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Role of iNOS in Osteoarthritis: pathological and therapeutic aspects

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Abstract

Accumulating evidence suggests that inflammation has a key role in the pathogenesis of Osteoarthritis (OA). Nitric oxide (NO) is established as one of the major inflammatory mediators in OA and drives many pathological changes during the development and progression of OA. Excessive production of NO in chondrocyte promotes cartilage destruction and cellular injury. Synthesis of NO in chondrocytes is catalyzed by inducible nitric oxide synthase (iNOS), which is thereby an attractive therapeutic target for the treatment of OA. A number of direct and indirect iNOS inhibitors, bioactive compounds, and plant-derived small molecules have been shown to exhibit chondroprotective effect by suppressing the expression of iNOS. Many of these iNOS inhibitors hold promise for the development of new, disease-modifying therapies for OA; however, attempts to demonstrate their success in clinical trials is not yet achieved. Many plant extracts and plant-derived small molecules have also shown promise in animal models of OA, though further studies are needed in human clinical trials to confirm their therapeutic potential. In this review, we discuss the role of iNOS in OA pathology and the effects of various iNOS inhibitors in OA.

Keywords

iNOS; nitric oxide; osteoarthritis; inflammation; iNOS inhibitor; Oxidative stress; Chondrocytes

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7. **Authors contributions**:

NA, MYA and TMH developed the main idea. NA and MYA performed the literature search and made the figures. NA, MYA and TMH wrote the manuscript. All the authors read and approved the final version of the manuscript.

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All cited references are available in Pubmed.gov

1. Introduction

1.1 Biosynthesis of NO and Nitric oxide Synthases

NO is a free radical and endogenously it is produced by the conversion of L-arginine to L-citrulline, with NADPH acting as an electron donor. The production of NO is catalyzed by a family of enzymes called nitric oxide synthases (NOSs) that includes three different isoforms: eNOS (endothelial NOS or NOS1), iNOS (inducible NOS or NOS2) and nNOS (neuronal NOS or NOS3) (Table 1). eNOS and nNOS were first characterized in endothelial cells and neurons respectively but were later characterized in other cell types also and were then renamed to NOS1 and NOS3, respectively (Nathan & Xie, 1994). NOS1 and NOS3 are expressed constitutively, while NOS2 is only expressed in activated cells and is induced by lipopolysaccharide (LPS) and inflammatory cytokines such as IL-1β, TNF-α, or IFN-γ (Coleman, 2001). Production of NO by NOS2 is induced after several hours of stimulation, but once induced, it lasts for up to five days (Kolios, Valatas, & Ward, 2004). The physiological half-life of NO ranges from seconds to minutes, depending on its concentration (Wink & Mitchell, 1998).

1.2 NO reactivity

The effects of NO can be divided into direct and indirect effects depending on whether NO directly interacts with the target, or first forms an effector molecule by reacting with a reactive oxygen species (ROS), which then interacts with the target (Kolios et al., 2004). The direct action of NO is exemplified by the reaction with guanylate cyclase leading to the formation of cGMP from GTP. Indirect effects of NO are characterized by the formation of a reactive nitrogen oxide species (RNOS) intermediate that is derived from the reaction of NO with either O_2 or O_2^-. NO is quickly oxidized to RNOS under aerobic conditions; it reacts with O_2^- to form peroxynitrite ($ONOO^-$), which is a potent oxidative and nitrosative agent and may lead to alteration in cellular pathways (Figure 1). Peroxynitrite is known to mediate most of the severe toxic effects of NO (Radomski & Herschorn, 1992). In biological fluids, the primary oxidative product is dinitrogen trioxide (N_2O_3), which is unstable and quickly nitrates to thiols or amines. NO regulates cell death and has apoptotic effects on cells as the production of RNOS like $ONOO^-$ and N_2O_3 can directly modify nitrogenous bases in DNA and can induce single-strand breaks in DNA (P. K. Kim, Zamora, Petrosko, & Billiar, 2001). However, regulation of cell death by NO is concentration-dependent, as at lower concentration, NO generally appears to be anti-apoptotic, but at higher concentration, it acts as a pro-apoptotic molecule (Kroncke, Fehsel, Suschek, & Kolb-Bachofen, 2001).

