Background document commissioned by ReAct for the seminar “Collaboration for Innovation – The Urgent Need for New Antibiotics”, Brussels May 23 2011.
The paper reflects the views of the author.

Scientific Obstacles to Discovery of Novel Antibacterials

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This document summarizes the salient points of a recently published review (Silver, L. L., Challenges of Antibacterial Discovery. Clin. Microbiol. Rev. 2011, 24, 71-109. [1]). Additionally, several other reviews are cited here, but for otherwise uncited specifics and supporting data, please refer to that review.

Introduction

While antibacterial resistance has increased over the past twenty years, the number of novel antibacterials discovered and developed has markedly decreased. In recent years, large pharmaceutical companies (Big Pharma) have greatly reduced their commitment to antibacterial discovery. Biotechnology companies have entered the area, but few of them have made any progress in discovery of developable novel agents. The low output of novel antibacterials over the past 20 years has a number of causes: commercial, regulatory and scientific. This report focuses on the scientific challenges:

The need for additional small molecule antibacterial agents to overcome resistance can be approached in two ways:

1. Discovery of completely novel chemical classes that do not cause cross-resistance to existing antibacterials.
2. Modification of existing classes to overcome resistance.

Before the current withdrawal from the antibacterial area by many in Big Pharma, both of these approaches were heavily pursued. The modification pathway was reasonably productive, although it has met with some regulatory obstacles. The pursuit of truly novel classes of antibacterials – both by discovery and exploitation of new antibacterial targets and by empirical screening (trying to find antibacterial activity without initial regard to mechanism of action) has been almost entirely unproductive. The last 4 novel classes to reach the clinic (including fidaxomicin which is expected to be approved this month) were all discovered (empirically) *before* 1987 (see Table 1).

This failure in novel discovery over the years since 1987 was not through lack of activity. It appears that, during this time, industrial discovery programs in Big and Small Pharma were turned toward utilizing high throughput technologies to find “novel” essential enzyme targets in bacteria. Chemical libraries were screened in an effort to make novel discovery a turnkey operation. First, find new, “unexploited” target, then find inhibitors of the target by screening or design (using biochemical or whole cell phenotypic screens), and then optimize the inhibitors to meet the pharmacological requirements for a drug. Unfortunately, this process does not work because it does not address the rate-limiting steps of the process, which require basic research and an investment of time (as well as money).
To simplify the obstacles:

1. Target choice by selection of essential, broadly conserved, gene products with no human homolog is not sufficient and must include the criterion of low resistance potential
2. Chemical libraries and understanding of the necessary physicochemical requirements for entry into and prevention of efflux (active expulsion) from bacteria are poor. Future success will depend on both identifying good targets (and recognizing that there are likely relatively few good targets) and understanding the nature of good antibacterial chemicals in order to create better libraries of chemicals and fragments and incorporate these elements into design of better inhibitors.

Unless efforts are made to solve these basic problems of antibacterial discovery, regulatory or financial incentives will not lead to greater output of novel classes of antibacterials to meet the challenge of antibacterial resistance. Support of basic research – whether academic, industrial, non-profit or a consortium of all – is required.

Recent history

Table I shows the outcomes of 19 New Drug Applications (NDAs) for antibacterial novel chemical entities (NCEs) evaluated by the FDA since 2000.

