

Antibiotic Development | 5

The fact that U.S. Food and Drug Administration (FDA) approved no new antibiotics in 1994 has led to fear that there are no new ideas for antibiotics or that there are insufficient financial incentives for new antibiotic development. Even the information that 13 new antibiotics are currently awaiting FDA approval, and that two-thirds of the 53 antibiotics developed by drug companies since 1960 received FDA approval after 1980 (*Modern Healthcare*, 1994) must be tempered by additional information. The 13 antibiotics awaiting approval are not “new” in terms of new mechanisms of action. They are derivatives or new applications or formulations of antibiotics already on the market.

As shown in figure 5-1 (and discussed below) several years elapse between the discovery of a chemical with antibiotic activity and its reaching the market. The scarcity or abundance of new antibiotics is dependent on many factors, some of which are described in this chapter, but some of the decisions necessary for the appearance of new antibiotics in 1995 were made years ago.

This chapter reviews general considerations in the development of new antibiotics and describes some antibiotics that are now in use and how researchers are attempting to modify them to

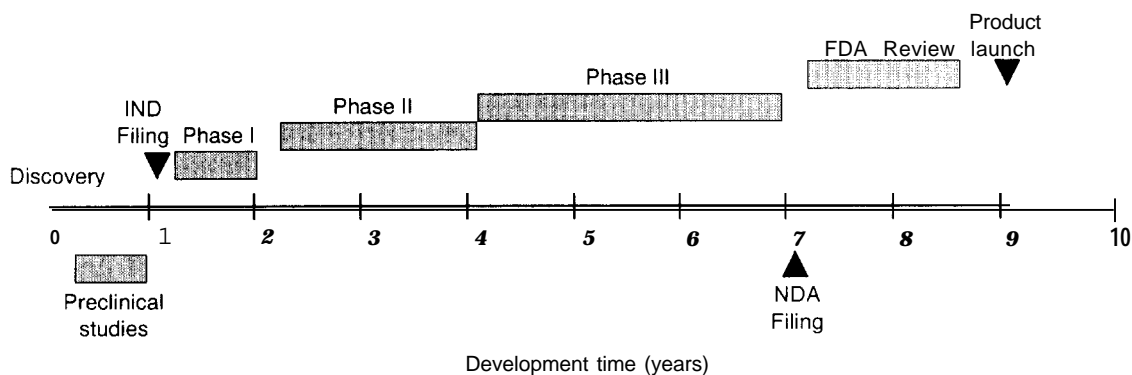
extend their usefulness. It also discusses the search for new antibiotics using new chemical and molecular biology knowledge and techniques as well as the search for new antibiotics in biological materials not formerly examined. It also reviews briefly some aspects of drug development and approval (those issues are covered in greater depth in OTA’s 1993 report *Pharmaceutical R&D: Risks, Costs, and Rewards*).

DESIGNING NEW ANTIBIOTICS

Development of almost any drug is a matter of science and serendipity, and antibiotics are no different. Traditional methods, like screening of soil and biological samples—“panning” for compounds—have been partly replaced by computerized modeling, recombinant DNA technologies, new methods of chemical synthesis, and other advances (Levy 1992, p. 39). Nevertheless, looking for antibiotic activity in biological materials as exotic as frogs and the silk glands of moths is a part of current research.

No matter how chemicals with antibiotic properties are derived, they must still be evaluated in the microbiology laboratory, laboratory animals, and ultimately, humans. “Preclinical studies” are tests for efficacy and toxicity in laboratory animals, and “phases I, II, and III” are

FIGURE 5-1: Approximate Timeline for the Development of a New Antibiotic



NOTE: IND = Investigational new drug; NDA = new drug application.

SOURCE: Gootz, 1990.

clinical trials in humans, with phase I being trials to establish the safety of the drug and phases II and III to establish efficacy (figure 5-1).

The creation of a new idea is the critical starting point for much research, and probably every company tries methods to encourage creativity. Once an idea is developed, the company can speed up the pre-clinical research by pouring additional resources into it, increasing the numbers of scientists committed to the project, and providing more and better equipment.

■ Toxicity

Toxicity tests in animals and humans identify what side effects may occur; but the occurrence of such effects does not mean that the developer will drop the drug or that FDA will not approve it. It does mean that the toxicity will be weighed against the benefits in deciding what uses will be sought by the developer and what uses will be permitted by FDA. For instance, greater toxicity would be acceptable in an antibiotic to treat vancomycin-resistant *Enterococcus* (VRE), for which there are few or no available antibiotics, than in one intended for routine use against respiratory infections for which there are many available antibiotics.

Most antibiotics inhibit or kill bacteria while remaining relatively non-toxic to humans

because of differences between the structures and metabolic characteristics of bacterial and animal cells (see chapter 2). One major difference is the presence of the cell wall that surrounds the plasma membrane in bacteria. Cell walls are missing from animal cells, and many antibiotics kill bacteria by interfering with cell wall synthesis.

Despite their generally low toxicity, antibiotics can cause allergic reactions and other side effects. Penicillin can be allergenic, and vancomycin can cause hearing loss and kidney damage. Many promising new compounds that inhibit or kill bacteria in the test tube are not useful as drugs because of allergenic or other toxic side effects.

■ Efficacy

The Infectious Disease Society of America, a professional medical organization, under contract to FDA, developed guidelines for clinical trials that outline the minimal acceptable information to be submitted to FDA. Because antibiotics are available for the treatment of almost all bacterial diseases, it is unethical to test a new antibiotic by comparison with a placebo. Instead, one half of the patient population is given the standard antibiotic treatment, and the other half is given the new antibiotic. This comparison of

efficacies necessarily requires more patients than if the antibiotic were evaluated against no treatment or a placebo. If the new antibiotic is equal to or more effective in treating the disease than the standard treatment, FDA will approve its use. Even if it is not quite so effective, FDA will approve the new antibiotic if it has lower toxicity than the standard to which it is compared.

FDA will consider the results of foreign trials when the makeup of the test population in the foreign country approximates the U.S. population, the distribution of antibiotic-resistant bacteria in the foreign country is about the same as in the United States, and the disease is caused by the same bacteria in the other country and in the United States. The Office of Technology Assessment (OTA) did not investigate how often, if ever, FDA has decided not to consider a foreign trial, but there appears to be some room for disagreement between a manufacturer and FDA about how closely the foreign conditions approach those in the United States. On the other hand, an FDA official stated that multi-national companies have done one trial in a European country and one in the United States, combined the results, and obtained approval for the new drug in both countries, and that FDA will make approval decisions based solely on foreign studies (FDA, 1995).

The time necessary for FDA review has decreased in the last few years. In the early 1990s, FDA took an average of 25 months to act on a New Drug Application (NDA). Through “The Prescription Drug User Fee Act of 1992 (P.L. 102-571),” Congress increased funds for FDA to staff and run the review process. That law requires that each manufacturer pay an annual fee based on the number of the company’s drugs that are in use and the number of its manufacturing plants. In addition, manufacturers may pay a fee at the time of submission of an NDA. These fees are used to hire additional reviewers at FDA to speed up the review process, not to speed up the review of the particular NDA. Since the Act’s implementation, the average time for FDA drug approval in 1994 had dropped to 19 months.

The time line on figure 5-1 is an approximation; some drugs move more quickly through the trials, and some move more slowly. More frequently, a drug fails some critical test and must be abandoned. Such hurdles have always been present. Scaling-up production of a drug from the small quantities needed for initial testing to the large quantities needed for phase III clinical testing and manufacture can also be significant hurdles in getting a new drug to market (box 5-1).

FDA regulations allow for an accelerated review process when a candidate drug is a possible treatment for a life-threatening disease (such as an antibiotic for use against VRE). FDA officials can meet with the drug sponsors at the end of the phase I trial and design a phase II trial that will be sufficient to make a decision about approval of the drug. Moreover, drugs that are entered into accelerated review go to the “head of the line” at all stages of the review process.

