QnAs with James J. Collins

Tinsley H. Davis
Science Writer

When it comes to administering antibiotics, two are not necessarily better than one. Instead of working in synergy, antibiotics intended to slow bacterial growth can undermine the effects of antibiotics intended to kill bacteria. In his Inaugural Article, James Collins, elected to the National Academy of Sciences in 2014, uses a systems biology approach to determine how different classes of antibiotics affect the cellular metabolism of their targets (1). The Termeer Professor of Medical Engineering and Science at Massachusetts Institute of Technology (MIT), Collins ultimately wants to preserve and enhance antibiotic efficacy by uncovering a nuanced understanding of how bacteria respond to antibiotics. A former physicist turned bioengineer, Collins has also worked toward developing paper-based synthetic gene networks intended to provide rapid, portable diagnostic field tests for infectious diseases like Ebola (2).

PNAS: What is antibiotic antagonism and what prompted you to look at antibiotics' effects on cellular respiration?

Collins: Antibiotic antagonism is a phenomenon wherein antibiotics work against each other. We were intrigued to study this phenomenon, primarily to see if we could gain insight into the underlying mechanisms.

Our laboratory is keen on using network biology approaches and bioengineering methods to better understand how antibiotics act, to examine how bacteria protect themselves against antibiotics, and to use insights from such studies to enhance our antibiotic arsenal. In the present study, we focused on understanding the relationship between bacterial metabolism—broadly defined—and antibiotic effectiveness.

There are significant differences between bactericidal antibiotics, drugs that kill bacteria, and bacteriostatic antibiotics, drugs that inhibit cell growth. In a recent paper (3) we reported that bactericidal antibiotics lead to elevated bacterial respiration rates, and in the present study we were interested in considering what would happen with bacteriostatic antibiotics.

PNAS: How was respiration affected when antibiotics were used in combination? What did you measure metabolically that might explain the results?

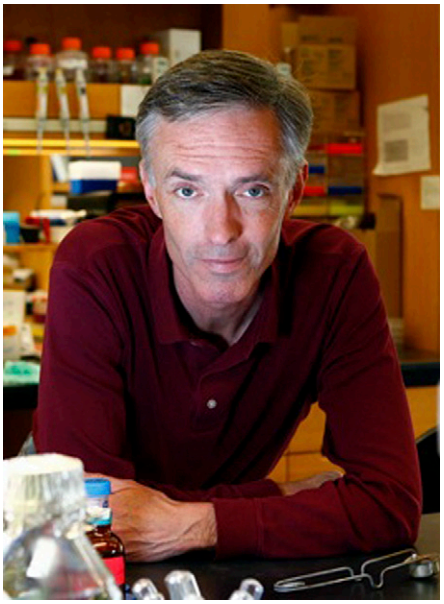
Collins: We confirmed that the bactericidal antibiotics elevate cellular respiration and, interestingly, we found that the bacteriostatics inhibit cellular respiration. When you put them together the bacteriostatics win, and the overall result is an inhibition of cellular respiration. Further, we found that when you deliver the bactericidals first, elevating respiration, subsequent delivery of a bacteriostatic will dominantly suppress the bactericidal phenotype. The combination basically turns the cidal drug static.

We then used a metabolite profiling approach to assess what was happening as a result, specifically, of the bacteriostatic treatments. We found that the inhibition of cellular respiration was projected back to the inhibition of metabolism. As we expected, we saw accumulation of the metabolites involved in the target of the drugs, in this case translation, so we saw a large accumulation of amino acids. In addition, we observed accumulation of central metabolites, specifically those that would feed into cellular respiration. By shutting down translation, the bacteriostatic drugs drive this build-up, or a kind of logjam, of metabolites in the cell.

We suspected that boosting respiration could be important for the bactericidal drugs. When we looked at a mutant with basally elevated respiration, it turned out that bactericidal drugs were much more effective at killing this mutant. The target, the F1F0 ATPase, is one for which drugs are being developed, and our findings suggest that using these in combination with bactericidal antibiotics may be a way to make such drugs more effective.

