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Discovery and Development of New Antimicrobial Agents

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INTRODUCTION

In the past two decades, there has been unprecedented activity in the discovery and development of new antimicrobial agents. One need only reflect upon the long list of new penicillins, cephalosporins, and quinolones that were not available in 1965 to form the opinion that such activity was the result of unrelated research and discovery efforts in many different chemical areas. A closer review of the events surrounding the evolution of antibiotic development, however, suggests a much more orchestrated series of events in which the development of several of the different structural classes of compounds was closely interdependent. Technical advances in penicillin chemistry, for example, helped to push the subsequent rapid development of cephalosporins and other beta-lactams. Advances in understanding of bacterial physiology led to development of structure-activity relationships (SAR) used to direct the chemical modification of antibiotics to improve their antibacterial activity. This approach also led to the discovery of new antibiotics that were active against bacteria that had acquired resistance to the older agents.

The development process for a new antibiotic involves studies that characterize its mechanism of action and extensively assess its safety and efficacy in both humans and other animals. This process can take over 8 years to complete and represents a sizable investment on the part of the sponsoring pharmaceutical company (162). Given this investment and the current competitive environment with many new agents being marketed, it is incumbent upon the pharmaceutical industry to find new agents that demonstrate distinct advantages over existing antibiotic therapies. This review will discuss some experimental aspects of new antibiotic discovery and describe the various developmental stages through which a new agent must pass to reach the marketplace. An historical perspective on past research efforts in the most prominent antibiotic classes will also be given, to provide insight into the many factors that have influenced the development of these agents.

PRECLINICAL TESTING STAGES

Discovery of New Antibiotics

The novel lead structures that have generated most new classes of antibiotics have been found by testing natural products. A number of these novel discoveries are listed in Table 1 and include penicillins, cephalosporins, cephamycins, aminoglycosides, clavulanic acid, glycopeptides, tetracycline, chloramphenicol, carbapenems, and monobactams. The discovery and isolation of the lead structures for each of these classes have provided a unique starting point for chemical modification in attempts to improve their spectrum, pharmacokinetics, and human safety. Three basic approaches have been used to find new antibiotics from natural sources: (i) direct isolation from soil and marine microorganisms, (ii) genetic modification of known antibiotic-producing organisms to induce their production of novel metabolites, and (iii) diversion of natural metabolic pathways of antibiotic-producing organisms by introduction of substrate precursors into the fermentation system (150).

The sophistication of these approaches has evolved in industry over the years; the first methods for testing fermentation broths prepared from soil samples relied on the detection of antibacterial activity in agar diffusion plates seeded with susceptible target organisms (87, 120). The specificity of this approach was increased by testing broths with isogenic strain pairs that differed only by their degree of susceptibility to a specific class of antimicrobial agent. Even with such improvements, the isolation of minute amounts of a potentially novel activity from complex fermentation broths involved a heroic effort, using large-scale fermentation methods and multiple chromatographic separations and biological assays to follow the antibiotic activity (120). Such fermentation discovery efforts initially rely on testing large numbers of soil samples by rapid, automated techniques. One early study, for instance, estimated that, of 21,830 fermentation cultures tested, 6,464 produced antibiotics, 490 of which possessed activities of interest; 6 were eventually submitted for structural determinations, from which two novel structures were isolated (178). Reports of new antimi-

TABLE 1. Antibiotics discovered by natural product screening

Antibiotic	Producing organism(s)
Pencillin G	*Penicillium notatum*, *P. chrysogenum*
Penicillin V	*P. chrysogenum*
Cephalosporin C	*Cephalosporium acremonium*
Cephamycin	*Streptomyces lactamdurans*
Thienamycin	*S. cattleya*
Clavulanic acid	*S. clavuligerus*
Sulfazecin (monobactam)	*Pseudomonas acidophila*
SQ 26,180 (monobactum)	*Chromobacterium violaceum*
Vancomycin	*S. orientalis*
Ristocetin (glycopeptide)	*Nocardia lurida*
Streptomycin	*S. griseus*
Erythromycin	*S. erythreus*
Oleandomycin	*S. antibioticus*
Chloramphenicol	*S. venezuelae*
Lincomycin	*S. lincolnensis*
Tetracycline	*S. viridifaciens*, *S. aureofaciens*
Polymyxin	*Bacillus polymyxa*

crobial agents published in the *Journal of Antibiotics* between 1981 and 1985 indicated that, on average, 50 new compounds worthy of communication were discovered per year (120). The majority of these new antibiotics isolated from fermentations have come from soil organisms of the genus *Streptomyces*, although in more recent efforts novel monobactams have been found in fermentations of *Agrobacterium* and *Pseudomonas* species (15, 150, 173).

Continued refinement of the process of antibiotic discovery from natural products has substituted biochemical-based assays for those that rely on killing of the bacterial target organism (120). The more specific assays have utilized enzyme-linked immunosorbent techniques (179), beta-lactamase induction (161), D,D-carboxypeptidase inhibition (143), receptor ligand binding competition (127), and monitoring of the specific morphological changes in cells that are characteristically associated with exposure to a particular class of antimicrobial agent (111). Several novel monobactams were first detected in bacterial fermentations by making use of their inherent property to induce beta-lactamase production in *Bacillus licheniformis* ATCC 14580 (161). When the hydrolysis of a chromogenic cephalosporin substrate was measured to detect induced levels of beta-lactamase, nanogram quantities of novel beta-lactams could be detected in fermentation samples (161). Extreme sensitivity has also been achieved with enzyme-linked immunosorbent assay methods, as described for the detection of novel aminoglycosides (179). In one typical system, an antibody with specificity for gentamicin was developed and used to coat the surface of a microdilution plate well. A gentamicin-alkaline phosphatase conjugate was then added to the plate, which was subsequently incubated before addition of substrate for alkaline phosphatase, *p*-nitrophenylphosphate (179). Competitive assays were run with fermentation broths potentially containing novel aminoglycosides. Following exposure to these broths, the amount of the original enzyme conjugate bound to the antibody in the well was quantitated by measuring the change in absorbance after addition of the enzyme substrate. Such assays are specific for a particular class of antimicrobial agent (in this case, aminoglycosides) and have been reported to be far more sensitive for detecting the presence of antibiotics than conventional plate diffusion assays that measure bacterial growth inhibition (179). The state of the art in natural product screening techniques for antibiotics is limited only by the ingenuity of the researcher.

Once activities are detected, their chemical source must be identified from a fermentation broth that might contain dozens of commonly known antimicrobial agents and only picogram to microgram amounts of a new agent. The numerous chemical procedures used to isolate novel antibiotics from fermentations include solvent extractions, ion-exchange methods, gel filtration, and adsorption and partition chromatographies (14). A complete isolation, together with the final structural assignment of the new molecule by nuclear magnetic resonance or X-ray crystallography, can take several months to complete. Given the complexity of isolating novel antibiotics from natural products, it is easy to understand why even more compounds have not been discovered from this source.

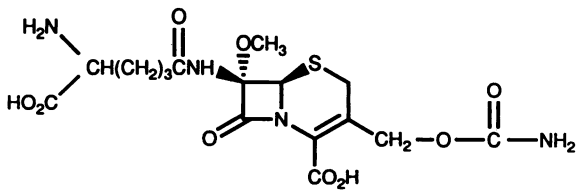
A more contemporary approach to natural product research aimed at finding new antibiotics involves the use of recombinant DNA technology for isolating genes involved with antibiotic production. As reviewed by Hunter and Baumberg (79), cloning of genes from streptomycetes and filamentous fungi has enabled the isolation and characterization of specific genes for several metabolic pathways involved with antibiotic production in these organisms. The isolation of these genes not only enables studies to understand better the pathways for antibiotic production, but also provides the opportunity to mix DNA isolated from different organisms and to transfer it into a new host cell in attempts to stimulate the production of structurally novel antibiotics (79). Efforts to obtain such genetic recombination between species was greatly facilitated by cloning antibiotic-producing genes on plasmids that could be readily transformed and maintained in recipient fungi (78, 97, 100). McAlpine et al. (100) used this strategy to transfer DNA from *Saccharopolyspora antibioticus*, which produced the macrolide oleandomycin, to an *S. erythraea* mutant that was blocked for production of erythromycin. This cross species mixture of DNA resulted in the production of a structurally novel antibiotic, 2-norerythromycin (100). The result of such recombinant DNA studies has been the production of antibiotics containing minor structural modifications of known agents. The commercial application of this technology may depend on devising genetic constructs that would produce more novel compounds with greater structural diversity (79).

The third approach to natural product research for finding new antibiotics involves biochemical diversion of normal synthetic pathways in antibiotic-producing microorganisms by introduction of substrate precursors. Two methods have been used to pursue this approach. The first involves supplying precursors to wild-type organisms in an attempt to drive secondary metabolic pathways toward the synthesis of new antibiotics (123, 150). A modification of this approach involves the selection of mutants blocked in the normal metabolic pathway for antibiotic production that can be given unnatural chemical moieties from external sources (34). The goal of this mutasynthetic approach is to permit the microorganism to complete the blocked synthetic pathway, using the new structural moiety, thereby increasing the chances for producing a new antimicrobial agent (34). This method has accounted for the discovery of many structurally novel aminoglycosides, macrolides, and beta-lactams; however, yields of antibiotic have often been too small to make commercial production realistic. These last two methods of antibiotic discovery have not met with the same success for finding new lead structures as those involving direct detection from natural products.

