# Correspondence

## Letter Regarding Article by Hu et al, "Heme Oxygenase-1 Inhibits Angiotensin II–Induced Cardiac Hypertrophy In Vitro and In Vivo"

*To the Editor:*

I read with great interest the work of Chien-Ming Hu et al,[1] in which they suggest that heme oxygenase-1 (HO-1) attenuates angiotensin II (Ang II)–induced cardiac hypertrophy both in vitro and in vivo, and that this effect may be mediated, at least in part, by bilirubin via inhibition of reactive oxygen species production (ROS) after Ang II stimulation. I agree with the authors' conclusions that HO-1–derived bilirubin may be partially responsible for the protective effect of the enzyme via ROS scavenging, which has been shown to play a key role in cardiac hypertrophy. Three points need to be raised, however, about alternative HO-protective pathways in Ang II–mediated hypertrophy. First, several lines of evidence suggest that p21, a well-known cell cycle inhibitor, plays an important role in the physiopathological process of Ang II–mediated cardiac hypertrophy.[2] In this regard, previous studies have extensively demonstrated a link between CO, p21, and cell proliferation, thus suggesting that HO-1–derived CO may represent an alternative pathway involved in cardiac hypertrophy.[3] Hu et al demonstrated that the use of 50 μmol/L CO-releasing molecule (CORM) failed to prevent Ang II–mediated hypertrophy. My experience with CORM in astrocytes, endothelial cells, and cancer cells suggests that different concentrations of CORM may have different and sometimes opposite effects, and that the appropriate concentrations must be determined each time (unpublished data observations). Second, because HO is the sole physiological pathway in heme degradation, it plays a crucial role in regulating the activity of other important heme proteins involved in Ang II cell signaling and ROS formation, such as guanylate cyclase and NAD(P)H oxidase.[4] Third, my laboratory's recent studies demonstrated that HO-1 translocates into the nuclear compartment during oxidative stress, thus suggesting that the enzyme itself may contribute to the cell adaptive response by activating the gene transcription process.[5] The familiarity of Hu et al with the literature on the role of bilirubin in the prevention of damage to DNA and the enhancement of cell proliferation may support their findings; however, CO is still a major factor for controlling hypertrophy. In conclusion, I do agree with the authors that HO-1 may represent an excellent therapeutic target for long-term protection of the heart and that the use of CORM in vivo may represent a good strategy for understanding the real protective effects of CO.

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### *Response*

We appreciate the interest of Dr Li Volti in our recent publication.[1] Although we share the view that pathways other than the inhibition of reactive oxygen species production by bilirubin may take part in HO-1–mediated protection against angiotensin II (Ang II)–induced cardiac hypertrophy, the role of CO is not evident from our study. The cellular responses to CO can be quite different in various cell types. We found that cardiomyocytes are less tolerant to CO toxicity as compared with vascular smooth muscle cells in culture. The greater vulnerability of cardiomyocytes is understandable because these cells demand higher energy production via oxidative phosphorylation from mitochondria, which is the major target of CO poisoning. We used 50 μmol/L CO-releasing molecule in our experiment because it is the highest concentration tested without causing significant cytotoxicity to cells. At this concentration, it still failed to protect cardiomyocytes from hypertrophic response, as shown in our article. Although CO has been shown to inhibit smooth muscle cell proliferation via upregulating the cell cycle inhibitor p21, it is unclear whether the same scenario would be observed in the case of cardiac hypertrophy. Some studies have shown that cyclin-dependent kinase inhibitors p21and p27 are induced by Ang II and may have a role in G1 arrest occurring in hypertrophic response.[2–4] Whether CO has an influence on the cell cycle reentry and G1 progression in Ang II–activated cardiomyocytes remains to be determined. With regard to the effect of HO-1 on heme metabolism, we agree that the depletion of heme may have an impact on the activities or functions of some important heme proteins involved in cell signaling and metabolic pathways. We have no intention of neglecting these important issues, but obviously more studies are required to provide the answers to these open questions.

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