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Review Article

# INFLAMMATION: THE ROLE OF DIFFERENT MEDIATORS

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## *ABSTRACT*

*Inflammation is a protective response for the purpose of removal of exogenous and endogenous harmful substances produced by injurious stimuli and is a part of the healing process in wounded tissues. Since proinflammatory mediators such as COX-2, iNOS, tumor necrosis factor alpha (TNF-α), interleukin IL-1 and IL-6, proteases, and oxidants produced during the typical response can cause damage to normal tissues regardless of how and where the inflammatory response is triggered, the substances involved in the inflammatory response need to be tightly regulated. If the scavenging reaction is delayed, the inflammatory response may evolve into a variety of chronic inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, asthma, and neurodegenerative diseases. A vast number of molecular studies have identified several target molecules involved in inflammatory changes, and most anti-inflammatory drugs currently used to suppress the biosynthesis of the inflammatory mediators mentioned earlier.*

***KEYWORDS:*** *COX-2, iNOS, NF-κB, MAPK, ROS, Arachidonic Acid, Nitric Oxide.*

## INTRODUCTION

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. It can be classified as either acute or chronic, and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues.

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Chronic inflammation concerns a progressive change in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue [1].

Regardless of the triggering factor, the mechanisms involved in the inflammatory process are common to all and the standard signs of inflammation are expressed by increased blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx [1]. Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed by release of arachidonic acid and inflammatory mediators such as cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase

vascular permeability, thus facilitating the migration of leukocytes to the site of inflammation [2,3].

**PRO-INFLAMMATORY MEDIATORS**

Inflammation may release or generate a diverse population of pro-inflammatory mediators like bradykinins, serotonin, histamines, prostaglandins, nitric oxide and reactive oxygen species. These substances contribute to the classic clinical picture of heat (calor), redness (rubor), pain (dolor), swelling (tumor) and diminished function associated with inflammation and may produce hyperalgesia or allodynia [4]. Inflammation can be classified into two categories: acute inflammation and chronic inflammation. Acute inflammation is the initial response of the immune system against pathogens and tissue injury. It is a rapid self-limiting process, mediated by eicosanoids and vasoactive amines which increase the movement of plasma and leukocytes into infected site [5]. The classical hallmarks of acute inflammation are reddening, heat, pain, oedema and loss of function [6]. Acute inflammation helps the body ward off infections; it lasts for short period and generally is regarded as therapeutic inflammation [7, 8]. Early in the inflammatory response, pro-inflammatory mediators such as prostaglandins and leukotrienes play an important role [9]. The progression from acute inflammation to chronic inflammation as in many widely occurring human diseases is widely viewed due to excess of pro-inflammatory mediators [10].

In chronic inflammation, various cytokines and growth factors are released, resulting recruitment of higher order immune cells such as leukocytes, lymphocytes and fibroblasts. The inflammation can lead to persistent tissue damage by these cells [7, 8]. In addition, chronic inflammation can also lead to a number of diseases such as hay fever, periodontitis, rheumatoid arthritis, arteriosclerosis, cardiovascular diseases, diabetes, obesity, pulmonary diseases, neurologic diseases and cancer [11]. Inflammation plays a critical role in the promotional stage of carcinogenesis. Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [12, 13]. Inflammatory response and tissue damage are induced by inflammatory mediators generated through up-regulation of inducible pro-inflammatory genes COX-2 and iNOS. During the inflammatory process, large amounts of the pro-inflammatory mediators like nitric oxide and prostaglandins are generated by the inducible iNOS and COX-2 respectively [14-16]. They have been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders. Continuous production of these molecules in chronic inflammation has been linked to the development of cancer [17].

Many other proinflammatory cytokines and inflammatory mediators are involved in physiological responses to chronic and acute inflammation. For example, nuclear transcription factor kappa-B (NF-κB) is transported into the nucleus to activate inflammatory gene expression [15]. In the inactive state, NF-κB is a heterodimer that consists of p50 and p65 proteins. It is constitutively localized in the cytoplasm, and its movement is restrained by the inhibitor of nuclear factor kappa B (IκB) [18]. When gram-negative bacteria infect the cell, LPS stimulates the activation of macrophages. This activation induces the phosphorylation of IκB, which releases NF-κB to move to the nucleus and stimulate the expression of proinflammatory cytokines, COX-2 and iNOS. The mitogen-activated protein kinase (MAPK) signaling pathway also plays an important role in upregulating inflammatory gene expression in LPS-stimulated macrophages [19]. Several studies found that phosphorylation of MAPKs could induce NF-κB activation and regulate inflammatory gene expression [20, 21]. Hence, suppressing NF-κB and MAPK pathways could ameliorate the inflammatory response.

Reactive oxygen species (ROS) production also plays an important role in the modulation of inflammatory reactions. Major ROS produced within the cell are superoxide anion, hydrogen peroxide and hydroxyl radical [22]. Extracellular release of large amounts of superoxide,

produced as respiratory burst in leukocytes, is an important mechanism of pathogen killing and also leads to endothelial damage resulting in an increased vascular permeability as well as cell death [23].