1.3 NO and OA

Osteoarthritis (OA) is a disabling joint disease that results in substantial morbidity and reduced quality of life (Castrogiovanni et al., 2019). OA is now considered as a disease of the whole joint, as it not only affects the articular cartilage but also affects the entire joint structure and is characterized by cartilage degradation, synovitis, osteophytes formation, and thickening of subchondral bone (Loeser, Goldring, Scanzello, & Goldring, 2012). The current therapy of OA involves interventions that provide symptomatic relief only and the only definite treatment for OA is the joint arthroplasty, which is expensive and requires revision in 10–15 years (Castorina et al., 2017). Excess NO is considered a pro-

inflammatory mediator that acts as a tissue-damaging molecule and is implicated in many inflammatory diseases (Chen et al., 2018). These results led to the prevailing hypothesis that NO is a pro-inflammatory factor, which is detrimental to cartilage if present in excess. Considering the pivotal role of inflammation in OA, novel anti-inflammatory therapeutics targeted against iNOS could form the basis of much-needed disease-modifying OA drug (DMOADs). Below we provide an overview of the involvement of iNOS in OA pathogenesis and the potential of various iNOS inhibitors in mitigating joint inflammation in OA. We also review here the importance of inflammation in OA and the transcriptional regulation of iNOS in chondrocytes. Additionally, we explored the chondroprotective and therapeutic potential of various plant-derived small molecules in the prevention or treatment of OA.

2. Inflammation and OA

Traditionally, OA was considered a degenerative disease resulting from the daily wear and tear in the joints, predominantly due to mechanical factors. This concept was based on the observation that chondrocytes, the only cell type present in cartilage are avascular, non-innervated, and have no ability to regenerate (Sophia Fox, Bedi, & Rodeo, 2009). However, with the advancement of molecular biology in the past century, there came a paradigm shift in our understanding of the pathological mechanisms underlying OA. Now, there is strong evidence that OA is a multifactorial disease, and the structural changes observed in OA are due to a combination of factors among which inflammation has a key role (Robinson et al., 2016). By the early twenty-first century, synovitis, occurring due to interactions between damaged tissue and the immune system, was established as a critical feature of OA and was accepted as a driver of the pathogenesis of OA (Zhuo, Yang, Chen, & Wang, 2012).

Inflammation in OA is characterized by the involvement of innate immune system mainly and to some extent, an adaptive immune system (Haseeb & Haqqi, 2013). Joint inflammation is clearly reflected in many of the clinical symptoms of OA, such as joint swelling, warmth, and pain (Sellam & Berenbaum, 2010). We have shown previously that ZCCHC6 knockout mice develop less severe OA due to reduced expression of IL-6 (Ansari et al., 2019). Inflammation of the synovium (synovitis) is a common finding in OA and is characterized by synovial hypertrophy and infiltration of the sub lining tissue with inflammatory cells (Scanzello & Goldring, 2012). The inflammation in OA is modest and less pronounced as compared to RA and also differs in terms of the cellular and molecular players involved (de Lange-Brokaar et al., 2012).

The most accepted hypothesis to explain the inflammation in OA is that once the degraded cartilage fragments come into contact with the synovium, they are considered foreign particles, and there is a protective inflammatory response by the synoviocytes (Berenbaum, 2013). The products of cartilage damage trigger the activation of inflammatory signaling pathways including nuclear factor-kappa b (NF-κB), that plays a central role in the inflammatory response (Scanzello, 2017). Inflammation in the joint can also be triggered by stress response, and obesity-related systemic inflammation might add to the local inflammation (Scanzello, 2017). Cytokines including IL-1β, TNF-α, IL-6, IL-17, IL-18, and IL-21 have been implicated in the pathogenesis of OA and are among the most widely studied mediators of inflammation (Kapoor, Martel-Pelletier, Lajeunesse, Pelletier, & Fahmi,

2011). These inflammatory mediators are deleterious for the joint and initiate or perpetuate the cartilage damage and amplify the low-grade inflammation, which may also induce other inflammatory diseases that are affected by systemic low-grade chronic inflammation (Berenbaum, 2013).