Table 1. NDAs reviewed by FDA 2000-2011

Compound	Usage	Class	Active versus resistance	Discovery of class	Fail at FDA	Pass at FDA
Linezolid	Systemic IV/oral	Oxazolidinones		1978		2000
Ertapenem	Systemic IV/IM	Carbapenem		1976		2001
Cefditoren	Systemic oral	Cephalosporin		1948		2001
Gemifloxacin	Systemic oral	Fluoroquinolone		1961		2003
Daptomycin	Systemic oral	Lipopeptide		1987		2003
Telithromycin	Systemic oral	Macrolide+	*Ery*[R] *S. pneumo*	1952		2004
Tigecycline	Systemic IV	Tetracycline+	Tet[R]	1948		2005
Faropenem	Systemic oral	Penem		1978	2006	
Retapamulin	Topical	Pleuromutilin		1952		2007
Dalbavancin	Systemic IV	Glycopeptide		1953	2007	
Doripenem	Systemic IV	Carbapenem		1976		2007
Oritavancin	Systemic IV	Glycopeptide+	VRE	1953	2008	
Cethromycin	Systemic oral	Macrolide+	*Ery*[R] *S. pneumo*	1952	2009	
Iclaprim	Systemic IV	Trimethoprim+	Trm[R]	1961	2009	
Besifloxacin	Ophthalmic	Fluoroquinolone		1961		2009
Telavancin	Systemic IV	Glycopeptide+	VRE	1953		2009
Ceftobiprole	Systemic IV	Cephalosporin+	MRSA	1948	2009	
Ceftaroline	Systemic IV	Cephalosporin+	MRSA	1948		2010
Fidaxomicin	Oral CDAD	Lipiarmycin		1975		Due soon

Only 4 (shaded pink) are representatives of novel chemical classes – and those 4 were successfully registered. However, those 4 classes were each discovered more than 24 years ago, and all were discovered by empirical screening (non-target based) of natural product or synthetic libraries. Of the remaining 15 candidates, 8 were modifications of earlier registered classes which were qualitatively different from the original classes, having been optimized to overcome antibiotic resistance mechanisms specific to their parents' classes (shaded blue). Of the 15 non-novel candidates, 9 were successfully registered and 6 were not. In general, the failures were due to the lack of demonstration of non-inferiority or other shortcomings of trial design according to the FDA criteria.

The public perception that Pharma has only worked on "me-too" modifications is incorrect. First, as noted above, many of the modified drugs were significantly and qualitatively different from their progenitors. Second, despite their early class-discovery dates, 2 of the 4 novel drugs (linezolid and retapamulin) were the product of extensive chemical optimization programs, while the other 2 (daptomycin and fidaxomicin) required long development times determine optimal efficacy and dosage regimens. Third, and most important, there has been an enormous amount of work toward discovery of novel antibacterial targets and inhibitors of those targets for >20 years without significant output. It is the lack of success in finding novel developable entities which, apparently, has led to the impression that pharmaceutical companies have only worked on easy me-toos. While not widely touted and often

poorly or not at all documented in the literature, literally thousands of programs, both industrial and academic have pursued inhibitors of many so-called “unexploited” targets by screening and rational design but have produced only a few clinical candidates, only one of which has even progressed to Phase II (and that only recently). No others have progressed beyond Phase I. These efforts that are reviewed in [1] in an effort to illustrate the specific challenges to discovery of novel antibacterials.

Discovery motifs: natural products and chemical libraries

It is easy to kill bacteria. It is hard to kill bacteria in ways that will affect only the desired spectrum of bacteria without toxicity to the host. Empirical screening of natural products (especially those produced by soil bacteria) was successful in discovering novel, relatively safe compounds that proceeded quickly to the clinic. But this source eventually became less productive and the emphasis on target-directed screening and high-throughput screening technologies (less well-suited to natural products) led to a drastic decrease in natural products sourcing (in Big Pharma) for antibacterials and the rise of the use of chemical libraries. With synthetic chemical libraries, empirical screening most often selects for compounds with cytotoxic or lytic activity [2]. Indeed, the potential for toxicity is one of the reasons that most modern antibacterial efforts have been directed toward inhibition of pre-selected targets. In contrast, microbial natural products have, most likely, evolved to be relatively target directed – and their origins in living cells have probably limited their outright toxicity. But the best way to exploit natural products, which needs to be done to a greater extent, is to screen for novelty without requiring hits on any specific pre-selected target (although targets or pathways may well be used in the process).

