Interactions between Cyclooxygenase–2 and Inducible Nitric Oxide Synthase: a New Prospect for Intervention

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ABSTRACT In the past years, inflammatory reaction and oxidative stress have been hot focus in many research fields. Cyclooxygenase–2 (COX–2) takes a great part in inflammatory reaction and inducible nitric oxide synthase (iNOS) plays a crucial role in oxidative stress, both of them participating in many pathological process. It is the alterations of COX–2 and iNOS that coincide with each other in a series of diseases. Increasing studies have been found that COX–2 pathway and iNOS pathway have "cross–talk". COX–2 and iNOS can be induced by the same stimuli. COX–2 and iNOS can be up–regulated by NF–κB. COX–2 and iNOS can react with each other directly. COX–2 can be induced and activated by iNOS binding as well as its resultant product, and meanwhile the product of COX–2, such as prostaglandin E2, can induce iNOS expression. In addition, when COX–2 or iNOS is induced, they can mediate the similar pathological processes. In a word, interaction between COX–2 and iNOS, a new crucial research trend in cancer, diabetes, autoimmune diseases and so on, represents a new prospect for intervention.

Key words Cyclooxygenase–2; Inducible nitric oxide synthase; Inflammation; Oxidative stress

Prostaglandins (PGs), especially prostaglandin E_2 (PGE_2), are inflammatory cytokines, playing a crucial role in inflammation. In the synthesis of PGs from arachidonic acid, cyclooxygenase is one key enzyme. There have been found at least three cyclooxygenase isoenzymes: cyclooxygenase–1 (COX–l), cyclooxygenase–2 (COX–2) and cyclooxygenase–3 (COX–3). It is reported that COX–3 might play ancillary roles in membrane–based COX signaling or when basal levels of COX–1 or COX–2 expression persist [1–3]. Although COX–l and COX–2 share approximately 60% amino acid identity, their expression and functions are not the same[4]. COX–l, expressed in a constitutive manner, has physiological functions and mainly expresses in the gastrointestinal tract, kidney, platelet and other cells and tissues [5,6]. While the inducible isoenzyme–COX–2, can be induced by a series of cytokines, such as tumor necrosis factor (TNF)–α, interleukin (IL)–1, interferon–γ (IFN–γ) and nitric oxide (NO) [7–10]. In the upstream of the COX–2 gene, has the regulation sequence for nuclear factor–kappa B (NF–κB). COX–2 leads to the dramatic and transient synthesis of PGE_2, mediating inflammation and taking an important part in many pathophysiologic processes. Over the past years, COX–2 has been hot focus for its significant action in inflammation and diseases.

There found at least three isoenzymes of nitric oxide synthases (NOS): nervous NOS (nNOS or NOS1), endothelial NOS (eNOS or NOS3) and inducible NOS (iNOS or NOS2). The former two are also called constitutive NOS (cNOS), taking a part in physiological processes. While iNOS expresses after stimulation, and plays a key role in oxidative stress. In pathological conditions, iNOS can be induced by stimuli to generate excessive NO and superoxide anion [11]. NO reacts with superoxide anion to form peroxynitrite ($ONOO^-$). $ONOO^-$ and its reactive derivatives with high energy state are one species with the strongest oxidation known at present, and its oxidative ability is 2000–fold stronger than hydrogen peroxide [12]. $ONOO^-$ might be the mediator of iNOS caused damages, playing a crucial role in a series of diseases.

Interestingly, the alterations of COX–2 and iNOS coincide with each other in many a pathological process. Whether their alterations are coincidental, or they have internal relationships? This phenomenon drives numerous researchers to investigate the relationship be-

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tween COX-2 and iNOS. Up to now, increasing studies have manifested that there exists close internal associations between COX-2 and iNOS: COX-2 and iNOS showed similar alterations in a series of diseases; share the same stimuli and have co-regulator; interact directly with each other; mediate the similar pathological effects; their inhibitors have synergistic effect.

