EDITORIAL

The essential role of a "healthy" relationship between caveolin-1 and endothelial nitric oxide synthase in counteracting vascular inflammation and atherothrombosis

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The recent study by Mierke et al,[1] published in *Kardiologia Polska* (*Kardiol Pol*, *Polish Heart Journal*), highlights the role of caveolin 1 (Cav-1) / constitutive endothelial nitric oxide synthase (eNOS) in adventitial inflammation and, subsequently, in the progression of vascular smooth muscle cell (VSMC) proliferation. This was assessed in a model of the denuded aorta from C57Bl6n (wild-type), Cav-1$^{-/-}$, eNOS$^{-/-}$, as well as Cav-1$^{-/-}$ and eNOS$^{-/-}$ (C/e$^{-/-}$) mice transplanted into common carotid arteries of wild-type mice.

This confirms previous background data which showed that the Cav-1 / eNOS system plays a protective role against VSMC proliferation, which is associated with several disease states, including diabetes, hyperlipemia, arterial hypertension, etc, leading to atherosclerotic plaque progression and atherothrombosis.[2] The activity of Cav-1 / eNOS protective mechanisms is attenuated by inflammatory stimuli associated with an overproduction of inflammatory cytokines which, in turn, reduce eNOS release and, via activation of inducible NOS (iNOS) and cyclooxygenase 2, generate large quantities of NO and prostanoids.[3] This effect, alongside oxidative stress, leads to apoptotic cell death of endothelial cells and VSMC proliferation, though the mechanisms still remains to be elucidated.

Evidence clearly showed that a continuous cross-modulation exists between eNOS and iNOS under basal conditions as well as in the development of vascular inflammation. Moreover, it is known that eNOS contributes, under basal conditions, to maintaining nuclear factor κB (NF-κB) / iNOS not activated.[3] Recent evidence demonstrates that nM concentrations of NO released by eNOS counteract the binding of NF-κB to its promoter response element, thereby modulating the activation and translocation of NF-κB.[4] On the other hand, NO generated by "healthy" eNOS inhibits NF-κB DNA binding through S-nitrosylation of the Cys 62 residue of p50 subunit, thereby maintaining NF-κB under subtoxic concentrations.[4] The maintenance of this equilibrium between Cav-1 / eNOS at one side and NF-κB / iNOS on the opposite side represents the basis for the physiological regulation of vascular functionality. Yet, the disruption of this relationship leads to endothelial dysfunction and vascular inflammation. This is confirmed in the model used by Mierke et al,[1] obtained by means of Cav-1 / eNOS knockout mice.

Beside the above-mentioned evidence, several aspects on the shift occurring at the level of NF-κB / iNOS system from the inactive "healthy" state into the inflammatory (activated) condition is still to be clarified. However, the involvement of Cav-1 in the regulation of eNOS / iNOS activation states seems to play a relevant role in vascular inflammation.

Compelling evidence shows that caveolae (which are 50–100 nm vesicular invaginations of the endothelial cell membrane) play a crucial role in vesicular trafficking and modulation of signal transduction including eNOS regulation.[5,6] This occurs via Cav-1, a 21-to-24-kDa

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protein, which works as a scaffolding protein and negatively modulates phosphorylation of eNOS by interacting with multiple molecules. This effect of Cav-1 on eNOS activation is also regulated by Ca^{2+}-calmodulin.[7]

In particular, it has been shown that the binding of Cav-1 to eNOS is a crucial negative regulator of eNOS activity and that inflammation-related decrease of NO production in endothelial cells is partially due to an increased interaction of Cav-1 and eNOS.[8] Thus, the mechanism is involved in inflammation-induced endothelial dysfunction and atherosclerosis, though it is still unclear.

Toll-like receptor (TLR) 4 is the major player in the regulation of the activity of Cav-1.[9] This receptor has been found to play a crucial role in inflammatory response occurring in vascular and nonvascular tissues. Its function requires Tyr14 phosphorylation of Cav-1 to exert its inflammatory role.[10] On the other hand, TLR4 starts early recruitment of the adaptor protein MyD88 leading to the activation of IκB kinase, which is involved in phosphorylation and degradation of IκB, leading, in turn, to the release and translocation of NF-κB to the nucleus.[2] Thus TLR4, once activated, leads to Cav-1 phosphorylation, thereby inhibiting a constitutive NO release by eNOS. Moreover, TLR4 activates NF-κB, which leads to activation of iNOS, thereby initiating vascular inflammation and VSMC proliferation. This fits very well with data provided by Mierke et al.[1] Indeed, they found that Cav-1 and eNOS knockout was accompanied by inhibition of vascular endothelial growth factor A expression and VSMC proliferation. It is likely that derepression of iNOS and NF-κB activation, in Cav-1 and eNOS knockout mice may contribute to this response. On the other hand, it is likely that other mechanisms may contribute in the consequences of imbalanced Cav-1 / eNOS regulation occurring in vascular inflammation. Indeed, evidence exists that metabolic disorders accompanied by enhanced formation of oxidized low-density lipoprotein lead to the upregulation of Cav-1.[2] This is associated with an overexpression of the lectin-like oxidized low-density lipoprotein receptor-1, which contributes to translocation of NF-κB with subsequent activation of iNOS and cyclooxygenase 2.

In conclusion, physiological regulation of eNOS and Cav-1 contributes to the maintenance of blood vessels into a noninflammatory state, which is characterized by low tissue concentrations of inducible enzymes, inflammatory mediators, and growth factors. An imbalanced regulation of these mechanisms produces overexpression of TLR4 / NF-κB which, in turn, is accompanied by larger concentrations of inflammatory mediators, mostly from the adventitia, which promote VSMC proliferation and vascular stenosis. Further exploration of these mechanisms should provide useful information for a better management of atherothrombosis.

ARTICLE INFORMATION

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