A company seeking approval to market an antibiotic for use against diseases caused by antibiotic-resistant bacteria must demonstrate efficacy against particular bacteria-disease combinations. For instance, an antibiotic effective

BOX 5-1: Quantities of Drugs Needed at Different Stages of Development

0.01 g-10 g: Discovery (performs initial bench-level discovery, creation, or isolation of the new entity).

10 g-100 g: Chemical process research (identifies possible ways to make the entity on a larger scale).

1,000 g-100,000 g: Chemical process development (a collaboration between research and development programs (R&D) and manufacturing; scales up manufacture for toxicology and clinical research; makes the process useful for manufacturing).

100,000 g-1,000,000 g: Manufacture (scales up once again to make the entity in commercial amounts).

SOURCE: Bristol-Meyers Squibb, 1995.

against VRE in laboratory tests would have to be shown effective against VRE-caused endocarditis to be marketed for that use, and it would also have to be shown effective against VRE-caused bacteremia to be marketed for use against that indication. This raises problems because the number of such diseases is relatively small, making it difficult to obtain as many cases for a clinical trial as are commonly required. According to a U.S. FDA official, however, the agency could adjust the number of cases required for the trial of an antibiotic for use against particular diseases caused by particular antibiotic-resistant bacteria.

ANTIBIOTICS IN CURRENT CLINICAL USE

Table 5-1 is a listing of the actions of antibiotics, a sampling of antibiotics that display those actions, and the development or use status of the antibiotics. Currently, research and development efforts are in place that seek to improve currently used antibiotics.

■ Sulfonamides¹

The sulfonamides are synthetic, not of natural origin, and are properly called “antimicrobials” and not antibiotics. They are included here because they were the first antibacterial drugs that were not overtly toxic to humans, and their chemical modifications foreshadowed much of the work to improve natural antibiotics.

In 1936, a year after German researchers reported that Prontosil (the first sulfonamide) cured bacterial diseases, British researchers set out to improve upon its usefulness (Colebrook and Kenny, 1936). The British researchers’ plans were based on the results of studies by French investigators, who noted that the antibacterial effects of compounds like Prontosil were lost when some parts of the chemical were removed, but that removal of other substituents had no effect on antibacterial properties in mice. They concluded that a metabolic product, para-aminobenzenesulfonamide, was responsible for

the activity of Prontosil, and that the full structure of the parent compound was not necessary for bacterial killing. The involvement of researchers from three different countries in this research points to the international flavor of antibiotic research from its very beginning.

The British researchers tested a dozen sulfonamide analogues for antibiotic activity, but, practically, their most important discovery was that para-aminobenzenesulfonamide was well tolerated when injected subcutaneously and that it could be given orally. Prontosil, on the other hand, was biologically active only when given by injection (Buttle et al., 1936; Mandell and Sande, 1990). This finding was another harbinger of research directions with antibiotics; low toxicity and ease of administration increased the acceptability of an antibiotic and reduced the medical care costs associated with it.

If bacteria were passive when faced with antibacterials, the sulfonamides would have remained potent therapy. Bacteria are not passive. Through mutation and selection, they become resistant to antibiotics. This sets up the struggle between antibiotic developers and bacteria—the biological war.

Sulfonamides inhibit one step in the bacterial synthesis of folic acid. Humans and other mammals do not synthesize folic acid; they obtain it from food. Hence, sulfonamides have no effect on mammalian cells. When, by the early 1960s, many bacteria had developed resistance to the sulfonamides, researchers postulated that the antimicrobial action of sulfonamides might be augmented by the co-administration of trimethoprim, which blocks another step in folic acid synthesis (Bushby and Hitchings, 1968). Blocking two sequential enzymes on the bacterial biosynthetic pathway of a vital nutrient (such as folic acid) was expected to act synergistically. The reasoning proved correct, and bacteria resistant to sulfonamide were inhibited by the sulfonamide/trimethoprim formulation. The preparation is still used widely.

¹ NOTE: An OTA mention of products and companies does not imply any endorsement, and products and companies are included only as examples.

Action	Family/Class	Example(s)	Source	Status
Antibiotics that inhibit cell wall synthesis	Beta-lactams			
	Natural penicillins	Penicillin G	<i>Penicillin notatum</i>	Used since 1940s
	Semi-synthetic penicillins	Methicillin Piperacillin	Semi-synthetic penicillin derivatives	In use since 1960s; among the most widely prescribed antimicrobials
	Cephalosporins	Cephalexin	<i>C. acremonium</i>	Widely used class of antibiotics
	Carbapenems	Imipenem	Derived from thienamycin, a compound produced by <i>Streptomyces catleya</i>	In use; wide spectrum (active against many species of bacteria including cephalosporin-resistant Enterobacteriaceae)
	Monobactams	Aztreonam	Derived from a compound produced by <i>Chromobacterium violaceum</i>	In use; tolerated by patients with penicillin allergies; spectrum limited to aerobic gram-negative bacteria
	Penicillinase inhibitors	Clavulanate potassium (used clinically with amoxicillin or ticarcillin) Sulbactam (used with ampicillin) Tazobactam sodium (used clinically with piperacillin)	<i>Streptomyces clavuligerus</i>	Used since 1970s; clavulanate combinations used for wide range of disorders Similar to amoxicillin/clavulanate
	Vancomycin	Vancomycin	Semi-synthetic penicillin derivative Semi-synthetic penicillin derivative	Tazobactam/piperacillin effective against intra-abdominal infections
	Teicoplanin	Teicoplanin	<i>Strep. orientalis</i> <i>Actinoplanes teichomyeticus</i>	Introduced in 1956; used against staphylococcal and enterococcal infections Experimental in the U.S., available for compassionate use
	Vancomycin derivatives with catalytic activity		Semi-synthetic	Experimental

(continued)

TABLE 5-1: Antibiotics in Use and Under Development (Cont'd.)

Action	Family/Class	Example(s)	Source	Status	
Antibiotics that increase membrane permeability	Peptides				
	Bactericidal/Permeability Increasing Protein (BPI)		Mammalian cells	Experimental	
	Magainins		African clawed frog	Experimental	
	Cecropins		Silk moth, other insects, mammals	Experimental	
	Defencins		Mammalian cells	Experimental	
	Steroids		Dogfish sharks	Experimental	
	Metabolic interference	Sulfonamides	Sulfamethoxazole	Azo dyes	In use since 1930s; first antimicrobial agent used in man
		Trimethoprim	Trimethoprim	Synthetic	Synthesized in 1968, commonly used together with sulfonamides
	Protein synthesis inhibitors	Aminoglycosides	Streptomycin	<i>Streptomyces griseus</i>	In use since 1940s; important class of antibiotics
			Kanamycin	<i>Streptomyces kanamyceticus</i>	
Tobramycin			<i>Streptomyces tenebrarius</i>		
Gentamicin			<i>Micromonospora purpurea</i> and <i>echinospora</i>		
Fusidanes	Fucidin		Sodium salt of fusidic acid, derived from the fungus <i>Fusidium coccineum</i>	In clinical use since 1962, but not available in US (except through compassionate release); active against some strains of methicillin-resistant <i>Staph. aureus</i> (MRSA)	
Tetracyclines		Chlortetracycline	<i>Streptomyces aureofaciens</i>	First introduced in 1948, found by screening soil samples for antibacterial activity	
		Oxytetracycline	<i>Streptomyces rimosus</i>		
		Minocycline	Semi-synthetic derivative		
		Doxycycline	Semi-synthetic derivative		

(continued)

TABLE 5-1: Antibiotics in Use and Under Development (Cont'd.)