3. Role of iNOS in OA pathogenesis

Production of NO is abnormally high in OA cartilage as high levels of NO are produced by OA chondrocytes (Scher, Pillinger, & Abramson, 2007). Moreover, elevated concentrations of nitrotyrosine, a marker of NO-dependent oxidative damage has been found in synovial fluid from OA patients, and excess NO production in OA cartilage has been illustrated by immunostaining with anti-nitrotyrosine antibodies (Loeser, Carlson, Del Carlo, & Cole, 2002). In tandem, expression of iNOS has been demonstrated by Western blot and immunohistochemical staining in OA cartilage (Vuolteenaho, Moilanen, Al-Saffar, Knowles, & Moilanen, 2001). Elevated levels of nitrite and iNOS mRNA and protein and have been found in the synovium of patients with OA (Ersoy et al., 2002; McInnes et al., 1996). However, cartilage is the major source of NO in OA joints as more enhanced expression of iNOS is found in chondrocytes as compared to the synovial cells in OA patients (Melchiorri et al., 1998). Chondrocytes isolated from OA cartilage show overexpression of iNOS mainly in the superficial zone, whereas chondrocytes isolated from patients without OA do not express iNOS (Amin et al., 1995). Aberrant expression of iNOS plays a crucial role in many inflammatory diseases like OA, colitis, asthma, multiple sclerosis, and psoriasis (Kroncke, Fehsel, & Kolb-Bachofen, 1998). iNOS generated NO seems to be crucially involved in the pathomechanisms of OA, and it contributes to the OA pathogenesis by modulating ECM homeostasis and cytokines expression, causing oxidative damage and chondrocyte apoptosis (Figure 2). Excess production of NO by iNOS leads to cartilage damage by enhancing MMP activity and downregulating biosynthesis of aggrecan and collagen (Lepetsos & Papavassiliou, 2016).

3.1 Cartilage destruction

iNOS is preferentially expressed in the superficial zone of OA cartilage, which is the surface zone that defines the site of initial damage, indicating that the damaged OA cartilage actively produces NO (Melchiorri et al., 1998). Well documented actions of NO in cartilage include acceleration of chondrocyte mediated matrix degradation and inhibition of cartilage matrix synthesis, and together these effects lead to the loss of cartilage matrix (Scher et al., 2007). The destructive action of NO is mediated by inhibiting the formation of ECM components such as type II collagen at a post-translational level by inhibiting prolyl hydroxylase, which is involved in hydroxylation of proline and collagen crosslinking (Hauselmann, Stefanovic-Racic, Michel, & Evans, 1998). Furthermore, NO is implicated in the inhibition of synthesis of proteoglycan such as aggrecan (Johnson et al., 2000). Earlier, matrix degradation in OA was considered as a consequence of mainly mechanical factors, but later it was established that active resorption of matrix components is also involved in matrix degradation. NO promotes degradation of ECM by enhancing the activity of matrix metalloproteinases (MMPs) that subsequently leads to joint destruction (Davies et al., 2013). Several molecules, such as endothelin 1 that regulate MMP expression in cartilage, are themselves controlled

by NO (Manacu et al., 2005). Inhibition of iNOS decreases the loss of glycosaminoglycan content in the cartilage in an OA model (Balaganur et al., 2014). Besides, NO plays a regulatory role in the activation of MMP-9 and inhibits the activity of TIMP-1 (Ridnour et al., 2007).

3.2 Pro-inflammatory reaction

NO acts as a proinflammatory and destructive mediator in OA and increases the production of PGE2 and inflammatory cytokines (Amin et al., 1997). Additionally, NO has been shown to disrupt the cytokine balance leading to a pro-inflammatory and destructive response by reducing the synthesis of endogenous interleukin-1 receptor antagonist (IL-1Ra) and anabolic mediator TGF-β (Vuolteenaho, Moilanen, Hamalainen, & Moilanen, 2003). It has been reported that NO also increases the susceptibility to injury by other oxidants like H_2O_2 and by upregulating IL-18 and TNF-α (Pelletier, Mineau, Ranger, Tardif, & Martel-Pelletier, 1996). IL-1β and TNF-α induce the production of NO as well as ROS, which can react with NO to form peroxynitrite and augment NF-κB activation (Scher et al., 2007), thereby providing persistent signaling for NF-κB dependent gene transcription (Clancy, Gomez, & Abramson, 2004).

3.3 Cytotoxicity

Inappropriate chondrocyte apoptosis is one of the central features of OA pathogenesis, and NO is a principal inducer of chondrocyte apoptosis, via the action of caspase-3 and tyrosine kinases (Maneiro et al., 2005), however, the exact mechanism of NO-induced apoptosis in OA has not been elucidated yet (Scher et al., 2007). Also, whether NO can independently trigger apoptosis is not established, and it is argued that ROS are the actual mediators of chondrocyte apoptosis (Del Carlo & Loeser, 2002).Whiteman et al. proposed that peroxynitrite ($ONOO^-$) rather than NO per se, triggers apoptosis in chondrocytes (Whiteman et al., 2004). Since the effect of NO in cartilage is dependent on concentration and duration of action, and the local concentration of NO is an important determinant of cytotoxicity, so the small amounts (picomolar concentration) of NO produced for a limited duration by eNOS and nNOS are sufficient for physiological signaling and homeostasis and is known to be protective and essential for chondrocyte maturation , but large amounts (micromolar concentration) produced by iNOS for a sustained period results in inflammation, cytotoxicity and chondrocyte catabolism causing damage to chondrocytes and surrounding tissues (Abramson, 2008).