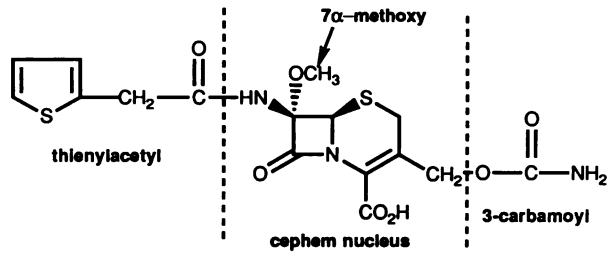
Establishment of SAR

The characteristics of most new antibiotics discovered from natural product testing are not of immediate commercial interest. This may be due to important deficits in spectrum of activity or potency, in vivo stability and pharmacokinetics, or an unacceptable safety profile observed in animal studies. To make the necessary structural changes in molecules to achieve commercially desirable goals, the SAR between chemical modification of a compound and the resulting biological activity must be understood. While this may appear to be a calculable endeavor, in reality, many of the relationships between the chemical structure of an antibiotic and its biological activity are never understood. Logical approaches to gaining this information are, however, well established for each class of antimicrobial agent and constitute the major research and development effort toward discovering new antibiotics that possess significant advantages over other marketed agents. This goal is particularly relevant in the current antibiotics marketplace, which is crowded with numerous, highly competitive agents. The approaches used to develop SAR usually rely on determining which substitutions made on a particular molecule will translate to improved antibacterial activity. An understanding of bacterial resistance mechanisms to antibiotics can be indispensable in developing SAR. If, for example, the compound under study is an aminoglycoside, knowledge of the many different bacterial enzymes that can chemically modify aminoglycosides and make them inactive as antibiotics is important in designing new agents that might avoid such modification. The most valuable approaches to developing SAR are those that have used biochemical data correlating the compound's resistance to inactivation by a bacterial enzyme or affinity for a lethal bacterial cell target with activity against the whole organism. The importance of establishing SAR can be demonstrated by some examples.

One of the clearest examples of the important relationship between antibiotic structure and biological activity is in the development of cefoxitin from cephamycin C. In 1972, when cephamycins A, B, and C were discovered in fermentation mixtures (154), few antibiotics were available with activity against gram-negative bacilli that produced large amounts of beta-lactamase. The most active component of the cephamycin mixture was cephamycin C, which possessed activity predominantly against gram-negative bacteria. Its structure (Fig. 1) differs from the classical cephalosporin nucleus by the presence of a methoxy group in the 7-alpha position which conferred on the molecule resistance to hydrolysis by beta-lactamases (152). Compared with the cephalosporin cephalothin, this stability led to potent in vitro activity against isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and indole-positive *Proteus* sp. A major effort was begun at Merck Sharp & Dohme to make chemical substitutions on the cephamycin C molecule to increase its activity against enteric organisms further and to broaden its spectrum of activity to include gram-positive pathogens. The chemical properties inherent in the cephamycin nucleus made such substitution difficult as compared with earlier efforts accomplished in the cephalosporin class (152). New chemical reactions were designed to permit side chain substitution, however, and a large number of analogs were made with substitutions at positions C-3 and C-7 (20) of the nucleus. One of these analogs, cefoxitin, contained a thienyl acetyl group at C-7 in place of the alpha-amino-adipic acid moiety of cephamycin C (Fig. 1). This substitution broadened the spectrum of cefoxitin compared with cephamycin C to include gram-positive organisms and also increased its activity against gram-negative organisms severalfold (11, 152). Further chemical substitution made it possible to study the SAR of the 7-alpha-methoxy group of cefoxitin. The analog possessing a hydrogen at the 7-alpha position in place of the methoxy group demonstrated increased potency against gram-positive isolates, with decidedly decreased potency against gram-negative bacteria (152). Studies measuring the stability of various substituted analogs to hydrolysis by beta-lactamase revealed that both the 7-methoxy and thienyl acetyl groups conferred resistance to hydrolysis and made cefoxitin highly active against many enteric organisms that expressed beta-lactamase. Interestingly, more recent studies have shown that, on a weight basis, cefoxitin is an excellent inducer of beta-lactamase in certain gram-negative bacilli in which enzyme expression is usually repressed (70). This may explain why cefoxitin is not highly active against many isolates of *Enterobacter*, *Citrobacter*, and *Serratia* species that possess this enzyme. With the understanding that the alpha-methoxy group of cefoxitin conferred beta-lactamase stability, efforts were undertaken to make substitutions in this position to find derivatives with even greater stability to the enzyme. Using the unsubstituted compound as standard, it was discovered that any group at the 7-position larger than methyl conferred enhanced stability to hydrolysis of the beta-lactam ring by beta-lactamase (152). The natural methoxy moiety, however, conferred the greatest degree of stability without adversely affecting antibacterial activity, and it has been hypothesized that substitution at this position sterically blocks the beta-lactam bond from attack by the enzyme (11, 152). Other lines of research established that the carbamoyl moiety at C-3 increases the metabolic stability of cefoxitin in vivo (11). Subsequent efforts attempting to transfer the beneficial properties of the 7-alpha-methoxy group to cephalosporins have not always met with success. 7-Alpha-methoxy-substituted analogs of cefuroxime, cefamandole, and cephapirin were shown to be less potent antibacterial agents than their unsubstituted parent mole-



Cephamycin C



Cefoxitin

FIG. 1. Structure of cephamycin C and cefoxitin illustrating important side chain substituents. Redrawn from reference 11 with permission of the publisher.

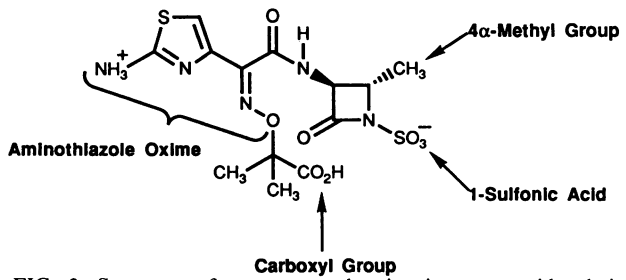


FIG. 2. Structure of aztreonam showing important side chain substituents for biological activity. Redrawn from reference 156 with permission of the publisher.

cules, and this effect was associated with a decreased affinity of the substituted analogs for several of the lethal penicillin-binding proteins (PBPs) in gram-negative bacteria (32).

The result of such structure-activity studies with cefoxitin illustrate how making changes in an active molecule can translate into major clinical benefits such as lower susceptibility to inactivation. The broad spectrum and beta-lactamase stability of cefoxitin constituted a major breakthrough at the time of its discovery, and its availability was a major advance in the treatment of infections caused by organisms that produce high levels of beta-lactamase.

As the SAR around cefoxitin helped to explain its stability to beta-lactamase, similar approaches were taken with monobactams isolated from natural products to improve their antibacterial activity. Monobactams have been isolated from fermentations of several bacterial species, and all contain a 2-azetidinone-1-sulfonic acid moiety (Fig. 2). The naturally occurring monobactams demonstrated only modest antibacterial activity that was limited to gram-negative organisms. Competitive binding studies with radiolabeled penicillin G and unlabeled monobactam indicated that this limited spectrum resulted from the poor affinity of monobactams for the PBPs of gram-positive organisms (60, 61, 156, 158). Attempts were made to broaden the spectrum of naturally occurring compounds by making side chain substitutions around the monobactam nucleus. Chemistry efforts at the Squibb Institute centered around making penicillin and cephalosporin side chain substitutions on the monocyclic beta-lactam ring (60, 156, 158). As indicated by Sykes and Bonner (156), however, because SAR do not translate well from penicillins to cephalosporins, chemical moieties from these compounds when substituted onto the monobactam nucleus do not impart the same degree of biological activity as observed for the other beta-lactams. The monobactam homologs of penicillin G, carbenicillin, and mecillinam were less active than their penicillin counterparts against gram-positive organisms, while the piperacillin analog had a spectrum and potency similar to those of piperacillin (60, 156). The cephalothin homolog had the same spectrum as cephalothin but was less potent, while the cefoperazone homolog was very similar in potency to cefoperazone against gram-negative organisms (60). These patterns of activity generally followed the affinities of each agent for the lethal PBPs in gram-positive and gram-negative organisms (60). Some of the best activity against gram-negative organisms was obtained by adding the aminothiazole oxime side chain (Fig. 2) derived from expanded-spectrum cephalosporins and through addition of a methyl group at position 4, which greatly increased stability to beta-lactamase hydrolysis (156). Several hundred monobactam derivatives were prepared, which led to the properties found in aztreonam (Fig. 2). The high activity of aztreonam against gram-negative organisms can be explained by the presence of the aminothiazole oxime moiety, which confers high affinity for the lethal PBPs 3 and 1a (60, 157). The presence of a carboxylic acid function on the oxime side chain was shown to improve activity against *Pseudomonas aeruginosa* (158). The α-methyl group at position 4 increases the stability of the beta-lactam ring to hydrolysis by beta-lactamase (156). In fact, aztreonam is reported to be a potent inhibitor of many type I cephalosporinases that hydrolyze cephalosporins (19). Aztreonam is also a poor inducer of these beta-lactamases (158), although some mutants that are overproducers of type I enzyme are resistant to the drug.

Thus, it can be seen that the use of in vitro PBP and beta-lactamase biochemical assays helped to develop the SAR that led to the discovery of aztreonam. The use of these types of specific, mechanism-based assays has helped pharmaceutical researchers develop other novel antibiotics that possess properties that have translated into significant clinical benefits. SAR have also been developed for studying parameters other than antibacterial activity. These include increasing the metabolic stability of compounds in vivo and improving the pharmacokinetics of antibiotics as measured by their distribution characteristics in animals. A number of animal models have been developed to perform these tests.