Action	Family/Class	Example(s)	Source	Status
	Chloramphenicol	Chloramphenicol	<i>Streptomyces venezuelae</i>	First introduced in 1949, currently second line antibiotic because of side effect of aplastic anemia
	Macrolides Azalides	Erythromycin Azithromycin	<i>Streptomyces erythreus</i> semi-synthetic derivative of erythromycin	Discovered in 1952 Usage began in 1992
	Lincosamides	Clindamycin	Semi-synthetic derivative of lincomycin derived from <i>Streptomyces lincolnensis</i>	Available since the mid 1960s; active against aerobic bacteria
	Mupirocin	Mupirocin	<i>Pseudomonas fluorescens</i>	Introduced in the mid 1980s; topical antibiotic
Interference with RNA synthesis	Rifamycins	Rifampin	<i>Streptomyces mediterranei</i>	First isolated in 1957, important tuberculosis drug
Toxic effect through DNA binding	Nitroimidazoles	Metronidazole	Synthetic	Introduced in 1959, active against anaerobes such as <i>B. fragilis</i>
Block DNA replication or RNA transcription	Antisense nucleotides		Laboratory	Experimental
Interfere with DNA replication	Quinolones Fluoroquinolones	Nalidixic acid Ciprofloxacin, Ofloxacin	Semi-synthetic	First identified in 1962 Usage began in 1980s; some of the most widely used antibiotics
Anti-tuberculosis drugs		Isoniazid (INH) Pyrazinamide (PZA) Ethambutanol	Synthetic Synthetic Synthetic	Shown to be effective in 1952 Important tuberculosis drug since 1980 Important tuberculosis drug since 1974
Decoy receptors	Carbohydrates		Laboratory	Experimental

SOURCE: OTA, compiled from G.L. Mandell, J.E. Bennett and R. Dolin (eds.) *Mandell, Douglas & Bennett's Principles & Practice of Infectious Diseases*. Churchill Livingstone: New York, NY, 1995; and A.G. Gilman, T.W. Rall, A.S. Nies, et al. (eds.) *The Pharmacological Basis of Therapeutics*. Pergamon Press: New York, NY, 1990.

■ Penicillins and Clavulanic Acid

Penicillin was the first true antibiotic. Its action involves binding to penicillin-binding proteins which are enzymes necessary for the synthesis of the bacterial cell wall, inhibiting those enzymes, which leads to the death of the cell, and uncovering or activating other enzymes that cause the bacterial cell to burst. Shortly after penicillin's introduction, resistant micro-organisms began to appear. By the mid-1940s, the enzyme penicillinase or β -lactamase, which degrades penicillin so that it has no effect on bacteria, had been isolated from a bacterium that was not specifically identified, and soon after, scientists found it was present in other bacteria such as *Staphylococcus aureus*. As early as 1948, 50 percent of *S. aureus* in hospitals were resistant to penicillin, rising to 80 percent in 1957 (Gootz, 1990).

Semi-synthetic Penicillins

Semi-synthetic penicillins—methicillin, nafcillin, and cloxacillin—are the product of searches for penicillins that could escape the action of penicillinase. They were made possible by the large-scale production of a part of the penicillin molecule, called 6-aminopenicillanic acid, to which chemists could add different chemical substitutions. These penicillins resist the degrading action of penicillinases, and they found immediate application in treating some penicillin-resistant bacteria. The extremely low toxicity of penicillin has fueled efforts to continue development of this antibiotic.

Penicillinase Inhibitors

Molds of the genus *Streptomyces* produce chemical compounds that “suicidally” tie up penicillinases. When administered with penicillins, the inhibitors bind the penicillinases, leaving the unbound penicillin free to kill bacteria (Reading and Cole, 1977). By the early 1970s, olivanic acid, produced by *Streptomyces olivaceus*, had proved a successful penicillinase inhibitor, and it

was used with ampicillin and amoxicillin in treating *S. aureus* and *Klebsiella pneumoniae*, both Gram-positive bacteria, but it was unable to penetrate the Gram-negative bacterial cell wall.² Clavulanic acid, from *Streptomyces clavuligerus*, proved more effective than the olivanic acids, and it extended the spectrum of penicillinase activity to Gram-negative bacteria. Amoxicillin/clavulanic acid is the mainstay of treatment for otitis media in children caused by *Hemophilus influenzae* and *Branhamella catarrhalis*.

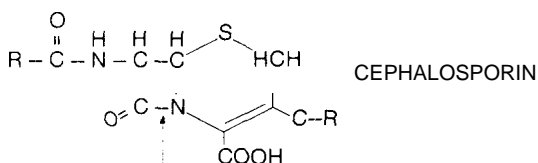
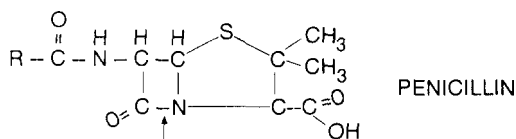
The success of the penicillin/clavulanic acid combination suggested that semi-synthetic penicillins—while promising as single-agent therapy—might not be the only solution to the problem of antibiotic resistance. More importantly, perhaps, the notion of identifying and attacking a specific bacterial target responsible for resistance (in this case, penicillinases) became a principle of antibiotic research.

■ Other Beta-Lactam Antibiotics

The cephalosporins (see figure 5-2) share a similar chemical structure (the beta-lactam ring) and similar mechanisms of action (inhibition of synthesis of the bacterial cell wall) with penicillin. Cephalosporin antibiotics were first isolated from the organism *Cephalosporium acremonium* in 1948 from the sea near a sewer outlet off the Sardinian coast (reviewed in Mandell and Sande, 1990). Chemists have modified the structure of the antibiotics and produced semisynthetic antibiotics with increased antimicrobial activity. The resulting so-called “third generation” cephalosporins, including ceftriaxone and ceftazidime, are widely used. Imipenem, yet another β -lactam antibiotic, is a chemical derivative of a compound first isolated from the organism *Streptomyces catleya*; it is the broadest-spectrum antibiotic commercially available (see Emori and Gaynes, 1993).

² Some bacteria take up a stain, called the Gram stain, and some do not. The difference depends on the structure of the cell wall in the two kinds of bacteria, and the permeability of the two kinds of bacteria differ as a result of the difference in the cell walls.

FIGURE 5-2: Core structure of penicillins and cephalosporins



NOTE The R groups specify the particular antibiotic; arrows indicate the bond broken during function and during inactivation by β -lactamases

SOURCE: Frankel, 1995

■ Vancomycin

Vancomycin is a naturally occurring glycopeptide [a protein (peptide) molecule with attached sugars (glyco-)] antibiotic that blocks synthesis of the bacterial cell wall. However, vancomycin inhibits the synthesis of the bacterial cell wall by binding to the peptidoglycan (cell-wall) precursor, a very different mechanism from that used by the penicillins, and it does not have the beta-lactam ring structure of penicillins. Vancomycin has become clinically important because it is sometimes the only drug that can be used to treat MRSA (methicillin-resistant *S. aureus*) infections, an increasingly prevalent pathogen in hospitals (see chapter 4).

Teicoplanin, a related glycopeptide antibiotic, is widely used in Europe, but is available only as an investigational drug in the United States. It is potentially an effective alternative to vancomycin; it requires less frequent dosing, and it is less toxic. It is not likely to be successful in treating bacteria resistant to vancomycin because bacteria resistant to vancomycin are usually resistant to teicoplanin as well (Fekety, 1995).

ANTIBIOTICS THAT INHIBIT OR BLOCK DNA REPLICATION OR PROTEIN SYNTHESIS

While the general features of DNA replication and protein synthesis are common to bacterial and animal cells, subtle differences exist, and some antibiotics inhibit bacterial DNA replication or protein synthesis without harming the analogous processes in animal cells.

■ DNA Synthesis—Ciprofloxacin, Other Quinolones, and Fluoroquinolones

The synthetic antibiotic ciprofloxacin has become one of the most widely prescribed antibiotics since its introduction in 1987 (Frieden and Mangi, 1990). Ciprofloxacin, other quinolones, and fluoroquinolones work by inhibiting the action of a bacterial enzyme necessary for DNA synthesis (“DNA gyrase”). Ciprofloxacin is derived from nalidixic acid, an antibiotic discovered 15 years earlier, but never widely used. Therefore, ciprofloxacin had a substantially “new” mechanism of action. It is not known whether quinolones bind to animal cell DNA gyrase, but these antibiotics are relatively nontoxic.

Although resistance to ciprofloxacin occurs at rates 100- to 1,000-times slower than resistance to nalidixic acid (Hooper and Wolfson, 1989), many strains of bacteria became resistant to ciprofloxacin over a period of three years (see table 5-2). This experience shows that resistance can develop rapidly even when the mechanism of action is substantially “new.”

