NEWS AND VIEWS

The complexities of antibiotic action

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Antibiotics are arguably the most successful medicine on the planet, but the one under huge threat from antibiotic resistance in the face of diminishing new antimicrobial discovery efforts (Hancock, 2007). One of the great hopes for discovering new antibiotics arose when whole-genome sequencing came of age in 1995 with the decoding of the Haemophilus influenzae genome, followed rapidly by those of many other pathogens. Although this offered antibiotics researchers a window into every possible antibiotic target and stimulated massive efforts in Pharma and Biotech to uncover and exploit these targets, we have not seen a single new antibiotic arising from such studies. The reason is elusive, but could relate to the concept that antibiotics have much more complex mechanisms and targets than previously hypothesized (see Brazas and Hancock, 2005a for discussion). Indeed, a plethora of microarray studies have indicated that all studied antibiotics induce or repress dozens to hundreds of genes at or below their minimal inhibitory concentrations (MIC), and these patterns of expressed genes (signatures) appear to relate to the general mechanism of action of a particular antibiotic, with signatures for cell wall synthesis inhibition, DNA synthesis inhibition, folate and fatty acid synthesis inhibition, and membrane damage that were recognized in one study of 28 antibiotics (Hutter et al, 2004). It seems likely that some of these gene expression signatures could arise from the complexities of antibiotic action or cellular-resistance responses in an attempt to counter these actions.

Two recent publications (Dwyer et al, 2007; Kohanski et al, 2007) have shed substantial light on how certain bactericidal antibiotics work in Escherichia coli by applying unbiased systems-wide approaches and deductive experiments derived from them. In particular, despite the textbook perspective that would suggest that the targets of traditional antibiotics like the fluoroquinolone norfloxacin (DNA gyrase involved in DNA replication), *B*-lactam ampicillin (penicillin-binding proteins involved in cell wall biosynthesis) and aminoglycoside kanamycin (30S ribosomes) are well characterized and their mode of action well understood, it seems likely that actual cell killing involves the induction of oxidative stress in E. coli (Figure 1). These results are consistent with the complexities of gene expression responses induced by antibiotics (Hutter et al, 2004; Brazas and Hancock, 2005a, b) and results in Pseudomonas aeruginosa which indicated that the actual mode of killing by fluoroquinolones depended on RecAmediated induction of a large phage-pyocin operon rather than DNA-gyrase-mediated damage (Brazas and Hancock, 2005b) per se. Clearly antibiotics are not simple.

The paper of Dwyer *et al* (2007) published in this journal carefully examined the influence of norfloxacin on *E. coli* gene expression. The anticipated DNA-damage response signature, as observed by many other researchers to be induced by quinolones (Hutter *et al*, 2004; Brazas and Hancock, 2005b), was evident and easily explained by the action of norfloxacin on a key enzyme of (error-prone) DNA synthesis. However, this represented only a small subset of the 800 genes with altered expression. Application of Gene Ontology classifications and information in the RegulonDB database to the pool of

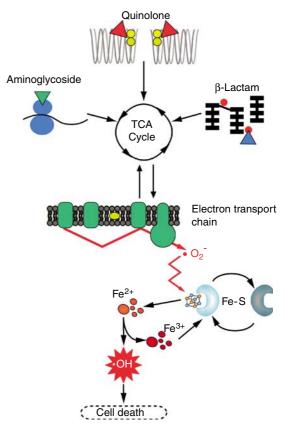


Figure 1 A proposed common mechanism of killing by bactericidal antibiotics. Antibiotics with diverse targets (ribosome for aminoglycosides, DNA gyrase for quinolone and penicillion-binding proteins for β -lactam) trigger NADH depletion and superoxide ($\bigcirc Q_2^-$) formation by hyperactivation of the electron transport chain. Free-radical damage of iron-sulfur clusters releases ferrous ion, inducing the generation of highly destructive hydroxyl radicals 1(\bigcirc OH) and cell death. (Figure courtesy of James J Collins).

genes with altered expression revealed a significant and meaningful upregulation of the genes involved in responses to oxidative damage, iron uptake and utilization, and ironsulfur cluster synthesis. The authors then applied a series of chemical and genetic tools to explain the presence of these signatures, demonstrating the importance of iron misregulation, hydroxyl radical formation and oxidative attack of iron-sulfur clusters, and the particular genes involved in these processes. Each was shown to influence killing by norfloxacin, providing a rather novel perspective, as it had been previously assumed that norfloxacin killed *E. coli* because it induced DNA gyrase to stall, leading to doublestranded DNA breaks.

The second paper of Kohanski *et al* (2007) went even further by looking at bactericidal antibiotics with diverse targets and asking whether there were commonalities in their killing mechanisms. They demonstrated using the indicator hydroxyphenyl fluorescein that norfloxacin, ampicillin and kanamycin, but not five other bacteriostatic antibiotics, induce hydroxyl radical formation in *E. coli* via the Fenton reaction using intracellular iron. Mutant studies indicated an analogous mechanism to that proposed previously (Dwyer *et al*, 2007) and the NADH depletion was the trigger for hydroxyl radical formation (Figure 1).

The authors conclude that induction of hydroxyl radical formation might be considered a new strategy for antimicrobial discovery efforts, and that there would be several ways and possible targets for achieving this. As no indications were provided about the effects on the MIC of various mutants in this process, we do not know if hydroxyl radical formation works in concert with other underlying mechanisms; however, our analogous study in *Pseudomonas* showed that phagepyocin operon induction had about an eightfold effect on MICs (Brazas and Hancock, 2005b).

These studies thus show that there is a lot more to understand about antibiotics, a frightening observation when one considers that research on antibiotics has malingered for decades, especially given the enormous importance of these medicines and the growing difficulties with multidrugresistant 'Superbugs'. The papers of Collins and co-workers have shown that there is much to learn about antibiotics, and the answers, which could well drive the next generation of drug discovery in this field, will involve a concerted systems biology approach.

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