3.4 Oxidative/Nitrosative stress

In addition to inflammation, catabolic cytokines, and other factors, recent studies have supported the role of oxidative/nitrosative stress in OA pathology (Alcaraz, Megias, Garcia-Arnandis, Clerigues, & Guillen, 2010). Free radicals are eliminated by antioxidants, such as superoxide dismutase and non-enzymatic agents, such as tocopherol that protect from oxidation. An imbalance created between the production of ROS/RNS and the cellular scavenging antioxidants, is defined as oxidative/nitrosative stress, which contributes to OA pathogenesis (Ziskoven et al., 2011). Excess of NO derived RNS and ROS can be detrimental in chondrocytes by causing mitochondrial dysfunction (Maneiro et al., 2005). RNS are able to react directly with ROS by producing highly reactive molecules that can

induce DNA fragmentation (Beckman & Koppenol, 1996). Additionally, both ROS and RNS are able to reduce the synthesis capacity of proteoglycans and collagen by chondrocytes (Franz et al., 2018).

4. Transcriptional regulation of iNOS expression in chondrocytes

Regulation of NO produced by iNOS is regulated by several mechanisms and varies according to species, cell type, and the inducer and is regulated both at the transcriptional and post-transcriptional level but is largely controlled by transcriptional mechanisms (Frasca et al., 2010). Induction of iNOS expression in most human cells requires the synergistic action of a complex mixture of cytokines (Kleinert, Pautz, Linker, & Schwarz, 2004). Interestingly, the production of NO in primary human chondrocytes can be induced by a single cytokine such as IL-1β, TNF-α, IL-17, or IFN- γ (Henrotin et al., 2000). Bacterial LPS, fibronectin fragment (FN-f) and shear stress have also been reported to induce NO production in human chondrocytes (Vuolteenaho, Moilanen, Knowles, & Moilanen, 2007). IL-1β induced NO synthesis in primary human chondrocytes is time and concentration-dependent and starts after a lag period of 6 hours (Palmer, Hickery, Charles, Moncada, & Bayliss, 1993). On the other hand, induction of iNOS in C-28/I2 chondrocytes depends on triple combination of cytokines (Kleinert et al., 2004). In human chondrocytes, iNOS is mainly regulated by MAPK (ERK, p38, JNK), NF-κB and JAK2-STAT1α pathway. In C-28/I2 chondrocytes also, iNOS induction is regulated by p38 MAPK, JAK2-STAT-1α and NF-κB pathways (Schmidt et al., 2010). The promoter region of the human iNOS gene contains binding sites for several TFs like NF-κB, AP-1, STAT1α, IL-6, and Interferon regulating factor (Kleinert et al., 2004). Binding sites for C/EBP, CREB, GATA, HF, p53, Sp1, and KLF-6 have also been validated (Pautz et al., 2010).

5. iNOS inhibition as a therapeutic strategy for OA

Currently, there is no approved DMOAD that could inhibit the structural progression of OA; however many potential agents are under investigation (Davies et al., 2013). Since NO is established as a major catabolic factor in OA pathogenesis, iNOS inhibitors are considered as a class of prospective DMOADs and are currently under investigation (Watt & Gulati, 2017). In animal models, iNOS inhibitors were shown to reduce NO levels as well as the levels of prostaglandins at the inflammation site (Clancy, Amin, & Abramson, 1998), indicating that inhibiting iNOS could be an attractive therapeutic target for OA (Chevalier, Eymard, & Richette, 2013). iNOS knockout mice have been used to study the importance of iNOS in OA pathology and development. Preclinical studies have demonstrated that iNOS KO mice are resistant to developing OA (Salerno, Sorrenti, Di Giacomo, Romeo, & Siracusa, 2002). In a classic study, OA was induced in iNOS deficient mice and cartilage damage, cartilage proteoglycan depletion, and osteophytes formation were evaluated (van den Berg, van de Loo, Joosten, & Arntz, 1999). They found that cartilage damage, osteophyte formation, and proteoglycan depletion were significantly reduced in the iNOS-deficient mice, suggesting that iNOS and NO play a significant role in the induction and progression of OA in this model. On the other hand, in another study it was found that iNOS deficient mice showed accelerated development of OA (Clements et al., 2003). The

authors suggested that this observation could be because of overexpression of eNOS or nNOS (Clements et al., 2003).