Ciprofloxacin and other quinolones are popular because they are effective against bacteria that have developed resistance to other antibiotics and because they can be taken orally rather than requiring parenteral administration (through injection or intravenously). Oral ciprofloxacin is equally or more effective than many parenteral antibiotics, and oral administration costs less, and can reduce or eliminate hospital stays.

TABLE 5-2: Resistance to Ciprofloxacin, 1988-1990

Organism	% Resistant		
	1988	1989	1990
<i>Acinetobacter anitratus</i>	0	34	40
Enterococcus (various species)	8	25	35
Methicillin-resistant <i>Staphylococcus aureus</i>	—	—	85
<i>Pseudomonas aeruginosa</i>	9	35	45
<i>Staphylococcus aureus</i>	6	10	20

SOURCE: Adapted from Husain, 1991.

■ RNA Synthesis—Rifampin

The first step in protein synthesis is the transcription of information in DNA into RNA (see chapter 2). Rifampin binds to bacterial RNA polymerase, inhibits bacterial RNA synthesis, and does not bind to animal cell RNA polymerase. Its principal use is in the treatment of tuberculosis (TB).

■ Protein Synthesis—Streptomycin and Other Aminoglycosides

The inactivity of penicillin G against Gram-negative bacteria led scientists to search for antibiotics with activity against those organisms. The 1944 discovery of streptomycin from a strain of the bacterium *Streptomyces griseus* was followed by discovery of related compounds such as neomycin, kanamycin, and gentamicin from other bacteria in later years. This family of antibiotics, the aminoglycosides, inhibits bacterial protein synthesis by binding to the small subunit of the bacterial ribosome, which differs from the corresponding subunit of the animal ribosome (see chapter 2). Aminoglycoside inhibition of protein synthesis is irreversible and lethal to the bacteria.

Other antibiotics that inhibit protein synthesis are the macrolides, such as erythromycin, clindamycin, and chloramphenicol, which bind to the large subunit of the bacterial ribosome. They inhibit bacterial growth, but they do not kill the bacteria. (Chloramphenicol is now seldom used

in medicine because of adverse side effects.) Tetracyclines, which are widely used in medicine, veterinary medicine, and animal husbandry (see chapter 7), are also inhibitors of protein synthesis with broad activity spectra. They, like chloramphenicol, are bacteriostatic rather than bactericidal.

DEVELOPMENT OF NEW ANTIBIOTICS FROM OLD

The development of semisynthetic penicillins and ciprofloxacin from nalidixic acid has demonstrated the usefulness of modifying existing antibiotics so they are active against resistant strains of bacteria. Modifications can reduce toxicity, make the antibiotic resistant to degrading enzymes, or improve penetration into bacterial cells.

Frankel (1995) contacted a number of large, established pharmaceutical companies and a number of smaller, startup or beyond, biotechnology firms and asked about their research and development programs in antibiotics. The section that follows is based on his report. It is an overview and should not be taken as exhaustive because not all firms were contacted, and not all firms were willing to discuss their research and development programs in antibiotics.

■ Streptogramins

Rhone-Poulenc Rorer (1995) announced that one of its antibiotics, now in phase III clinical trials, is effective against antibiotic-resistant bacteria, including some strains of VRE (*Journal of Antimicrobial Chemotherapy*, 1992). The antibiotic is currently available from the company in an FDA-reviewed program, and it is usually shipped within 24 hours of request.

This drug is a combination of two semisynthetic derivatives of streptogramin, an antibiotic from *Streptomyces pristinaespiralis*. One such antibiotic, pristinamycin, has been available in Europe for many years as an oral anti-staphylococcal antibiotic. It inhibits protein synthesis by affecting ribosome function, but was never widely used, partially because it cannot be

made in an injectable form due to low water solubility. The new derivatives of pristinamycin—quinupristin/dalfopristin (used in combination)—are injectable.

■ Tetracycline Analogs

The first clinically useful tetracycline, chlortetracycline, was introduced in 1948. It was isolated from the micro-organism *Streptomyces aureofaciens* and was discovered after screening samples of Missouri farm soil (Levy, 1981). Following this discovery, other researchers identified more tetracyclines by further screening of soil micro-organisms or by synthesis in laboratories. As with the penicillins, manipulation of the tetracycline molecule has brought different spectrums and properties of antibiotic activity. While all of the tetracyclines now used in the United States are generally considered broad-spectrum agents, bacterial resistance to this family of agents is widespread.

“Active efflux,” which transports tetracyclines out of the bacteria, is a major mechanism of bacterial resistance. Since its description (Levy, 1981), it has also been shown to be a mechanism of resistance to several other antibiotics including chloramphenicol, fluoroquinolones, erythromycin, and β -lactams (Nikaido, 1994), and it is present in both Gram-positive and Gram-negative bacteria. Nikaido (1994) reviews evidence about permeability barriers to antibiotic entry into bacteria and active efflux, which can bestow resistance to many antibiotics, and states that, “It will be a major challenge for the pharmaceutical industry to produce compounds that are able to overcome mechanisms of this type.”

Such research is underway. Nelson et al. (1993) tested 30 tetracycline analogues and identified two chemical substitutions that block active efflux. Subsequently, Nelson et al. (1994) determined the part of the tetracycline molecule that is essential for its antibacterial activity and which substitutions inhibit efflux. This information may increase the usefulness of tetracycline, an old antibiotic.

Minocycline, the last tetracycline to reach the market, was introduced in the 1970s, and it was the starting point for researchers who took another look at the tetracyclines in the late 1980s. This new tetracycline research program, a multidisciplinary effort by chemists, molecular biologists, biochemists and microbiologists, has produced the semisynthetic glycycline antibiotics. These are active against both Gram-positive and Gram-negative bacteria and evade resistance mediated by six of the known mechanisms of tetracycline resistance. Researchers are continuing to modify the glycyclines to optimize their antibacterial properties (Bergeron et al., 1994; Sum, Lee, Peterson et al., 1994), and have recently introduced modifications that may lead to the production of “later-generation” glycyclines (Sum, Lee, and Tally, 1994). When and whether they will reach clinical application is unknown.

■ Dual-Action Cephalosporins

One approach to evading bacterial resistance to cephalosporins or quinolones is to chemically couple the two to produce conjugates that have a dual mechanism of action (hence the name “dual-action” cephalosporins), reflecting the actions of both the β -lactam, cephalosporin, and quinolone components.

The first of these conjugates, as reported by Georgopapadakou et al. (1989), was found to act initially as a cephalosporin by binding to appropriate penicillin-binding proteins, and then to inhibit DNA replication, as would be expected from the quinolone function. Some conjugates appeared to act primarily as cephalosporins, while others acted primarily as quinolones (Georgopapadakou and Bertasso, 1993). The pharmaceutical company that sponsored Georgopapadakou’s work is no longer supporting research in dual-action cephalosporins, but such research is reportedly continuing in at least one other company.

■ Vancomycin Research

Vancomycin is the antibiotic of last resort in some specific situations, and it is a popular one, accounting for a quarter of the budget for antibiotics in some hospitals. The appearance of some strains of VRE that are resistant to all antibiotics³ leaves physicians with no currently approved antibiotic treatment for infections caused by those organisms. Intravenous vancomycin is the first choice for the antibiotic treatment of MRSA, and the probably inevitable appearance of vancomycin-resistant MRSA will leave physicians with no marketed antibiotic effective against that serious nosocomial infection.

Currently, however, some strains of MRSA are reportedly susceptible to other antibiotics: Novobiocin, which is available only in oral form, is active against many strains of MRSA. Minocycline (a tetracycline) has been used in successful treatment of a few cases of endocarditis caused by MRSA. Most isolates of MRSA are susceptible to fusidic acid. Used in combination with other antibiotics, fusidic acid has been part of successful therapy for a variety of MRSA-caused diseases, but the role of fusidic acid is not entirely clear. Emergence of resistance to all of these antibiotics has been reported, and it is especially a problem with fusidic acid. The problems with resistance have led to the recommendation that alternatives to vancomycin be used in combination—such as rifampin with fusidic acid—to treat MRSA (Mulligan, Murray-Leisure, Ribner et al., 1993). While these alternatives to vancomycin exist, they are less than the first choice for treatment of MRSA.

