Bacteria-killing dispute casts doubt on antibiotic development

Antibiotic drugs are one of the cornerstones of modern medicine, but, surprisingly, scientists still don't understand all of the ways in which they work. So when biomedical engineer James Collins and his team at Boston University announced several years back that they had discovered a common mechanism of cell death underlying all major classes of antibiotics—and that the pathway could be used to combat resistance, an increasingly growing problem—the report generated a lot of excitement. It even spawned a new company, called EnBiotix, which aims to develop antibiotic 'adjuvants'—agents designed to weaken the defenses of superbugs and resensitize them to existing antimicrobials.

But in recent months, several different researchers have tested Collins's idea and found it wanting. "When you look at bacteria killed by different antibiotics, it's hard to believe there is a common mechanism," says Frédéric Barras, a bacterial geneticist at Aix-Marseille University in France.

The three major classes of bacteria-killing antibiotics all attack bacterial cells in different ways—fluoroquinolones inhibit DNA replication, aminoglycosides attack the ribosome to disrupt protein synthesis and beta-lactams disrupt the cell wall. Bacteriologists agree on that. But in 2007, Collins and his colleagues found that all three types of antibiotics also generated harmful amounts of reactive oxygen species (ROS), toxic free radical molecules that helped wipe out the bacteria[1]. Antibiotics push cellular respiration into overdrive, they showed. This, in turn, produces dangerous amounts of hydroxyl radicals that help kill the bacterial cell by disrupting its DNA[2,3].

Not so, say the authors of three independent papers published this year in *Science*, all of which have raised doubts about the ROS model[4–6]. In one report, from March, James Imlay and his graduate student Yuanyuan Liu found that antibiotics were just as effective at killing bacteria whether oxygen—a prerequisite for ROS to form—was present or not. There was no increase in the rate at which hydrogen peroxide formed and no evidence that the bacteria were suffering from DNA damage[4]. "Cell death was still happening, but there was no evidence of oxidative stress," says Imlay, a microbiologist at the University of Illinois at Urbana-Champaign.

Barras similarly found no connection between antibiotics and the production of ROS. Reporting on 28 June, his team showed instead that the most important variable is the permeability of the cell membrane[6]. Under low-oxygen conditions, the membranes allowed less of the antibiotic into the cell. That, rather than the lack of ROS, could explain why cells under those conditions are less susceptible to the drugs, he says.

Just a misunderstanding?

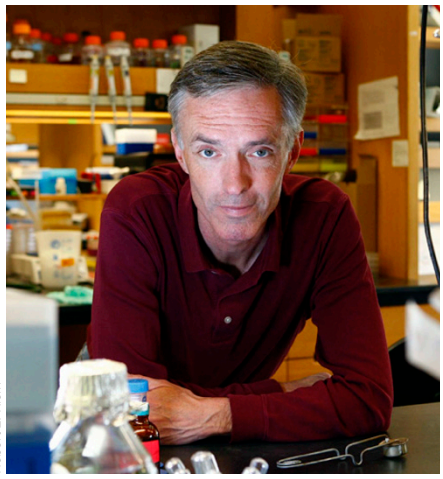
Collins counters that there has been "a fundamental misunderstanding of the model" he proposed. He never suggested that oxidative stress was the sole way that antibiotics kill bacteria; rather, that ROS complemented the drugs' main activity, which could be different from agent to agent. "As a result of antibiotics interacting with their primary targets, they induce a general stress response that creates ROS that contribute to cell death," Collins told *Nature Medicine*.

Collins recently published two papers in *Science Translational Medicine* further supporting this mechanism. In June, he reported that silver induces oxidative stress that can be harnessed to potentiate antibiotic activity[8]. And last month he showed that antibiotics increase the production of ROS not just in bacteria but in human cell lines, too, leading to mitochondrial dysfunction[9].

Together with Graham Walker, a microbiologist at the Massachusetts Institute of Technology in Cambridge, Collins has now completed a series of experiments that the two scientists intend to publish as a rebuttal to the criticisms of the ROS model. "I don't know how to explain my results without ROS," says Walker.

But for Collins, this debate is not just of academic interest. He believes that a deeper understanding of how antibiotics work and the part that oxidative stress plays will help scientists develop either new bacteria-killing drugs or compounds that can make existing drugs work better. To take these ideas forward, Collins, together with two drug development companies and a life sciences-focused investment bank, last year helped launch EnBiotix, based in Boston.

Jeff Wager, EnBiotix's chief executive, says the company remains "unshaken" by the recent challenges to Collins's papers. "I don't think this affects our work at all," he says. Confident that the ROS model will be vindicated when all the data are in, Wager notes that the added scrutiny ultimately "will be a positive thing for the credibility of Jim's research."



Robert E. Klein

A reactive backlash: A unified mechanism of antibiotic action, proposed by James Collins (pictured), is under attack.

No matter who's right about the role that ROS have in the killing of antibiotics, focusing on trying to rationally develop drugs that promote oxidative stress in this way may be a fool's errand, says Brad Spellberg, an infectious disease specialist at the Harbor-University of California–Los Angeles Medical Center. "Anybody who thinks mechanism-led discovery is effective [against bacteria] hasn't learned anything from the past 80 years." For example, the UK pharmaceutical giant GlaxoSmithKline spent much of 1990s trying to use genomic data to zero in on new and more effective targets for antibiotics but met with limited success and essentially abandoned the project[10]. "It just hasn't worked," Spellberg says.

So, instead of focusing on ROS, Spellberg offers one unradical suggestion: continue scouring small libraries of compounds for those with bacteria-killing effects. "We have to go back to traditional drug screening," he says.

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