In target-directed programs in which chemical libraries are screened for inhibitors of specific target enzymes in vitro, the great majority of inhibitors either have no antibacterial activity or any antibacterial activity they possess has not been proven to be due to inhibition of the desired target enzyme. This appears to be a function of the limitation of synthetic chemical libraries used for antibacterial screening and to lack of understanding of the chemical parameters that define the ability of compounds to enter bacteria and not be pumped out [1-2].

TYPES OF ANTIBACTERIAL SCREENING STRATEGIES

1. Empirical: whole cell screening for activities that inhibit growth of bacteria
2. Target-based
    a. Phenotypic: whole cell screening for inhibition of specific pathways or targets by asking for production of specific phenotypes such as:
        i. Morphological changes
        ii. Induction of a specific reporter gene at sub-MIC levels
        iii. Hypersensitization to an inhibitor of an enzyme lowering its level of expression
        iv. Synergy with another antibiotic
    b. In vitro screens such as:
        i. enzyme inhibition
        ii. target-binding
        iii. disruption of protein:protein interactions

Compounds that have been shown to have antibacterial activity due to specific inhibition of the “desired” single enzyme target and that are non-cytotoxic, where tested, have all been shown to be subject to relatively high level resistance through single mutational changes (generally in the target)

under laboratory conditions [1]. This is somewhat of a vicious cycle or "catch-22": the best way to prove that a compound is antibacterial due to inhibition of a specific intracellular target is to find a mutation that leads to significant resistance in the gene encoding the target. But the existence of in vitro resistance development is often viewed as a detriment to further development. Whether this has led to curtailment of programs is unknown, but it is likely to have played a part.

The nature of "good" targets and how to identify them *a priori* or after the fact

As developed in a number of reviews [3], it appears that successful monotherapeutic systemic agents are not inhibitors of single enzymes but target the products of multiple genes or structures that are the products of pathways. It is my theory that these so-called "multitargeted" agents are successful at monotherapy precisely because they do not fall prey to rapid development of high level endogenous resistance while single enzyme-targeted compounds will [1, 3e]. Thus, a main difficulty of target-directed discovery is the identification of targets that will not be subject to single-step high-level resistance due to mutation in the pathogen.

These are examples of already defined multitargets:

1. Pairs of essential enzymes with highly homologous active sites (such as DNA Gyrase and Topoisomerase IV, the targets of the fluoroquinolones, and the penicillin binding proteins [PBPs], the targets of the β-lactams).
2. Structures that are the products of the action of many gene products acting in a pathway (such as the cell wall intermediate Lipid II, target of the glycopeptides and the membrane, target of daptomycin).
3. Products of multiple, essentially identical, genes – where a resistance mutation in any one will be recessive (such as ribosomes, the target of many drugs including tetracycline, linezolid, chloramphenicol, macrolides, streptogramins, etc.).

It may be possible to find more potential multitargets of the first type by computational means. This may be approached by analysis of ligand-target networks, as is being done to identify polypharmacology in other human health areas, such as oncology.

The multitarget hypothesis posits that single enzyme targets are likely to be poor candidates for systemic monotherapeutic antibacterials because there is a high probability that a single mutational change in the target would lead to high-level resistance. But, does the documentation of such resistance in the laboratory actually presage clinical outcomes? Will in vitro resistance lead to resistance in the clinic? It is known that resistance can lead to lowered fitness, slower growth, lowered virulence in vivo and poor competitiveness with other, sensitive bacteria. This lowered fitness can often be at least partly reversed by the occurrence of secondary compensatory mutations. Thus, selection of pre-existing resistance mutations leading to high-level resistance would give at least temporary growth advantage (over dead peers) in the presence of a selecting drug and this edge could provide time for the occurrence and selection of compensatory mutations. This may be exacerbated by the presence (and selection for) mutators in the challenged population and mutations induced by free radicals produced during challenge by the drug [4]. However, as most in vitro compensatory mutations do not fully restore fitness, it is important not to disregard a prospective new antibiotic based only on the frequency of these mutations.

