ANTI-INFECTIVES

Antibiotics and death — the Fenton connection

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The discovery of antibiotics has been heralded as one of the triumphs of modern medicine. The various cellular pathways that antibiotics inhibit, which include the synthesis of the cell wall and macromolecules such as DNA, RNA, proteins and folic acid, have been pinpointed by researchers. But how the inhibition of these processes actually leads to cell death has remained a mystery. Now, a paper published in *Cell* documents the extraordinary finding that bactericidal antibiotics that target different cellular pathways kill cells by a common mechanism — the production of hydroxyl radicals.

Antibiotics can be classified as either bactericidal (those that kill cells) or bacteriostatic (those that prevent cell division). James Collins and colleagues previously showed that the treatment of *Escherichia coli* with a quinolone antibiotic induces the cell to generate reactive oxygen species that contribute to cell death. The reactive oxygen species superoxide can be dismutated to form hydrogen peroxide, which is converted into hydroxyl radicals using the Fe^{2+}-dependent Fenton reaction. Hydroxyl-radical species damage proteins, lipids and DNA, resulting in cell death. In this paper, Kohanski and colleagues investigated whether a selection of bactericidal and bacteriostatic antibiotics induce the formation of toxic hydroxyl radicals.

Using a hydroxyl-radical-sensitive fluorescent dye, the researchers showed that treatment with lethal doses of bactericidal, but not bacteriostatic, antibiotics induced hydroxyl-radical formation in both Gram-negative (*E. coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. Blocking the production of hydroxyl radicals by treatment with an iron chelator, or quenching hydroxyl radicals with thiourea, increased the survival of antibiotic-treated bacteria.

Further, using *E. coli* mutants defective in iron transport and iron–sulphur cluster formation, Kohanski and colleagues demonstrated that the lethal hydroxyl radicals are formed using intracellular iron sources and the Fenton reaction.

The inspection of the transcriptomes of antibiotic-challenged *E. coli* revealed that NADH-coupled electron transport was specifically affected by bactericidal antibiotics. Assays confirmed that the intracellular concentrations of NADH plummet following treatment with bactericidal antibiotics. The authors speculate that the depletion of NADH stimulates free-radical damage of iron–sulphur clusters by superoxide, which releases the Fe^{2+} that is then used in the Fenton reaction to form hydroxyl radicals that damage cells, resulting in death.

Bacteria fight back against the action of hydroxyl and other free radicals by the induction of the DNA-damage response system (the SOS response). Significantly, Kohanski and colleagues found that the SOS response was induced by bactericidal antibiotics, indicating that the hydroxyl radicals that are generated damage DNA. Blocking the SOS response increased the amount of cells that are killed by bactericidal antibiotics.

Understanding how antibiotics kill bacteria is essential if we are to overcome the huge problem of antibiotic resistance.This study suggests not only new approaches to killing bacteria, by the design of drugs that induce hydroxyl-radical formation, but also how auxiliary drugs that target bacterial responses to hydroxyl-radical formation (including the SOS response) could be used to boost the killing power of antibiotics.

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ORIGINAL RESEARCH PAPER Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. & Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797–810 (2007)