

Antibacterial and antifungal drug discovery

Companies are racing to keep pace with the emergence of drug-resistant microorganisms.

Infectious diseases caused by bacteria and fungi affect millions of people worldwide, and in the United States alone cause a disease burden of more than \$20 billion annually. Concerted and systematic programs to discover and develop new antibiotics and antifungals have been driven to a considerable extent by the development of resistance by these organisms to the drugs commonly used against them. According to the Pharmaceutical Research and Manufacturers of America (PhRMA), there were 27 antibiotics, 12 antifungals, and 14 vaccines under development in 1998 against bacterial and fungal infections (this excludes more than 50 antiviral drugs) in all stages of clinical development. This illustrates the seriousness of the effort to develop new and improved medicines against this vast array of diseases. In this article, I summarize small molecule efforts in the antibacterial and antifungal areas.

Historical perspective

Infectious diseases are as old as life itself. They have certainly played a major part in shaping human history, not only because of the decimating effects of the various plagues through the centuries, but also because of the intense efforts made to find cures for them, thus advancing medical science.

In the past 200 years, empirical science and serendipity have combined to bring us to our current state of knowledge. For example, Paul Ehrlich is credited with the concept of selectivity, when, early in this century, he postulated the existence of molecules that would bind to microbes, but not to host cells. Bacteria and yeast were actually first observed under the first microscopes in the 18th century, and in 1877 Pasteur and Joubert reported how a culture could be inhibited by the products of a contaminating microorganism.

The most celebrated of these observations is Fleming's, who in 1929 observed the lysis of staphylococci colonies adjacent to colonies of the mold *Penicillium notatum*¹. The first sulfonamide, *p*-aminobenzene sulfamide, was characterized in the mid-1930s by Trefouel in France, and streptomycin was discovered by Waksman, a soil microbiologist, in 1944. Penicillin was being mass-produced successfully by the mid-1940s, in time to save the lives of millions of soldiers in World War II. The accelerating pace of antimicrobial drug discovery has resulted in thousands of molecules screened, from natural sources and from organic chemistry modifications of natural compounds, and combinatorial chemistry has pushed that number considerably higher. At present, about 100 or so antibiotics are in the clinic. Microbial resistance and new strains necessitate the substitution of many compounds that simply no

Table 1. Selected companies and their antibacterial and antifungal programs

Company	Program	Status
Aronex (The Woodlands, TX)	Liposomal nystatin against fungal infections	Phase III completed
Atrix (Fort Collins, CO)	Chronic adult periodontal disease	Market, 9/98
Bayer (West Haven, CT)	Respiratory tract infections	Phase II
Biosyn (Philadelphia, PA)	Glymiox for oral candidiasis	Phase I/II, 1999
Bristol-Myers Squibb (Princeton, NJ)	Gatifloxacin for a wide variety of infections	Phase I
Cubist (Cambridge, MA)	Endocarditis and blood stream bacterial infections	Phase II, 1999
DepoTech (San Diego, CA)	Amikacin for bacterial infections	Phase I, 12/95
Eli Lilly and Co. (Indianapolis, IN)	Vancomycin- and teicoplanin-resistant enterococcal antibiotics	Phase II
F. Hoffmann-La Roche Ltd. (Basel, Switzerland)	Peptide deformylase inhibitors	Preclinical, 1998
Hoecsh Marion Roussel (Bridgewater, NJ)	Ketolide for respiratory infections	Phase III
Janssen Pharmaceutica (Titusville, NJ)	Itraconazole for dermatomycoses	Application submitted
IntraBiotics (Mountain View, CA)	Protegrins for oral mucositis	Phase II, 11/98
Magainin (Plymouth Meeting, PA)	Infection in diabetic foot ulcers	FDA action taken, 9/98
Merck (Whitehouse Station, NJ)	Various bacterial infections	Phase II
NABI (Rockville, MD)	Staphylococcus aureus infections in newborns	Phase I/II, 1/98
Novartis (East Hanover, NJ)	Terbinafine for subcutaneous and systemic fungal infections	Phase III
PathoGenesis (Seattle, WA)	Rifalazil for tuberculosis	Phase II, 10/97
Penederm (Foster City, CA)	Butenafine for onychomycosis	Phase III
Pfizer (New York)	Azithromycin for various infections	Phase III
Pharmacia & Upjohn (Peapack, NJ)	Gram-positive antibacterials	Phase III
R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ)	Levofloxacin for various infections	Phase III
Rhône-Poulenc Rorer (Collegeville, PA)	Streptogramin against vancomycin-resistant enterococci	Application submitted
Schering-Plough (Madison, NJ)	Ziracin for resistant Gram-positive infections	Phase III
SmithKline Beecham (Philadelphia, PA)	Broad spectrum antibiotics	Phase II
Warner Lambert (Morris Plains, NJ)	Clinafloxacin for various infections	application submitted
Wyeth-Ayerst (Philadelphia, PA)	Glycylcyclin against resistant bacteria	Phase I
XOMA (Berkeley, CA)	Recombinant bacteriocidal permeability increasing protein for chlamydia infections	Preclinical, 1999