## ROLE OF ARACHIDONIC ACID IN INFLAMMATION

The potent mediators of inflammation are derivatives of arachidonic acid (AA) a 20-carbon poly unsaturated fatty acid produced from membrane phospholipids. Arachidonic acid, the major poly unsaturated fatty acid present in mammalian systems is the precursor for PGs synthesis by cyclooxygenase pathway. Under normal conditions the concentration of free AA within the cells is low. Most of it is stored as part of phospholipids in the membranes of the cells [24].

The availability of free AA is essential for the biosynthesis of eicosanoids. Therefore, this mediator is released from the phospholipids membranes by the action of various phospholipase enzymes, which are activated in response to multiple stimuli such as mechanical trauma, cytokines and growth factors [25]. In most cells, arachidonic acid may be released at the endoplasmic reticulum and nuclear membrane, predominantly via the translocation of type IV cytosolic phospholipase A2. Arachidonic acid released from the membrane is rapidly metabolized in several enzymatic and non-enzymatic pathways to yield an important family of oxygenated products, collectively termed eicosanoids [26, 27]. The arachidonic acid metabolism generally occurs via one of four major avenues (i) the cyclooxygenase (COX) pathway, involved in the formation of prostaglandins (PGs), thromboxanes (Txs), and prostacyclin, (ii) the lipoxygenase (LOX) pathway, which produces leukotrienes (LTs) and lipoxins, (iii) the cytochrome P450 monooxygenase pathway, which produces epoxyeicosatrienoic and hydroxyeicosatetraenoic acids and (iv) non-enzymatic lipid peroxidation which produces isoprostanes [6].

## CYCLOOXYGENASE PATHWAY

Cyclooxygenase converts arachidonic acid to endoperoxide containing intermediates to produce prostaglandins and thromboxanes. Two distict active catalytic sites exist on COX: the cyclooxygenase active site (CAS), which converts arachidonic acid to prostaglandin G2 ($PGG_2$) and the peroxidase active site (PAS) which transform $PGG_2$ to $PGH_2$. The $PGH_2$ is the precursor of several bioactive prostanoids, which are formed by the action of specialized tissue isomerases. The five prostanoids synthesized by this pathway include $PGE_2$, $PGD_2$, $PGF_{2\alpha}$, $PGI_2$, and $TxA_2$. The production of individual prostanoids is catalyzed by specific synthases and they have distinct biological functions [28].

## ISOFORMS OF CYCLOOXYGENASE

Cyclooxygenase also known as Prostaglandin endoperoxide H synthase (PGHS) and exists in two isoforms; PGHS-1 (COX-1) and PGHS-2 (COX-2), which catalyses the oxidation of AA to prostanoids. COX-1 and COX-2 enzymes are heme proteins, homodimers that are widely distributed [29]. These enzymes are located in the lumenal portion of the endoplasmic reticulum membrane and the nuclear envelope [30]. COX-2 is twice more abundant at the nuclear envelope than within the endoplasmic reticulum, whereas the concentration of COX-1 is equal at both locations [31]. COX-1 functions predominantly in the endoplasmic reticulum and COX-2 mostly in the nucleus [32]. Therefore, it appears that COX-1 and COX-2 are two distinct prostanoid biosynthetic systems with separate biological functions for their products. COX-1 is expressed constitutively in most mammalian tissues and plays a role in the production of PGs that control normal physiological processes such as regulation of gastric response. Therefore, it is kept responsible for the housekeeping prostaglandins synthesis. In contrast, COX-2 is an inducible enzyme responsible for the production of pro-inflammatory PGs causing inflammation and pain [33].

## ROLE OF COX-2 IN INFLAMMATION

COX-2 is the more important source of prostanoid formation in inflammatory processes [34]. The prostanoids are metabolites that exert their biological effects in the proximity of the sites of their synthesis, in autocrine or paracrine manner. These mediators play an important role in the inflammatory process. In inflamed tissues, their biosynthesis is significantly increased, and they contribute to the development of the main signs of acute inflammation. Moreover, during an inflammatory response, the level and profile of PG production change significantly [35].

COX-2 is also expressed constitutively, in few tissues such as in brain, kidney and seminal vesicles, but is induced by various inflammatory and mitogenic stimuli [36]. It is highly induced by various growth factors, cytokines, endotoxins, pro-inflammatory molecules and tumor promoters in various cell types and has emerged as the isoform primarily responsible for PGs production in acute and chronic inflammatory conditions [37]. Over-expression of COX-2 is associated with high levels of $PGE_2$ and has been demonstrated in several malignancies of breast, lung, prostrate, skin, cervix and head and neck [38]. Higher prostaglandin levels have been shown to stimulate proliferation of cells and mediate immune suppression [39]. Recently, COX-2 has been shown to be involved in the suppression of apoptosis, which is critical in tumor cell death [40]. In parallel with the COX enzyme family, there also exists constitutive isoforms of Nitric oxide synthase (NOS), produce NO to maintain physiological functions, including regulation of vasodilation and neurotransmission. Like COX-2, iNOS also plays an important role in the mediation of inflammation [41].

