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Antibiotic resistance: can we beat the bugs? ▼

It is becoming clear that antibiotic resistance can emerge and disseminate rapidly. For example, in France, penicillin-resistant isolates of *Streptococcus pneumoniae* increased from 5% to 25% within five years[1]. It also took only five years for ampicillin-resistant *Shigella dysenteriae* in Bangladesh to increase from 2% to 90%[2]. To beat the bugs, we need a better understanding of how to use therapeutics to minimize the development of resistance.

Antimicrobial agents are generally used at concentrations that kill or block the growth of susceptible pathogens. Resistant mutants must then be overcome by host defence systems, which usually prevail because mutant numbers are low. However, enrichment of the mutant fraction of pathogen populations can occur during each infection. Spread to new hosts or non-compliance with therapy allows bacterial populations to expand; additional mutant enrichment occurs when selective pressure is reapplied. Such repeated cycles of selection and population outgrowth lead to resistance despite most patients being cured.

Patient response and resistance

The classic measure of antimicrobial potency is the minimal inhibitory concentration (MIC) for a particular agent with a given pathogen. By relating the MIC to concentrations of the pathogen in human tissues, it has been possible to develop empirical relationships between drug exposure, potency and patient response. With the fluoroquinolones, for example, both the ratio of the maximal serum concentration (C_{max}) to MIC, and the ratio of the area under the concentration–time curve (AUC) to MIC, relate to disease outcome[3,4]. Maintaining these ratios above empirically determined values has been suggested as an approach for restricting the selection of resistant mutants[3–5]. However, these pharmacodynamic tests have involved few patients[3,4] and/or low inoculum sizes[5]. Such factors could have focused resistance analyses on non-topoisomerase mutants whose loss of susceptibility is modest. These mutants occur much more frequently than topoisomerase mutants[6]; thus we cannot conclude that dosing-to-cure will limit the high-level clinical resistance normally associated with drug-target (topoisomerase) mutants.

Beyond cure

An approach that goes beyond cure would set the drug concentration high enough so that mutants are less likely to be selectively enriched during treatment, that is, above the MIC of the most resistant, first-step mutant. Growth of resistant mutants would then require the presence of two concurrent mutations, which would in turn require more cells ($>10^{12}$) than are present during most infections. A mutant prevention concentration (MPC) can be defined as the concentration that allows no mutant to be recovered when at least 10^{10} cells are applied to drug-containing agar plates[7]. New fluoroquinolones that have tissue-achievable MPC for bacteria are described in a recent issue of *Drug Discovery Today*[8].

Conclusion

We might be able to control the development of resistance by more effectively dosing antimicrobial agents above MPC during monotherapy. Compounds that cannot be dosed in this way could still be used, but only as parts of combination therapy. Clinical studies are needed to determine whether dosing above the MPC will severely restrict the selection of resistant mutants and still be tolerated by patients. However, for this strategy to ultimately be effective, we must see its rapid implementation before the prevalence of first-step mutants in the community gets too high.

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Bacterial fatty-acid biosynthesis: an antibacterial drug target waiting to be exploited ▼

Fatty-acid biosynthesis has been validated as a target for antimicrobial agents through extensive work in *Escherichia coli*[1]. This has demonstrated that fatty-acid biosynthesis is essential to the survival of the organism, is distinct from the mammalian pathway and is generally highly conserved among bacteria. Thus, there exists the potential for the generation of a broad spectrum of novel antibacterial drugs. To date, triclosan, isoniazid, cerulenin, thiolactomycin and diazaborines are known fatty-acid-synthase inhibitors with proven efficacy.

New leads described recently in *Drug Discovery Today*[2], target the enoyl-acyl carrier protein (ACP) reductase step. The FabI reductases of Gram-negative and Gram-positive bacteria are inhibited by the consumer antimicrobial, triclosan, whereas the protein from Mycobacteria is inhibited by isoniazid. However, the enoyl-ACP reductase step has some drawbacks that limit enthusiasm for further developments. Two novel isoforms of this enzyme have been discovered: FabL, identified in *Bacillus subtilis* by its weak homology to FabI, has overlapping, redundant functions *in vivo* with FabI; and FabK, which was identified as the sole enoyl-ACP reductase in *Streptococcus pneumoniae* as a triclosan-resistant flavoprotein with no homology to FabI (Refs 3,4). The existence of these enzymes will limit the activity spectrum of drugs targeted against FabI.

Triclosan had been thought to kill bacteria by disruption of the cell membrane, an effect that has now been disproved for Gram-negative bacteria. Overexpression of either the FabK or FabL isoforms renders *E. coli* refractive to triclosan inhibition[3,4]. Expression of these isoforms in Gram-positive bacteria, however, merely increases triclosan resistance by approximately eightfold. Payne and co-authors[2] conject that this remaining sensitivity is a result of membrane-perturbing effects in Gram-positive bacteria. However, inhibition of a second, undefined target in Gram-positive bacteria remains a plausible hypothesis.

Bacterial fatty-acid-synthase inhibitors have a potentially wider usage, as antimalarial chemotherapeutics. *Plasmodium falciparum*, a unicellular eukaryotic parasite, contains a bacterial-like fatty-acid synthase system in a vestigial chloroplast. Thiolactomycin and triclosan have been demonstrated to have antimalarial activity *in vitro*[5,6], and thus new inhibitors of the bacterial pathway could also be effective against this organism.

Two targets in particular stand out as potential candidates for further development. The condensing enzymes, FabH, FabB and FabF, are essential, highly conserved and inhibited by thiolactomycin and cerulenin[7]. Despite different substrate specificities, their similar active-site architecture makes them ideal candidates for the design of new drugs that can inhibit both isoform activities. Thus, the bacteria would need to mutate two loci to become resistant. The second possibility lies with the FabG β-ketoacyl-ACP reductase[8]. From a bioinformatic analysis, it is observed that this enzyme is highly conserved in all bacteria, has no known isoforms and is probably essential. Thus, inhibitors of FabG should possess broad-spectrum activity.

Bacterial fatty-acid synthesis is a pathway with a high potential for the discovery of new antimicrobial drugs. The time is now ripe for further exploitation by the pharmaceutical industries, which will hopefully result in much needed new chemotherapeutics.

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