The role of COX-2 and Nrf2/ARE in anti-inflammation and antioxidative stress: Aging and anti-aging

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Abstract

Oxidative stress and inflammation are constant features of many chronic diseases and complications, and have been linked to carcinogenesis. Cyclooxygenase 2 (COX-2), a rate-limiting enzyme for the synthesis of prostaglandins, plays important roles in physiology and pathology, but has been a source of controversy within the scientific and clinical community. However, recent work has shown that nuclear factor erythroid-2-related factor-2 (Nrf2) confers protection against oxidative stress. Furthermore, COX-2-dependent electrophile oxo-derivative (EFOX) molecules have been shown to act as anti-inflammatory mediators via activation of the Nrf2-dependent antioxidant response element (ARE). These studies have provided more insight into COX-2-mediated events. The function of all tissues, especially epithelial and endothelial tissues, declines with age, leading to the production of reactive oxygen species (ROS). COX-2 expression increases with aging in most tissues, due in part to ROS, chemical reactions, physical shearing, and dietary molecules. Here we discuss new findings related to COX-2 inflammatory and anti-inflammatory responses. Taken together, we hypothesize that COX-2 levels increase during the aging process because increasing levels of ROSs necessitate the involvement of COX-2-dependent EFOXs for anti-inflammation and Nrf2/ARE signaling for antioxidation. We also propose that COX-2 may act as an intrinsic biological aging clock due to its role in balancing inflammatory and anti-inflammatory responses.

Introduction

Oxidative stress and chronic inflammation play critical roles in neoplastic development, though they are not necessarily linked to malignancy in most cases. Cyclooxygenase (COX)-2 is an inducible enzyme, which is constitutively expressed in some tissues and a number of organs [1–4]. COX-2 is well known for its inflammatory roles, but the use of COX-2 inhibitors as painkillers for arthritis has been controversial due to extremely sensitive regulation of COX-2, strong end amplification, unrestrictive substrate specificities, and its multiple functions. As opposed to lipoxygenase (LOX), which generates low-acting leukotrienes, the downstream products of COX-2 are rapid acting and versatile molecules, which are involved in almost all physiological and pathological activities. Inhibition of COX-1 by aspirin has been proven to reduce cardiovascular risk, whereas selective COX-2 inhibition increases cardiovascular events, such as stroke or myocardial infarction [7,8]. However, in recent years the discovery of COX-2’s multiple sub-strates, in addition to COX-2-dependent electrophilic oxo-derivative (EFOX) molecules and their regulation of nuclear factor erythroid-2-related factor-2/keap1 and antioxidative response elements (ARE), has revealed some of the anti-inflammatory mechanisms of COX-2 [9,10]. Anti-inflammatory lipid autacoids, such as COX-2-derived 15-deoxy PGJ2 [11] and 15-LOX-derived LXA4, have emerged as central regulators of leukocyte function and the active resolution of inflammation. The receptors of these lipid autacoids are highly expressed in the mucosa, epidermis, and cornea [12]. The roles of COX-2-mediated lipid molecules in inflammation and anti-inflammation provide compelling evidence of the molecular mechanisms of COX-2, as well as promising potential therapeutic targets and agents.

