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The Death of the "Three Ms"

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Conventional wisdom has it that anti-infective research is one of the easier arenas in which to practice drug discovery. Like many urban legends, this view is mostly promulgated by drug hunters in other therapeutic areas as actual industrial anti-infective programs are few and far between. A sober assessment of our progress against pathogens important to the developed world has recently led to the Infectious Disease Society of America concluding that we are falling well short of where we need to be[1] and to the establishment of a Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria in late 2014. Why are we failing in this easiest area to discover new medicines? The real answer is that this enterprise is simply not as easy as it seems.

Tuberculosis (TB) is, of course, not a prime concern in the developing world, although the U.S. Centers for Disease Control listed highly drug-resistant TB as one of the top five global threats due to infectious agents in 2012.[2] A large outbreak of extensively drug-resistant TB in the United States would be devastating and seems increasingly likely. Although we have had two new drugs approved by regulatory authorities in the past few years, neither has been shown to be curative and resistance to both is simply inevitable. There are a number of problems associated with drug discovery for TB that require innovative solutions to move the field forward.

In the 1990s, when the complete genomes of TB and many other bacteria were first unveiled, there was a euphoric rush to classify genes as "essential" and develop high-throughput enzyme-based screens against these targets with the hope of enabling the tools of structure-based drug design. The closure of many anti-infective efforts in the pharmaceutical industry can perhaps be understood, in part, as a crisis in confidence after the spectacular failure of this approach to provide any useful new classes of drugs in the clinic. The problem was not that potent inhibitors of various enzymes were not developed; the problem was that this rarely translated into whole cell activity.[3]

In TB drug development, this gave rise to a movement away from considering targets at all. Whole cell screening for minimum inhibitory concentration (MIC) was the only approach considered worthwhile, and any suggestion of interest in the mechanism of action was deeply frowned upon and declared "academic". The Nobel Laureate developer of prontosil, Gerhard Domagk, who worked on TB drug development at Bayer Pharmaceuticals during World War II, highlighted the "three Ms" as the mantra for antibacterial drug development. These three Ms include (1) MIC, does it kill the bug?; (2) mouse, does it clear infection in mice without killing them?; and (3) man. Nearly a century of science was deemed to have been of no use in the development of new drugs to treat TB.

The problem with focusing only on MIC as a read-out for antibacterial activity is that inhibitory activity is a function of more than just drug binding to and inhibiting a specific target. Antibacterial efficacy relies on many other factors affecting the drug including permeation through the thick cell envelope, efflux, metabolism by the bacteria, binding to other proteins, and all of the pharmacodynamics of getting to where the bacteria are in the body. Interpreting the impact of chemical changes in a series of leads using only MIC as an output can be very challenging as target-engagement effects can be difficult to assess if influx, efflux, and/or metabolism are changing simultaneously. There is also a significant chance of slipping between multiple low-affinity targets/mechanisms, resulting in uninterpretable trends in structure−activity relationships (SAR), particularly when one is working with less potent compounds early in discovery.

The role of drug efflux in mycobacteria has been chronically understudied, a fact that was driven home by the discovery of the TB-active spectinamides last year by Lee et al.[4] Recognition that spectinamides were substrates of a specific efflux pump permitted the simultaneous monitoring of efflux and protein synthesis inhibition, therefore allowing the authors to identify compounds that both bound the ribosome potently and were poor substrates for efflux. This type of two-pronged screening can facilitate lead optimization in a way that simple MIC screening cannot.

Target-based whole cell screening presents a viable option to target-agnostic screening. Combining target-based whole cell screening and the powerful tools of biochemistry and structural biology can conceptually provide a way to simultaneously track SAR against the target, as well as SAR trends against the whole organism. Nonetheless, not all whole-cell screening is created equal. We have increasingly come to understand that the physiology of TB in the human host is fundamentally different from the physiology of TB growing rapidly on glucose under aerobic conditions, and these differences affect the intrinsic drug-susceptibility of the organism to many inhibitors. High-throughput screening of a large chemical library against TB replicating inside macrophages revealed that only 10% of the hits were equipotent under standard growth conditions and that 10% of the hits were active only against intracellular bacteria.[5] Most of these selective compounds regained activity against TB in vitro if the growth medium was simply switched to provide cholesterol, rather than glucose, as the primary carbon source. Although inhibiting TB growth in macrophages

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is important, the situation in human patients with TB is more complex, and the bacteria exist in a variety of different locales, both intracellular and extracellular, and under a variety of different environmental conditions.

As if the bacterium was not complicated enough, the host introduces a whole new layer of complexity due to the nature of the immune response to TB infection. TB induces the formation of granulomas at sites of infection. Superficially, these granulomas are organized masses of immune cells that physically sequester the bacteria away from the body. In the process of doing this, blood vessels collapse and the interior tissue becomes necrotic. Drugs therefore have to diffuse into this necrotic material to kill these reservoirs of bacteria. The ability of different drugs to penetrate into these lesions was the subject of a recent study that used imaging MALDI mass spectrometry on human lesions following surgical removal of drug-resistant lesions.[6] This study found that the two most clinically useful TB drugs share an ability to penetrate and accumulate in the necrotic center of these lesions.

An important and rapidly unfolding renaissance is occurring in TB drug discovery and perhaps in anti-infective drug discovery more generally. As we begin to figure out how to merge the deluge of information about the biology of microbes with our ability to use structural biology, our chances of identifying new targets and drugs are increasing dramatically. One can only imagine the level of motivation and dedication that led Domagk to continue to trek to the Bayer laboratories to synthesize and test new compounds against TB as Allied bombs exploded nearby. Our challenge is to show enough fortitude to work through the seemingly endless explosions of biological data and identify ways to engage this in drug discovery to bring a new generation of antibiotics into play before these organisms get the upper hand. We have come a long way since World War II; it is time to move beyond the three Ms.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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