Source: Biovista (www.biovista.com)

longer work with newer variants, showing that more is not always better.

Interestingly, ideas about new antibiotics come from a variety of sources. For example, in 1985, antibiotic peptides released by normal human neutrophils were characterized. These peptides, called defensins, kill *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* in vitro². They are now being developed as potential antibiotics, and are a good example how present-day research in this area is evolving constantly.

Current state

As the market for antimicrobial agents is so large, it is not surprising that dozens of biopharmaceutical companies have a wide variety of drugs in various preclinical and clinical trials. A small selection is shown in Table 1. In addition, many efforts aimed at modifying the molecular characteristics of existing antibiotics to make them even better, in terms of reduced side-effects and avoidance of resistance. A good example are the 4H-4-oxoquinolizine derivatives. These antibacterials were developed in response to growing bacterial resistance to fluoroquinolones among Gram-positive, Gram-negative, and anaerobic pathogens. The 4H-4-oxoquinolizine derivatives are being modified to improve their characteristics, by making substitutions at specific locations, such as the C-8 position³. It is believed that these modifications will improve the antibacterial efficacy and spectrum, as well as the physicochemical and pharmacokinetic properties of these compounds. Early results confirm this. Furthermore, uncultivable *Streptomyces* from soil are being mined for novel antibiotics by biodiversity-focusing companies, such as Terrangen Discovery (Vancouver, BC, Canada) and others⁴.

In addition, one of the key issues that characterize the current state of antibiotic drug discovery is the ongoing arms race in academic and corporate laboratories to defeat the emergence of antibiotic resistance. It is widely accepted that the increased availability and use of antibacterial and antifungal agents in recent years has resulted in the control and even eradication of diseases, but it has also led to the development of resistant strains. Much effort is being expended to not only develop new drugs faster than resistance develops, but also understand the basics of resistance itself. For example, antifungal agents are often classified into three groups, depending on their site of action: the polyenes interact with fungal membrane-building blocks; azoles inhibit the synthesis of ergosterol, the key fungal sterol; and 5-fluorocytosine is an inhibitor of fungal macromolecular synthesis. These agents were developed based on an understanding of the fungal life cycle and weaknesses, and the

same approaches are used by the organisms themselves to develop resistance.

A number of different mechanisms contribute to the development of resistance. In the case of antifungals, they include molecular changes in the drug target itself; overexpression of the drug target—thus swamping the antifungal agent; the reverse of overexpression, namely reduction in the concentration of the drug target—thus eliminating it as a site of action; changes in molecule biosynthesis; and pumps that actively eliminate the antifungals⁵. Dissection of each of these mechanisms reveals new weaknesses in the pathogens, and is used as a strategy to combat the problem of resistance.

Finally, there is an emerging trend to look at host defense mechanisms for specific tools that may be added to the arsenal. For example, epithelial cells are known to release peptides, known as defensins, that act as antibiotics. The physiology and local mode of action of these peptides is under intense investigation⁶ and will no doubt lead to significant new antibiotics based on optimized forms of these natural molecules.

Industry challenges

A key challenge to the antibiotics industry is that constant innovation is necessary not only because of resistance, but also because of side effects. For example, erythromycin is a well-known macrolide antibiotic used as an alternative for patients who are allergic to penicillins. There are three analogs of erythromycin on the market—azithromycin, clarithromycin, and dirithromycin—which last longer, are more stable in acid, and have better tissue distribution than erythromycin. In addition, they cause less gastrointestinal problems. Azithromycin and dirithromycin also do not interact with the same liver drug-metabolizing enzymes as does erythromycin, and thus do not have the same drug interactions⁷. The development of these analogs was driven essentially by the need for drugs with reduced side effects, so it is often possible to develop multiple analogs of successful antibiotics that achieve the same results with less adverse side effects.