## NITRIC OXIDE SYNTHASE PATHWAY

NOS enzyme catalyzes the conversion of L-arginine into L-citrulline with stoichiometric formation of NO, a gaseous free radical. It acts as a novel transcellular messenger molecule in many key physiological and pathological processes [16, 42]. The nitric oxide synthases (NOSs) catalyze the oxidation of the terminal guanidine group of L-arginine to NO [43]. This conversion occurs in two steps, a two electron oxidation of L-arginine to Nω-hydroxy-L-arginine followed by a three-electron oxidation of Nω-L-hydroxy arginine to NO and L-citrulline [44]. Nitric oxide formation is catalyzed by three homologous NOS isozymes, constitutive isozymes function to produce low levels of NO predominantly for blood pressure regulation and nerve function. In contrast, iNOS induced by cytokines produces high quantities of NO in activated inflammatory cells [45].

## ISOFORMS OF NITRIC OXIDE SYNTHASE

NOS classified into subfamilies according to the location of expression in the body and the manner of expression namely constitutive or inducible. Three quite distinct isoforms of NOS have been identified, referred by the most common nomenclature as nNOS (also known as Type I, NOS-I) being the isoform first found (and predominating) in neuronal tissue, iNOS (also known as Type II, NOS-II) being the isoform which is inducible in a wide range of cells and tissues and eNOS (also known as Type III, NOS-III) being the isoform first found in vascular endothelial cells. These isoforms have in the past been also differentiated on the basis of their constitutive (eNOS and nNOS) versus inducible (iNOS) expression [16].

The human NOSs exist on distinct genes, with different localization, regulation, catalytic properties and inhibitor sensitivity, and with 51±57% homology. They are homodimers with each monomer composed of two distinct catalytic domains, N-terminal oxygenase domain containing binding sites for heme, $BH_4$ and L-arginine and are linked by a CaM-recognition site to a C-terminal reductase domain that contains binding sites for FAD, FMN and NADPH [46]. The nNOS, iNOS and eNOS genes were located on human chromosomes 12, 17 and 7 respectively. The gene for constitutive nNOS is >200kb in length, contains 29 exons, 28 introns and for eNOS is 21-22kb in length, contains 26 exons, 25 introns, whereas iNOS gene is about 37kb, contains 26 exons, 25 introns. The molecular weight of nNOS, iNOS and eNOS proteins is approximately 161kD, 131kD and 133kD containing 1434, 1153 and 1203 amino acids respectively [47-52]. NOSs

have essential roles in the maintenance of homeostasis, e.g., regulating blood vessel tone (eNOS), and providing neurotransmitter and neuromodulator (nNOS) functions. On the other hand, numerous reports have implicated that sustained and/or excess NO generation, most of which is attributable to iNOS expression, often occurs in pathogenic conditions. In particular, iNOS has drawn considerable attention for its critical functions in inflammation-related diseases [53-55].

## ROLE OF INOS IN INFLAMMATION

NO synthesized from L-arginine by iNOS is a multifunctional mediator involved in the vasodilatation observed during inflammatory responses and an important biological signaling molecule and cellular cytotoxin. The excessive production of this free radical is pathogenic to the host tissue, since NO can bind with other superoxide radicals which directly damages the function of normal cells [56, 57]. iNOS is expressed in a variety of cell types under both normal and pathological conditions, including macrophages, microglial cells, keratinocytes, hepatocytes, astrocytes and vascular endothelial and epithelial cells. With infectious and pro-inflammatory stimuli, iNOS protein is highly induced to produce NO in a micromolar range, whereas NO generation from nNOS and eNOS enzymes is constant and within the nanomolar range [16]. The expression of iNOS can be transcriptionally regulated by factors such as interferon-γ (IFN-γ), IL-1β and TNF-α, LPS and oxidative stress (hypoxia) [58]. iNOS has been implicated in different stages of cellular changes that lead to malignancy: transformation of normal cells, growth of transformed cells, angiogenesis triggered by angiogenic factors released from tumor cells or from the surrounding tissue, and metastasis of malignant cells [59]. NOS activity has been observed in human tumor cell lines and cells from tumor biopsies. In a variety of human malignant tumors, e.g, breast, lung, prostate, bladder, colorectal cancer and malignanat melanoma, expression of iNOS can be observed. An initial study on iNOS expression in human breast cancer suggested that iNOS activity was higher in less differentiated tumors in invasive breast carcinomas [58, 59]. Patients with iNOS positive breast carcinomas were found to have significantly poorer overall survival rates than those with iNOS negative tumors [60].