COX-2-dependent EFOX(s) in the Nrf2/ARE pathway

Initially, COX-2 is mainly expressed in activated macrophages and other inflammatory cells and is up-regulated after exposure to growth factors or inflammatory stimuli. In addition, COX-2 is expressed at elevated levels in malignant cells. COX-2 is widely
recognized as a classic mediator of pro-inflammatory activities, though it can also mediate physiological effects, such as regulating the contraction and relaxation of smooth muscle tissue. The standard substrate of COX-1 and COX-2 is the membrane-derived polyunsaturated fatty acid, arachidonic acid (AA). However, COX-2 can oxygenate a broad spectrum of fatty acid and fatty ester substrates [13]. For instance, COX-2 converts the ω-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) into prostaglandins, and further converts them into 13-EFOX-D_6 and 13-EFOX-D_5, respectively, via dehydrogenase and non enzymatic reactions [14]. These COX-2-derived oxidative metabolites possess both anti-inflammatory and antioxidant properties. Some EFOXs and lipoygenase-dependent lipoxins, as well as members of the resolvin and protectin families, provide potent signals to selectively stop neutrophil and eosinophil infiltration, stimulate non-phlogistic recruitment of monocytes, activate macrophase phagocytosis of microorganisms and apoptotic cells, increase the exit of phagocytes from the inflamed site through the lymphatics, and stimulate the expression of molecules involved in antimicrobial immune defenses [15]. More significantly, EFOX levels were significantly increased in the presence of aspirin, suggesting another mechanism for aspirin's cardiovascular protection and beneficial anti-inflammatory effects. In addition, COX-2-dependent EFOXs have been shown to reversibly adduct to cysteine and histidine residues of proteins via thiols and activate Nrf2- and ARE-dependent gene expression [16]. Under oxidative or electrophilic stress, adduction disrupts critical cysteine residues in Keap1, resulting in a dissociation of the Keap1-Cul3 ubiquitination system and a build-up of Nrf2 in the cytoplasm [17,18]. Free Nrf2 can translocate to the nucleus and regulate the expression of various genes that are cytoprotective against oxidative and inflammatory stress. In the nucleus, Nrf2 heterodimerizes with a small Maf protein and binds to the upstream promoter ARE(s) to activate many antioxidant genes (Fig. 1). These genes constitute the so-called phase-II antioxidant and antixenobiotic response genes, and include heme oxygenase-1 (HO-1), NADPH quinine oxidoreductase (NQO1), glutathione S-transferase (GST), γ-glutamylcysteine synthase (GCS), glutathione reductase (GR), superoxide dismutases (SODs), UDP-glucuronosyl transferases (UGTs), and γ-glutamyl synthase [19]. Under normal or unstimulated conditions, Nrf2 is tethered in the cytoplasm by Keap1. The tramtrack and Bric-a-Brac (BTB) domain of Keap1 contains the Cys151 residue required for stress sensing [20] and the intervening region domain contains two cysteine residues, Cys272 and Cys288, critical for the repression of Nrf2 activity. In addition, the double glycine repeat and C-terminal region domains of Keap1 contribute to its interaction with Nrf2. The induction of Nrf2 target genes has a profound impact on combating oxidative stress and inflammation [20].

In addition to specific lipid autacoids, prostaglandin E_2 (PGE_2) and PGD_2 extruded from certain inflamed tissues have proinflammatory activities after acute inflammation or inflammatory diseases and promote a switch from an inflammatory phenotype to a pro-resolution homeostasis characterized by infiltrating neutrophils [21]. Via their receptors, PGE_2 and PGD_2 increase intracellular cyclic adenosine monophosphate (cAMP) levels, resulting in a switch to an anti-inflammatory response [22]. In addition, the non-enzymatically modified products 15-deoxy-delta-12,14-PGJ_2 from PGD_2 and the analogs of cyclopentaenones can enhance resolution by promoting leukocyte apoptosis and macrophase clearance from inflamed sites [23,24], possibly by inhibiting NF-κB activation [25]. These anti-inflammation and antioxidative activities are subject to cross-regulation, as evidenced by the fact that the levels of pro-inflammatory mediators, such as COX-2, inducible nitric oxide synthase (iNOS), interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNFα), are significantly elevated in the colonic tissues of Nrf2−/− mice compared to their wild-type counterparts [26].