Like penicillin and other antibiotics before it, vancomycin is a starting compound in efforts to produce new and more effective antibiotics.

Semisynthetic Vancomycin

Eli Lilly and Company (1995) has prepared a semisynthetic vancomycin (LY333328) specifi-

cally for use against vancomycin-resistant organisms. The drug has demonstrated activity against VRE in animal tests and against MRSA and penicillin-resistant *Strep. pneumoniae* in in vitro tests. According to a company spokesperson, more animal tests of safety and efficacy are required, and, if they are successful, human trials may begin in 1996. This new compound is the product of research centered on development of antibiotics for use against vancomycin-resistant organisms.

Catalytic Antibiotics

Shi and Griffin (1993) discovered that vancomycin has a catalytic (chemical-degrading action) activity, and they are chemically altering vancomycin to develop a molecule that will not only bind to the cell-wall precursor and inhibit cell-wall synthesis, the normal activity of vancomycin, but destroy the precursor as well. If this is achieved, it should increase the potency of vancomycin; the catalytic antibiotic should be able to move to another cell-wall precursor after destroying the first, and so on. Griffin (1994) is also seeking to alter the vancomycin molecule so that it regains its binding affinity to the altered cell-wall precursors that are present in vancomycin-resistant bacteria. Once affinity is restored, the antibiotic can bind to the cell wall precursor, inhibit the synthesis of the wall, and kill the bacteria. If researchers develop the catalytic function so that it destroys the cell-wall precursor, that activity could be added.

■ The Macrolides

The macrolide antibiotics inhibit protein synthesis. Erythromycin, the most commonly used member of the class, is effective against a broad range of Gram-positive and Gram-negative bacteria, and is available for oral, intravenous, and topical uses. While resistance has been noted in the United States, it is more common in other

³ Not all vancomycin-resistant enterococcus are resistant to all antibiotics. *Enterococcus faecalis* remains susceptible to ampicillin, as do some strains of *E. faecium*.

countries, and the level of resistance appears related to the level of use (Steigbigel, 1995).

Azithromycin, a closely related molecule, is now being marketed with advertised advantages in being effective against more strains of bacteria than erythromycin, but it is being marketed on the basis of other positive attributes as well. Because it persists in human white blood cells for a few days (rather than a few hours as with some other antibiotics), two tablets of azithromycin on the first day of treatment and one tablet a day for four more days is sufficient for most applications (Pfizer, Inc., 1993). The convenience of this schedule is contrasted with those for other antibiotics that require three or four daily doses for up to 10 days. According to studies referenced in the advertising literature (Pfizer, Inc., 1993), compliance is better, there are fewer side effects, and patient costs are lower. This example illuminates some of the factors, including convenience and cost, as well as effectiveness, that go into marketing of antibiotics.

NEW RESEARCH TOOLS

New techniques in chemistry and molecular biology have immediate application to research and development of antibiotics. Box 5-2 discusses some of those techniques.

ANTIBIOTICS FROM NEW SOURCES

In addition to using new laboratory tools, antibiotic researchers are also exploring new biological sources for antibiotic activities. Unlike the traditional searches that have looked at products from micro-organisms, some current ones are looking at materials from humans and other animals.

■ Carbohydrates

Carbohydrates called oligosaccharides [“oligo-” a few, “saccharides” sugars] (OS), are ubiquitous on the surface of mammalian cells, and bacteria and viruses adhere to host cell OS as the first step in the process of recognition, adhesion, and infection (Rosenstein et al., 1988). Individual OS

are structurally specific for different organisms, and microbial adherence has been referred to as a “lock and key” phenomenon, in which only certain keys (microbial proteins, called “lectins” or “adhesions”) “fit” into specific locks (host-cell OS receptors).

Until recently, the complexity of OS structure and the resulting inability to synthesize sufficient OS at reasonable cost hindered OS drug design. The simplest OS—a disaccharide that is composed of only two sugars—can take any of 20 different forms. The problem increases with size; there are 35,560 possible ways to arrange four sugars into tetrasaccharides. In comparison, four amino acids can create only 24 distinct tetrapeptides (Hughes, 1994). These complexities contributed to the formerly high costs that ranged up to \$2 million per gram of OS. New techniques have lowered the cost of some OS by 10,000 times to \$200 per gram, and OS drug design has accelerated (George, 1994; Glaser, 1994) with applications in treating bacterial diseases, including ulcers.

The bacteria *Helicobacter pylori* causes gastric and duodenal ulcers, and the usual treatment eradicates it and prevents the reappearance of ulcers with a success rate of 70 to above 90 percent. Resistance of *H. pylori* to antibiotics used in the usual therapy is a factor in lower treatment success rates.

Neose Pharmaceuticals (Roth, 1995) has perfected the synthesis of the OS to which *H. pylori* binds, and animal studies have shown that administration of the OS competes with the *H. pylori* binding sites in the digestive tract, causing the *H. pylori* to release from those sites with the bacteria then being eliminated from the body. The OS is identical to an OS found in mothers’ milk, and it has extremely low toxicity in animal tests. Phase I clinical trials for toxicity were underway in March 1995.

Up to 80 percent of all hospital-acquired bacterial pneumonias are caused by one of six bacterial species. According to Roth (1995), all six of those bacterial species bind to the same OS, which opens the possibility of treating those infections with a soluble form of the OS. Another

BOX 5-2: Some New Methods for Research in Antibiotics

Structure-Based Drug Design

Traditionally, that is, for 50 or so years, scientists have discovered new antibiotics by screening thousands of natural, synthetic, or semi-synthetic compounds for antimicrobial properties, analyzing the structures of active ones, and modifying active compounds for greater utility. Scientists have discovered many antibiotics serendipitously, usually an expensive and time-consuming process and always an unpredictable one, and many have been discovered and tested in laboratories and in humans long before researchers understood their mechanism of action.

Structure-based drug design (SBDD), on the other hand, begins with an understanding—or physical model—of the drug mechanism, especially the ligand:receptor interaction (Kuntz, 1992). This interaction occurs at the “active site” where the “ligand,” in this case the antibiotic, binds to some structure, the “receptor” (or “target”) in the bacteria. SBDD employs newer research tools, such as X-ray crystallography, nuclear magnetic resonance spectroscopy, and supercomputer combinatorial chemistry to design new compounds that will bind more tightly to the active site (Knox, 1993; Fan et al., 1994; Balbes et al., 1994; Boyd and Milosevich, 1993).

Targeted Replacement of Segments of Antibiotic Proteins

The bacterium *Bacillus subtilis* produces an antibiotic called surfactin. Stachelhaus, Schneider, and Marahiel (1995) isolated the DNA segments that code for surfactin from *B. subtilis*, and DNA segments from another bacterium, *Bacillus brevis*, and from the fungus, *Penicillium chrysogenum*. Using recombinant DNA techniques, they constructed hybrid *B. subtilis*-*B. brevis* and hybrid *B. subtilis*-*P. chrysogenum* DNA molecules that they reinserted into *B. subtilis*. Hybrid DNAs of the first kind coded for recombinant proteins in which some segments of the protein came from *B. subtilis* and some from *B. brevis* hybrids of the second kind resulted in the production of proteins with some segments from *B. subtilis* and others from *P. chrysogenum*.

This experiment demonstrates a method to construct hybrid molecules, and it may have an application to the development of new antibiotics. Because the DNA segments can come from unrelated organisms, or even from chemical synthesis, the structure of the recombinant DNA, and the resulting protein, can be specified. Better understanding of ligand:receptor interactions may provide the information for the construction of recombinant DNA molecules that will code for new antibiotics.