THE SIMILAR ALTERATIONS OF COX-2 AND INOS

COX-2 and iNOS are both inducible isoenzymes, and involved in the pathogenesis of a number of diseases, including cancer, atherosclerosis, diabetes, neurodegenerative, arthritis, respiratory, hepatic and intestinal diseases [13-21]. In the beginning, people found an interesting phenomenon that the alterations of COX-2 and iNOS were consistent with each other. COX-2, as well as iNOS, is usually expressed at low or undetectable levels in most tissues and cells but can be significantly increased by stimuli in pathological state.

Significant increase of COX-2 and iNOS protein levels was observed in human colon cancer tissues as compared with control tissue [22]. Several lines of evidence suggested the critical role of iNOS and COX-2 in tumorigenesis[23]. COX-2 and iNOS may play a role in the pathophysiologic processes in colitis [24]. In diabetes, advanced glycation end products (AGEs) can up-regulate the expression of COX-2 and iNOS [25,26]. Both COX-2 and iNOS increased with the severity of the inflammatory reaction in the lipopolysaccharide (LPS) treated animals and macrophage cells [20,27]. COX-2 and iNOS have been reported to play important roles in pathophysiology of ARDS [19]. Estrogen treatment up-regulates IFN-γ-inducible-iNOS gene expression, iNOS protein, and COX-2 protein, resulting in female-predominant autoimmune diseases [28]. A marked expression of COX-2 and iNOS in rat brains after traumatic brain injury (TBI) is observed [29].

COX-2 AND INOS SHARE THE SAME STIMULI

Increasing studies have shown that both COX-2 and iNOS can be provoked by cytokines, such as IL-1, IL-1β, TNF-α, IFN-γ and chemicals. TNF-α [1ng/ml] induced a transient increase of COX-2 and iNOS mRNA accumulation and protein expression in VSMC, and the kinetics of COX-2 and iNOS mRNA accumulations were similar after challenge with TNF [7]. LPS/IFN-γ is believed to up-regulate COX-2 and iNOS expression[30]. Furthermore, NF-κB is a transcription factor necessary for COX-2 and iNOS expression. And stimuli can provoke COX-2 and iNOS expression through the activation of NF-κB-specific DNA-binding protein complex formation[31]. Blocking NF-κB activation, nuclear translocation and I-κB phosphorylation can inhibit COX-2 and iNOS expression[32,33].

COX-2 AND INOS INTERACT DIRECTLY

Currently, the effect of iNOS and its product NO on COX-2 has been further studied. Sangwon F. Kim, et al find that iNOS can bind, S-nitrosylate, and activate COX-2 [34]. Co-immunoprecipitation showed that iNOS can directly bind COX-2, and NO derived from iNOS is mainly responsible for the S-nitrosylation of COX-2. This effect can be inhibited by an iNOS inhibitor, but not a NO scavenger, which showed in deed that the S-nitrosylation of COX-2 does not appear to be elicited by freely diffusible NO. This binding and S-nitrosylation are selective and specific, as iNOS does not bind COX-1, and hydrogen peroxide does not elicit S-nitrosylation of COX-2.

S-nitrosylation is a specific posttranslational modification [35], closely related to COX-2 activity. The NO donor SNP elicited a twofold increase in COX-2 activity, reflecting S-nitrosylation. NO activates COX-2 by increasing its apparent Vmax without changing its Km. The iNOS inhibitor, at drug concentrations that provide 50% inhibition of iNOS activity, reduces 50% formation of PGE_2. Thus, about 50% of induced COX-2 activity is determined by S-nitrosylation. Generally, a physiological binding of iNOS and COX-2 brings NO in proximity to COX-2, facilitating its S-nitrosylation and activation, which may explain earlier findings that NOS inhibitor decreases PG formation.

Furthermore, COX-2 has the ability to enhance iNOS-induced S-nitrosylation, and the synergistic interaction between COX-2 and iNOS could contribute to PGE_2 production in carcinogenesis [36].