## SYNERGISTIC ROLE OF COX-2 AND INOS IN INFLAMMATION

The ubiquitous nuclear factor kappa B (NF-κB) signalling pathway plays central role in regulating inflammation through transcription of pro-inflammatory genes COX-2 and iNOS. Although this factor is expressed in an inactive state in most cells, cancer cells express an activated form of NF-κB. This activation is induced by a wide variety of pro-inflammatory stimuli (such as mitogens, inflammatory cytokines and LPS), carcinogens, and the gene products regulated by it mediate tumorigenesis [61]. NF-κB is composed of a range of homo or heterodimeric combinations of NF-κB /Rel proteins, such as Rel (cRel), RelA (p65), RelB, NF-κB1 (p50), and NF-κB2 (p52) in mammals. The main inducible form is a heterodimeric consisting of the p50/p65 subunit. NF-κB is present in the cytoplasm as an inactive complex associated with an inhibitory protein called IκB. Exposure of cells to pro-inflammatory stimuli causes the dissociation of NF-κB/IκB complex by phosphorylation of IKK (IκB kinase), which in turn phosphorylates IκB at Ser-32 and Ser-36 followed by proteosomal degradation of IκB. Then subsequent translocation of NF-κB from cytoplasm to nucleus occurs via specific machinery. In the nucleus, NF-κB induces the transcription of a large variety of target genes that encode inflammatory enzymes, by binding to the cis-acting κB element. Among the transcription regulators in the promoter regions of iNOS and COX-2, NF-κB seems to work as the most essential transcription factor for the expression of these inflammatory enzymes in LPS induced cells [62-65]. Since the expression and activity of both iNOS and COX-2 is induced by the same pro-inflammatory agents and their similarities in terms of pathophysiological phenomena, it has been proposed that inhibition of both iNOS and COX-2 would provide the most potent anti-inflammatory effect [41]. Therefore, the targeted inhibition of iNOS and

COX-2 is a promising approach to inhibit inflammation as well as preventing cancer.

## ROLE OF REACTIVE OXYGEN SPECIES IN INFLAMMATION

Intracellular ROS production plays a key role in modulation of release of other mediators of inflammation. This is related mainly to the constitutive expression of NAD(P)H oxidases (termed NOXs- non-phagocytic oxidases) in various tissues [66, 67]. ROS produced by this family of enzymes can regulate adhesion molecule expression on endothelium and inflammatory cells, thus affecting cell recruitment to the sites of inflammation [68, 69]. They also increase chemokines and cytokine expression [70, 71]. At least part of these effects results from the ability of ROS (in particular H2O2) to stimulate MAP-kinases activity which leads to activation of several transcription factors. It is possible that intracellular ROS may act as second messengers in inflammatory signal transduction. Inflammatory cytokines (like TNF-a) may in turn increase NAD(P)H oxidase activity and expression which closes vicious circle of inflammation [72]. While loss of NAD(P)H oxidase activity in cells leads to diminished inflammation in the vascular wall several humoral factors may affect constitutive NAD(P)H oxidase expression in the vascular wall and therefore intracellular ROS production. These include angiotensin II, endothelins, high glucose or high cholesterol levels [66, 73]. Their effects on baseline ROS production may therefore mediate modulatory effects of these factors (traditionally not considered) on inflammation. Accordingly, attempts were undertaken to inhibit intracellular ROS production in order to limit inflammatory responses. Apocynin, an NAD(P)H oxidase activation inhibitor has been successfully used in limiting inflammation in animal model of rheumatoid arthritis [74, 75] while decoy peptide, which prevents an association of NAD(P)H oxidase subunits was shown to be effective in inflammation related to atherosclerosis [76]. It is important to note that antioxidant properties of nitric oxide are also important in mediating anti-inflammatory properties of NO [77, 78]. NO inhibitory effects on NAD(P)H oxidase can explain successful application of nitric oxide gene transfer to limit the extent of vascular inflammation [79, 80].

## ANTI-INFLAMMATORY DRUGS

A number of drugs have been developed to cure the diseases of chronic inflammation origin. These drugs can be divided into two groups; steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs. Steroids are the chemical compounds released by the adrenal gland and have anti-inflammatory action by different mechanisms. As an example, glucocorticoids are the steroidal hormones which enhance the expression of nearly 130 genes which include the anti-inflammation, phagocytosis, antioxidative stress and suppress the pro-inflammatory genes [81, 82]. In addition, glucocorticoids may express non genomic pathways by restricting ATP consuming activities and these effects are much more rapid than genomic effects. Corticosteroids, another type of steroid hormones, inhibit the activity of phospholipase $A_2$ and diminish the production of AA upon activation of cells by pro-inflammatory molecules. PGs and LTs are thus inhibited by corticosteroids through the action of phospholipase $A_2$. However, a number of side effects are revealed as a result of glucocorticoid use in inflammatory diseases. Glucocorticoids enhance glucose levels by degrading proteins and modulating fatty acid metabolism partly. This catabolic interference by corticosteroids leads to tissue remodeling, osteoporosis, insulin resistance and diabetes. Long term use of glucocorticoids increases the apoptosis of hypertrophic chondrocytes in growth plate which reduces the longitudinal growth of bones [83].