“Unnatural” natural products

The bacterium *Streptomyces coelicolor* produces the antibiotics tetracyclines and erythromycin, which are members of a class of compounds called polyketides. Scientists have discovered more than 10,000 polyketides, including many useful drugs, but the percentage of medically useful compounds in the total number of discovered natural polyketides has decreased in recent years (Lipkin, 1995). McDaniel et al., (1995) have categorized the enzymes involved in the synthesis of polyketides and constructed plasmids that contain genes for those enzymes. When expressed in *S. coelicolor*, the genes on the plasmids resulted in the synthesis of new polyketides.

Based on their understanding of the activities of the enzymes, McDaniel et al., (1995) devised rules for the bioengineered synthesis of polyketides, and they suggested that chemists will be able to generate bioengineered (unnatural) products that will be as diverse as the thousands of polyketides already seen in nature. The expectation is that medically useful compounds will be generated.

(continued)

BOX 5-2: Some New Methods for Research in Antibiotics (Cont'd.)

In vivo Expression Technology

Traditional research has sought microbial virulence factors by culturing and growing microbes in the laboratory and then examining the products of bacterial growth that are present in the culture broth. Well-known examples of such products are diphtheria and cholera toxins which were used for vaccine development.

Mekalanos and his colleagues (Mahan, Slauch, and Mekalanos, 1993) acted on the idea that bacteria are “Trojan Horses,” hiding their virulence factors and toxins until specific host signals cause them to be released. Such genes would cause the production of proteins that could be the targets for antibiotics or antigens for the production of vaccines. Mahan et al., (1993) call the technology to explore such hidden bacterial strategies “in vivo expression technology” (IVET), which has been heralded as “revolutionary” (Barinaga, 1993).

IVET may be applied to the problem of antibiotic resistance in at least two ways. First, it can identify new antimicrobial targets. Nearly half of the *Salmonella* genes detected with IVET were previously unknown. The products of these genes are potential targets for new antibiotic design. Second, IVET may guide production of new vaccines, as previously unknown products of IVET-identified genes give vaccine developers new immunogens against which humans can be inoculated.

Antibiotics Targeted Against a Bacterial Regulatory System

In bacteria, some RNA synthesis depends on a two-step regulatory system. The first component is a sensor protein in the bacterial membrane that can detect a signal in the environment, say, a sugar or other nutrient of use to the bacterium. In response, the sensor chemically adds a phosphate to itself and to another protein, the transducer. The phosphorylated transducer then activates RNA synthesis from specific sites on the DNA, and the RNA is used to direct synthesis of enzymes necessary to transport the nutrient into the bacterial cell, for its metabolism, or for some other aspect of biochemistry associated with the nutrient.

Virulence genes, as detected by IVET or other methods, are probably regulated by a two-component system, with the sensor detecting some chemical in the host animal or host cell. A substance that interferes with the regulatory system might be a useful antibiotic, and such substances have been described. The two-component regulatory system does not exist in mammalian cells, making toxic side effects from such antibiotics unlikely (Salyers and Whitt, 1994).

OS designed to lower the risk of infant infections is modeled after naturally occurring OS found in mothers’ milk (Neose Pharmaceuticals, 1994).

Microbial resistance to OS is predicted to be small because two independent genetic events would have to take place. First, the bacterium would have to mutate so that it would no longer bind to the OS; that would also make it non-infective because it could not bind to OS on cell surfaces. Only a second mutation that produced a

mechanism to bind to another molecule on the surface of the stomach cell could restore bacterial infectivity.

■ Antibiotic Peptides

Among the most widely studied of the “new” antibiotics are peptide antibiotics. Within this large group of molecules are bactericidal/permeability increasing proteins (BPI), magainins, and cecropins.⁴ Their common antimicrobial activity

⁴These agents are included here to be illustrative; this list is not inclusive. J.E. Gabay provides a short description of these and some other antimicrobial peptides as well as a useful reference list in “Vbigitous and natural antibiotics,” *Science* 264:373–374, 1994.

results from increasing bacterial permeability, and in this regard they are similar to the topical peptide antibiotic polymyxin B, produced by the bacterium *Bacillus polymyxa*. Scientists, however, know few specifics about their mechanisms of action (Gabay, 1994). New technologies that allow researchers to synthesize and screen “combinatorial libraries” consisting of tens of millions of natural and synthetic peptides (Blondelle et al., 1994) have increased the capacity to make and test candidate peptide antibiotics.

Bactericidal/Permeability Increasing Peptide

Weiss et al. (1978) reported isolation of a bactericidal protein from human and rabbit cells that appeared to cause an “almost immediate” breakdown of the bacterial permeability barrier to the entry of the antibiotic actinomycin D. While BPI was bactericidal to several strains of *E. coli* and *Salmonella typhimurium*, both Gram-negatives, it had no effect on Gram-positive bacteria or the yeast *Candida*.

Using molecular biology techniques, scientists produced a fragment of the BPI molecule (called rBPI-23) that increased bactericidal activity, including activity against penicillin-resistant strains of *Streptococcus pneumoniae* (Lambert, 1994), and enhanced the efficacy of co-administered antibiotics (Meszaros et al., 1994). Human subject testing has recently begun with another fragment (rBPI-23). When administered along with low doses of endotoxin, a toxin produced by Gram-negative bacteria, rBPI-23 blunted the adverse effects of the endotoxin, was well tolerated by the volunteers, and was not immunogenic (von der Mohlen, 1994).

Magainins

Science, like all human pursuits, has its own folklore, and the discovery of the magainins passed immediately into the legends of science. In the late 1970s, a researcher at the National Institutes of Health was studying RNA expression in the African clawed frog, *Xenopus laevis*. He noted that the frogs never developed post-operative inflammation or wound infections—

even though surgical procedures were performed under non-sterile conditions—and he wondered if “there might be a ‘sterilizing’ activity in the skin.” Zasloff (1987) isolated two closely related peptides with broad-spectrum bactericidal activity that were also active against some single-celled parasite species. He named the two peptides “magainin 1” and “magainin 2” (Hebrew for “shield”).

The magainins are short peptides that insert into the bacterial cell membrane and open up channels that lead to the death of the bacteria. Thousands of magainin analogues have been synthesized with the goal of increasing antimicrobial activity (Cuervo et al., 1988). One magainin, MSI-78, is now in phase III trials, which are expected to be completed in mid-1996. If that schedule is kept, Magainin Pharmaceuticals expects to file an NDA at the end of that year for the sale of MSI-78 as a topical antibiotic (Magainin Pharmaceuticals, 1994); however, an earlier trial of this magainin against impetigo was suspended because of disappointing results. Other magainins are undergoing toxicity tests in animals in expectation that they will find application as systemic antibiotics.

Cecropins

Cecropins are peptides from the North American silk moth, *Hyalophora cecropia*. They are similar in size to the magainins, and like the magainins, they increase bacterial permeability. Researchers have chemically combined cecropin with another natural peptide antibiotic, mellitin, derived from bee venom. The resulting product demonstrated activity against *S. aureus* and *Plasmodium falciparum* (Blondelle and Houghten, 1992). More recently, a recombinant cecropin/mellitin hybrid was shown to be bactericidal against *Pseudomonas aeruginosa*. Other antimicrobial cecropins and cecropin-like molecules have been recently isolated from the hemolymph of the silk worm *Bombyx mori*, the male reproductive tract of the fruitfly *Drosophila melanogaster*, and from the intestines of pigs.

Defensins

Defensins are broad-spectrum antimicrobial peptides isolated from mammalian cells, including epithelial cells lining the human small intestine (Blondelle and Houghten, 1992). Although similar in size to magainins and cecropins, defensins differ in chemical structure. The isolation of a related group of molecules isolated from cow airways, called “ β -defensins,” has added to the theory that defensins form a natural, primary mucosal defense against microbial pathogens and are therefore potentially powerful new antimicrobial agents (Taylor, 1993).