Expression of iNOS and NO production can be inhibited by a wide variety of compounds, which are either direct or indirect inhibitors of iNOS. Direct inhibitors bind directly to the iNOS enzyme and inhibit its activity, whereas indirect inhibitors mainly target NF-κB and STAT-1 signaling pathways thereby downregulating iNOS expression. Based on the site of binding, iNOS inhibitors are classified into four categories. The first category of iNOS inhibitors bind to the arginine-binding site, and examples include L-NMMA, L-NIL, 1400W, GW274150, and L-NAME. The second category includes iNOS inhibitors like Cindunistat that mimic the tetrahydrobiopterin cofactor. The third class includes a set of compounds that interact directly with the heme, and the fourth class includes compounds that interact with the calmodulin or flavine cofactors (Alderton, Cooper, & Knowles, 2001). Studies published with various iNOS inhibitors and OA are presented analytically in Table 2.

In animal models, the use of iNOS inhibitors significantly reduced cartilage degeneration and osteophyte formation (Pelletier et al., 1998). In a dog model of OA, inhibition of iNOS by L-NIL decreased general MMP activity, incidence and size of osteophytes and the size of cartilage lesions (Martel-Pelletier, Wildi, & Pelletier, 2012). Methylisothiourea attenuated monosodium acetate induced histopathological changes in knee joint (More et al., 2013). Conversely, in a recent 2-year randomized, double-blind, placebo-controlled clinical trial of another iNOS inhibitor, cindunistat, there was no significant reduction in joint space narrowing (JSN) and progression of OA in patients with severe OA (Hellio le Graverand et al., 2013). Many pharmaceutical drugs act as indirect iNOS inhibitors and have been shown to exert their chondroprotective effects by inhibiting iNOS expression at the transcriptional level. For example, in a double-blinded, placebo-controlled RCT, it was found that doxycycline decreased Joint space narrowing (Brandt et al., 2005). Another indirect iNOS inhibitor, Ursodeoxycholic acid (UDCA) reduced the expressions of MMP-3, MMP-13, and ADAMTS-5 in IL-1β-stimulated OA chondrocytes (Moon et al., 2014).

Plant-derived polyphenolic compounds are well known for their anti-inflammatory and antioxidant properties. Previous studies from our laboratory have shown that plant polyphenols like Epigallocatechin-3-gallate (EGCG) and Wogonin inhibit NO production in IL-1β-stimulated human OA chondrocytes by inhibiting the expression of iNOS mRNA (Ahmed et al., 2002; Akhtar & Haqqi, 2012; Khan et al., 2017). We showed that EGCG inhibited the IL-1β-induced inflammation in primary human OA chondrocytes (Akhtar & Haqqi, 2011). Many dietary polyphenols like Gallic acid, Silibinin, Carvacrol, and Sinapic acid have also been shown to inhibit iNOS and have anti-inflammatory effects in chondrocytes as shown in Table 3 (Huang et al., 2018; Wen et al., 2015; Xiao, Li, Liu, & Ma, 2018; Zheng et al., 2017). Additionally, chondroprotective and OA suppressive effects of Pomegranate extract in a rabbit model of OA have also been shown (Akhtar, Khan, Ashruf, & Haqqi, 2017). We have demonstrated the chondroprotective effects of pomegranate extract in IL-1β-stimulated primary human chondrocytes (Haseeb, Khan, Ashruf, & Haqqi, 2017). We also found that the mechanism behind the chondroprotective activity of Pomegranate extract was via suppression of the IL-1β-induced activation of

p38α-MAPK (Rasheed, Akhtar, & Haqqi, 2010). Studies reporting the chondroprotective effects of selected plant extracts via inhibition of iNOS are presented in Table-4.