These new findings have renewed our interest in the role of COX-2, especially in the context of the Nrf2/ARE pathway. Nrf2 can induce genes important in combating oxidative stress, thereby activating the body's own protective response, even in situations where the administration of exogenous antioxidants, such as vitamin C and E, have failed. Therefore, this system might represent an innate aging maintenance system. The full extent of COX-2's functions likely have not been explored. For instance, there are likely additional COX-2-dependent, short half-life eicosanoids to

Fig. 1. A diagram briefly describes a plausible mechanism of COX-2's antioxidation via EFOX molecules, where ROS(s) are hypothesized to trigger the antioxidation and anti-inflammation largely based on the observation that COX-2 expression is increased in aging tissues. The abbreviation, AA: arachidonic acid, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, LNA: linolenic acid, ROS: reactive oxygen species.
be discovered. However, the current data strongly suggest that con-
tinuous chronic up-regulation of pro-inflammatory mediators oc-
curs during the aging process due to an age-related redox imbalance that activates many pro-inflammatory signaling path-
ways, including NF-κB and COX-2, as well as anti-inflammatory and anti-oxidant pathways such as Nrf2/ARE (Fig. 1).

COX-2 in epithelial and endothelial aging

The only difference between the three-dimensional structures of COX-1 and two occurs at the 523 position where the COX-1 se-
quence contains a bulky isoleucine. At this position, COX-2 con-
tains a valine residue, which creates a bigger loop, making design of a selective inhibitor possible. Unfortunately selective COX-2 inhibitors have so far been associated with an increased risk for cardiovascular diseases. One of the speculative reasons for this is that these agents reduce levels of prostacyclin (PGI₂), an important vasodilator that is released by healthy endothelial cells and acts through a paracrine signaling cascade without affecting thrombox-
ane (TXA₂) levels. Despite this speculation, the factors underlying the development of vascular disease associated with COX-2 inhibi-
tion and the development of hypertension, the most common cardio-
vascular complication associated with COX-2 inhibition, largely remain unknown [4]. Both COX-1 and COX-2 are expressed during hemostasis of the endothelium, including arteries, but COX-2 lev-
eels are elevated during this process, while COX-1 levels remain un-
changed in aging rats [27]. The expression of COX-2 varies, and age is apparently one of the major factors influencing its expression levels. For instance, acetylcholine-induced vasodilation affected rats aged 24 months differently from those 4-month-old; a differ-
ence reflected in COX-1 and COX-2 levels. Specifically, COX-2 was more active when AA concentrations were low, while COX-1 was active when substrate concentrations were high in the aged rats [27]. The maximum phenylephrine-induced contraction was in-
creased in arteries from older rats after IL-1β treatment, but inhibi-
tion of iNOS and COX-2 with 1400W and NS398, respectively, abolished age-related differences in phenylephrine contraction [28]. Furthermore the intrinsic antioxidative Nrf2/ARE can be mod-
ulated or activated by naturally-derived compounds. For example, resveratrol has been shown to activate Nrf2-dependent antioxid-
ant enzymes in hepatocytes, primary cardiocytes, endothelial cells, and epithelial cells, and is capable of inducing protection against oxidative stress or carcinogen-induced cell death [29]. Sim-
ilar to quercetin, resveratrol augmented Nrf2 RNA levels and pro-
tein stability in hepatocytes. In addition, resveratrol attenuated cigarette smoke extract (CSE)-induced ROS and restored CSE-de-
pleted glutathione (GSH) levels by up-regulating glutamate–cy-
teine ligase via Nrf2 activation in human alveolar epithelial A549 cells [30]. Grapes and olives are rich in resveratrol, which is likely why people in the Mediterranean region have a lower incidence of age-related cardiovascular diseases, cancer, diabetes and neurodegeneration.

The functional integrity of the endothelium is essential for pre-
venting vascular leakage and atherosclerotic lesions. Endothelial COX-2-dependent EPOXs possesses anti-coagulatory, anti-adhe-
sive, and anti-proliferative properties and generate a number of vasoactive substances. The complex interactions between relax-
ing/dilating and constricting factors derived from endothelial cells and smooth muscle cells result in a dynamic regulation of vascular smooth muscle cell activity that regulates blood flow to tissue. It is clear that COX-2-dependent PGE₂, PGF₂α, and other endothe-
lium-dependent relaxing factors (EDRFs) result in hyperpolariza-
tion and dilation of smooth muscle cells, whereas COX-2-
dependent TXA₂ and other endothelium-dependent contracting factors (EDCFs) result in smooth muscle depolarization and vessel