Animal Models for Studying the Pharmacokinetics and Efficacy of Antibiotics

As mentioned previously, mechanism-based studies are frequently used in antibiotic research to guide the SAR which defines those biological characteristics that make a compound effective against a broad range of bacterial pathogens. In vitro activity, however, does not guarantee in vivo efficacy. All new antibiotic compounds with an interesting antibacterial spectrum must be tested in standard animal models to assess their safety, distribution in various tissues (pharmacokinetics), and efficacy against experimental bacterial infections. Since these studies are the final assessment before testing a new compound in humans, a major issue that has confronted pharmaceutical researchers is to understand how well test results in animals predict the performance of the antimicrobial agent in humans. This question can perhaps be answered best by considering some standard methods for performing and evaluating pharmacokinetic studies of antibiotics in animals.

A number of sources provide details on the standard methodologies for performing pharmacokinetic and metabolic studies with experimental antimicrobial agents in animals (63, 104, 149). While the parameters measured and the methods used in animal studies are similar for all classes of antibiotics, each experimental compound can present special problems with respect to its chemical stability, solubility limitations, toleration by animals, and ease of quantitation in body fluids. At an early stage of testing, when supply of experimental compound may be limiting, the first animal studies ensure that the compound is not rapidly metabolized in vivo and that it is well tolerated. Administering antibiotics in rodents via the intravenous, subcutaneous, or oral route is usually the simplest way to determine the preliminary pharmacokinetics and safety of experimental compounds. It should be emphasized that in each case appropriate control antibiotics must be run for comparison. The best controls to use are those for which extensive published data are available in both test animals and humans (149). Usually 8 to 12 mice or rats are used per administration route per dose, and

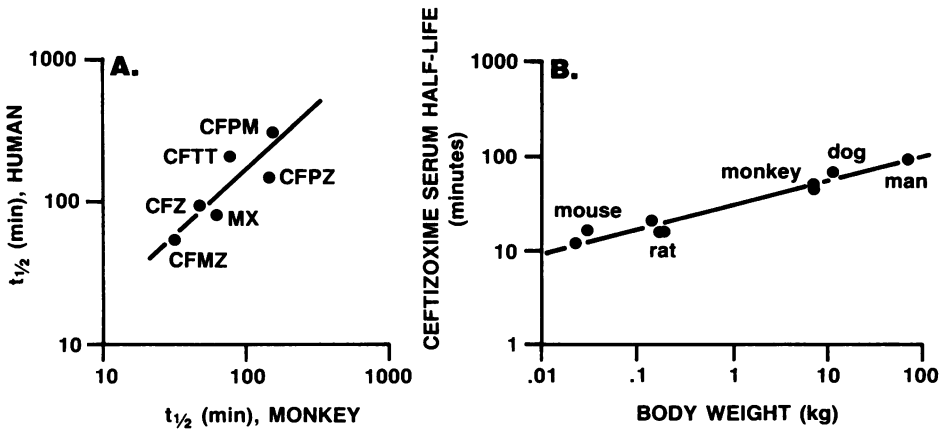


FIG. 3. Comparison of serum elimination half-lives for cephalosporins between monkey and humans (A) and relationship between species body weight and serum elimination half-life of ceftizoxime (B). (A) CFMZ, cefmetazole; CFZ, cefazolin; MX, moxalactam; CFPZ, cefoperazone; CFTT, cefotetan; CFPM, cefpiramide. Redrawn from reference 140 with permission of the publisher. (B) Redrawn from reference 109 with permission of the publisher.

the dose ranges selected reflect the anticipated use levels of the agent in clinical trials in humans. As an example, a cephalosporin that will be given in 1-g doses by the intravenous route to a 70-kg adult would be tested in rodents at around 14 to 20 mg/kg by injection into the tail vein. Strict adherence to protocol is an important part of obtaining reproducible animal pharmacokinetic data, since blood samples must be taken from all subjects at precise intervals, usually 15, 30, 60, 90, 120, and 150 min after dosing (149). Rodents are usually bled through the orbital sinus, and serum or plasma samples are prepared to test for antibiotic. Biological assays which rely on the diffusion of test antibiotic into a confluent lawn of a susceptible test organism grown on an agar plate are usually the simplest ways of measuring antibiotic levels in body fluids (145). With this method, the test agent must incubate overnight in the assay plate, and this can be problematic if stability data for the experimental compound are not available. The current trend is to assay the level of drug in test samples immediately after collection or following storage at −70°C. Analytical chemical methods such as high-pressure liquid chromatography are now used to quantitate drug levels in body fluids since the assay time is short (73). This method of analysis is also important for identifying metabolites of the parent compound excreted in urine. This is important since metabolites are often inactive in microbiological assays, yet they must be identified for safety evaluation purposes.

When the mean plasma drug levels determined for each time point are plotted against sampling time, a pharmacokinetic curve is generated from which important information can be obtained (149). The peak drug concentration (C_{max}), time to C_{max} (T_{max}; particularly relevant for drugs given orally), elimination half-life ($t_{1/2\beta}$), and area under the plasma concentration-versus-time curve (AUC) are all parameters that can be compared with control compounds tested in a given animal species. As an experimental antimicrobial agent is evaluated further, pharmacokinetic studies are carried out in additional species, usually including beagle dogs and monkeys. The goal of such studies is to obtain multiple pharmacokinetic "models" with which to compare the experimental drug with data obtained from well-characterized antimicrobial agents belonging to the same structural class. More advanced studies are performed on compounds of developmental interest to identify clinically relevant characteristics that would distinguish the compound from existing therapeutic agents. An example of such animal studies involved experiments performed in mice, rats, dogs, and cynomolgus monkeys with a new macrolide antibiotic, azithromycin (65). These pharmacokinetic models indicated that azithromycin possesses a very long elimination half-life in vivo and achieves high tissue levels compared with the macrolide erythromycin (65). Additional studies with azithromycin in mice have characterized the extensive accumulation of this compound inside phagocytic cells that are involved with the resolution of experimentally induced infections (66). These tissue distribution studies define some potential advantages of azithromycin over the standard macrolide, erythromycin, and help with the design of clinical trials in humans (65). Among numerous other animal pharmacokinetic studies that have evaluated new antibiotics are studies to measure the penetration of azole antifungal compounds into the eyes of rabbits (139), models to study drug penetration into surgical wounds of dogs (133), and novel sampling methods to measure spinal fluid levels of antibiotics in rats (103).

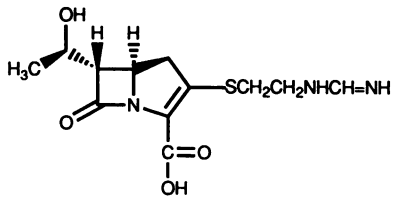
One of the most frequent uses of animal models is in making pharmacokinetic predictions of an experimental antibiotic in humans. This is extremely important since new and efficacious antibiotics with long elimination half-lives can be administered less frequently and are therefore more convenient and cost effective to use. In one published study, the long-acting cephalosporin ceftriaxone was administered for surgical and ambulatory use in 42 patients with osteomyelitis (48). Since ceftriaxone can be given once a day by intramuscular injection, patients can leave the hospital once postsurgical improvement is seen, receiving the remainder of their therapy at home. The use of this long-acting antimicrobial agent resulted in an estimated savings of $204,340 in hospital room costs alone (48). Efforts to find such long-acting agents from research and development programs present a dilemma. It simply is not cost effective to test all interesting new antibiotics in human clinical trials to determine whether they have significant pharmacokinetic advantages over existing agents.

To approach this problem, investigators have developed mathematical models for predicting various pharmacokinetic parameters in humans from data collected for antibiotics tested in a single animal species (16, 140). This approach

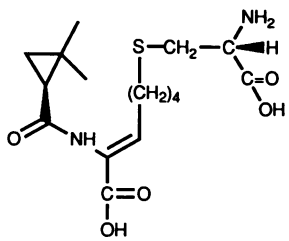
(Fig. 3A) relies on analyzing data collected for several structurally related antibiotics in a single animal species (140). For example, by including many cephalosporins that have been tested in both humans and other animals in the analysis, a mathematical relationship can be made between monkey (in this case) and humans for individual pharmacokinetic parameters such as elimination half-life. When the animal model is found that generates the best data "fit," the half-life of experimental agents can be predicted for humans from data obtained in the animal model.

Another approach often used to predict the pharmacokinetics of a new agent in humans makes use of a mathematical procedure for "scaling" data obtained in several different animal species (39, 110). While small mammals tend to eliminate drugs more rapidly than large mammals, pharmacokinetic data can be mathematically compared between species as a function of body weight (110). This process is referred to as allometric analysis of pharmacokinetic scaling and has been used to compare the data obtained with antibiotics in humans and other animals (Fig. 3B). While allometric analysis has been successfully used for predicting the elimination half-lives of new beta-lactams in humans from animal data (26, 109), it appeared to be insufficiently precise for making such predictions with macrolides (42). Despite potential limitations, this approach holds an important place in discovery research.