6. Concluding remarks

It is now well established that inflammation plays a central role in the pathogenesis of OA, and NO acts as a major pro-inflammatory mediator during joint inflammation. NO plays a destructive role during the pathomechanism of OA and leads to cartilage damage and matrix degradation. A number of studies suggest that inhibition of NO has chondroprotective effects in OA. Hence, inhibitors of iNOS are attractive molecules for the development of DMOADs. iNOS inhibitors like L-NMMA, L-NIL and 1400W have shown chondroprotective effects, and they need to be assessed further in human phase I trials. Despite the promising results obtained with most of the iNOS inhibitors *in vitro* and *in vivo*, no successful outcome has been observed in clinical trials yet. Studies from our group and others have demonstrated that plant-derived compounds and herbal medicines have positive effects on OA in animal models, but further human studies need to be conducted to confirm the beneficial effects of dietary and bioactive compounds in OA.

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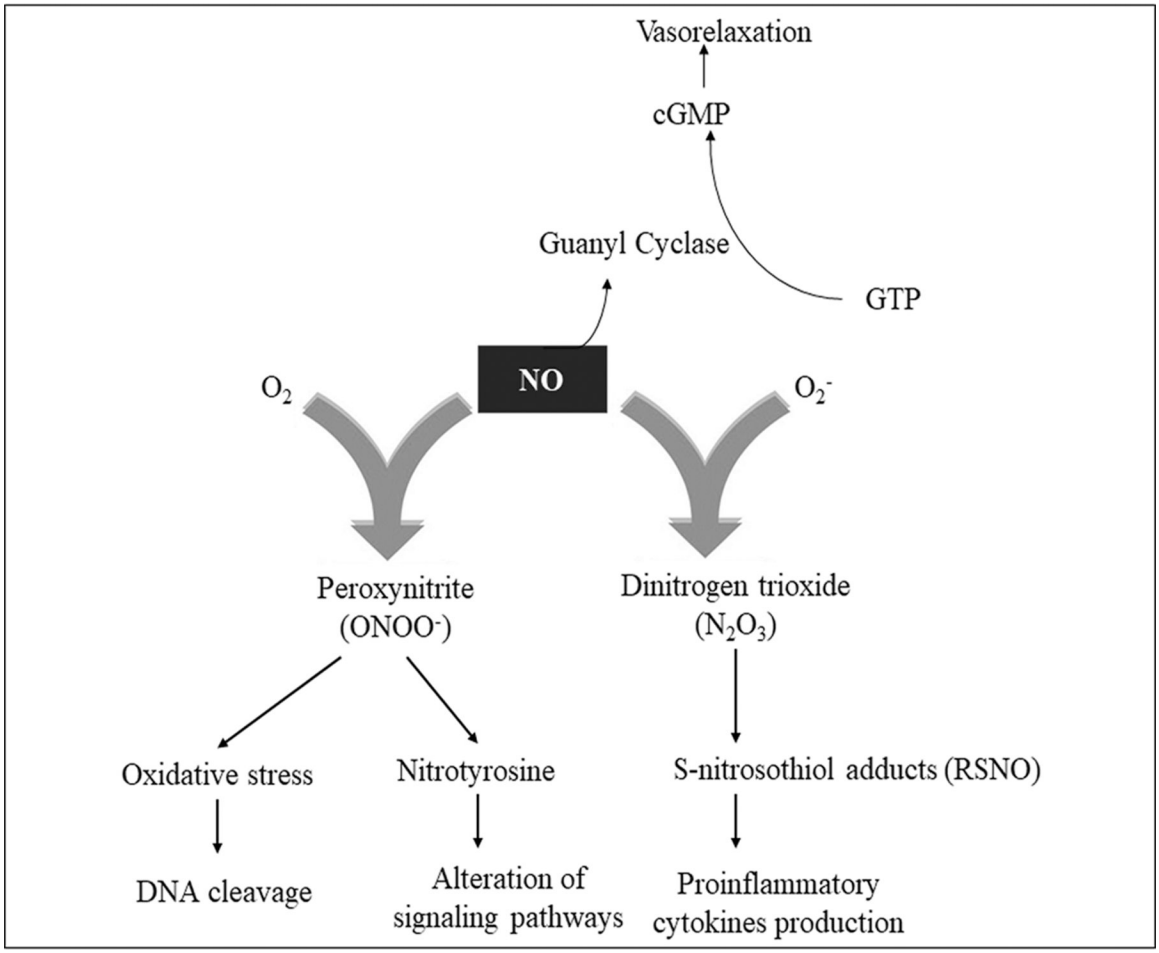