The second category of anti-inflammatory drugs is NSAIDs. Approximately, 60 million Americans use the non steroidal anti-inflammatory drugs annually to treat inflammation related diseases and especially rheumatological disorders and arthritis. NSAIDs show their effect by inhibiting the action of COX instead of phospholipase $A_2$ and do not prevent the activity of LOX [84]. NSAIDs block the production of PGs by inhibiting both COX-1 and COX-2. It is known that about 1% of

chronic users of NSAIDs, such as patients with chronic inflammatory diseases develop gastrointestinal (GI) complications such as mucosal damage and bleeding. Moreover, some researchers have found acute renal failure as a result of NSAIDs use [85]. NSAIDs inhibit the production of renal prostaglandins and negatively affect glomerular filtration rate and salt excretion. These drugs appear to produce at least some of their beneficial effects by inhibiting COX-2 and their deleterious side effects by inhibiting COX-1. Hence, synthetic anti-inflammatory drugs are more associated with negative effects rather than positive effects. Thus, selective inhibition of the induced enzyme, without affecting the homeostatic one, might avoid the side effects of currently available NSAIDs. NSAIDs have also been shown to inhibit iNOS but the pharmacological inhibitors of iNOS are not yet in clinical use while selective inhibitors of COX-2 have recently been launched on the market [86]. Selective COX-2 inhibitors (COXibs) have same anti-inflammatory benefits as traditional NSAIDs with little effect on COX-1, but as inhibitors of the enzyme responsible for the production of most inflammatory PGs, their drug efficacy is upheld. COXibs have proven to be effective in suppressing experimental tumorigenesis. Furthermore, several recently reported randomized clinical trials have shown that COXibs significantly reduce the incidence of colorectal adenomas in humans. Dismayingly, these trials also identified an increased risk for cardiovascular events associated with COXib use, suggesting that COXibs may not be sufficiently safe for general use as cancer chemopreventive agents [86]. In view of the gastric side effects of conventional NSAIDs and the recent market withdrawal of rofecoxib and valdecoxib due to their adverse cardiovascular side effects there is need to develop alternative anti-inflammatory agents with reduced gastric and cardiovascular problems [87].

## CONCLUSIONS

Nitric oxide, COX-2 and other proinflammatory mediators play important roles in the modulation of inflammation and immune regulation. Their effects are achieved through interactions with numerous signal transduction pathways and transcription factors. Therefore the exact effects of these mediators on individual cells participating in inflammation may be ambiguous, and depend on cellular environment, NO concentration as well as other factors.

## REFERENCES


1. *Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE, Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation, Clin, Exp, Immunol,, 2007;147: 227–235.*
2. *Dassoler M, Schwanz M, Busseto F, Moreira EA, Gutierrez L, Perfil fitoquímicoensaio farmacológico de Averrhoa carambola L, (Oxalidaceae), J, Bras, Fitom,, 2004; 2: 4–8.*
3. *Falcão H, Lima IO, Santos VL, Dantas HF, Diniz MFFM, Barbosa-Filho JM, Batista LM, Review of the plants with anti-inflammatory activity studied in Brazil, Braz, J, Pharmacogn,, 2005; 15: 381–391.*
4. *Howard OZ, Autoantigen signalling through chemokine receptors,Current opinion in rheumatology, 2006; 18(6): 642-646.*
5. *Charles M, Belcram H, Just J, Huneau C, Viollet A, Couloux A & Chalhoub B, Dynamics and differential proliferation of transposable elements during the evolution of the B and A genomes of wheat, Genetics, 2008; 180(2): 1071-1086.*
6. *Hortelano S, Molecular basis of the anti-inflammatory effects of terpenoids, Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy), 2009; 8(1): 28-39.*
7. *Aggarwal BB, Harikumar KB, Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases,The international journal of biochemistry & cell biology, 2009;41(1): 40-59.*
8. *Lin WW, Karin M, A cytokine-mediated link between innate immunity, inflammation, and cancer, Journal of Clinical Investigation, 2007;117(5): 1175.*
9. *Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN, Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, Science, 1987; 237(4819): 1171-1176.*
10. *Serhan C N, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Spite M, Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions, The Journal of experimental medicine, 2009;206(1): 15-23.*
11. *Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G, Inflammation and cancer: how hot is the link?, Biochemical pharmacology, 2006; 72(11): 1605-1621.*
12. *Mantovani A, Sica A, Locati M, Macrophage polarization comes of age, Immunity, 2005; 23(4): 344-346.*
13. *Coussens LM, Werb Z, Inflammation and cancer, Nature, 2002;420(17): 860-867.*
14. *Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-Bailey D, Croxtall J, Willoughby DA, Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation, Proceedings of the National Academy of Sciences, 1994; 91(6): 2046-2050.*

15. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD, Antioxidant xanthones from the pericarp of Garcinia mangostana (Mangosteen),Journal of agricultural and food chemistry, 2006; 54(6): 2077-2082.
16. Murakami A, Ohigashi H, Targeting NOX, INOS and COX-2 in inflammatory cells: Chemoprevention using food phytochemicals, International journal of cancer, 2007; 121(11): 2357-2363.
17. Israf DA, Khaizurin TA, Syahida A, Lajis NH, Khozirah S, Cardamonin inhibits COX and iNOS expression via inhibition of p65NF-κB nuclear translocation and Iκ-B phosphorylation in RAW 264,7 macrophage cells, Molecular immunology, (2007), 44(5): 673-679.
18. Oeckinghaus A, Hayden MS, Ghosh S, Crosstalk in NF-[kappa] B signaling pathways, Nature immunology, (2011), 12(8): 695-708.
19. Hinz M, Scheidereit C, The IκB kinase complex in NF-κB regulation and beyond, EMBO reports, (2014), 15(1): 46-61.
20. Bony E, Boudard F, Dussossoy E, Portet K, Brat P, Giaimis J, Michel A, Chemical composition and anti-inflammatory properties of the unsaponifiable fraction from awara (Astrocaryum vulgare M,) pulp oil in activated J774 macrophages and in a mice model of endotoxic shock, Plant foods for human nutrition, (2012), 67(4): 384-392.
21. Wun ZY, Lin CF, Huang WC, Huang YL, Xu PY, Chang WT, Liou CJ, Anti-inflammatory effect of sophoraflavanone G isolated from Sophora flavescens in lipopolysaccharide-stimulated mouse macrophages, Food and Chemical Toxicology, (2013), 62: 255-261.
22. Salvemini D, Ischiropoulos H, Cuzzocrea S, Roles of nitric oxide and superoxide in inflammation, Methods Mol Biol, (2003), 225: 291-303.
23. Tiidus PM, Radical species in inflammation and overtraining, Can J Physiol Pharmacol (1998), 76: 533-538.
24. Brash AR, Arachidonic acid as a bioactive molecule, Journal of Clinical Investigation, (2001), 107(11): 1339.
25. Stratton MS, Alberts DS, Current application of selective COX-2 inhibitors in cancer prevention and treatment, Oncology, (2002), 16(5 Suppl 4): 37-51.
26. Stables MJ, Gilroy DW, Old and new generation lipid mediators in acute inflammation and resolution, Progress in lipid research, (2011), 50(1): 35-51.
27. Simmons DL, Botting RM, Hla T, Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition, Pharmacological reviews, (2004), 56(3): 387-437.
28. Davies G, Martin LA, Sacks N, Dowsett M, Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention, Annals of Oncology, (2002), 13(5): 669-678.
29. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Smithies O, Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse, Cell, (1995), 83(3): 473-482.
30. Chandrasekharan NV, Simmons DL, The cyclooxygenases,Genome biology, (2004), 5: 2004-5.
31. Bonventre JV, Huang Z, Taheri MR, O'Leary ELiE, Moskowitz MA, Sapirstein A, Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2, Nature, (1997), 390(6660): 622-625.
32. van Rees BP, Saukkonen K, Ristimäki A, Polkowski W, Tytgat GN, Drillenburg P, Offerhaus JA, Cyclooxygenase-2 expression during carcinogenesis in the human stomach, The Journal of pathology, (2002), 196(2): 171-179.
33. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Seibert K, Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic, Proceedings of the National Academy of Sciences, (1994), 91(8): 3228-3232.
34. Baigent C, Patrono C, Selective cyclooxygenase 2 inhibitors, aspirin, and cardiovascular disease: a reappraisal, Arthritis & Rheumatism, (2003), 48(1): 12-20.
35. Ricciotti E, FitzGerald GA, Prostaglandins and inflammation,Arteriosclerosis, thrombosis, and vascular biology, (2011), 31(5): 986-1000.
36. Méric JB, Rottey S, Olaussen K, Soria JC, Khayat D, Rixe O, Spano JP, Cyclooxygenase-2 as a target for anticancer drug development, Critical reviews in oncology/hematology, (2006), 59(1): 51-64.
37. Minghetti L, Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases, Journal of Neuropathology & Experimental Neurology, (2004), 63(9): 901-910.
38. Dannenberg AJ, Altorki NK, Boyle JO, Dang C, Howe LR, Weksler BB, Subbaramaiah K, Cyclo-oxygenase 2: a pharmacological target for the prevention of cancer, The lancet oncology, (2001), 2(9): 544-551.
39. Ji CHUAN, Marnett LJ, Oxygen radical-dependent epoxidation of (7S, 8S)-dihydroxy-7, 8-dihydrobenzo [a] pyrene in mouse skin in vivo, Stimulation by phorbol esters and inhibition by antiinflammatory steroids,Journal of Biological Chemistry, (1992), 267(25): 17842-17848.
40. Pardhasaradhi BV, Ali AM, Kumari AL, Reddanna P, Khar A, Phycocyanin-mediated apoptosis in AK-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS, Molecular cancer therapeutics, (2003), 2(11): 1165-1170.
41. Weinberg JB, Nitric oxide synthase 2 and cyclooxygenase 2 interactions in inflammation, Immunologic research, (2000), 22(2-3): 319-341.
42. Moncada SRMJ, Palmer RML, Higgs E, Nitric oxide: physiology, pathophysiology, and pharmacology, Pharmacological reviews, (1991), 43(2): 109-142.
43. Kerwin Jr JF, Lancaster JR, Feldman PL, Nitric oxide: a new paradigm for second messengers, Journal of medicinal chemistry, (1995), 38(22): 4343-4362.
44. Stuehr DJ, Cho HJ, Kwon NS, Weise MF, Nathan CF, Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD- and FMN-containing flavoprotein, Proceedings of the National Academy of Sciences, (1991), 88(17): 7773-7777.
45. Kwon OK, Lee MY, Yuk JE, Oh SR, Chin YW, Lee HK, Ahn KS, Anti-inflammatory effects of methanol extracts of the root of Lilium lancifolium on LPS-stimulated Raw264, 7 cells, Journal of ethnopharmacology, (2010), 130(1): 28-34.
46. Knowles RG, Moncada S, Nitric oxide synthases in mammals,Biochemical Journal, (1994), 298(Pt 2): 249.
47. Nakane M, Schmidt HH, Pollock JS, Förstermann U, Murad F, Cloned human brain nitric oxide synthase is