Animal models have also been used to study the metabolism of new antimicrobial agents, as illustrated by the example of imipenem. After the initial clinical testing of this carbapenem antibiotic in humans, it became apparent that the urinary recovery of intact imipenem varied considerably between subjects, ranging from 6 to 33% (86). Despite this variability, the plasma elimination half-life of the drug in all subjects was around 60 min, and it was calculated that its renal clearance rate was only a fraction of the known rate of human glomerular filtration (86). These findings, supported by additional metabolic studies, suggested that imipenem was metabolized to a high degree in the human kidney. Similar results obtained in animal studies led to the finding that the enzyme renal dehydropeptidase I, located in the brush border of the renal tubule, was responsible for the high degree of renal metabolism of imipenem in both humans and other animals. For reasons that are still not completely understood, some humans are high metabolizers of imipenem, while others destroy it to a lesser degree in the kidney. The low urinary recovery and metabolic instability of imipenem in the kidney were of clinical concern with respect to achieving optimal therapeutic levels of drug in urine (86). For this reason, a program was started at Merck Sharp & Dohme to synthesize compounds containing the dehydropeptide bond that might act as inhibitors of renal dehydropeptidase I in vivo and could therefore be coadministered with imipenem. Many analogs were tested and found to be competitive inhibitors of the human enzyme in vitro. This approach addressed only part of the problem, however, since a potent inhibitor of renal dehydropeptidase I would still have to match the pharmacokinetic characteristics of imipenem to be effective in humans. In considering this issue, it was determined that the chimpanzee most closely parallels humans with respect to its metabolism of imipenem. An integral part of the program, then, was to find the most potent dehydropeptidase I inhibitors and attempt to modify them chemically so that they would have a pharmacokinetic profile similar to that of imipenem, as assessed experimentally in the chimpanzee (86). By having an animal model that was closely representative of a human, the



Imipenem



Cilastatin

FIG. 4. Structures of imipenem and the renal dehydropeptidase I inhibitor cilastatin.

dehydropeptidase I inhibitor cilastatin was discovered (Fig. 4). It is striking that, while the dehydropeptide bond of both compounds is structurally related, the remainder of the cilastatin molecule is dissimilar in structure from the antibiotic imipenem. Coadministration of cilastatin with imipenem in humans has been shown to increase uniformly the recovery of intact antibiotic in the urine, and the combination is marketed in the United States under the trade name Primaxin.

Animal models have also been used to evaluate how well a new antimicrobial agent cures experimental infections prior to clinical testing in humans (12, 183). These experiments are referred to as in vivo protection studies, and they are most often performed in mice, rats, or rabbits given lethal infections with human pathogens. Routine protection tests are usually run in mice since a quantitative 50% protective dose measured in milligrams per kilogram for most experimental antimicrobial agents can be determined by using a relatively small amount of compound (63). The pathogenic microorganisms serving as challenge are grown in culture broth, washed in buffer, and suspended in physiological saline or some other appropriate medium. Tenfold dilutions of organism are made and then inoculated intraperitoneally into groups of mice with 10 individuals per challenge group. The minimum number of cells required to kill all of the mice in a group within 24 to 48 h postchallenge is referred to as the lethal dose for 100% of the animals. Once the 100% lethal dose is established for each microbial pathogen, test doses of antimicrobial agent are given in twofold increments to individual groups of mice challenged with 1 100% lethal dose. The route of drug administration and length of treatment can vary depending on the virulence of the microorganism (27). The drug dose in milligrams per kilogram that protects 50% of the animals in a group from death is termed the 50% protective dose (PD_{50}). PD_{50}s can easily be determined for several different pathogens, and the quantitative endpoints found for an experimental antimicrobial agent can be compared with those obtained with control compounds. The finding that the new antimicrobial agent is

very effective and produces low PD_{50}s may correlate with data indicating that the agent also has high antibacterial activity in vitro. Pharmacokinetic data obtained in rodents and other animals may further indicate that the bioavailability of the new agent is greater than that of any other available compound in its class. These parameters, when considered together, indicate how well a new antimicrobial agent cures experimental infections relative to existing agents that have been tested in animals and used in humans.

More complex animal protection experiments are designed to test the efficacy of new antimicrobial agents for clearing infecting organisms from specific tissues. Infection models that represent abscess formation, pneumonia, pyelonephritis, meningitis, osteomyelitis, and endocarditis have been developed in mice, rats, and rabbits (27, 58, 62, 105). Furthermore, with the increased incidence of immunosuppressed patients seen in hospitals today, there is a major need for new antimicrobial agents that provide adequate therapy in patients lacking an intact immune system (57, 98, 134). Therefore, new infection models have been developed in immunosuppressed animals that measure the efficacy of antimicrobial agents against bacterial and fungal pathogens found in immunosuppressed patients (6, 91, 132). Usually, the animals are chemically immunosuppressed with repeated doses of agents such as dexamethasone or cyclophosphamide before challenge with a lethal inoculum of test organism (91, 132). The antimicrobial treatment period for such infection models may be longer than the routine PD_{50} experiments, since many infections in immunosuppressed patients are chronic in nature and require treatment over a period of time longer than 48 h. Chronic protection studies of this type often require different criteria for measuring successful therapy compared with routine PD_{50} studies that usually record lethality. These criteria can include counting viable microbial cells in one or more infected organs of animals sacrificed during, or at the completion of, a course of therapy (91). Measurement of microbial cell clearance from infected tissues is another way of assessing how well a test antibiotic might work in many of the serious infections encountered in humans.

To this point, a number of approaches have been described that aid in the discovery and early characterization of new antibiotics. Obviously, the process for discovering a new antibiotic with advantages over existing therapies can take years, with no guarantee of success. Once such a candidate is identified, however, a recommendation for development document is written which comprehensively reviews all of the in vitro and in vivo data generated and includes a plan for testing the safety of the compound in animals and clinical efficacy in humans. This document is reviewed internally by the pharmaceutical sponsor to determine whether the perceived advantages of the compound are sufficient to warrant the time and expense necessary for its clinical development. Before a new antibiotic can be tested in humans, however, it must first pass an extensive series of safety evaluations in other animals.

Safety Studies in Animals

In his discussion of the early history of cephalosporins, E. P. Abraham recounted that Giuseppi Brotzu, the discoverer of cephalosporin C activity in a fungus, "appeared to have dispensed with toxicity experiments in animals" and tested culture filtrates of the producing organism directly in infectious skin lesions in humans (1). He subsequently made a crude extract of filtrate and "boldly injected it intramuscularly and intravenously into patients with brucellosis, paratyphoid infections, and typhoid fever" (1). While these in vivo experiments were performed over 40 years ago, clinical investigators of today can ill afford to be so optimistic. Thorough safety studies of experimental antibiotics are now performed in animals before they are given to humans. As reviewed by Hirschman (77), the federal government has taken an increasingly active role in regulating studies that evaluate the safety of prescription drugs marketed in the United States. In 1906, the Pure Food and Drug Act was passed by Congress to more closely monitor the chemical purity and efficacy claims of new drugs. The Federal Food and Drug and Cosmetic Act was then enacted as law in 1938 as a response to the accidental deaths of over 70 people given sulfanilamide that was contaminated with a poisonous substance. This law provided that new drugs could not be marketed through means of interstate commerce unless they had been described in a new drug application and had undergone extensive safety testing in animals. The responsibilities of the Food and Drug Administration (FDA) broadened in 1962 with the passage of the Kefauver-Harris Amendments which placed additional governmental controls over the safety testing, distribution, advertising, and clinical monitoring of new drugs.

The protocols for routine toxicology testing of new antibiotics have been established in the United States by the FDA based on safety observations made historically from drugs already in clinical use (4). Each country has its own regulatory board, with most European nations bound by the European Economic Community set of regulations (4). There is no set protocol that specifically covers antibiotics; the types and duration of safety studies are generically dictated based on the anticipated duration of clinical exposure to the drug.

The types of animal toxicology tests conducted include both acute and chronic dosing studies, tests to assess the genetic safety of the drug, and studies to determine whether it has any adverse effects on reproductive capability. Acute toxicity testing involves administering a single or multiple doses of drug over a 24-h period at levels considerably above the anticipated use level in humans. These studies monitor behavioral changes along with lethality and define the maximum tolerated dose of antibiotic. Chronic safety testing experiments can involve several different antibiotic dosing schedules in animals depending on the stage of development of the compound in humans (4). As an example, safety studies in two animal species, one rodent and one nonrodent (including equal numbers of males and females), of 30 days in duration at daily doses severalfold above the expected human use level are required before a phase I multiple-dose study can be run in humans. For antibiotic candidates that have progressed to phase II therapeutic trials in humans, 6-month animal safety studies performed in two species must be completed before the drug is approved for marketing. The dose of antibiotic administered and the duration of dosing in chronic testing are guided by the FDA's desire to identify a toxic dose for each new drug (4). This information defines the maximum level of drug allowable in humans, which for most antibiotics is usually far above the projected use level needed to achieve efficacy. It should be remembered, however, that some antibiotics, such as aminoglycosides, have a narrow therapeutic index, and animal safety tests are essential for identifying the maximum dosing range allowed for each new agent (126). A number of parameters are monitored in conjunction with chronic safety tests. Blood is drawn during and after the completion of dosing, and the