Figure 1.
Direct and indirect effects of NO

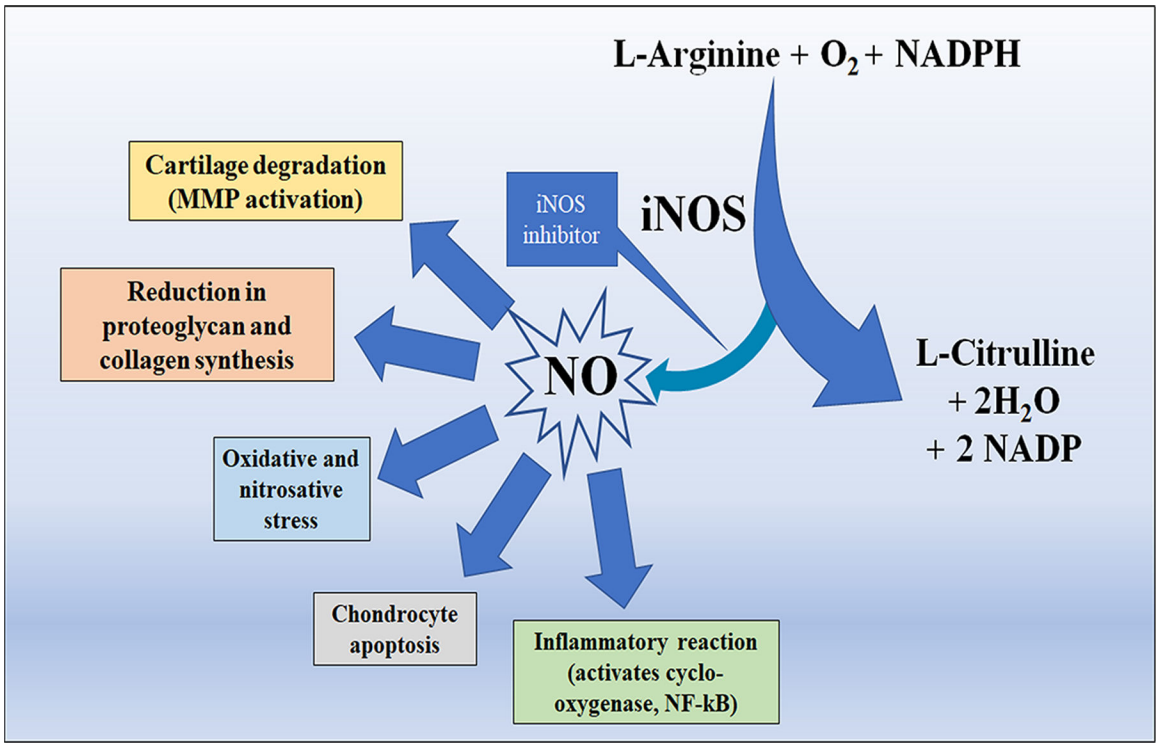

Figure 2.
Synthesis of NO and its deleterious effects on cartilage.

Table 1.

Isoforms of nitric oxide synthases

	eNOS	**iNOS**	**nNOS**
Alias	endothelial NOS, NOS I	inducible NOS, NOS II	neuronal NOS, NOS III
Function	Regulates vascular tissue, blood pressure, inhibits platelet aggregation and leukocyte adhesion	Response to stress and inflammation, involved in host defense and tissue destruction	Involved in neurotransmission, modulates response to pain and neuronal sensitization
Gene expression	Constitutively expressed	Induced in response to Immunostimulatory cytokines or bacterial pathogens	Constitutively expressed
Dependency on Calcium	Calcium- calmodulin dependent	Calcium- calmodulin independent	Calcium- calmodulin dependent
NO production	Small amount (pico to nanomolar)	Large sustained amount of NO (nano to micromolar)	Small amount (pico to nanomolar)
Signal for discontinuation of NO production	Self-limited	NOS degradation	Self-limited
Expression/localization	Endothelium	Chondrocyte, synoviocytes, hepatocyte, macrophages, other	Brain