THE INTERACTIVE EFFECTS OF THE COX-2 AND INOS PRODUCTS

NO could increase PGE_2 production and induce COX-2 protein expression in a dose- and time-dependent manner. Higher concentration of NO also inhibited cell growth and induced apoptosis, regardless of COX-2 expression/activities. Inhibiting PGE_2 production did not further improve the inhibitory effect of NO [14], which implied that NO could have effects through COX-2 or not [37]. It is found that $ONOO^-$ increased the activity of COX without affecting its expression [38]. Others reported that $ONOO^-$ induced COX-2 protein expression in a dose-dependent manner as well as iNOS protein levels[39,40]. Still Fujimoto Y, et al. found that $ONOO^-$ both activated and inhibited the COX-1 and COX-2 activities, depending on $ONOO^-$ concentration. At a low concentration (5 mM), $ONOO^-$ enhanced COX-1 and COX-2 activities, but suppressed the activities of these two enzymes at higher concentrations (COX-1, at 200 mM; COX-2, >50 mM) [41].

On the other hand, PG modulated the iNOS pathway. Treatment with lipoteichoic acid (LTA) caused a time-dependent increase in PGE_2 release. PGE_2 induced iNOS expression in a concentration-dependent manner. Furthermore, the LTA-induced iNOS expression was inhibited by a non-selective COX inhibitor and a selective COX-2 inhibitor. These results suggest that LTA-induced iNOS expression involves COX-2-generated PGE_2 production [31]. Inhibiting COX-2 and NF-κB significantly could diminish the free radicals´ signal intensity [42]. It has been reported that the COX inhibitor-aspirin and aspirin-like drugs, such as sodium salicylate, can inhibit NO synthesis[43]. However, there is opposite view, believing that the aspirin-like drugs enhance NO production by IL-1β - stimulated in VSMCs. And the enhancement of iNOS transcription was independent of NF-κB activation. At therapeutic plasma concentrations, all these drugs significantly enhanced IL-1β induced NO production, although they by themselves had no effect on nitrite accumulation [44].

COX-2 AND INOS MEDIATE SIMILAR EFFECTS

There exists "cross talk" between the two enzyme systems, COX-2 and iNOS. Then inflammation and oxidative stress form a vicious cycle, aggravating their damaging effects. These damaging effects may be mediated by the formation of $ONOO^-$. The co-distribution of COX-2 and iNOS with nitrotyrosine suggests the active production of NO and formation of $ONOO^-$ [15,45]. Mercaptoethylguanidine (MEG) acts as a dual inhibitor of iNOS and COX-2 with scavenging effect on $ONOO^-$, and it can attenuate the pathological alterations [29]. It is implied that $ONOO^-$ might be the co-mediator of the effects of iNOS and COX-2, causing extensive pathological damages[15, 46].

COX-2 inhibitors have attained widespread use as anti-inflammatory agents, although they elicit potentially side effects, whereas iNOS inhibitors are not presently employed therapeutically[34,47]. Researchers have found inhibitors of COX-2 and iNOS can have synergistic effects, and are trying to find out new drugs that can inhibit both COX-2 and iNOS [48-52]. The molecular synergism between COX-2 and iNOS may represent a major mechanism of drug development. Drugs blocking the iNOS-COX-2 interaction may be anti-inflammatory, synergizing with COX-2 inhibitors and permitting lower doses. As the binding site on iNOS is in the catalytic domain, derivatives of iNOS inhibitors that also prevent binding to COX-2 may decrease both NO and PG formation [34]. Selective inhibitors of the inducible isoforms are one approach to the treatment of diseases, and an improved approach is to scavenge or remove excess $ONOO^-$.

IN CONCLUSION

All the related articles published suggest that COX-2 and iNOS share a lot of similarities, and there exist interactions between the two pathways. To investigate the interactions between COX-2 and iNOS is a prospective direction, and it is significant to clarify the molecular mechanisms of their interactions. Furthermore, their interaction represents a new prospect for intervention, helping us to explore drugs, with more effectiveness and fewer side effects.

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