Antioxidant 1 in Hypertension
More Than Just a Copper Chaperone

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Oxidative stress, defined as an increase in reactive oxygen species, has been implicated in a number of diseases, including hypertension. Superoxide is a particularly detrimental reactive oxygen species in the cardiovascular system because it reacts directly with NO, thereby limiting the amount of NO normally available for processes such as endothelium-dependent relaxation. Superoxide dismutases (SODs) represent a first-line defense against oxidative stress by tempering increases in superoxide via its metabolism (dismutation).

Three distinct mammalian isoforms of SOD exist, CuZn-SOD (localized mainly to the cytoplasmic space), MnSOD (the predominant isoform in mitochondria), and extracellular SOD (SOD3), a CuZnSOD found exclusively in the extracellular compartment (Figure). The fact that there are 3 different gene products that catalyze the same reaction underscores the importance of limiting cellular superoxide.

Both CuZnSOD and SOD3 require copper for full catalytic activity; however, free copper is essentially nonexistent (<1 copper ion per cell copper). Thus, copper-containing enzymes are dependent on a number of proteins, including copper transporters, copper scavengers, and copper "chaperones" for appropriate delivery of copper. To date, 2 mammalian copper chaperones have been identified, copper chaperone for CuZnSOD delivers copper as its name would imply to CuZnSOD, whereas antioxidant 1 (Atox1) delivers copper to the transgolgi network and SOD3.

Although oxidative stress reflects both production and metabolism of superoxide, a majority of studies have focused primarily on those enzymes that produce superoxide, most notably NADPH oxidase. In contrast, no previous studies have considered the relationship between Atox1 and SOD3 expression/activity in hypertension. In this issue of Hypertension, Ozumi et al provide some of the first data regarding the role of Atox1 in limiting hypertension and endothelial dysfunction produced by angiotensin II (Ang II). The authors found that acute (7-day) Ang II infusion was associated with a selective increase in vascular SOD3 expression and activity in wild-type mice. Although these data are consistent with previous findings from this group, there is also evidence that Ang II infusion for longer periods (and at higher concentrations) has no effect on SOD3 expression. Such differences may reflect the fact that early increases in SOD3 may represent an initial compensatory response to increased oxidative stress that is lost with longer, more chronic Ang II exposure.

Nonetheless, how would a selective increase in SOD3 produced in response to Ang II be functionally important? This is a key question, because the increase in SOD3 in response to Ang II does not appear to be sufficient to attenuate the pressor response or to limit the degree of endothelial dysfunction observed in the present study. In addition, the increase in SOD3 activity is not of sufficient quantity to increase total SOD activity. Perhaps the increase in SOD3 activity produced by Ang II is simply masked by activity of the other SOD isoforms. This is probable, because SOD3 activity accounts for only 12% of total SOD activity within the vascular wall. Irrespective of the contribution of each isoform to total SOD activity, the SODs are among the most catalytically efficient enzymes known. Thus, even slight alterations in SOD activity would be predicted to have a profound influence on both superoxide levels and endothelial function.

In addition to increased SOD3 expression, Ang II infusion was associated with increases in vascular Atox-1, most notably within the nucleus. Why would Ang II promote nuclear accumulation of an enzyme normally involved in copper transport to the transgolgi network and to SOD3, the latter of which is found outside the cell? Recent evidence suggests that, in addition to its role as a copper chaperone, Atox1 may also function as a transcription factor for a number of genes, including SOD3. In exploring this possibility further, Ozumi et al found that Ang II promoted nuclear Atox1 expression in cultured smooth muscle, supporting their in vivo findings. In addition, mutagenesis of the putative Atox1 binding site in the SOD3 promoter (−312/−307) was associated with an absence of Atox1 binding and a marked reduction in SOD3 promoter activity in cultured cells, providing convincing evidence that Atox1 serves to promote SOD3 transcription. Whether any or all of these events are Ang II receptor dependent remains an important unanswered question.

If Atox1, in addition to its role as a copper chaperone, is acting as a transcription factor to promote SOD3 expression, what role does endogenous Atox1 play in blood pressure homeostasis and vascular function? To address this question directly, the authors examined the effect of Ang II infusion in
Atox1-deficient (Atox1<sup>−/−</sup>) mice. Atox1 deficiency was associated with a greater pressor response, higher levels of vascular superoxide, and additional impairment of endothelial responses after Ang II infusion as compared with Ang II–infused wild-type mice. SOD3 expression and activity was markedly reduced in Atox1<sup>−/−</sup> mice both under baseline conditions and after Ang II infusion, most likely reflective of the loss of Atox1-mediated SOD3 transcription. Cursory examination of expression of other copper-containing enzymes (eg, lysyl oxidase) was found to be unaltered in Atox1<sup>−/−</sup> mice, providing some evidence that the reduction in Atox1-mediated SOD3 transcript in the present study was selective.

Measurements of cellular copper revealed that Ang II infusion was associated with reductions in copper content in wild-type mice. In contrast, deficiency of Atox1 was associated with higher basal copper content, consistent with previous findings. Finally, Ang II did not influence copper content in Atox1<sup>−/−</sup> mice. The authors suggest that the reduction in cellular copper with Ang II is attributed to increased efflux to the extracellular matrix. However, increased cellular copper content with Atox1 deficiency is most likely a result of loss of Atox1:SOD3 coupling with the copper exporter ATP7A, which is normally required for appropriate trafficking of SOD3 and cellular efflux of copper.

Considering the data presented, several important questions arise. For example, do findings from the present study translate to other forms of hypertension, such as non-Ang II–dependent hypertension? Does Atox1-mediated increases in SOD3 only appear in the early stages of hypertension development? What about long-term, more established, hypertension? How does loss of Atox1-mediated SOD3 transcription, per se, promote hypertension, especially when one considers the fact that homozygous SOD3 deficiency is not associated with a hypertensive phenotype? Are polymorphisms in the Atox1 gene associated with human hypertension? Finally, how can one manipulate Atox1 expression as a clinical therapy for hypertension or other diseases associated with oxidative stress?

Although future studies are needed to provide additional answers, the study by Ozumi et al<sup>5</sup> provides new insight into Atox1 as not only a copper chaperone and a transcription factor for SOD3 but also as a mediator of copper efflux through the interaction of Atox1 with the copper exporter ATP7A (Figure). The present findings serve to further our understanding of mechanisms that contribute to the metabolism of superoxide, mechanisms that can have profound and sometimes underappreciated consequences on levels of oxidative stress in the cardiovascular system. Clearly, Atox1 delivers and then some.
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References
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