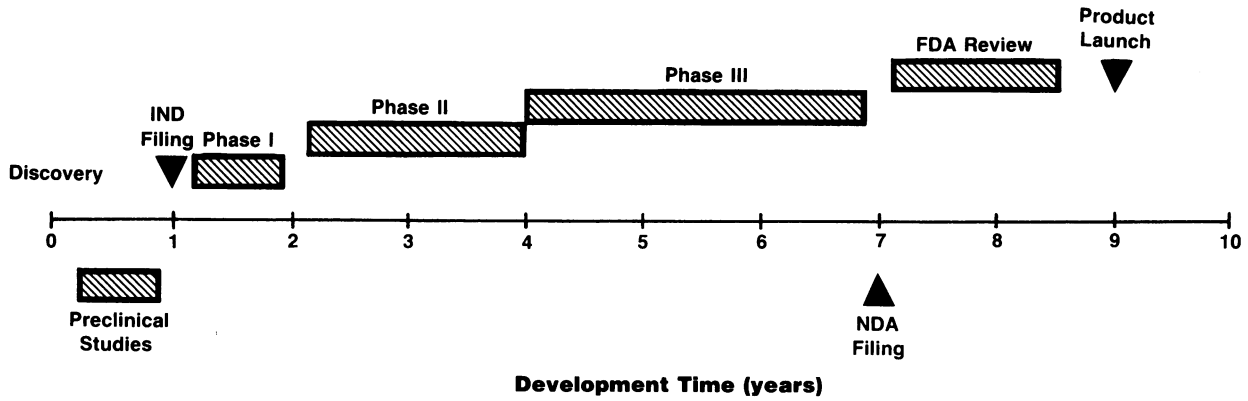


FIG. 5. Time line for the development of a new antibiotic. IND, Investigational new drug; NDA, new drug application.

samples are analyzed for hematological and blood chemistry abnormalities. Tests are run to monitor liver and kidney function as well as effects on the central nervous system. At the completion of antibiotic dosing, all animal subjects are sacrificed and multiple tissues are examined for gross and microscopic histological abnormalities. For antibiotics given parenterally, injection sites are examined for irritation that may have resulted from the drug. Other aspects of chronic safety testing involve determining whether the new agent has any effect on the reproductive capability in animals. These studies are done in three segments that assess safety over each phase of reproduction (112). The first stage involves dosing of male and female rats prior to mating to look for unusual effects on spermatogenesis in males and embryogenesis in females. Newborns are also studied for gross abnormalities in these general stage I tests. The second stage of reproductive tests involves a formal teratology test in which soft tissue and skeletal development of animal fetuses is examined, while the mother is given drug during the period of fetal organogenesis. These studies must be completed before a new antibiotic can be given to women of childbearing age. The last stage of reproductive testing involves giving the drug to animals during the last trimester of pregnancy to detect any effects on late fetal development, labor, lactation, or neonatal growth. For antimicrobial agents that give any abnormal response in standard mutagenicity tests, a 2-year carcinogenicity study is run in rodents. Due to the time and considerable expense associated with the carcinogenicity test, it is usually performed last in the safety evaluation phase for a new antimicrobial agent. Several short-term genetic toxicity tests are usually run to determine whether there is a need to examine an antimicrobial agent in a 2-year test. The scientific community is not in agreement, however, about the reliability of these short-term tests to predict compound carcinogenicity (181).

All acute and chronic toxicology tests are subject to rigid rules of design and execution, including recording of data. Results of the required tests, along with all other experimental data pertaining to the antibiotic candidate, must be submitted to the FDA before any human trials begin in the United States. Planned clinical trials abroad are subject to submission of these data to the regulatory agency covering the country in question (4).

In addition to these standard acute and chronic toxicology tests, a number of other safety studies may be required that assess whether the drug has any unusual effects in vivo that can be detected in a battery of general pharmacology tests or in laboratory experiments that may be specific for a given class of antibiotics. With quinolones, for example, tests that measure their effects on developing joint cartilage in young dogs, central nervous system and ocular toxicity in adult dogs, and mutagenicity in both cell culture and animals are usually required (25, 76, 80, 99). Other examples of supportive drug safety tests include in vivo immediate hypersensitivity tests for beta-lactams and tests evaluating the nephrotoxicity of aminoglycosides (126, 141).

The successful completion of a series of safety studies on an antibiotic candidate fulfills part of the criteria necessary to allow its clinical testing in humans. At this point, the sponsoring pharmaceutical group is required to compile all data on the compound pertaining to antibacterial spectrum, mechanism of action, experimental efficacy, and safety into a document termed the investigational new drug filing. The various studies included in this document can, in some instances, take up to two years to complete. The investigational new drug filing, in addition to being a comprehensive review of the new antimicrobial agent, also outlines the initial clinical plans to assess its safety and pharmacokinetics in healthy human volunteers. Once such a filing is submitted, the FDA has 30 days to review and approve it before such clinical trials can take place. The investigational new drug filing further serves as the reference document for the outside clinical investigators chosen to study the drug in humans.

CLINICAL TESTING PHASES

The three clinical testing phases required for a new antibiotic are designed to assess its efficacy and safety. A fourth phase can also be delineated, and such clinical tests are conducted to characterize the antimicrobial agent more fully after it has been introduced into the market. The total cost of discovering and developing a new antibiotic can approach 100 million dollars, with 60% of this cost resulting from safety and clinical studies (162). The phase I to III clinical trials take 5 years to complete on average and involve thousands of subjects (64) (Fig. 5). As discussed in a recent review (64), the rules governing the execution of phase I to III antibiotic trials were defined initially in two publications compiled by the FDA in 1977 (52, 53). These documents provide for the medically safe and scientifically accurate evaluation of new antimicrobial agents in clinical trials and set specific criteria for testing efficacy (64).

Phase I clinical trials are designed to study the safety and pharmacokinetic profile of a new agent in healthy volunteers. Such studies initially involve at least six individuals

and verify pharmacokinetic parameters in humans as projected from earlier experimental results obtained in animals. Blood levels, elimination half-life, and urinary recovery are all determined. Such data give an early indication of the distribution and metabolism of the drug under "optimum" conditions of health in humans. The oral absorption of a compound can be studied (if appropriate) by dosing 6 to 12 subjects orally and comparing the plasma concentration curves obtained with those derived from subsequent intravenous administration of the agent in the same subjects. This experimental format provides the most accurate determination of the oral bioavailability of an investigational drug and usually requires only a small number of subjects (10). More extensive multiple-dose bioavailability studies, using escalating dose levels of an oral antibiotic, are important for establishing dose proportionality (the proportional increase in peak plasma level seen with increasing dose) of the new drug in humans (10). Understanding the absorption characteristics of an antimicrobial agent can be important, since ampicillin and amoxicillin, for example, demonstrated decreased rates of absorption at high dose levels (146). The total number of subjects needed in phase I oral absorption studies may vary depending on the absorption characteristics of the particular antimicrobial agent in question. Macrolides, for instance, characteristically show erratic degrees of absorption between individuals, thereby necessitating studies enrolling up to 25 individuals to gain meaningful data (10). Other orally absorbed compounds such as beta-lactams can be tested in phase I trials with fewer subjects since their absorption characteristics usually vary less between individuals. Dose-dependent pharmacokinetic changes can be seen with parenteral agents as well. Ceftriaxone is a parenteral cephalosporin with a long elimination half-life, which is highly bound to plasma proteins. At high doses of ceftriaxone, the percentage of unbound drug increases and is therefore subject to more rapid elimination from the body by glomerular filtration in the kidney, thereby decreasing the overall elimination half-life (121).

The pharmacokinetic data generated from phase I studies help to set the appropriate dosing schedule for subsequent clinical trials that will evaluate the efficacy of a new antibiotic. The phase I trials may eventually involve several hundred subjects and take as long as 2 years to complete (64). The data generated on a new antibiotic are carefully reviewed at this point, since subsequent efficacy trials aimed at gaining FDA approval for the drug are costly and can take over 5 years to complete. It is not unusual, therefore, for a candidate to be discontinued should the pharmacokinetic or safety data obtained in phase I trials be less than that desired to encourage further development.

Phase II trials begin to assess the efficacy of a new antibacterial agent in treating patients with diagnosed bacterial infections. The FDA guidelines suggest that each new agent be directly compared with an antimicrobial agent with an established efficacy record for the particular therapeutic indications in question (53, 64). There is provision, however, for running noncomparative trials for those indications when the degree of efficacy observed with standard agents is well established (64). Early phase II trials are usually run in patients whose infections are not life-threatening, such as urinary tract and soft-tissue infections in which the causative agent is also easily identified by culture. In these studies, the efficacy of the new agent is carefully assessed by monitoring clinical cures, i.e., a relief of symptoms and a resolution of the signs associated with infection. The bacteriological cure is also measured by documenting the eradication of the causative organism after treatment and, when appropriate, by a follow-up culture several weeks posttherapy. In the types of infections included in phase II trials, clinical cure rates of 80 to 90% are routinely recorded (64). These studies can take up to 2 years and involve several hundred patients. The safety and toleration of new antibiotics continue to be closely monitored at this stage to detect any side effects that would advise against further clinical development. It is customary for the sponsoring pharmaceutical firm to meet periodically with representatives from the FDA to monitor the progress of the new agent and to approve plans for the broader phase III clinical program.

The design of phase III clinical studies is similar to those in phase II, and such studies are carried out to show efficacy for multiple therapeutic indications with the new antimicrobial agent. To achieve this goal, results from several thousand patients must be compiled. The FDA prefers that efficacy be demonstrated for each organism at each infection site at which an indication for therapy is desired (64). This means, for example, that for a new antibiotic to include *K. pneumoniae* in its indication list, a sufficient number of patients must be evaluated who have infections with this pathogen in the lung, urine, blood, and any other body site for which approval is sought. Several years can elapse before sufficient numbers of cases are reported and, therefore, phase III programs require the greatest amount of time and expense from the pharmaceutical sponsor. Many small clinical trials are usually conducted simultaneously in medical centers throughout the world. It is customary for the pharmaceutical sponsor to design the clinical trials in order to gain information about specific indications for the new drug. Hospital-based infectious disease specialists, however, are taking a more active role in designing advanced-stage clinical trials in view of their extensive experience in evaluating previous antibiotic products that often possess some efficacy characteristics similar to those of the new experimental agent (64).