Table. 2

iNOS inhibitors and OA

	iNOS inhibitor	Specificity	Model	Main finding	References
1	**L-NMMA**	Not selective for different isoforms of NOS	Human chondrocytes	Expression of iNOS was inhibited by L-NMMA	(Mathy-Hartert et al., 2002)
2	**1400W**	Selective iNOS inhibitor	Human OA cartilage	Treatment with 1400W reduced MMP-10 in IL-1β treated OA cartilage	(Jarvinen et al., 2008)
3	**L-NIL (n-iminoethyl-L-lysine)**	Selective iNOS inhibitor	ACLT induced canine model	L-NIL treatment showed a reduction in the size of osteophytes and decrease in the severity of cartilage lesions and the progression of experimental OA	(Pelletier et al., 2000)
4	**GW 274150**	selective	Collagen-induced arthritis in mice	GW 274150 attenuated inflammation and tissue damage associated with collagen-induced arthritis in mice	(Cuzzocrea et al., 2002)
5	**L-NAME**	selective	Adjuvant induced arthritis in rats	L-NAME suppressed arthritis progression and increase weight gain	(Ialenti, Moncada, & Di Rosa, 1993)
6	**SMT (S-methyl isothiourea)**	Preferential iNOS inhibitor	MIA-induced arthritis in rats	SMT reduced NO production in synovial fluid and reduced	(More et al., 2013)
7	**Cindunistat**	Selective iNOS inhibitor	Double-blinded, placebo-controlled Randomized controlled trial	Cindunistat slowed joint space narrowing in patients with mild OA but had no effect on patients with severe OA.	(Hellio le Graverand et al., 2013)

Table 3.

iNOS inhibition by plant-derived molecules in chondrocytes and animal OA models.

	Molecule	Source	Outcome	Model	Reference
1	**EGCG**	Green tea	EGCG inhibited the IL-1 beta-induced activity and expression of iNOS via blocking the NF-κB pathway activation	Human chondrocytes	(Ahmed et al., 2002; Singh, Ahmed, Islam, Goldberg, & Haqqi, 2002)
2	**Wogonin**	Scutellaria baicalensis	Wogonin suppressed the IL1-β- induced NO production and iNOS expression via activation of the ROS/ERK/Nrf2 signaling pathway	Human chondrocytes	(Khan et al., 2017)
3	**Gallic acid (GA)**	Gallnuts	GA suppressed the AGE-induced expression of iNOS in articular chondrocytes, playing a chondroprotective role against OA progression	Rabbit model of knee OA	(Wen et al., 2015)
4	**Silibinin**	Siltbum Marianum	Silibinin inhibited the IL1-β-induced NO production and iNOS expression via inhibiting the NF-κB pathway	Human chondrocytes	(Zheng et al., 2017)
5	**Carvacrol**	Oregano and thyme	Carvacrol inhibited the IL1-β-induced NO production and iNOS expression via inhibiting the NF-κB pathway	Human chondrocytes	(Xiao et al., 2018)
6	**Sinapic acid**	Vinegar and wine	Sinapic acid inhibited the IL1-β-induced NO production and iNOS expression via inhibiting the MAPK pathway	Rat chondrocytes	(Huang et al., 2018)
7	**Sulforaphane**	Broccoli, cabbage	Sulforaphane treatment inhibited NO synthesis and cartilage matrix degradation	Human chondrocytes and DMM model of osteoarthritis in mouse	(Davidson et al., 2013)

Table 4.

Use of plant extracts and herbal medicine to inhibit iNOS in OA.

	Plant extract/ herbal medicine	Plant source	Outcome	Model	Reference
1	**Ginkgo biloba extract (EGb)**	Ginkgo biloba	EGb inhibited NO production, iNOS expression, chemokine production, and the JNK-AP1 signaling pathway.	Human OA chondrocytes	(Ho et al., 2013)
2	**Cherry seed extract (SCE)**	Prunus avium	Patients who were treated with SCE topically showed significant decreased inflammatory cytokines levels, decreased pain and decreased serum CRP levels.	Double-blind, placebo-controlled RCT	(Mahmoud, Al-Awadhi, & Haines, 2015)
3	**Herbal-Leucine mixture (HLM)**	Uncaria tomentosa, Boswellia spp., Lepidium meyenii and L-Leucine	HLM showed chondroprotective and anti-inflammatory activity in OA.	Primary OA chondrocytes or OA cartilage explants	(Akhtar, Miller, & Haqqi, 2011)
4	**Ginsenoside Rb1 (G-Rb1)**	Panax ginseng	G-Rb1 reduced the levels of IL-1β, TNF-α, NO, MMP-1 and MMP-13, by suppressing iNOS expression and reducing ROS production.	Rat articular chondrocytes	(S. Kim et al., 2012)
5	**Duhuo Jisheng decoction (DHJSD)**	Radix angelicae pubescentis and Herba taxilli	DHJSD inhibited NO-induced apoptosis in rat articular chondrocytes. DHJSD combined with Western medicine or sodium hyaluronate injection appeared to have benefits for knee OA.	Rat articular Chondrocytes, Randomized clinical trials	(Zhang et al., 2016)