■ **Lactoferrin, a Substance with Antibiotic Properties from Human Milk**

Lactoferrin, the second most abundant protein in human milk, is bacteriostatic *in vitro* and in tissue culture tests against a variety of bacteria, including MRSA. Three different mechanisms contribute to the bacteriostatic activity of lactoferrin: It binds iron, thereby depriving bacteria of that essential element, it increases bacterial permeability, and it activates immunological defenses. Ward et al., (1995) recently described a method to produce human lactoferrin in the laboratory, and the product has the same antibiotic properties as the human protein. Pre-clinical studies are now under way with the laboratory-produced chemical (Ward et al., 1995; Wyatt, 1995).

Human milk has antibacterial properties, and some of those properties reside in lactoferrin. Lactoferrin is also found in other external secretions—tears, nasal secretions, saliva, and genital secretions—all of which have antibacterial properties. Those secretions have been around for millions of years and they are still effective against bacteria. Development of lactoferrin, or other substances with antibiotic activity from humans, as antibiotics might provide therapies that will not elicit resistance.

Like all the protein antibiotics, lactoferrin presents administration difficulties because they cannot be absorbed from the digestive tract, thereby eliminating oral uses. They can be used

topically, as polymyxin B, and they may find use against enteric infections and pulmonary infections, where they might be administered by aspiration.

■ **Steroid Antibiotics**

The discoverer of magainins also wondered over the rarity of infections in fetal dogfish sharks (*Squalus acanthis*), despite the fact that mother sharks flush their fallopian tubes regularly with seawater to remove fetal wastes. Moreover, he noted that the sharks rarely became infected after surgery. Using the same methodology as the one used for magainins, he and co-workers successfully isolated squalamine from shark stomach, liver, gall bladder, spleen, testes, gills, and intestine. Squalamine is a steroid compound, closely-related to cholesterol (Moore et al., 1993) and has antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi and protozoa. Testing of squalamine is now at the pre-clinical stage.

■ **“Anti-Sense” Nucleotides**

One of the more frequently proclaimed “magic bullets” against drug-resistant bacteria is “anti-sense” molecules (Stein and Cheng, 1993) that bind to critical DNA or RNA segments in the bacterial cell and disrupt their functioning. A variety of new technologies, many developed for application in the federally funded Human Genome Project, allow for simpler and more rapid DNA sequencing and have made investigations of anti-sense therapy feasible.

Like many new therapies, the oligonucleotides (ON), the segments of DNA and RNA molecules that would be used as anti-sense molecules, present many challenging problems. New technologies need to be developed for the bulk synthesis of ON and to transport ON through the body and inside bacterial cells, and methods may have to be developed to deliver the ONs to their complementary DNA or RNA target (Rahman et al., 1991). “Oligonucleotide-like” molecules will be required to circumvent the instability and rapid degradation of ON in the body, and some

such molecules have been synthesized and shown to have improved stability.

GETTING NEW ANTIBIOTICS TO MARKET

This chapter reviews some ideas for new antibiotics, and any of those ideas will require significant investments to support the research and development necessary to bring it through clinical trials and to market. In 1993, OTA (1993) comprehensively reviewed the return on investments in pharmaceutical research and development. This section contains a brief review of some of the issues related to pharmaceutical developments specifically focused on antibiotics.

Antibiotics are used for short periods of time, and representatives of some pharmaceutical companies claim that greater profit is to be made in developing drugs for chronic illnesses such as heart disease and arthritis, for which drugs may be necessary every day for years at a time. The counter-argument to that contention is that a life-saving drug with no alternative, even if used only rarely, can command a high price. Resistance limits the market life of antibiotics: As they lose some of their efficacy, they become less profitable. At the same time, antibiotic resistance opens up new markets.

Participants at OTA advisory panel meetings said that major pharmaceutical companies are not likely to mount a research and development effort for potential annual markets of less than \$100 million. They also stated that some smaller companies, generally lumped under the rubric of “biotech firms,” could do very well on a market of \$20 to \$30 million a year.

Some antibiotics, however, have generated major markets. As shown in box 5-3, a single antibiotic can account for 15 percent of a major manufacturer’s sales. Such a percentage is probably unusual, but it indicates that an antibiotic can be a major source of revenue.

A new antibiotic that overcomes resistance has a ready market. There are approximately 19,000 VRE cases yearly. If an antibiotic effective against VRE were developed, OTA assumes the company that marketed it could charge a high price because no other antibiotic is available for that use, but OTA did not try to estimate that price. There are about 60,000 MRSA cases annually, and some proportion of those are treatable only with vancomycin. For illustrative purposes, OTA assumes that all 60,000 cases are now treated with vancomycin, that the antibiotic costs \$100 per day, and that the treatment requires 10 days. That market is then \$60 million annually (60,000 cases per year × \$100 per day × 10 days of treatment per case), and the new antibiotic would be competing for that market with vancomycin.

A major company might not be interested in this market; it is well below \$100 million per year. But the new antibiotic could probably be used for many other infections, and the market could be much larger, with, most likely, earlier emergence and spread of resistance than if the antibiotic were restricted to use against MRSA.

Whatever the size of market for an antibiotic, it is expected to erode with the development of antibiotic-resistant bacteria. Control of the emergence and spread of resistance would result in a longer market life and greater sales and profits. However, the major way known to slow down resistance is to minimize the use of the antibiotic, which would have an adverse effect on sales and profits, at least in the short run. To return to the hypothetical example of an antibiotic to treat MRSA, restricting the use of the drug would prolong its effectiveness before resistance developed. That restriction would also reduce sales compared to those expected if there were unrestricted use against all respiratory infections, for example. This tradeoff is discussed further in the following section.

BOX 5-3: Patent Protection and Post-Patent Hurdles for Competitors
(News media clips)

“Generic Erosion for Ceclor?”

“When Lilly’s Ceclor (cefactor) comes off patent in the U.S. in 1992, unit sales of the antibiotic, which account for roughly 15 percent of the company’s total sales, could be eroded by 70 to 80 percent by generic competition in the first 18 months, according to Kidder, Peabody analyst James Flynn.

“This erosion will take place despite the fact that Lilly holds process patents for Ceclor which expire between 1994 and 2006, and plans to introduce a sustained-release formulation, Ceclor AF, the analyst predicts.

“Recent legal action in Japan, where Lilly has filed suit against 10 companies for alleged infringement of its cefactor patent, suggest that the company intends to defend its patents vigorously.... However, Mr. Flynn argues that Lilly’s process patents will not be recognized in a number of countries (e.g., Italy) which are likely to be used as manufacturing sites for generic companies planning to import formulations of cefactor on expiration of the product patent.

“Barr and Biocraft, which have valid cephalosporin manufacturing facilities in the U.S., may also try to ‘skirt’ Lilly’s process patents, Mr. Flynn says. Such a strategy would give these companies a ‘meaningful cost advantage’ over importing firms, he adds.

“Ceclor AF is unlikely to be introduced in the United States much before the cefactor product patent expires, Mr. Flynn says. A preferred dosing regimen is the only benefit he is aware Ceclor AF would have over generic competition. The analyst notes that Lilly’s keftabs formulation of Keflex (cefalexin) gained less than 15 percent of Keflex’ sales after the 1987 product patent expired.”

SOURCE: “Generic Erosion for Ceclor,” *Scrip World Pharmaceutical News* 1594:25, 1991.

“Ceclor Market Dominance Will Continue Past Dec. 1992 Patent Expiration, Lilly Contends: Process Protection Thru 1994

“Lilly’s dominant position in the oral antibiotic market will survive the expiration of the U.S. patent on Ceclor in December 1992, the company maintained at a meeting with financial analysts in New York on Feb. 28. Based on a process protection for cefactor and a pending NDA application for the follow-up compound loracarbef, Lilly is forcefully declaring its intention to hold its place in the oral antibiotic field....

“Asked to comment on the impact of the upcoming patent expiration on Ceclor sales, Lilly Pharmaceutical President Gene Step said the relevant questions should be what will be Lilly’s overall position in the oral antibiotic market and what is the likelihood of generic versions of cefactor reaching the market.

“‘You really have to [ask] what is our participation in the oral antibiotic market and to what extent will that be affected’ by generic cefactor or ‘by other products that we may or may not be selling’ in the future, Step said.