The most meaningful clinical data are obtained from randomized, double-blind, comparative trials in which the performance of the new agent can be evaluated against the standard therapy (64). Since all modern antibiotics demonstrate a high degree of efficacy in humans, other exploitable characteristics of new compounds are frequently considered in clinical trials. These include demonstration of an improved side effect profile, reduced dosing frequency, or reduced cost over existing therapies. In this regard, comparative trials of imipenem versus gentamicin plus clindamycin, for example, have proven the advantage of the single antimicrobial agent over the standard combination used clinically (142). Thus, the recent trend towards development of antibiotics with broad spectrum or long half-life makes single-agent therapy and reduced dosing frequency new parameters to consider in comparative clinical studies that have traditionally compared efficacy only.

The importance of phase III studies in demonstrating the advantages of a new antimicrobial agent cannot be overemphasized. While it is common practice to conduct many small clinical trials throughout the world, this tradition presents a problem of data interpretation, since in some instances the number of patients evaluated for a given clinical indication is too small to provide convincing proof of a clinical advantage over existing therapy. It has been suggested that results from these many small studies be combined into a multicenter collaborative study format, in which a larger number of clinical cases per indication can be evaluated in a more significant manner (64). This is impor-

tant to the pharmaceutical sponsor because clinical trials can take several years to complete and cost tens of millions of dollars. The magnitude of this investment can be appreciated from the developmental time line presented in Fig. 5. This time line can be compared against the traditional 17-year patent life granted to a new compound, which guarantees the pharmaceutical sponsor sole marketing rights to the product. It is easy to see that much of this exclusive lifetime can be consumed by developmental periods during which no income from the new agent is generated.

After completion of the phase II and III studies, the sponsoring pharmaceutical company must organize all of the case report data in preparation for filing these results with the FDA. No predetermined number of studies must be run to qualify a new antibiotic for FDA approval, and the decision to submit a new drug application is based on whether the sponsor, outside clinical investigators, and the FDA agree that a convincing body of safety and efficacy data has been compiled to warrant approval for the indications sought (64). Once this point is reached, a new drug application is compiled and all data must be reviewed by the FDA, a process that takes an average of 21 months for a new antibiotic (54). An effort has been made by the FDA to shorten the development time of new therapeutic agents, including antibiotics. In a published revision of the rules and regulations governing development, the FDA has suggested ways for the pharmaceutical sponsor to prepare and submit higher-quality new drug applications and improve the efficiency with which the FDA reviews them (180).

After approval of the new drug application, the new antibiotic is permitted to be sold in the United States. At this point, a large effort is placed behind organizing the worldwide introduction of the new antimicrobial agent into the marketplace. This includes publication of a symposium (86, 155, 176) that reviews all of the relevant preclinical and clinical studies on the new agent. This type of peer review helps to support efforts to gain hospital formulary acceptance for the new agent. In today's highly competitive antibiotics marketplace, this is not an easy task. In order not to overburden the pharmacy budget of a hospital, acceptance of a new antibiotic onto the formulary often signals the discontinuation of an older agent. This is accomplished with great difficulty unless overwhelming published evidence documents the clinical efficacy, safety, convenience, or cost advantages of the new agent.

It is expected that the total scientific and medical information available describing a new antibiotic supports its use clinically. In this regard, basic information concerning the mechanism of antibacterial activity, human pharmacokinetic profile, and clinical efficacy all contribute to an understanding of the relative advantages of the new antimicrobial agent over existing therapy. The accuracy and thoroughness with which these studies are performed and their results disseminated to clinicians and other health care personnel are important factors in determining how well a new antibiotic will be accepted in this crowded therapeutic marketplace.

The remainder of this review will give an historical perspective on the discovery and development trends observed for certain classes of antibiotics. Some of the clinical and industrial influences that helped determine the direction of new antibiotic discovery and development will be discussed. The improvements made in specific antibiotics that supported their introduction into clinical practice will also be considered.

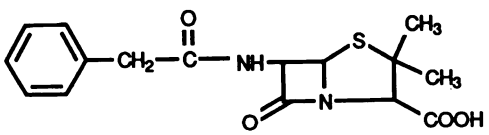
HISTORICAL TRENDS IN ANTIBIOTIC DISCOVERY AND DEVELOPMENT

Discovery of Penicillin G

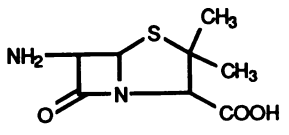
The discovery of penicillin G (Fig. 6) marked the beginning of the modern antibiotic era and served as a model for the development of future agents in at least two ways. First, the isolation in 1957 of the active nucleus of penicillin G, 6-aminopenicillanic acid (6-APA) (Fig. 6), provided a key intermediate for production of a large number of new semisynthetic derivatives. The trend toward making chemical side chain substitutions around an active lead nucleus was subsequently followed for several other classes of beta-lactams, including the cephalosporins, carbapenems, penems, and monobactams. The second way in which penicillin G influenced antibiotic research resided in its ability to promote the spread of bacterial resistance genes that limited its own clinical effectiveness. The development of penicillin G was a result of pure necessity since the treatment of infectious diseases at that time was limited essentially to sulfonamides. Other penicillins, however, were later designed to combat resistance encountered from microbial elaboration of penicillinase.

The penicillins isolated from fermentation broths of *Penicillium chrysogenum* were found to be very active against gram-positive and gram-negative cocci. Upon its initial introduction in the early 1940s, over 85% of *Staphylococcus aureus* isolates were susceptible to <0.1 μg of penicillin G per ml. However, penicillin-resistant staphylococci appeared within 3 years of the widespread use of this antimicrobial agent, and by 1948 up to 50% of hospital strains were resistant (51, 116, 131). This trend continued as the level of resistance rose to 80% by 1957, the year that the structure of 6-APA was elucidated (131). In 1944, Kirby identified the enzymatic mechanism responsible for penicillin resistance in bacteria (90). When other agents such as erythromycin, chloramphenicol, and tetracyclines were chosen more frequently to treat penicillin-resistant staphylococci, bacterial resistance to these agents also increased, limiting their clinical use by the mid-1950s (92, 93). Penicillin G also served as an alternative therapy for infections by *Streptococcus pyogenes* since, by the 1950s, resistance to tetracyclines and sulfonamides was common in this organism (50, 102, 129). Despite the occasional isolation of resistant strains, pulmonary isolates of *Streptococcus pneumoniae* remain susceptible to penicillin G and synergy is still commonly demonstrated with penicillin G, in combination with aminoglycosides, against *Enterococcus faecalis* (43, 50). With the appearance of sporadic sulfonamide resistance in *Neisseria meningitidis* in the 1960s, penicillin G was used empirically in suspected cases of meningitis (43, 116). In the 1940s, isolates of *N. gonorrhoeae* demonstrated resistance to sulfonamides and penicillin G became the drug of choice. By 1975, however, the appearance of penicillinase-producing strains required the use of alternative therapies, including cefoxitin, spectinomycin, and tetracycline (175).

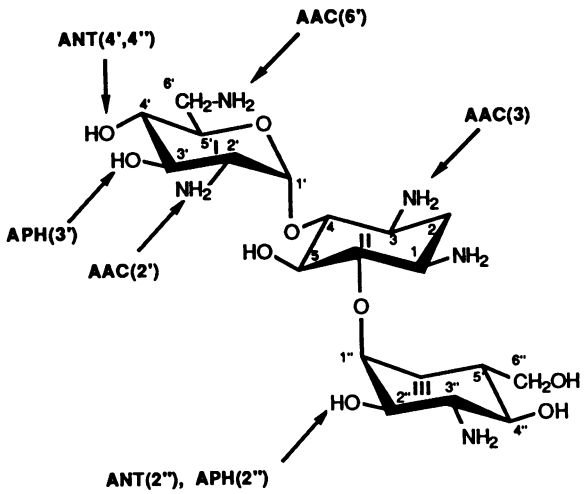
Semisynthesis around 6-APA produced methicillin, oxacillin, nafcillin, and the cloxacillins, and the resistance of these compounds to inactivation by penicillinase earned them a prominent position in the treatment of staphylococcal infections. However, by 1961 the first methicillin-resistant strain of *Staphylococcus aureus* was isolated (84) and became a source of nosocomial infections in Europe in the 1960s and 1970s. This type of resistance was rare in the United States until the late 1970s (75, 163). Methicillin-



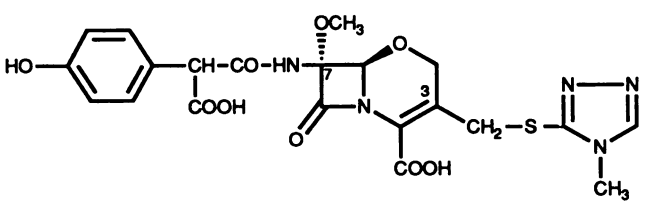
Penicillin G



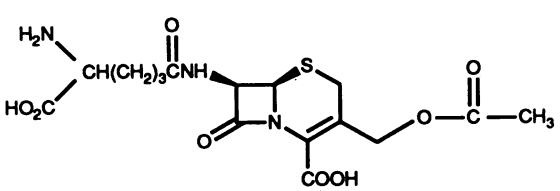
6-aminopenicillanic acid
(6-APA)



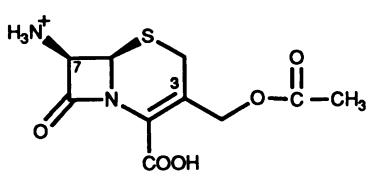
Kanamycin B (shown with sites of modification)



