Nitric oxide-mediated negative regulation of cyclooxygenase-2 induction in vascular inflammation

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ATHEROSCLEROSIS AND OTHER metabolic disorders such as obesity, traditionally considered as mere lipid store disorders and strongly associated with increased cardiovascular risk and morbidity, are currently accepted as inflammatory diseases that lead to a chronic inflammatory state of the vascular wall (12). Cyclooxygenase (COX)-derived prostaglandins are important mediators of the local inflammatory response and increase vascular permeability, promote chemotaxis and monocyte adhesion, and favor cell proliferation (6). In healthy blood vessels, most prostanooids are formed by the constitutive COX-1 isoform, present in the endothelium and platelets and considered the classic target of aspirin. In contrast, COX-2 is usually expressed at undetectable levels in the vascular wall and is upregulated by inflammatory, mitogenic, and physical stimuli, hence being regarded as a mediator of inflammation and pain (6, 11). The targeting of the COX-2 pathway was initially designed to exploit the benefits of the classic nonsteroidal anti-inflammatory drugs while evading their side effects (6). However, both protective and deleterious effects have been demonstrated for COX-2 in the context of vascular inflammation. Thus, while both pharmacological inhibition of COX-2 through the administration of COX-2 inhibitors (Coxibs) and COX-2 gene deletion reduced atherosclerosis in experimental rodent models (2), a predisposition to thrombosis and an increased risk of cardiovascular events have been shown in COX-2 knockout models and in patients following Coxib therapy, respectively (13, 14). Given the controversy over the therapeutic value of the selective COX-2 inhibitors, a better understanding of the signaling pathways that regulate COX-2 expression and activity in the vascular system continues to be essential to design novel anti-inflammatory compounds.

COX-2 is coexpressed with inducible nitric oxide (NO) synthase (iNOS) in proinflammatory contexts, including native and transplant artherosclerosis, as a result of their induction in macrophages and vascular cells by various stimuli including cytokines, growth factors, and pathogens (1, 2). The concurrent production of prostaglandins such as PGE2 and NO strongly augments COX-2 expression by inflammatory stimuli, the effect of NO on COX-2 induction has earlier been shown in the vascular wall under conditions of chronic NO synthase blockage, which unveiled a compensatory role of COX-2 upregulation coupled to the synthesis of relaxant prostaglandins to maintain flow-induced vasodilatation (5).

In this issue of the *American Journal of Physiology-Heart and Circulatory Physiology*, the work by Lamon et al. (7) provides further insight into the interactions between iNOS and COX-2 in vascular inflammation and demonstrates a feedback loop in which iNOS-derived NO negatively regulates COX-2 induction through an attenuation of autogenous signals in vascular smooth muscle cells (VSMCs). Based on the considerable capacity of de novo COX-2 synthesis and on the fact that COX-2 is an immediate gene whose expression is coupled to cyclic changes in the levels of inflammatory stimuli, the authors explored the role of endogenous NO in COX induction in VSMCs in an inflammatory setting by using a double pharmacological and genetic approach. They demonstrated that a conventional pharmacological inhibition of NO synthesis augments COX-2 protein expression by inflammatory stimuli as early as 2 h poststimulus, indicating the involvement of NO in the immediate early phase of COX-2 induction. Confirmation of this observation in iNOS-deficient mice identifies the iNOS isoform as a critical source of endogenous NO to modify VSMC COX-2 expression in response to inflammatory stimuli. The nature of the regulation of COX-2 induction by NO has been found to vary widely in different cells and tissues and under different pathophysiological conditions. In the context of inflammation, NO can both amplify and downregulate COX-2 induction by inflammatory stimuli, as demonstrated in mesangial cells, neurons, microglia, macrophages, and experimental models of inflammation (9, 10, 15). The focus of Lamon’s work is that the negative regulation of COX-2 induction by iNOS-derived NO is first demonstrated in vascular smooth muscle, which is of relevance since both enzymes are induced in inflammation-driven vasculopathies such as atherogenesis (1) where VSMCs proliferate, migrate, and secrete extracellular matrix under the influence of COX-derived prostanoids, thus contributing to the advanced fibrous plaque and the progression of lesions (1, 12). Interestingly, the inhibitory effect of NO on COX-2 induction has earlier been shown in the vascular wall under conditions of chronic NO synthase blockage, which unveiled a compensatory role of COX-2 upregulation coupled to the synthesis of relaxant prostaglandins to maintain flow-induced vasodilatation (5).