“Lilly is emphasizing the *de facto* protection of a difficult production process and a patent position on a late-stage intermediate... Step declared that when all factors are considered Ceclor should ‘remain a viable product for Eli Lilly beyond expiration of the patent.’

“As the company often has been pointing out recently, Step told the Feb. 28 meeting that Ceclor has yet to face generic competition outside the U.S., even in markets where there is no patent protection. ‘While we cannot know what the actions of everybody else in the world will be,’ Step said, ‘it is very interesting to observe that while there isn’t patent coverage in a large part of the world for Ceclor, there isn’t any generic Ceclor.’

(continued)

**BOX 5-3: Patent Protection and Post-Patent Hurdles for Competitors
(News media clips) (Cont'd.)**

"Lilly Research Labs President Mel Perelman explained the process protection during question-and-answer. 'The Ceclor synthetic route is so long and so complex' that it will be difficult to duplicate, Perelman said....

"A producer of cefaclor can take a number of different routes to get to the intermediate, Perelman explained, 'but they can't go through it without violating our patent. So an ethical or legal end-run seems extremely improbable.' The patent on the intermediate runs until December 1994. Step further pointed out that establishing a cefaclor manufacturing process 'will require very considerable capital investment...we haven't seen that yet'...."

SOURCE: Ceclor market: Quoted from "Ceclor Market Dominance Will Continue Past Dec. 1992 Patent Expiration, Lilly Contends: Process Protection Thru 1994, Lorabid NDA Filed as Backup," FDC Reports: Prescription and OTC Pharmaceuticals, March 4, 1991. p. 15.

"Ivax Corp. faces lawsuit from Eli Lilly"

In 1995, Eli Lilly sued Ivax Corporation, a pharmaceutical company that announced that it had received FDA approval to manufacture cefaclor capsules, a generic version of Lilly's Ceclor. Lilly claimed that Ivax's supplier of a raw material used a process that infringes on Lilly's process patents.

SOURCE: Ft. Lauderdale, FL *Sun-Sentinel*. 1995. April 29, 1995. p. 8B.

PATENTS

Patents provide the primary protection for a pharmaceutical company's investment in research, development, marketing, and production costs. The 1991 OTA report, *Biotechnology in a Global Economy*, described the patent process for pharmaceuticals:

Drug companies usually secure patent protection early in drug development, before the drug enters the regulatory process. Regulatory approval for new drugs takes, on average, 7 to 10 years to complete. This translates into a 7- to 10-year reduction in [the usual 17-year] patent protection for pharmaceutical products when they reach the market, leaving such products with, on average, 9 years of protected life....

[T]he Drug Price Competition and Patent Term Restoration Act of 1984... restores part of the patent life lost due to lengthy regulatory approval. The act allows extension of the patent term for up to 5 years, but it does not allow extension beyond 14 years for effective patent life. The actual extension granted is equal to the total time taken by the Food and Drug Administration (FDA) to review the New Drug Applica-

tion, plus one-half of the clinical testing time. In addition, the act promotes generic competition by providing FDA with an Abbreviated New Drug Application (ANDA) process. This process facilitates the approval of generic drugs by eliminating the need for costly clinical studies. An ANDA does require the sponsoring company to demonstrate its generic's bioequivalence to the pioneer drug. This is much less costly and time-consuming than complete clinical trials and facilitates the market entrance of generic drugs.

The GATT (General Agreement on Tariffs and Trade) legislation changed patent terms from 17 years from issuance to 20 years from filing (OTA, 1991, discusses the nuances of these terms), and in March 1995, the U.S. Patent and Trademark Office (PTO) announced a preliminary policy statement that extensions would be added to the new 20-year patent term. In June 1995, however, PTO reversed its position and presented manufacturers a choice between adding any extension they had to the 17-year term or accepting the 20-year term under GATT. Manufacturers are expected to challenge this decision in court.

Members of the OTA advisory panel discussed the pluses and minuses of a negotiated agreement between a manufacturer and the PTO to extend the patent life of an antibiotic in exchange for restrictions on its use. Again, consider the example of an antibiotic effective against MRSA. Could PTO, FDA, and the manufacturer work out an agreement so that the antibiotic was marketed only for use against MRSA? Such an agreement would have a positive impact on the emergence of resistance, but it would present supervision or enforcement problems to assure that the restrictions were followed. It would also present problems for the manufacturer in estimating its returns from unrestricted sales over a few years—until resistance becomes common—as compared to restricted sales over more years. How soon resistance would arise in both cases is difficult to estimate, as are the chances of another company developing a comparable or better drug.

Many compounds are patented but never brought to market. If, subsequently, it was discovered that such a compound was useful against antibiotic-resistant bacteria, probably no firm would be interested in conducting the tests and trials necessary to bring it to market. Without patent protection, the firm that paid for the tests and trials would be unable to recover its costs. Fusidic acid, an antibiotic that has been used in Denmark and other countries since 1962 (Mandell and Sande, 1995), provides a real-life example of such a drug. Fusidic acid is active against at least some strains of MRSA, and it is used against those bacteria in other countries. It has never been marketed in the United States, although it can be made available under compassionate use procedures to physicians in this country. Because it is off-patent, the company that developed and sells it elsewhere is not willing to fund clinical trials that would be necessary to obtain FDA approval for its being marketed for use against MRSA here.

Patent protection of the chemical substance is not the only method by which companies can maintain their markets. OTA (1993, p. 82-87) describes how complicated and expensive pro-

duction methods and facilities can be a major hurdle for competitors, especially when the methods and facilities are protected with process patents. For example, in 1995, Ivax Corporation announced it had received FDA approval to manufacture a generic version of a cephalosporin on which the patent had expired in 1992. Eli Lilly sued Ivax, claiming that Ivax's supplier of a raw material used a process that infringed upon Lilly's process patents (Fort Lauderdale *Sun-Sentinel*, 1995).

PRICING OF DRUGS DEVELOPED IN PART BY FEDERAL RESEARCH

The Federal Technology Transfer Act of 1986 (P.L. 99-502) authorized the establishment of CRADAs (Cooperative Research and Development Agreements) between federal intramural laboratories and private industry to bring inventions and discoveries in federal laboratories to market. In exchange, the private industries would receive the profit from sales of the developed products. In 1989, Congress directed the National Institutes of Health (NIH) to require "reasonable pricing" of any drugs that were developed in cooperation between its laboratories and industry. Industry objected to the restrictions on pricing, and, in April 1995, NIH relinquished its right to require reasonable pricing.

This change is expected to have little effect on antibiotics. While the federal government conducts research on antiviral and antifungal agents, it has supported little research on antibacterials, leaving that research to the pharmaceutical firms, and none of the six products that had been developed as of April 1995 through CRADAs was a drug (*Health News Daily*, 1995).

CONCLUSIONS

Antibiotic research and development, as almost all drug research and development in the United States, is carried out and sponsored by pharmaceutical companies. Recent years have seen the introduction of few new antibiotics into the mar-

ket, which may reflect a diminished research effort in antibiotics five, 10, and more years ago.

Currently, there is a great deal of activity in looking for substances with antibiotic properties in biological sources that have not been exploited in the past and in applying new molecular biologic and chemical techniques to the synthesis of antibiotics and to understanding their mechanisms of action. On the positive side, some of the compounds being considered as possible antibiotics have mechanisms of action different from those of currently used antibiotics, and they should be especially useful against bacteria now resistant to many or all currently available antibiotics. Despite that promise, there is great uncertainty about if and when there will be a pay-off from the research efforts, and few experts expect commercial availability of any antibiotics with new mechanisms of activity in this century. The uncertainty about availability of new antibiotics underlines the importance of efforts to reduce the emergence and spread of bacteria resistant to now-used antibiotics.

The emergence of antibiotic-resistant bacteria produces new market opportunities, and it can be expected that pharmaceutical firms will be interested in developing products for it. Some experts argue, however, that the profits to be expected from an antibiotic are smaller than those from other drugs and that pharmaceutical firms will focus their efforts on other, more profitable drugs. On the other side of that argument, an antibiotic that is effective against an infection resistant to all other antibiotics could probably be sold at a very high price.

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