Heme Oxygenase-1–Mediated Protection: Potential Role of Nonheme Iron–Nitric Oxide Complexes

To the Editor:

In a recent paper, Tulis and coworkers reported that transfection overexpression of heme oxygenase-1 (HO-1) inhibits arterial remodeling after balloon angioplasty via a mechanism involving apoptosis. HO-1 is a stress response protein that degrades heme to carbon monoxide, biliverdin, and nonheme iron. In general, overexpression of HO-1 plays a cytoprotective role against a variety of oxidative stimuli. Interestingly, cells overexpressing HO-1 exhibit low levels of free iron because of the upregulation of ferritin and via the extraction of iron into the extracellular space. The elimination of pro-oxidant iron from the cell is considered to be an important mechanism of HO-1–mediated protection against oxidative stress. How HO-1 overexpression stimulates apoptosis in the medial wall of injured artery is unclear.

We believe that, among potential mechanisms of HO-1–mediated stimulation of apoptosis in injured vascular tissue, at least one may be related to nitric oxide (NO). Vascular injury is associated with the expression of inducible NO synthase (iNOS) leading to an overproduction of NO, which is known to stimulate apoptosis and inhibit neointima formation after balloon angioplasty. In nonvascular cells, the content of intracellular free nonheme iron plays an important role in protecting against NO apoptosis. The mechanism of this protection involves the interaction of NO and free iron leading to the formation of dinitrosyl iron complexes (DNIC), which in turn inhibit caspase(s) and suppress NO-dependent apoptosis. An additional mechanism may be proposed for HO-1–mediated apoptosis on the basis of recent cell culture experiments from our laboratory. These results demonstrate that adenovirus-mediated HO-1 gene expression in rat vascular smooth muscle cells (SMCs) stimulates apoptosis in a dose-dependent fashion, as measured by DNA fragmentation, positive annexin V labeling, and caspase-3 activation. In addition, HO-1 overexpression upregulated cellular levels of the pro-apoptotic transcription factor p53. Direct administration of the HO-1–derived antioxidants biliverdin or bilirubin stimulated SMC apoptosis, whereas administration of the HO-1 products CO or free iron did not induce apoptosis. Under these in vitro conditions, HO-1 overexpression directly enhanced cellular apoptosis without the influence of any iNOS isoform, thereby eliminating the influence of the inhibitory actions of DNIC complexes. These results corroborate several recent findings that suggest that antioxidants can induce vascular SMC apoptosis, possibly through p53-related mechanisms. Moreover, these findings imply that multiple signaling pathways exist for the regulation of vascular SMC apoptosis by HO-1 under conditions of vascular trauma.

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Response

Kleschyo and colleagues propose an intriguing hypothesis about the increase in medial wall smooth muscle cell (SMC) apoptosis after adenovirus-mediated heme oxygenase-1 (HO-1) gene delivery to balloon-injured rat carotid arteries observed in our study. In their letter titled “Heme Oxygenase-1–Mediated Protection: Potential Role of Nonheme Iron–Nitric Oxide Complexes,” they theorize that an increase in vessel wall HO-1 would lead to a reduction in intracellular levels of free iron through the upregulation of the iron-sequestering compound ferritin and via augmentation of iron efflux into the extracellular space. The decreased levels of intracellular iron would then inhibit the formation of antiapoptotic dinitrosyl iron complexes (DNIC), formed from the interaction between nitric oxide (NO) and free iron. Therefore, under conditions of elevated HO-1, DNIC-mediated caspase inhibition would be reduced, and cells may be subsequently sensitized to caspase-mediated apoptosis, thereby abrogating ensuing neointima development. This hypothesis provides an interesting and physiologically feasible mechanism whereby HO-1 and NO synthase play interactive roles in attenuating the arterial remodeling response to vascular injury through enhanced cellular apoptosis.

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