Copper Trafficking and Extracellular Superoxide Dismutase Activity
Kinky Hair, Kinky Vessels
Volker Rudolph, Tanja K. Rudolph, Bruce A. Freeman

In 1962, Menkes et al. first described a pediatric disorder characterized by hypopigmented brittle hair, doughy skin, connective tissue fragility, failure to thrive, progressive neurological damage, and various defects of the arterial wall. This was termed “kinky hair syndrome” and later was found to be associated with a disturbance in copper metabolism due to mutations in the ATP7A gene.

The ATP7A (or Menkes ATPase) is 1 of 2 membrane-bound copper-transporting ATPases (ATP7A and ATP7B) essential for controlling intracellular copper homeostasis. ATP7A is of particular importance for the transfer of copper through the membrane of the trans-Golgi network and hence is important for the so-called secretory pathway of copper. In Menkes syndrome, in which the function of the ATP7A transporter is disturbed, systemic copper levels are low because copper export from enterocytes is greatly impaired and copper thus accumulates in the intestine. A critical manifestation of impaired ATP7A function is the limited incorporation of copper into the tyrosinase of patients with Menkes disease. Tyrosinase is the rate-limiting enzyme of melanin formation with defects in its activity leading to low melanin levels in skin, albinism, and the hypopigmentation that is a clinical hallmark of Menkes syndrome. The study by Qin et al., published in the current issue of Hypertension, is of interest and significance because a critical linkage has been made between cardiovascular function and the biochemical defects in copper trafficking that are a hallmark of altered ATP7A function.

Qin and colleagues have discovered that ATP7A plays an important role in the regulation of extracellular superoxide dismutase (EC-SOD) activity and the influence that altered steady-state concentrations of the substrate for EC-SOD, superoxide (O$_2^-$), has on vascular NO signaling. The copper and zinc-containing enzyme EC-SOD is typically concentrated in the vessel wall, where it is electrostatically bound to the endothelial glyocalyx and extracellular matrix proteins in the subendothelial layer. There, it scavenges O$_2^-$ and limits the facile radical–radical reaction between O$_2^-$ and NO, a reaction that both inhibits NO-dependent smooth muscle cell relaxation and generates the secondary oxidizing and nitrating species peroxynitrite. The anatomic distribution of EC-SOD is convenient in that it is in close apposition to sites of inflammatory O$_2^-$ generation and other components of the oxidative gauntlet that endothelial-derived NO must traverse to reach the molecular targets that transduce its physiological signaling actions. For this reason, EC-SOD has emerged as a crucial determinant of vascular NO bioavailability and an important antagonist of angiotensin II (Ang II)-derived reactive oxygen species in the control of blood pressure.

The authors convincingly show that mice carrying a mutation in the ATP7A gene exhibit reduced EC-SOD-dependent O$_2^-$-scavenging activity, although EC-SOD apoprotein levels were elevated in the mutant mice when compared with expression levels in wild-type mice. Interestingly, no changes in cytoplasmic CuZn-SOD activity or expression were noted in ATP7A mutant mice. After chronic treatment with Ang II, the ATP7A mutant mice showed no enhanced EC-SOD activity despite immunoreactive EC-SOD protein levels increasing. This imbalance between O$_2^-$ production and its scavenging translated into a more pronounced increase in blood pressure upon Ang II treatment of mice as well as higher detectable aortic O$_2^-$ levels and a deterioration in aortic endothelial function under both basal and Ang II “activated” redox conditions in mutant mice. All of the adverse Ang II-dependent effects could be rescued on addition of the antioxidant and O$_2^-$ scavenger Tempol. Of important mechanistic relevance, the authors also convincingly show that ATP7A physically associates with EC-SOD in the aorta and cultured smooth muscle cells after treatment with Ang II.

Previous work from Qin and colleagues revealed that ATP7A is proximally responsible for the incorporation of copper in EC-SOD. These investigators now extend this insight into a more physiological realm by discovering that ATP7A is intimately involved in blood pressure regulation by maintaining sufficient EC-SOD activity and hence the “preservation” of competent NO signaling. Their findings underscore the important role that EC-SOD fulfills as an antagonist of vascular O$_2^-$, especially in the context of the regulation of NO signaling and blood pressure in Ang II-dependent hypertension. Because mutation of ATP7A can lead to direct impairment of the catalytic activity of EC-SOD, apparently without affecting its protein levels, this model confers even more compelling support for the role of EC-SOD in blood...
pressure regulation than data stemming from EC-SOD knock-out models.

Considering the exquisitely rigid regulation of copper levels in organisms, the question arises whether the findings of Qin et al might reveal new strategies for the pharmacological manipulation of ATP7A that would result in desirable effects on EC-SOD activity and its cardiovascular actions. ATP7A distribution is not only restricted to the vasculature, but instead is generally ubiquitous; thus, the net consequences of the upregulation of ATP7A activity are difficult to fathom. Even if one used a more targeted therapeutic strategy that modulated only vascular ATP7A, a localized increase in copper supply would not be expected to result in an upregulation of EC-SOD synthesis or activity. Additionally, during oxidative and inflammatory conditions, EC-SOD is expressed in such abundance that no further beneficial effects were observed in a murine model of hypertension when recombinant EC-SOD was administered to wild-type mice with this benefit only evident in EC-SOD knockout mice.

The analysis of the differential expression and activity of ATP7A and how it impacts vascular function provides valuable fundamental insight into the pathophysiology of oxidative stress-related hypertension. New understanding provided by this report may also reveal new therapeutic strategies for treating hypertension. For example, ATP7A expression influences hormonal regulation inasmuch as treatment of human placental Jeg-3 cells with insulin and estrogen promote increased mRNA and protein levels of ATP7A. Considering the role of these hormones in influencing cardiovascular risk profiles, it would be of great interest to investigate their linkage with the expression and activity of vascular ATP7A and downstream consequences on EC-SOD activity.

Another intriguing and related issue is that the activity of ATP7A is largely influenced by its redox state and thus is susceptible to regulation by the cellular and inflammatory milieu. Specifically, the copper-binding site of ATP7A consists of a motif containing 2 oxidation-sensitive cysteine residues that accept copper only when in a reduced state. For this reason, copper transport by ATP7A depends on appropriate concentrations of reducing agents and is favored at low pH. Furthermore, the thiol-reducing agent glutaredoxin interacts with ATP7A and is essential for its function, supporting that fully reduced cysteine residues in ATP7A are critical. These findings also suggest that shifting of the cellular redox state toward increased oxidant production and a greater degree of thiol oxidation might impair the copper transport function of ATP7A and disrupt the biosynthesis and net activity of EC-SOD, thus further amplifying the biochemical and functional sequelae of oxidative stress.

In summary, this elegantly conceived and solidly executed study conveys important new insight into the physiology of the vessel wall by elucidating the critical roles that the Menkes copper ATPase, ATP7A, plays in regulating the catalytic activity and vascular cell signaling actions of EC-SOD both in vitro and in vivo. Although these findings might not immediately be translated into new therapeutic options, they reveal important mechanistic insights into the pathogenesis of oxidant-induced hypertension and how we might tame this beast in the future through redox-based strategies.

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Disclosures
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