Copper as a Magic Bullet for Targeted Microbial Killing

Jennifer S. Cavet[1,*]
[1]Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK
*Correspondence: jennifer.s.cavet@manchester.ac.uk
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The innate toxicity of copper can be exploited as an antimicrobial. In this issue of *Chemistry & Biology* Festa and colleagues report the use of QBP, a prochelator form of the metal-chelate 8-hydroxyquinolone, which allows for targeted copper-dependent microbial killing at sites of infection.

Copper is vital to most life forms as a key component of numerous enzymes and other proteins. However, excess copper is lethal and has led to the widespread exploitation of this transition metal for its antimicrobial properties. Indeed, copper is used today in numerous settings including healthcare, where its oldest recorded medical use dates back to the Smith Pyrus Egyptian text written around 4,500 years ago (Grass et al., 2011).

Recently, it has come to light that this innate toxicity of copper is also a component of our innate immune response. Although the precise mechanism of how copper contributes to these defenses is unknown, there is increasing evidence that the influx of copper into microbe-containing phagosomes of macrophages is a major factor (Hodgkinson and Petris, 2012). However, pathogens can sense high copper levels within these compartments and trigger expression of copper-resistance systems that allow their survival (Osman and Cavet, 2011). In this issue of *Chemistry & Biology*, Festa et al. (2014) present a new approach for treating microbial infections that harnesses copper mobilization in phagosomes in combination with another key aspect of the antimicrobial defenses there: the rapid release of reactive oxygen species. This association allows the prodrug QBP to become activated to form antimicrobial 8-hydroxyquinolone (8HQ)-copper complexes. Crucially, these complexes can overcome microbial copper defenses to result in pathogen killing (Figure 1).

The study focused on the fungal pathogen *Cryptococcus neoformans*. This fungus can live in both plants and animals, but, when inhaled by humans, can disseminate from the lungs to the bloodstream and cause life-threatening systemic disease. The brain is particularly vulnerable to *C. neoformans* infections, and there are approximately one million cases of cryptococcal meningitis each year, over 60% of which result in death (Sabiiti and May, 2012). Treatment of cryptococcal infections is notoriously difficult, and new antifungal regimens are urgently required.

The ability of the compound 8HQ to form lipophilic complexes with metals that translocate across cell membranes and exert antimicrobial activity is well documented. However, the toxicity of 8HQ-copper complexes is not pathogen-restricted, thus limiting its application. To mitigate the "off-target effects," Festa et al. (2014) exploited the use of QBP, which is an inactive boronate-based form of 8HQ and unable to bind copper unless its boronic ester group is removed by interaction with hydrogen peroxide or peroxynitrite (Dickens and Franz, 2010; Sikora et al., 2011). Because these reactive oxygen species are a key feature of phagocyte antimicrobial defenses, it was hypothesized that QBP would be converted to 8HQ by activated macrophages. This was indeed demonstrated, and the toxicity of QBP toward these cells is substantially reduced compared to that of 8HQ.

Crucially, the copper-binding ability of 8HQ causes a substantial potentiation of copper toxicity toward *C. neofomans*. Thus, by converting QBP to 8HQ by treatment with hydrogen peroxide, Festa et al. (2014) were able to conditionally convert QBP to a copper-dependent antifungal agent. Moreover, 8HQ-copper complexes were found to be fungicidal as a result of *C. neofomans* over-accumulating copper. As with other microbial pathogens, *C. neofomans* possesses copper-resistance proteins. Such proteins represent key microbial virulence factors (Osman and Cavet, 2011). In *C. neoformans*, they include small cysteine-rich proteins known as metallothioneins, and mutants lacking these proteins are particularly vulnerable to 8HQ. Notably, treatment of activated macrophages with QBP caused a substantial increase in their antifungal activity, despite an increase in *C. neofomans* metallothionein gene expression. This implies that the switch of QBP to 8HQ by these phagocytes induces copper-overloading sufficient to overcome *C. neofomans* copper defenses and elicit antifungal activity. Moreover, QBP was used successfully to reduce the fungal burden in the lungs of infected mice.