Additionally, Lamon et al. (7) present evidence for an inhibitory influence of NO on the mitogen-activated protein kinase (MAPK) signaling cascade, which propagates signals from extracellular ligands to intracellular and nuclear targets such as those regulating COX-2 induction (16). The authors found that COX-2 induction by inflammatory stimuli in VSMCs is dependent on the three main MAPK pathways, the extracellular signal-regulated kinase, the p38, and the c-Jun NH2-terminal kinase (JNK), and confirmed the link between NO and COX-2 upregulation by showing that both pharmacological inhibition and gene deletion of iNOS enhanced MAPK activation. Whereas the involvement of the MAPK signaling cascade in COX-2 induction by inflammatory stimuli is well established in inflammatory cells and cardiac and vascular myocytes, NO has been reported to have both stimulatory and
inhibitory influences on the MAPK signaling pathway (10). The work of Lamon et al. (7) demonstrates that iNOS-derived NO negatively regulates MAPK activation, particularly of p38 MAPK and JNK, both important mediators of the inflammatory response. However, this negative regulation of MAPK activation by NO in the vascular wall does not seem to be restricted to the context of inflammation, since recent investigations have reported that endogenous NO inhibits basal MAPK phoshorylation and regulates angiotensin II-stimulated MAPK activation in VSMCs in a redox-sensitive manner dependent on the formation of peroxynitrite (17).

NO can regulate gene expression by mechanisms independent of or dependent on its downstream mediator cGMP. Specifically, on increased intracellular cGMP can enhance or decrease the activity of all three MAPK pathways and thus regulate the expression of many genes involved in cell proliferation, differentiation, and apoptosis (10). Interestingly, additional information provided by the work of Lamon et al. (7) is that the regulation of COX-2 induction by NO through the MAPK pathway is cGMP independent, which suggests that interactions through nitration and S-nitrosylation of critical amino acid residues of the MAPK signaling proteins are responsible for the regulation of their activity, similar to that found for the posttranslational regulation by NO of the COX catalytic activity (3). Since NO can also have cGMP-dependent stimulatory effects on the MAPK cascade, the possibility that NO may initiate parallel actions with opposing biological actions is suggested by the authors (7). MAPKs, in particular p38 and JNK, are regulated by deactivation by MAPK phosphatases (MKPs), whose activity is in turn primarily regulated at the level of expression (8). Interestingly, Lamon et al. (7) demonstrate that both the pharmacological and genetic inhibition of iNOS-derived NO impairs MKP-1 induction by inflammatory stimuli, thus linking the endogenous inhibitory effect of NO on MAPK activity/COX-2 expression to the regulation of MKP-1 expression in vascular inflammation. Since the downregulation of COX-2 induction by NO was cGMP independent, MKP-1 is proposed as a candidate for the study of the regulation of the MAPK pathways by NO, though interactions with critical protein residues in MPK-1, besides its role in the regulation of MKP-1 induction. This would represent a similar mechanism to that underlying the redox state-dependent cGMP-independent regulatory influence of NO on COX-2 induction through S-nitrosylation of cysteine residues of transcription factors such as the nuclear factor-κB (4, 10).

In summary, the interactions between NO and COX-2 in the context of vascular inflammation are many, complex, and not completely understood. Lamon et al. (7) first provide evidence for a mechanism by which NO inhibits the activation of the COX-2 gene by inflammatory stimuli through the MAPK signaling, which represents a feedback loop that may contribute to the negative regulation of inflammation in vascular smooth muscle. These findings, along with the ability of NO to induce posttranslational modifications of the activity of COX-2 and of other prostaglandin synthases and to alter substrate bioavailability (3), draw a complex picture of the role of NO as modulator of the COX-2-mediated inflammatory response in the vasculature. An important effort needs to be made to clarify how these feedback loops by which NO regulates COX-2 biosynthetic pathway operate together to integrate a net inflammatory response in the vascular wall, as well as the functional consequences of these interactions. On the other hand, the study reinforces the concept that a better understanding of the complex interactions between NO and COX-2 in the context of vascular inflammation will improve the search for new and safer therapeutic anti-inflammatory agents with reduced cardiovascular risk and side effects.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


