Apocynin, Reduced Nicotinamide-Adenine Dinucleotide Phosphate Oxidase, and Vascular Cells

A Complex Matter

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Apocynin (4-hydroxy-3-methoxyacetophenone), isolated from the traditional medicinal plant Picrorhiza kurroa, is a naturally occurring methoxy-substituted catechol, experimentally used as an inhibitor of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase.1 Activated neutrophil NADPH oxidase, a multisubunit complex composed of membrane-associated gp91phox (Nox2) and p22phox and cytosolic subunits, p47phox, p67phox, and p40phox, produces superoxide anion, a precursor of microbicidal reactive oxygen species (ROS), thereby playing a crucial role in host defense. Apocynin inhibits the release of superoxide anion by NADPH oxidase by blocking migration of p47phox to the membrane, critically involved in initiating assembly of the functional NADPH oxidase complex.1 The underlying chemistry of apocynin’s actions has been elucidated in phagocytic cells.2 Apocynin needs to be activated (oxidized) for it to inhibit the oxidase. In the presence of H2O2 and myeloperoxidase (MPO), abundant in neutrophils, apocynin is oxidized and has as products thiol compounds and trimer derivatives resulting in diapocynin formation, the metabolically active compound of apocynin. The reactivity of apocynin radical with thiol compounds is possibly the mechanism involved in the inhibitory effect of apocynin on the NADPH oxidase complex.2,3

In the early 1990s, with the discovery that cardiovascular cells possess functionally active NADPH oxidase critically involved in ROS generation and oxidative stress in the vascular wall, many investigators extended neutrophil findings and used apocynin as a pharmacological agent to specifically inhibit the vascular cell oxidase. Convincing data from numerous in vitro studies demonstrated that, in endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts, apocynin blocks NADPH oxidase activity and superoxide anion generation.4,5 In vivo studies, in which mice and rats were treated with apocynin, confirmed these cellular findings.6,7 Such experiments facilitated progress in the field of ROS and vascular biology and elucidated the importance of NADPH oxidase in oxidative stress and cardiovascular, renal, and cerebrovascular disease.

The above studies were all based on the presumption that apocynin regulation in nonphagocytic cells is similar to that in phagocytic cells. However, this may not be true, as highlighted in the article by Heumueller et al8 in the current issue, where it is shown that apocynin is not an inhibitor of vascular NADPH oxidase but an important antioxidant. These provocative conclusions were based on the premise that vascular cells (and other nonphagocytic cells) do not possess MPO, and, therefore, activated apocynin is not formed. In their comprehensive experiments, using HEK 293 cells overexpressing Nox subunits, Nox1, Nox2, or Nox 4, and in endothelial and vascular smooth muscle cells, in which MPO is absent, the investigators demonstrate that apocynin does not inhibit NADPH oxidase activation or the generation of superoxide. Exposure to MPO induced apocynin dimer formation and, as predicted, inhibition of the oxidase. Despite no effect on NADPH oxidase, apocynin did reduce ROS bioavailability, probably through an antioxidant mechanism.

A number of important questions arise from the study by Heumueller et al, as described below.8 First, is apocynin activity MPO specific? Peroxidases-deficient cells appear to be insensitive to apocynin. However, peroxidases other than MPO might influence apocynin activity. Vejrazˇk ae ta l9 showed that horseradish peroxidase, like MPO, induces apocynin dimer formation, with consequent NADPH oxidase inhibitory effects. Hence it is possible that vascular cells possess peroxidases, other than MPO, which may activate apocynin. This is supported by findings in endothelial cells, where apocynin dimers were identified and found to dose-dependently inhibit NADPH oxidase activity, ROS formation, and cell proliferation.4

Second, can apocynin inhibit vascular cell NADPH oxidase even if these cells do not possess MPO themselves? In vivo studies demonstrated that neutrophils secrete MPO, which can be taken up by endothelial cells, in which apocynin can then be metabolized to active dimers to inhibit vascular cell NADPH oxidase (Figure). Cytokeratin 1 has been shown recently to be the protein responsible for MPO internalization in endothelial cells.10 Such a process may explain the NADPH oxidase inhibitory effects observed in many animal studies and does not necessarily refute the use of apocynin as a specific inhibitor in this context. However, such a situation is different in cultured cells, which are not exposed to...
leukocytes. What is difficult to explain in in vitro studies is the consistent data derived from many investigators, using different vascular cell preparations and various cell types exposed to many stimuli, which consistently show that apocynin inhibits activity of NADPH oxidase, measured by various techniques, including lucigenin chemiluminescence, p47phox phosphorylation, or p47phox/p67phox translocation.

Third, if apocynin is indeed an antioxidant, what are the mechanisms involved? Heumueller et al. demonstrate that, although apocynin does not inhibit activation of NADPH oxidase in vascular cells, it does reduce H$_2$O$_2$ and hydroxyl formation. Based on these results, it is concluded that, in this context, apocynin acts as a radical scavenger. The apocynin dimer undergoes a 2-electron transfer reaction itself, implying redox potential. However, the authors do not provide explanations as to how apocynin may have antioxidant potential.

To add to the complexity, some studies demonstrated that in endothelial cells apocynin is actually a pro-oxidant that increases, rather than decreases, ROS formation. The main product seems to be H$_2$O$_2$, because stimulatory effects of apocynin were abolished by superoxide dismutase and tiron. Moreover, apocynin has been shown to decrease the intracellular reduced/oxidized glutathione ratio in stimulated monocytes, further suggesting a pro-oxidant action. Hence, apocynin seems to have opposite actions: whereas it is inhibitory in phagocytic cells, it may be stimulatory in nonphagocytic cells in certain conditions.

The findings of Heumueller et al. are thought provoking because they challenge the now-accepted dogma that vascular NADPH oxidase is a major source of vascular ROS and oxidative stress. However, it should be stressed that these conclusions were based on data derived not only from apocynin-based studies but from myriad investigations in which NADPH oxidase was probed by various strategies, including transgenic and knockout mice and gene-targeted, molecular, and pharmacological approaches, and, therefore, do not minimize the importance of vascular NADPH oxidase in the vasculature. Nevertheless, the new findings under discussion alert us to the fact that apocynin effects on redox status in vascular cells may not be NADPH oxidase specific and that actions of this methoxy-substituted catechol could vary depending on the cell type studied and whether MPO (or other peroxidases) is functionally present. Future studies using apocynin as an inhibitor of NADPH oxidase in nonphagocytic cells should be conducted with caution and with the knowledge that, in the absence of peroxidases and H$_2$O$_2$, effects on redox status may be through NADPH oxidase-independent processes, such as through radical scavenging.

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**References**


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