or derived species, inhibit the glycolytic enzyme glyceraidehyde. 3-phosphate dehy drogenase (GAPDH) and the mitochondrial enzymes aconitase, complex I and comp/ex II. Furthermore, NO might limit ATP formation by inhibiting creatine kinase, an enzyme that catalyses the transfer of the high-energy phosphate group between phosphocreatine and ATP. NO impairs DNA replication by inhibiting ribonucleotide reductase, the rate-limiting enzyme for DNA synthesis. The effect is mediated by the NO induced loss of the Fe-tyrosyl radical located in the R2 subunit of the enzyme. Finally, NO leads to DNA damage by base deamination. DNA damage

activates poly(ADP-ribose) synthetase (PARS) which, in turn, results in cellular depletion of energy-rich substrates. DNA damage is one of the mechanisms by which NO could induce apoptosis.

On the other hand, they suggest NO might facilitate collateral blood flow to the ischemic area by producing vasodilation. NO produces relaxation of cerebrovascular smooth muscles by activating soluble guanylate cyclase and increasing cGMP. NO might also cause vasodilation by activating $Ca^{2+}$-dependent $K^+$ channels, an effect probably mediated by nitrosylation of the channel protein, NO could improve microvasculor flow by inhibition of platelet aggregation and leukocyte adhesion, actions mediated through complex mechanisms including activation of guanylate cyclase, inhibition of 12-1ipoxygenase, interactions with the CD11-CD18 glycoprotein complex, and inhibition of gene expression of adhesion molecules. The protective effect of NO might also be related to inhibition of the NMDA receptor. The mechanisms of the effect, however, remain controversial. One hypothesis is that NO nitrosylates thiol residues of the radox modulatory site leading to the formation of disulfide bonds. Such modification would permanently inhibit $Ca^{2+}$ flux through the channel and decrease NMDA-mediated neurotoxicity. Another hypothesis is that NO, or a derived species, perhaps a NO-metal complex, exerts an allosteric action on the NMDA-receptor protein that facilitates the blockade of the receptor by divalent ions. NO has also been reported to ameliorate the neurotoxicity produced by $H_2O_2$. Although well known to promote oxidative damage, NO, under certain conditions, can counteract the deleterious effect of reactive oxygen species. NO might divert superoxide anion from other cellular targets. NO reacts with alkoxyl (LO•) and peroxyl (LOO•) radicals thereby inhibiting radical chain propagation reactions. Finally, under certain conditions, NO might act as OH. scavenger.

We studied the production of NO in the ischemia-reperfusion (IR) of brain in Mongolian gerbil stroke model. NO trapped by DETC-ferrous complex detected by ESR significantly decreased by about 19.17% and ROS trapped by PBN in the brain homogenate significantly increased by about 36.89% in the IR group comparing with the sham group, which caused seriously injury for the brain tissue. The RT-PCR products from total RNA of iNOS in the hippocampus were analyzed by electrophoresis with ethidium bromide in agarose gel and a single band was detected in RT-PCR products from RNA of the iNOS. It was found that the level of mRNA in sham group was very weak and ischemia-reperfusion enhanced the levels. This suggests that decreased NO free radical detected in the IR may be caused by reaction with ROS to form peroxynitrite (76).

## 7. ANTIOXIDANT AND NO RELATED NEURODEGENERATIVE DISEASES

Oxidative stress is a main mediator in NO induced neurotoxicity and has been implicated in pathogenesis of many neurodegenerative disorders. Antioxidants are usually expected as a potent chemopreventive agent due to their ability of scavenging

free radicals and chelating metal ions. However not all the actions of antioxidants are necessarily beneficial. From the study in my laboratory, and it was found that the effects of antioxidant on the biological function of NO were different in different cases.

Nicotinic cholinergic receptor stimulation induces neuroprotection against glutamate cytotoxicity by its inhibitory action on NO-formation. We demonstrated that nicotine could scavenge free radicals *in vitro* system (77), and that nicotine rescued the apoptosis of hippocampal neuron cells (50). However, there are different reports about the effect of nicotine on the generation of NO. It was reported nicotine evoked NO release in the rat hippocampal slice (78) and NOS expression in the ventromedial hypothalamic nucleus increased by nicotine treatment. However, it was found that nicotine administration was effective in limiting the enhancement on NOS expression following food restriction in another report. While a mismatch result was reported about the effect of nicotine on NO production and NOS expression determined between biochemical and histological method was obtained (79). The contradictory indicates that there is no conclusion and its mechanism is not clear.