Is there a way to predict which single-enzyme targets will be insusceptible to rapid resistance development in the clinic? If so, it is not obvious and will require much basic research to uncover. The best avenue for a target based screening approach at present is to obtain inhibitors that can be shown to be antibacterial via inhibition of the target in question and then rigorously test that the target-

inhibitor combination for resistance potential with regards to both frequency and fitness of resistant mutants.

There have been a number of methods, in vitro and in vivo, used to model the potential for resistance to antibacterials under conditions that simulate the pharmacokinetic (PK) parameters expected in the human host. What is required is a set of standardized models with which to reliably predict the probability of occurrence of mutations leading to resistance under in vivo conditions and the further probability of their persistence, virulence, and potential for compensation. In the best case, resistant strains carrying single-step mutations will be so impaired as not to survive. Another possible outcome of such studies would be the identification of a "mutant prevention" dosing regimen allowing persistence of sufficiently high drug concentration to prevent the growth of even highly resistant mutants. Of course, high dosing would require very safe compounds. One problem of developing good models is that there is little clinical data with which to compare since most of the monotherapeutic agents are not subject to single-step high-level resistance. Without the optimization and use of such models, development of single-enzyme targeted inhibitors will remain a high-risk proposition.

The chemistry conundrum and how to solve it

As noted above, chemical collections in use are not well designed for finding molecules that can enter and stay in bacteria. Additionally, they often contain problematic compounds that are cytotoxic, aggregating, detergent or promiscuously interfering (due generally to broad protein binding qualities). A review of an extensive GlaxoSmithKline antibacterial screening program describes the results of 67 high-throughput screens of the GSK chemical library designed to find inhibitors of essential bacterial enzyme targets [2]. Only 16 targets yielded "hits" (chemically tractable compounds with good potency and low activity against related human enzymes). None of these hits were considered "leads", which required in addition to "hit" criteria, having antibacterial activity due to inhibition of the desired target. Aside from the problematic compound classes mentioned above, the physicochemical nature of most industrial or commercial chemical libraries developed for drug discovery in other human health areas is quite different from that of existing antibacterials and these libraries appear non-optimal for new antibacterial discovery [2, 5]

In general, antibacterials require less hydrophobic, more polar compounds than for non-antibacterials. For the sub-class of gram-negative bacteria, the needs are more particular, requiring small size and favoring compounds with both charged and uncharged forms at physiological pH [1-2, 5]. In order to improve the potential for finding novel antibacterials by empirical or targeted screening or by structure based design, it seems important to establish sets of rules for entry into bacteria (the barriers being different in gram positives and gram negatives) and for avoiding efflux. This is an undertaking that would profitably be done by a consortium as a basic research effort with publically available output. The application of rules to design of new libraries and specific compounds could then be undertaken by all – separately or together.

The pursuit of natural products for antibacterials has been largely abandoned, but should be revived. I believe the best sources are microorganisms, especially actinomycetes and other bacteria. But the major problem in natural product discovery of antibacterials is the constant re-discovery of previously described compounds. To discover novel antibacterial classes, efficient methods of dereplication are required using chemical and/or biological methods to quickly determine if activity is due to a novel compound. The use of directed phenotypic screens that can detect a signal below the concentration at which bacterial growth is inhibited (hypersensitive screens) as briefly reviewed in [1] and in the text box above, can efficiently detect novel compounds.

Summary
Discovery of new antibacterials for development requires solving two basic problems:

- Define good antibacterial targets in terms of their resistance potential
  - Favor multitargets
  - Validate targets by obtaining inhibitors and determining potential for clinical resistance by developing and using standardized PK/PD models
- Improve chemical sources for antibacterials
  - Formulate rules for entry into and lack of efflux from bacteria in order to improve
    - chemical libraries
    - fragment based approaches
    - optimization of enzyme inhibitors
  - Pair optimized chemical sources with screening strategies (empirical and target-based)
  - Revive microbial natural products as a screening source
    - use hypersensitive phenotypic screening to find novelty
    - explore new sources

Literature cited

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