highly expressed in skeletal muscle, FEBS letters, (1993), 316(2): 175-180.
48. Geller DA, Lowenstein CJ, Shapiro RA, Nussler AK, Di Silvio M, Wang SC, Billiar TR, Molecular cloning and expression of inducible nitric oxide synthase from human hepatocytes, Proceedings of the National Academy of Sciences, (1993), 90(8): 3491-3495.
49. Sherman MP, Aeberhard EE, Wong VZ, Griscavage JM, Ignarro LJ, Pyrrolidine dithiocarbamate inhibits induction of nitric oxide synthase activity in rat alveolar macrophages, Biochemical and biophysical research communications, (1993), 191(3): 1301-1308.
50. Charles IG, Palmer RM, Hickery MS, Bayliss MT, Chubb AP, Hall VS, Moncada S, Cloning, characterization, and expression of a cDNA encoding an inducible nitric oxide synthase from the human chondrocyte, Proceedings of the National Academy of Sciences, (1993), 90(23): 11419-11423.
51. Janssens SP, Shimouchi A, Quertermous T, Bloch DB, Bloch KD, Cloning and expression of a cDNA encoding human endothelium-derived relaxing factor/nitric oxide synthase, Journal of biological chemistry, (1992), 267(21): 14519-14522.
52. Marsden PA, Schappert KT, Chen HS, Flowers M, Sundell CL, Wilcox JN, Michel T, Molecular cloning and characterization of human endothelial nitric oxide synthase, FEBS letters, (1992), 307(3): 287-293.
53. Cedergren J, Forslund T, Sundqvist T, Skogh T, Inducible nitric oxide synthase is expressed in synovial fluid granulocytes, Clinical & Experimental Immunology, (2002), 130(1): 150-155.
54. Donnelly LE, Barnes PJ, Expression and regulation of inducible nitric oxide synthase from human primary airway epithelial cells, American journal of respiratory cell and molecular biology, (2002), 26(1): 144-151.
55. Cross RK, Wilson KT, Nitric oxide in inflammatory bowel disease, Inflammatory bowel diseases, (2003), 9(3): 179-189.
56. Griffith OW, Stuehr DJ, Nitric oxide synthases: properties and catalytic mechanism, Annual Review of Physiology, (1995), 57(1): 707-734.
57. Moncada SRMJ, Palmer RML, Higgs E, Nitric oxide: physiology, pathophysiology, and pharmacology, Pharmacological reviews, (1991), 43(2): 109-142.
58. Weiming XU, LIU LZ, Loizidou M, AHMED M, CHARLES IG, The role of nitric oxide in cancer, Cell research, (2002), 12(5): 311-320.
59. Thomsen LL, Miles DW, Happerfield L, Bobrow LG, Knowles RG, Moncada S, Nitric oxide synthase activity in human breast cancer,British Journal of Cancer, (1995), 72(1): 41.
60. Loibl S, Buck A, Strank C, von Minckwitz G, Roller M, Sinn HP, Kaufmann M, The role of early expression of inducible nitric oxide synthase in human breast cancer, European journal of cancer, (2005), 41(2): 265-271.
61. Aggarwal BB, Nuclear factor-κB: the enemy within, Cancer cell, (2004), 6(3): 203-208.
62. Baeuerle PA, Baltimore D, NF-κB: ten years after, Cell, (1996), 87(1): 13-20.
63. Pahl HL, Activators and target genes of Rel/NF-kappaB transcription factors, Oncogene, (1999), 18(49): 6853-6866.
64. Yeh CB, Hsieh MJ, Hsieh YH, Chien MH, Chiou HL, Yang SF, Antimetastatic effects of norcantharidin on hepatocellular carcinoma by transcriptional inhibition of MMP-9 through modulation of NF-kB activity,PLoS One, (2012), 7(2): e31055.
65. Winston JT, Strack P, Beer-Romero P, Chu CY, Elledge SJ, Harper JW, The SCFβ-TRCP–ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination in vitro, Genes & development, (1999), 13(3): 270-283.
66. Guzik TJ, West NEJ, Black E, Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors, Circ Res (2000), 86: e85-e90.
67. Guzik TJ, Mussa S, Gastaldi D, et al, Mechanisms of increased vascular superoxide production in human diabetes mellitus: Role of NAD(P)H oxidase and endothelial nitric oxide synthase, Circulation (2002), 105: 1656-1662.
68. Niu XF, Smith CW, Kubes P, Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion molecules to neutrophils, Circ Res (1994), 74: 1133-1140.
69. Fraticelli A, Serrano CV, Bochner BS, et al, Hydrogen peroxide and superoxide modulate leukocyte adhesion molecule expression and leukocyte endothelial adhesion, Biochim Biophys Acta, (1996) 1310: 251-259.
70. Kimura T, Iwase M, Kondo G, et al, Suppressive effect of selective cyclooxygenase-2 inhibitor on cytokine release in human neutrophils, Int Immunopharmacol (2003), 3: 1519-1528.
71. Brzozowski T, Konturek PC, Konturek SJ, et al, Implications of reactive oxygen species and cytokines in gastroprotection against stress-induced gastric damage by nitric oxide releasing aspirin, Int J Colorectal Dis (2003), 18: 320-329.
72. Decleva E, Dri P, Menegazzi R, et al, Evidence that TNF-induced respiratory burst of adherent PMN is mediated by integrin alpha(L)beta(2), J Leukoc Biol (2002), 72: 718-726.
73. Griendling KK, Sorescu D, Ushio-Fukai M, NAD(P)H oxidase: role in cardiovascular biology and disease, Circ Res (2000), 86: 494-501.
74. Lafeber FP, Beukelman CJ, van den Worm E, et al, Apocynin, a plant-derived, cartilage-saving drug, might be useful in the treatment of rheumatoid arthritis, Rheumatology (Oxford) (1999), 38: 1088-1093.
75. van den Worm E, Beukelman CJ, van den Berg AJ, et al, Effects of methoxylation of apocynin and analogs on the inhibition of reactive oxygen species production by stimulated human neutrophils, Eur J Pharmacol (2001), 433: 225-230.
76. Rey FE, Cifuentes ME, Kiarash A, et al, Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(-) and systolic blood pressure in mice, Circ Res (2001), 89: 408-414.
77. Kwiecien S, Brzozowski T, Konturek P, et al, The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions, J Physiol Pharmacol (2002), 53: 761-773.
78. Kwiecien S, Brzozowski T, Konturek SJ, Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury, J Physiol Pharmacol (2002), 53: 39-50.
79. Channon KM, Qian HS, George SE, Nitric oxide synthase in atherosclerosis and vascular injury: insights from experimental gene therapy, Arteriosc Thromb Vasc Biol (2000), 20: 1873-1881.
80. West NEJ, Guzik TJ, Black E, et al, Enhanced superoxide production in experimental venous bypass graft intimal hyperplasia: role of NAD(P)H oxidase, Arteriosc Thromb Vasc Biol (2001), 21: 189-194.