COX-2 is associated with neurodegeneration

COX-2 is constitutively expressed in the cerebral cortex, and has lately been recognized as an important player in brain function. Specifically, in terms of neurodegeneration COX-2 expression has been shown to be involved in inflammation in neurons, glia, and endothelial cells in the spinal cord and brain. Neurodegeneration associated with Alzheimer’s (AD) and Parkinson’s diseases (PD) in-
volves multiple mechanisms. COX-2 has been linked to dopaminer-
genic cell death in PD due to the fact that COX-2 protein levels are increased in dopaminergic neurons in PD brain tissue [34]. In addi-
tion, levels of PGE₂ were higher in the substantia nigra of PD brains when compared to normal brains [35]. In mice with a conditional COX-2 deletion, the peripheral inflammation-induced COX2 expression in the spinal cord was reduced, and mechanical hyper-
sensitivity after both peripheral soft tissue and periarticular inflammation was abolished [36]. In vivo studies showed that the pharmacological inhibition and genetic deletion of COX-2 attenu-
ated dopaminergic neuronal death in mice treated with 1-methyl-
4-phenyl-1,2,3,6-tetrahydropyridine [37]. Furthermore, a recent study showed that the formation of 3,4-dihydroxyphenylacetic acid and homovanillic acid was attenuated in the striatum of COX-2 deficient mice treated with the herbicide parquat, implicating COX-2 in paragquat-induced pathology [38]. These data suggest that COX-2 plays important roles in the central and peripheral nervous system and may be involved in neurodegeneration.

In Nrf2 knockout mice the expression of a number of inflamma-	ory markers is increased, while that of anti-inflammatory factors is reduced, which indicates that Nrf2 might modulate microglial fate in neurodegenerative diseases such as PD. In addition, Nrf2 has been shown to be involved in healing dopaminergic neuron injury and modulating the balance between microglial phenotypes in response to a prototypical PD toxin whose mechanism of action is related to oxidative stress [39]. Inhibition of COX-2 reduces the age-dependent increase in hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat. These results indi-
cate that there is a natural tendency to offset the age-dependent in-
crease in brain inflammatory processes via the homeostatic increase of circulating glucocorticoid hormone [40]. The inhibition of COX proteins reduces pain and inflammation peripherally through inhibi-
tion of PGE₂ at the site of peripheral inflammation, which contrib-
utes to pain hypersensitivity by reducing the threshold and increasing the excitability of peripheral terminals of nociceptor sen-
sory fibers. After peripheral inflammation, COX-2 was induced in CNS neurons, where it aided in the development of a central compo-
ment of inflammatory pain by increasing neuronal excitation. Mechanical pain is a major symptom of most inflammatory condi-
tions, and induction of COX-2 in neural cells in the CNS seems to con-
tribute to such postoperative pain and arthritis [36].
COX-2 expression in organs is influenced by aging and external factors