We have studied the protective effect of nicotine on hippocampal neurons against the $A\beta_{25-35}$ induced toxicity. It was found that nicotine protected cultured hippocampal neurons against the A$\beta$- induced apoptosis and increase of caspase activity. Measurements of cellular oxidation and intracellular free $Ca^{2+}$ showed that nicotine suppressed A$\beta$-induced accumulation of free radical and increase of intracellular free $Ca^{2+}$. Cholinergic antagonist mecamylamine inhibited nicotine-induced protection against A$\beta$-induced caspase-3 activity and ROS accumulation (80). We are studying the production of NO free radicals in the transgenic mice and the effect of nicotine and the primary result showed that nicotine may be involved in protection of the brain against AD by regulation of NO and ROS.

Green tea polyphenols are usually expected as a potent chemopreventive agent due to their ability of scavenging free radicals and chelating metal ions (81,82) and we found they could prevent the cell against apoptosis caused by 6-OHDA (83, 56). However we demonstrated that high concentration green tea ployphenols significantly enhanced the neurotoxicity by treatment of sodium nitroprusside (SNP), a nitric oxide donor. SNP induced apoptosis in human neuroblastoma SH-SY5Y cells in a concentration and time dependant manner as estimated by cell viability assessment, FACScan analysis and DNA fragmentation assay, whereas treatment with green tea polyphenols alone had no effect on cell viability. Pretreatment with low dose green tea polyphenols (50μM and 100μM) had only slightly deleterious effect in the presence of SNP, while high dose green tea polyphenols (200μM and 500μM) synergistically damaged the cells severely. Further research showed that co-incubation of green tea polyphenols and SNP caused loss of mitochondrial membrane potential, depletion of intracellular GSH and accumulation of reactive oxygen species and exacerbated NO-induced neuronal apoptosis. It was found that the levels of NFκB, $P^{53}$ and c-jun were up regulated after treatment while Bcl-2 was down regulated. Bax expression shown no obvious change (76).

We studied the level of intracellular NO in SH-SY5Y cells after treated with 6-OHDA and pre-treatment with different concentrations of green tea polyphenols. Treatment with 6-OHDA resulted in a 1.2-fold increase of NO and pre-treatment with green tea polyphenols showed inhibitive effect. Green tea polyphenols alone had little effect on the intracellular NO level. The expression of nNOS was detected and it was found that 6-OHDA induced an up-regulation of nNOS, and green tea polyphenols co-incubated decreased the expression of nNOS caused by 6-OHDA.

Crataegus (hawthorn) is one of the oldest medicinal plants and is described by many pharmacopoeias and evidences show that Crataegus flavonoids (CF) had antioxidant effect *in vitro* or *in vivo* (84). The effects of CF on brain ischemic insults were investigated in Mongolian gerbil stroke model and results showed that pretreatment of the animals with CF decreased ROS production, TBARS content, and nitrite/nitrate concentration in brain homogenate, increased the brain homogenate-associated antioxidant level in a dose dependent manner. And pretreatment with CF increased the amount of biological available NO. Comparing with the IR group, NO significantly increased about 44.67% and 77.56% in low dose (32.5mg/kg/day) and high dose (167mg/kg/day) group pretreated with CF for fifteen days, respectively. At same time, oral pretreatment with CF decreased the nitrite/nitrate content in the brain homogenate and increased the biological available NO concentration. iNOS was implied in delayed neuron death after brain ischemic damage and it was found that the NO concentrations in those groups were also significantly lower than that of the IR group. And it was also found that pretreatment with CF could decrease the protein level of TNF-α and NF-κB, and increase the mRNA level of NOS estimated by Western blotting and RT-PCR. There were more neurons survived and less cells suffered apoptosis in the hippocampal CA1 region of CF treated animal brain tested (85). Shutenko *et al* found that the NO levels were increased by treatment with Quercetin in brain ischemic results, but they didn't measure nitrite/nitrate content (86).

## 8. CONCLUSION

From above review, it can be found that NO plays an important role in degenerative diseases even there are some contradictory results. However, it needs to be studied further to understand the exactly mechanism of NO in the induction of degenerative diseases and how to regulate NO generation. In some case, brain nitric oxide showed dual role in neurodegeneration/neuroprotection.

## 9. ACKNOWLEDGEMENT

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