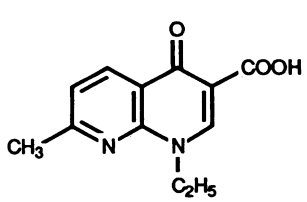
Moxalactam



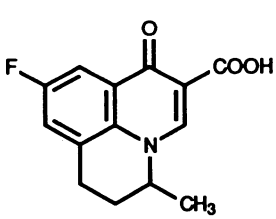
Cephalosporin C



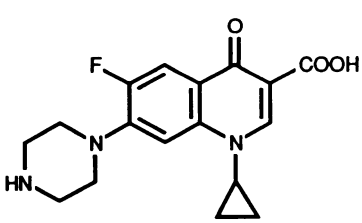
7-Aminocephalosporanic Acid
7-ACA



Nalidixic Acid



Flumequine



Ciprofloxacin

FIG. 6. Chemical structures of some representative antimicrobial agents. Sites of adenylylation (ANT), acetylation (AAC), and phosphorylation (APH) of kanamycin B are indicated.

resistant staphylococci have increased in frequency steadily, yet they remain a nosocomial pathogen primarily at institutions such as large tertiary care hospitals and nursing homes with a large percentage of chronically ill patients (3, 163). Strains that show intrinsic resistance to methicillin are also resistant to other beta-lactams and frequently contain additional resistance determinants for several other classes of antibiotics (3, 163). The mechanism responsible for intrinsic methicillin resistance in staphylococci has been described in several reviews (23, 68, 96, 165) and involves the expression of PBP 2a, which has a lowered affinity for methicillin and other beta-lactams. Due to its activity against these organisms, vancomycin has become the agent of choice for clinical indications involving methicillin-resistant isolates (124).

The development of penicillin for use against susceptible gram-positive and gram-negative cocci was a major advance in the treatment of infectious diseases when physicians had few agents from which to choose. Its broad use has been limited by the transfer among these pathogens of plasmid-encoded penicillinases, a prime example of how quickly selective pressures from antibiotic use in the hospital can promote the spread of resistance. The trend toward development of resistance to penicillin G prompted research in the area of the penicillinase-stable penicillins and eventually to agents with a broader antibacterial spectrum.

Expanded-Spectrum Penicillins and the Aminoglycosides

A major impact of the discovery of penicillin on the pharmaceutical industry was the subsequent successful use of 6-APA as a nucleus for the synthesis of many other new antibiotics. A natural extension of the synthetic chemistry work with 6-APA involved efforts to expand the spectrum of penicillin to include gram-negative bacilli. This was accomplished in 1961 with the introduction of the semisynthetic analog ampicillin. Ampicillin was effective against many enteric bacilli that did not elaborate high levels of beta-lactamase. Ampicillin also gained favor in the 1960s for the treatment of *Haemophilus influenzae* infections, since concerns over the hematological toxicities of chloramphenicol restricted its use. Plasmid-encoded beta-lactamase in *H. influenzae* was first noted in 1974, however (7, 164), and by 1976 between 2 and 15% of isolates worldwide were resistant to ampicillin by this mechanism (47, 116, 144).

The spectrum of penicillin derivatives was further expanded to include *P. aeruginosa* when carbenicillin was introduced in 1967 (131). This was followed by ticarcillin, and the novel ureido penicillins azlocillin, mezlocillin, and piperacillin were introduced in 1981. All of these agents had an extended spectrum of activity over ampicillin and carbenicillin, with piperacillin being the most potent agent against *P. aeruginosa* (45, 46, 72, 82, 108). Thus, the trend for discovering penicillin derivatives up to this point has been to determine SAR that lead to development of molecules that are resistant to inactivation by beta-lactamases and that penetrate the outer membrane surrounding gram-negative cells. The trend toward development of broader-spectrum agents has continued with the aminoglycosides.

The aminoglycoside (aminocyclitol) antibiotics have had a major impact on the treatment of infectious diseases. Most clinically useful members of this class belong to the 4,6-disubstituted deoxystreptamine antibiotics of the kanamycin and gentamicin group (37) and are produced by members of the genus *Streptomyces*. Two of the best semisynthetic derivatives, amikacin and netilmicin, have been widely used clinically. The initial efforts in aminoglycoside research were directed toward discovery of potent compounds. In 1944, streptomycin was the first such agent (introduced for the treatment of tuberculosis) and it was followed by kanamycin in the 1950s, gentamicin in 1963, and tobramycin in 1968, all of which were used predominantly to treat serious infections with gram-negative bacilli (21, 37, 38, 172). A number of factors have limited the introduction of new aminoglycosides, including their inherent potential to cause ototoxicity and nephrotoxicity. A large number of modifying enzymes have also been identified that spread among clinical isolates, limiting the antibacterial potency of these agents (35, 36, 81, 106, 126). Aminoglycosides can be inactivated by more than 12 different classes of bacterial enzymes that can modify a reactive hydroxyl or amino group by phosphorylation, acetylation, or adenylylation (Fig. 6). The 2″-adenylyltransferase, 6′-acetyltransferase, and 3-acetyltransferase enzymes are particularly prevalent among clinical strains in the United States (18). With the rapid development of resistance to kanamycin that occurred in the late 1960s, this agent was replaced by gentamicin for empiric therapy of serious infections (44, 55). The discovery of the aminoglycoside-modifying enzymes changed the direction of aminoglycoside research after the early 1960s. The development of new aminoglycosides followed a pattern aimed at identifying SAR that blocked susceptible sites for enzymatic modification on the molecule while retaining the acceptable safety profile of the earlier agents (9, 17). While many new analogs were tested clinically (33, 126, 138), amikacin remains one of the most useful due to the relatively infrequent occurrence of resistance encountered (13, 107). Amikacin use was often restricted in many hospitals in hopes of preventing the emergence of resistant strains, but its use for empiric therapy in combination with beta-lactams has been widely adopted in centers where resistance to the older aminoglycosides is common.

The effort behind continued aminoglycoside development has largely been shifted to other classes of antimicrobial agents perceived to have a better safety profile and broader spectrum of activity against susceptible organisms. Despite the discovery of some new agents, it is generally believed that the incentive for research and development of aminoglycosides has reached its end (126).

Cephalosporins, Carbapenems, Penems, and Monobactams

Unquestionably, the cephalosporins have taken a preeminent position in the research and development efforts of the pharmaceutical industry over the past two decades. The discovery of the fungus *Cephalosporium acremonium* by Brotzu in 1945 enabled Abraham to isolate cephalosporin C in 1953 (1, 2). The rapid discovery of new semisynthetic derivatives of cephalosporin C (Fig. 6) was in a large part a result of previous synthetic chemical expertise gained with 6-APA. As pointed out by G. N. Rolinson (131), the removal of the side chain from cephalosporin C was necessary to produce the nucleus 7-aminocephalosporanic acid, the commercial starting material for synthesis of the semisynthetic cephalosporins (Fig. 6). Since chemical removal of the side chain was a difficult task in the early days of cephalosporin research, Rolinson credits the earlier success with 6-APA analogs as the motivation behind the cephalosporin effort (131). This early work laid the foundation for the discovery of an unprecedented number of structurally novel and clinically useful cephalosporins. The relatively broad spectrum and excellent safety characteristics of cephalosporins, as compared with aminoglycosides, led to chemical exploita-

tion of the two modifiable substituent positions on the 7-aminocephalosporanic acid nucleus.

The first semisynthetic cephalosporin developed for clinical use was cephalothin in 1962, followed 2 years later by cephaloridine. These compounds were followed by agents that were better tolerated upon injection, such as cefazolin, cephapirin, and cephacetrile, and the orally absorbed agents cephalexin, cefaclor, and cefadroxil. A major thrust of cephalosporin research was then aimed at expanding their antibacterial spectra by increasing their resistance to hydrolysis by beta-lactamase. The broader-spectrum compounds such as cefamandole, cefuroxime, and cefonicid possessed greater activity against the members of the family *Enterobacteriaceae* but often lost some activity against gram-positive organisms (24, 137). Perhaps the best representative of these agents was the novel cephamycin derivative cefoxitin (28, 89, 113, 114, 136, 153). The improved resistance of this compound to beta-lactamase and the timing of its introduction into the marketplace established it as one of the most successful of the broader-spectrum agents. A similar cephamycin analog, cefotetan, possesses greater potency than cefoxitin against isolates of *Enterobacter cloacae* and *Citrobacter freundii*, although it is less potent against some gram-positive bacteria (118).

The expanded-spectrum cephalosporins were distinguished from the earlier compounds based on the inclusion of *P. aeruginosa* in their activity spectra (137). A great number of SAR have been determined with expanded-spectrum cephalosporins, and these have been covered in several reviews (56, 117, 118, 137). Cefotaxime and cefoperazone were among the first expanded-spectrum cephalosporins tested clinically, cefotaxime having a relatively high resistance to beta-lactamases and cefoperazone characterized by its good activity against *P. aeruginosa* (24, 85, 115, 147, 170). In addition, the twice-daily dosing frequency achievable for cefoperazone presented an economic advantage over competitor compounds which required administration every 6 h (170). This improved dosing schedule occurred at a time when cost containment was becoming a major issue in health care. Another expanded-spectrum cephalosporin, moxalactam (Fig. 6), is a structurally unique 1-oxocephalosporin containing an oxygen in place of sulfur at position 1 of the cephalosporin nucleus. This substitution confers high beta-lactamase resistance on the molecule (117). Moxalactam use, however, was complicated by a relatively high incidence of a vitamin K-responsive coagulopathy, and this halted its use in the United States (88).