81. *Franchimont N, Canalis E, Management of glucocorticoid induced osteoporosis in premenopausal women with autoimmune disease,Autoimmunity reviews, (2003), 2(4): 224-228.*
82. *Yona S, Gordon S, Glucocorticoids turn the monocyte switch,Immunol Cell Biol, (2007), 85: 81-2.*
83. *De Luca F, Impaired growth plate chondrogenesis in children with chronic illnesses, Pediatric research, (2006), 59(5): 625-629.*
84. *Vane JR, Botting RM, Mechanism of action of nonsteroidal anti-inflammatory drugs, The American journal of medicine, (1998), 104(3): 2S-8S.*
85. *Griffin MR, Yared A, Ray WA, Nonsteroidal antiinflammatory drugs and acute renal failure in elderly persons, American Journal of Epidemiology, (2000), 151(5): 488-496.*
86. *Simon LS, Weaver AL, Graham DY, Kivitz AJ, Lipsky PE, Hubbard RC, Geis GS, Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial, Jama, (1999), 282(20): 1921-1928.*
87. *Reddy RN, Mutyala R, Aparoy P, Reddanna P, Reddy MR, Computer aided drug design approaches to develop cyclooxygenase based novel anti-inflammatory and anti-cancer drugs, Current pharmaceutical design, (2007), 13(34): 3505-3517.*