These findings clearly have clinical implications in the treatment of *C. neofomans* infections. However, the use of QBP as a targeted antimicrobial is likely to have applicability against a broad spectrum of pathogens, because it is based on two key aspects of phagocyte antimicrobial defenses: the respiratory burst and the mobilization of copper. Indeed, Festa et al. (2014) demonstrated the efficacy of 8HQ against other fungal and bacterial pathogens. However, while similar susceptibility to *C. neoformans* was generally observed for other fungi and Gram-positive bacteria, some Gram-negative bacteria appeared less susceptible, suggesting limitations in the use of this treatment strategy for some pathogens.

The study raises important questions. First, what is the mechanism of toxicity of the 8HQ-copper complexes? Because 8HQ toxicity is associated with increased intracellular copper, pathogen killing may result from copper displacing other

metals from the metal binding sites of proteins, particularly exposed iron-sulfur clusters, because these represent a primary target for inhibition by copper (Macomber and Imlay, 2009). Alternatively, liberated intracellular 8HQ may bind other metals and inhibit metalloenzymes, and/or intact 8HQ-copper complexes may act directly, for example by inhibiting the proteasome as has previously been observed (Zhai et al., 2010). Second, what is the source of copper for the formation of the 8HQ-copper complexes in phagocytes? This may be derived from the mobilization of intracellular copper stores and/or extracellular recruitment, for example from ceruloplasmin, which is the major copper-containing protein in plasma. Third, does QBP activation occur in professional phagocytes other than macrophages, such as dendritic cells, microglia, and neutrophils? If so, this may further broaden the spectrum of susceptible diseases.

In conclusion, Festa et al. (2014) present exciting work that lays the foundation for the utilization of molecules that specifically target sites of phagocytosis and thereby minimize cytotoxicity to the host. The requirement for both copper and reactive oxygen species for the activation of the prodrug QBP to 8HQ endows it with a unique conditionally activated antimicrobial activity. 8HQ has potentiated efficacy against a broad spectrum of microbial pathogens and appears particularly well suited for the treatment of recalcitrant fungal infections such as cryptococcosis.

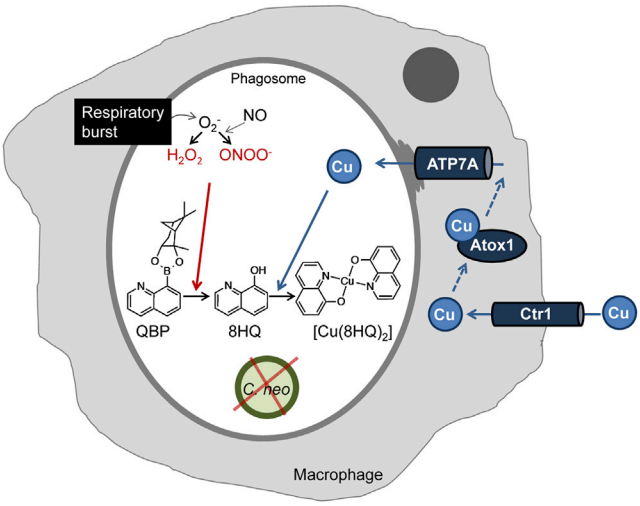

Figure 1. Conditional Activation of the Prodrug QBP Allows Targeted Microbial Killing

QBP can only bind copper when converted to 8HQ via removal of its boronic ester group by interaction with hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^-$). These reactive oxygen species are generated within macrophages from superoxide (O_2^-), produced by the respiratory burst, and by its interaction with nitric oxide (NO). The influx of copper into phagosomes occurs in response to infection and is associated with increased expression of the Ctr1 copper import protein and localization of the ATP7A copper pump to phagosome-associated vesicles, with Atox1 acting to shuttle copper to ATP7A. The resultant lipophilic 8HQ copper complexes overcome microbial copper defenses, resulting in pathogen (*C. neo*) killing.

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