Unlike the case with many of the preceding classes of antimicrobial agents, bacterial resistance to the expanded-spectrum cephalosporins has not occurred to a high degree. This is due in part to their high affinity for lethal PBPs in the bacterial cell and their comparatively high resistance to inactivation by a broad variety of beta-lactamases. Problem organisms, however, include *Enterobacter* sp., *Citrobacter* sp., *Serratia marcescens*, and certain indole-positive *Proteus* sp., all of which produce chromosomally mediated cephalosporinase whose expression is under an inducible form of genetic regulation (70, 94, 95, 135, 160). Under certain laboratory (and clinical) conditions, mutants that constitutively produce high levels of beta-lactamase can be selected upon exposure of the cells to cephalosporins (31, 69, 71). The enzyme hydrolyzes the cephalosporin beta-lactam ring or simply binds the drug, impeding its progress across the periplasmic space to the lethal PBPs on the cytoplasmic membrane surface (67, 166). Such mutants have been associated with clinical failure of cephalosporins, but the overall incidence of associated clinical failure has been low in comparison to the spread of plasmid-mediated forms of resistance observed with penicillin G (50, 116). Certain isolates of *Pseudomonas* sp. have also remained refractory to therapy with the newer cephalosporins, and this has been attributed to high beta-lactamase production, decreased outer membrane permeability, or lowered affinity for lethal PBPs in the cell (67).

In the 1980s, the developmental trend for new cephalosporins has been directed toward improving their pharmacokinetic performance as much as increasing their potency against problem pathogens. Ceftazidime has demonstrated an increased potency against *P. aeruginosa* compared with cefotaxime and, like cefoperazone, has the additional economic advantage of twice-daily dosing (118). The development of ceftriaxone further capitalized on this advantage, requiring only a once-daily dosing frequency as a result of its 6- to 8-h elimination half-life in humans (118). This advantage allows for ease of administration in the hospital and encourages its use in outpatient settings for the treatment of chronic infections such as osteomyelitis. In these situations substantial cost savings to patients are achieved, giving ceftriaxone a significant competitive marketing advantage, an important factor in the current atmosphere of cost control legislated by the diagnosis-related-group system of health care payment. The advantage of cost savings is an important one because expanded-spectrum cephalosporins are expensive to synthesize. For this reason, they must show a significant advantage in a crowded marketplace in order to gain acceptance on the hospital formulary. Research and discovery efforts for new cephalosporin agents have been guided by this objective. In this sense, some of the emphasis in recent years has changed from finding compounds active against resistant organisms to finding those with improved pharmacokinetics and convenient dosing characteristics that distinguish them from other therapies. The change is also in response to economic pressures to reduce the costs associated with antibiotic therapy.

Other research approaches have been to identify new compounds that could compete with the cephalosporins for certain therapeutic indications. Beta-lactamase inhibitors have been discovered that are active primarily against the TEM-type, plasmid-encoded beta-lactamases (22, 83, 130). Clavulanic acid and sulbactam are the most notable examples that have been introduced to improve the activity of amoxicillin and ampicillin, respectively, when used in combination against strains possessing plasmid-encoded beta-lactamases (22). Monobactams are monocyclic beta-lactams that possess activity predominantly against gram-negative organisms, including *P. aeruginosa* (156, 159). The first of these agents, aztreonam, has proven to be a safe and efficacious agent in clinical studies. A number of new oral cephalosporins and orally active prodrug esters of parenteral agents show an improved spectrum or pharmacokinetic characteristics over cephalexin and cefaclor (168). These include BMY-28271, cefixime, FK-482, cefpodoxime, and the carbacephem analog of cefaclor (168).

Some of the broadest-spectrum antibiotics have been derived from the carbapenem and penem structural classes. Penems are totally synthetic beta-lactams, while the carbapenems were derived from fermentation products, with thienamycin being the first member of the group identified (86, 151, 182). Unlike carbapenems, the penems discovered to date lack activity against *P. aeruginosa*. Carbapenems such as imipenem also have an advantage over expanded-spectrum cephalosporins in that they demonstrate high ac-

tivity against staphylococci, anaerobes, and almost all gram-negative bacilli, including *P. aeruginosa* (5, 86, 167). The broad spectrum and potency are unprecedented compared with any other class of antibiotics, leading to broad clinical indications for imipenem (5, 169). Several penem compounds have undergone clinical trials, but none has had the wide spectrum of imipenem. For reasons relating to unacceptable side effects, the remaining penems evaluated so far have not progressed to the marketplace (119, 122, 128, 182). One of the drawbacks of carbapenems and penems is their unique susceptibility to degradation by eucaryotic renal dehydropeptidase I found in the brush borders of the kidney (86). As described previously, imipenem was found to be so susceptible to dehydropeptidase I inactivation that an inhibitor of this enzyme, cilastatin, was developed for coadministration in order to stabilize the urinary metabolism of imipenem (86). While the combination of imipenem-cilastatin has been widely used, research continues in an attempt to identify new agents of this class, such as SM-7338, that are potent antibacterial agents and have the added advantage of being resistant to destruction by the renal enzyme dehydropeptidase I.

The penems have also demonstrated another highly desirable characteristic, oral absorption. SCH-29482, FCE-22891, and SUN-5555 have shown relatively good oral absorption in humans (74, 168, 182). This characteristic could result in a dual mode of dosing for these agents: an intravenous formulation for treating serious infections in the hospital and a less expensive oral formulation to be used as follow-up therapy once clinical improvement has occurred. While the first oral penem, SCH-29482, has not been developed for clinical use, it remains to be determined whether or not other agents of this interesting class will become successful products.

Quinolones

Quinolones are derivatives of nalidixic acid, which was first introduced in the 1960s. Historically, nalidixic acid (Fig. 6) has been reserved for use only in urinary tract infections due to its poor tissue distribution and the rapid emergence of bacterial resistance (59, 101). Many new fluoroquinolone derivatives of nalidixic acid have been developed, and these compounds possess much greater antibacterial potency than the original compound. The primary target of the new fluoroquinolones in the bacterial cell is the A subunit of DNA gyrase; inhibition of gyrase function elicits a rapid bactericidal effect accompanied by induction of DNA repair responses (8, 29, 125, 148). Since the A subunit of bacterial gyrase is a unique site of attack for these antimicrobial agents, many resistance mechanisms that have limited the susceptibility of bacteria to other agents do not affect susceptibility to quinolones. High-level resistance to the new quinolones has been observed but not with the same frequency as is characteristic for nalidixic acid (49). These desirable characteristics, along with good oral absorption properties, have prompted continued efforts in the areas of synthetic quinolone research. Derivatives such as oxolinic acid, cinoxacin, and flumequine followed nalidixic acid but lacked sufficient antibacterial potency to be viewed as significant therapeutic advances. Flumequine (Fig. 6) was a breakthrough compound in synthesis efforts because it was the first analog to contain a fluorine in the quinolone nucleus. This change was found to impart improved spectrum and potency (174) and furthered efforts such as addition of active substituents (e.g., the piperazine moiety) on the fluoroquinolone nucleus, which in turn led to the discovery of the broader-spectrum agents in development today (30). Norfloxacin has been introduced recently as an orally active agent for urinary tract infections, but lacks the potency necessary for the treatment of serious systemic infections (176, 177). More potent compounds such as fleroxacin, ofloxacin, enoxacin, and ciprofloxacin are markedly improved over the earlier quinolones and meet the criteria required for therapy of serious infections, including bacteremia, osteomyelitis, and pneumonia, as well as *P. aeruginosa* in cystic fibrosis patients (30, 40, 41, 171). Perhaps the most useful property of the quinolones is their good oral absorption in humans. Ciprofloxacin is the broadest-spectrum, orally administered antibacterial agent developed. This compound fills a need for oral outpatient therapy of osteomyelitis and pulmonary infections associated with cystic fibrosis, as well as many other indications, and is available worldwide.

Even though a large number of fluoroquinolones have been synthesized and tested, the SAR needed for optimal antibacterial potency are not as well established as for other antibiotic classes such as the beta-lactams (30). In fact, the specific interaction among DNA, gyrase, and quinolone that leads to bacterial cell death is poorly understood. Despite these limitations, however, the quinolones represent an evolving area of synthesis research that is expected to produce improved broad-spectrum antibacterial agents able to treat a wide variety of bacterial infections.

CONCLUSION

Over the last two decades, the introduction of many new antibiotics has drastically changed the available therapy for infectious diseases. Early trends in antimicrobial discovery and development were directed toward finding agents with improved activity and an expanded spectrum, including bacterial strains that had developed resistance to the older antimicrobial agents. The development of some classes such as the beta-lactams and aminoglycosides occurred at a rapid rate, characterized by the marketing of a large number of compounds that represented incremental improvements over existing therapies. Further understanding of bacterial physiology aided the development of SAR that produced compounds with high antibacterial activity and that were not as affected by the multitude of resistance mechanisms found in clinical microorganisms. More recent trends in antibiotic discovery and development have included placing greater emphasis on finding efficacious new agents with pharmacokinetic advantages that can be administered in ways that help to address the rising costs of health care. The development process for such new antibiotics takes many years and involves extensive safety and efficacy studies in humans and other animals. While this situation is not likely to change significantly, more extensive collaboration between the FDA and pharmaceutical sponsors may help to shorten the development time for new antibiotics that offer important therapeutic advantages. Such agents are likely to be those that are well tolerated and possess improvements in areas that are not covered well by existing therapies. New agents of this type would include those that are efficacious in chronically ill individuals such as immunocompromised patients and possess potent antibacterial activity against a wide variety of clinical pathogens. Although many effective antibiotics are available, the constant evolution of bacterial pathogens and the changing nature of health care needs will create opportunities for significantly improved agents.

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