PNAS: This study looks at effects on cellular respiration in vitro. Are there significant ways that the effect of antibiotics might differ during an actual infection?

Collins: There will, of course, be some differences in vivo as the bacteria will need to deal with the host, specifically the immune response. It will be interesting to extend our studies to in vivo settings to better understand how the metabolic state of bacteria, as part of an active infection site, is influenced by the microenvironment of the host and how such changes to the physiological state of the bacteria could impact the effectiveness of antibiotics.



James J. Collins. Image courtesy of Robert E. Klein (Howard Hughes Medical Institute, Chevy Chase, MD).

An underlying message of our work is that the metabolic state of bacteria influences their susceptibility to antibiotics and, correspondingly, antibiotics alter the metabolic state of bacteria, which in part dictates the outcome of the drug treatment: death or bacteriostasis.

PNAS: Your Inaugural Article asserts that "bactericidal" and "bacteriostatic" are insufficient descriptors of antibiotic action. What terms might you use instead?

Collins: There appear to be a range of phenotypes that come out of antibiotic treatments, and using these two simple bins may not be enough to capture all of the effects. It may be more useful to look at other functional consequences of the antibiotics, including their effects on cellular respiration or metabolism in general.

One of the more interesting observations from our study is that rifampin, a transcription inhibitor, works by inhibiting cellular

This is a QnAs with a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article 10.1073/pnas.1509743112.

respiration so it actually served to block the killing effects of other antibiotics, even though it is more commonly viewed as a bactericidal antibiotic. This serves as an important example that bacterial cell death is complex and may come in various forms; in this case, rifampin's metabolic effect was a predictor that it would be antagonistic to other bactericidals, which turned out to be the case.

PNAS: Could this study lead to ways to predict the clinical effects of antibiotic combinations?

Collins: There is debate about the utility of "static-cidal" combinations for treating human infections. Unfortunately, the increase in multidrug-resistant infections has pushed us to use combinations more commonly, perhaps not always to the better. A next step is to think about infections where antibiotic combinations are the norm, such as tuberculosis. Our study shows that if the goal is to kill the bacteria at the infection site, adding antibiotics that inhibit cellular respiration may end up with a disappointing outcome.

PNAS: How might the results address antibiotic resistance or increase efficacy, given a limited antibiotic arsenal?

Collins: Our work suggests a new way of thinking about effective combinations of antibiotics. It also points to certain ineffective treatments that are being used which may, in some cases, not be the result of underlying resistance, but instead the result of tolerance due to the physiological state of the bacteria, sometimes induced by the antibiotics. Our work points to the need to better understand the relationship between bacterial metabolism, cellular respiration, and antibiotic effectiveness. There is much more to be discovered as to how the physiological state of bacteria influences their susceptibility to antibiotics and how antibiotics influence the physiological state of bacteria. Importantly, we also identify a potential mechanism—namely, boosting cellular respiration—which may be a viable means to increase the killing efficacy of our existing antibiotics.

What we have done as a laboratory and with our collaborators, notably Graham Walker of MIT, is to use systems biology techniques and network biology approaches to more comprehensively characterize the cellular response of bacteria to antibiotic treatment, with the goal of more completely understanding what happens physiologically and biochemically to bacteria as a result of antibiotic treatment. It turns out that even death in single-celled organisms can be remarkably complex.

1 Lobritz MA, et al. (2015) Antibiotic efficacy is linked to bacterial cellular respiration. *Proc Natl Acad Sci USA*, 10.1073/pnas.1509743112.

2 Pardee K, et al. (2014) Paper-based synthetic gene networks. *Cell* 159(4):940–954.

3 Dwyer DJ, et al. (2014) Antibiotics induce redox-related physiological alterations as part of their lethality. *Proc Natl Acad Sci USA* 111(20):E2100–E2109.