COX-2 has different expression profiles in different organs, and its expression is influenced by age. For instance, it has been shown that periodontal ligament cells obtained from older donors have significantly greater COX-2 expression levels and PGE$_2$ production in response to mechanically compressive force than those from younger donors, with the turning point of aging, where significantly larger amounts of COX-2-derived factors were present, found to be approximately 35 years of age [41]. One study demonstrated that COX-2 was a limiting factor in delayed endochondral bone healing [42]. Similarly, COX-2-dependent PGE$_2$ is up-regulated in the replicative senescence of dermal and prostate fibroblasts and in H$_2$O$_2$-induced premature senescence of IMR-90 lung fibroblasts [43]. Similarly, the selective COX-2 inhibitor, NS-398, was shown to inhibit the replicative senescence of cultured dermal fibroblasts. In a human study, both COX-1 and COX-2 gene expression levels were found to be elevated in older kidneys [44]. In addition, it would appear that increased COX-2 expression in the aging kidney also differs between sexes, as glomerulosclerosis and tubulointerstitial damage in the renal cortex and medulla were also significantly enhanced in male, but not female, aged, COX-2 inhibitor treated rats [45]. COX-2 is constitutively expressed in pancreatic β-cells, and plays a role in insulin secretion, and possibly β-cell destruction, when insulin-dependent diabetes mellitus occurs [46]. In obese Zucker rats, increased COX-2 and oxidative stress may contribute to obesity-related kidney damage [47]. Furthermore, during caloric restriction, the oxidative stress-induced pro-inflammatory transcription factors, altered signaling transduction for inflammatory processes, which was mediated through ROS-induced NF-κB and AP-1 and their dependent genes [48]. The increased COX-2 expression observed in aged cells was responsible for age-related suppression of Leydig cell testosterone production. However, COX-2 is constitutively highly expressed in the distal end of the vas deferens of Sprague–Dawley rats from the time of puberty until 15 months of age, when COX-2 expression gradually fades but COX-1 expression remains [34,49,50].

Aging not only involves finite life expectancy, but cellular senescence as well. Inhibition of COX-2 activity through p38 MAPK inhibitors or specific siRNA attenuates the H2O2-induced increase in senescence-associated beta-galactosidase in human fibroblasts in vitro [51]. Aging is extremely complex, and involves oxidative damage associated with cellular metabolism and genome instabilities, such as telomere shortening, mitochondrial mutations, and chromosomal pathologies. Aging-related senescence remains in immortalized cells in vitro, though the process and mechanisms may differ. The emerging data show that COX-2 is associated with aging, which may accelerate pathogenic processes such as cancer [52]. Similarly, stress response and heat shock proteins are up-regulated after middle-age and/or are associated with age-related diseases. The molecular mechanisms involving COX-2 are not completely known, but the recent findings discussed above suggest that the intrinsic antioxidative system maintained by COX-2 and Nrf2/ARE signaling constitute an interaction important to the aging process due to the fact that increasing levels of COX-2 expression likely act to maintain antioxidative homeostasis as ROS increases occur during cell senescence, acute and chronic inflammation, and carcinogenesis. In addition, COX-2 expression may be a biological aging clock, though likely not the only such clock present. This hypothesis predicts that aging is an irreversible outcome, but that the COX-2 and Nrf2/ARE pathways do respond to internal and external inflammation/anti-inflammation as well as oxidation/antioxidation. However, the complex roles of these factors require extensive further study.

Future work

COX-2 and the Nrf2/ARE pathways interact through EFOX lipid molecules. COX-2 has been shown to be constitutively expressed in non-small cell lung cancer (NSCLC) A549, and activation of Nrf2 by 15d-PGJ2 was observed in acute lung injury initially [53]. A549 cells respond well to COX-2 inhibitors and environmental anti-oxidant molecules that trigger the Nrf2 pathway. Since we have studied microarrays of human lung cancer with patients ranging in age from 38 to 82, and NSCLC accounts for 80% of all incidents of lung cancer [54], we can determine the age-related gene expression of COX-2 and Nrf2-controlled ARE genes via qRT-PCR. The genes described in this study can be compared to those obtained from anti-oxidative assays (total antioxidant capacity of intrinsic and external redox) of cultured A549 cells in the presence and absence of the COX-2 inhibitor NS-398. The results may further extend existing findings and test our hypothesis that COX-2 increases with age and is involved in both inflammatory and anti-inflammatory processes.

Conflicts of interest

None declared.

Acknowledgements

This research is supported by EU LifeSpan – FPG grant #36894, a grant from the Estonian Science Foundation for human genome variability and its association with longevity: ETF7859, a grant to study genetic variations, lifestyle and environmental factors in shaping human health (Targeted Financing from Estonian Government: SF0180142Cs08). This research was also supported by the European Union through the European Regional Development Fund directed toward the Centre of Excellence in Genomics.

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