Another challenge to the industry is that in some cases, infection becomes so rampant and systemic that antibiotics alone are not enough to combat what becomes a multifactorial disease. For example, newborn babies are susceptible to bacterial and fungal sepsis that are major causes of morbidity and mortality in the immunocompromised, or in those with underdeveloped immune systems. In these cases, it is absolutely necessary to complement antibiotic therapy with other factors, such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), both of which counteract the various

neutropenias associated with newborns or the immunocompromised⁸. Therefore, in considering antibiotic development and application, it is important to do so in the larger context of the total clinical picture, as antibiotic treatment of infection is only one of many issues. A direct corollary of this is that interference between drugs used for different purposes becomes a major problem, as the antibiotic and the anti-neutropenia agent in the previous example need to be able to operate simultaneously in the body. This is why every new antibiotic is developed while taking into account as many scenarios as possible where it would be used in the presence of other drugs.

Future directions

In addition to focusing on the development of better antibiotics against infectious organisms and combatting the growing problem of resistance, we are likely to see some interesting side benefits from research into the infectious microbes themselves, and their broader context. For example, the bacteria that line the human intestine serve a vital catabolic role, and the perturbation of the intestinal microbiota that can occur after heavy antibiotic use can lead to a number of disease conditions. In addition, a growing body of evidence suggests links between the development of this microbiota and susceptibility to allergies. A close relationship may exist between the development of the intestinal microflora that occurs in infancy, and allergic sensitization to specific foods, for example⁹. It is postulated that intestinal microbes could down-regulate allergic responses by attenuating T-helper cell responses or by increasing immunoglobulin (Ig)A responses. This has led to the idea that helping the development of these microbes in infancy with certain foods, called probiotics (as opposed to antibiotics), may help reduce the risk of developing certain food allergies later in life. This type of work, whereby microbes are not always treated as foe, will likely increase in the future.

Another emerging trend for the future is the increasing correlation between infections and other diseases. For example, numerous studies have shown that infection with *Chlamydia pneumoniae* is a contributing factor in the pathogenesis of atherosclerosis¹⁰. *C. pneumoniae* and its constituents, such as specific antigens and even DNA, have been detected in atherosclerotic plaques and also in endothelium, smooth muscle cells, and macrophages of arterial walls with atherosclerosis, but have not been found in normal arteries. Treatment with the antibiotic azithromycin appears to have protective effects against atherosclerosis complications. It is important to note that the evidence to date does not say that *C. pneumoniae* causes

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atherosclerosis, but simply that it may enhance the disease progression in as yet unspecified ways. This kind of significant correlation will be taken into increasing account by antibiotics developers in the future, as the repercussions of infection, and therefore treatment, extend beyond the microbe itself.

As antimicrobial resistance increases into a major public health problem, the race will intensify between microbes and novel drug discovery and development efforts. Antimicrobial peptides are the starting point of multiple efforts to develop new agents that are effective against a variety of microbes but not against mammalian cells, which isn't always easy because of the similarities between the cell membranes that these agents target. There are many examples of different approaches, including protegrin lytic peptides and their analogs¹¹, and peptides derived from synthetic combinatorial libraries¹². Additional examples include the defensins described above, several peptide bacteriocins isolated from lactic acid bacteria¹³, and the ubiquicidins, which show antimicrobial activity against *Listeria monocytogenes* and *Salmonella typhimurium*, and have recently been characterized in murine macrophages¹⁴. Although these new molecules have yet to be

converted into clinical grade antibiotics, their prospects seem very promising.

In addition, specific microbial target enzymes will continue to attract attention for novel antimicrobial discovery. For example, peptide deformylase is a critical bacterial enzyme discovered almost 30 years ago, but it has not been possible to target it effectively until recently because it is an unstable enzyme¹⁵. Recent advances, however, have allowed this enzyme to be revisited as a valid target, and novel anti-bacterials, such as low-molecular-weight beta-sulfonyl- and beta-sulfinylhydroxamic acid derivatives are being developed as inhibitors with significant potential anti-bacterial activity¹⁶. Advances in deciphering the genome of a variety of microbes will further help the development of agents against them by providing a wider selection of potential targets.

Conclusions

Bacterial and fungal infections cause an enormous disease and societal burden. They have resulted in significant efforts to innovate constantly in the area of drug discovery, and in the period 1998–2000 alone, more than 70 new medicines were in various clinical stages of development. Although the issue of resistance together with the discovery and

evolution of new strains poses significant health threats worldwide, there is a constant effort to innovate around these issues, and the genomic decoding of many of these organisms will help accelerate the validation of multiple targets against which the new generations of antimicrobials are beginning to be developed.

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