

## MINIREVIEW

# Animal Models in the Evaluation of Antimicrobial Agents

O. ZAK\* AND T. O'REILLY

*Pharmaceutical Research Department, CIBA-GEIGY Limited, CH-4002 Basel, Switzerland*

### INTRODUCTION

The testing of potential antimicrobial agents in animal models of infectious disease is a long-established practice and is acknowledged as an essential prerequisite of anti-infective therapy, but the ultimate utility of such experiments in the development of antimicrobial agents is still debated. Although there are guidelines specifying appropriate models and relevant parameters for evaluating the safety of antibiotics, no clear guidelines exist for the evaluation of their efficacies in animals. This deficit appears to reflect the recognized difficulties in the design and interpretation of such experiments.

Animal models of infectious disease bridge the gap between the *in vitro* characterization and the clinical evaluation of antimicrobial agents. In contrast to *in vitro* testing, which determines the inherent susceptibilities of microorganisms to chemical agents (7), *in vivo* testing places antimicrobial agents into a realm where their *in vitro* activities are altered by a variety of host factors, most critically by metabolic processes and anti-infective defense mechanisms. The majority of agents active *in vitro* are devoid of significant activity *in vivo*, and while some may also exert demonstrable effects *in vivo*, the correlation is generally considered poor (49). Although the final judgment of the efficacies of antimicrobial agents must depend on the results of controlled clinical trials, the findings made in experimental models can permit reasonably accurate predictions of clinical efficacy, provided that the limitations of these models are clearly realized. This review outlines the role that animal experimentation can play in the evaluation of antimicrobial (largely antibacterial) therapy, with emphasis on the assessment of efficacy, and indicates the restrictions, which, if ignored, will compromise the conclusions that are reached. Experimental details already dealt with in previous, more extensive reviews (1, 3, 7, 20, 21, 28, 31-33, 39, 45-52) are omitted.

### ETHICAL ISSUES

The ethical issues involved in animal experimentation have been treated comprehensively elsewhere (e.g., see references 5, 9, and 48) and need no more than a brief mention here.

At present, two positions are developing and are apparently leading to intense and impassioned conflict. Doctors, scientists in general, and a majority of the public are in favor of the greater stringency regarding the demonstration of the efficacy and safety of new antibiotics that is being demanded by drug regulatory agencies worldwide, even though such demands require more extensive experimentation on ani-

mals. On the other hand, condemnation of the use of animals in any sphere of medical research is growing not only among the public but also within the scientific community. Although these views are fundamentally irreconcilable, ideally the two opposing pressures should converge to foster the development of improved models for evaluating antibiotic efficacy that will require fewer animals to give clear indications. At present, the interests of both parties can best be satisfied if experiments are planned and executed with meticulous care, in full awareness of the limitations of each infection model used, and with special attention to statistical requirements, which will dictate the minimum number of animals needed to yield unambiguous conclusions.

### ANIMAL MODELS IN THE EVALUATION OF THE TOXICITY OF ANTIMICROBIAL AGENTS

Studies with animals to determine the toxicity of antibiotics must be performed to ensure their innocuousness, and guidelines that describe relevant parameters and the interpretation of the results obtained are available (e.g., see references 2, 8, 34, 43, and 48). These studies are usually carried out in stages, preceding each successive phase in the clinical evaluation of new drugs (19). Clearly, the limitations of these experiments must also be considered in order to assess the toxicity of new anti-infective agents accurately. Differences in pharmacokinetic profiles, drug metabolism, the susceptibilities of nontarget bacterial flora, and anatomy between humans and animals can lead to incorrect predictions of antibiotic toxicity in humans on the basis of data obtained from toxicity studies with animals (see reference 48). Despite these limitations, toxicity testing in animals can give clear indications of possible short-term and long-term toxic effects and the maximum tolerable doses, and is thus a prerequisite to clinical trials.

### ANIMAL MODELS IN THE EVALUATION OF THE EFFICACIES OF ANTIMICROBIAL AGENTS

For the demonstration of the efficacies of antimicrobial agents *in vivo*, in contrast to the demonstration of their toxicity *in vivo*, there are no detailed or definitive guidelines (19, 42, 48). Those published by the World Health Organization concerning the preclinical testing of antibacterial drugs (42) merely state that "the behavior of the substances in infected experimental animals can give some guidance to the future possible effects in man. The data usually available comprise: results obtained in acute experimental infections with fatal outcome to establish the ability of the agent to control the infection *in vivo*; and results of experiments which mimic the infection in man to evaluate the possible therapeutic role of the agent in man; models of meningitis,

\* Corresponding author.

endocarditis, renal infection and abdominal sepsis may be relevant in some instances." However, no specifications regarding the design, execution, or interpretation of such experiments are given. Perhaps the lack of stringent guidelines reflects the awareness of the limitations of the available models, the difficulty of interpreting the results obtained, and the high degree of interlaboratory variation in the data derived from such experiments.

Nonetheless, it is expected that the efficacy of any new antimicrobial agent will have been demonstrated *in vivo*. Models used to demonstrate efficacy can be classified according to the nature of the infection, as follows: basic antimicrobial screening models, *ex vivo* models; monoparametric models, and discriminative models (45, 49).

**Basic antimicrobial screening models.** The model most commonly used in evaluating antimicrobial agents is systemic infection in mice. This model lends itself to the routine evaluation of antimicrobial agents, since it satisfies the following principal requirements established by O'Grady (33): simple one-step infection and simple technique and regimen of treatment, short duration of the experiment (especially to minimize environmental influences), reproducible course of infection and results of therapy, and results that are readily amenable to evaluation, preferably on the basis of a single "all-or-nothing" parameter.

This category of models also includes the thigh lesion test, intra-abdominal sepsis originating from the large-bowel flora, and meningitis or pneumonia in mice (45). Infection is usually induced with an inoculum that ensures death (or thigh swelling) in all the animals, which are treated according to a standard regimen usually initiated at the time of infection or soon afterward. Therefore, these models do not accurately evaluate the *therapeutic* efficacy of antibiotics; as in clinical practice, antibiotics are usually administered to combat established infections most likely originating from low inocula. The ability of various doses of the agents to promote or prolong survival (or reduce thigh swelling) in infected animals is determined, and the estimated median effective dose (in milligrams per kilogram of body weight), the dose that protects 50% of the animals from death (or that inhibits thigh swelling), can be taken as a basis for comparing the effectiveness of various agents. These models are routinely used for the early evaluation of antibiotics because they have several advantages (28, 31, 45, 49): the techniques of infection and treatment are simple, and the endpoint is clearly defined; they are economical in the amount of substance required, the duration of treatment, and the overall costs; administration by various routes can indicate the relative oral and parenteral efficacies of an agent or its ability to reach infected sites located distally from the site of injection; and parallel treatment of uninfected animals gives an indication of the toxicity of the test compound.

The capacity of these models to predict clinical efficacy, however, is compromised by several inherent limitations, usually representing discrepancies between the experimentally simulated and the naturally occurring disease in humans (31, 45, 49, 52). (i) In these models, the progress of the infection is uncharacteristically fulminant; (ii) owing to the rapid course of the disease, antibiotics are usually administered at the time of infection, and the results therefore more closely represent prophylactic rather than therapeutic efficacy; (iii) these models are highly sensitive to the size of the infective inoculum, which can result in false-positive and false-negative efficacies; (iv) pharmacokinetic differences, compounded by the use of only a single or a few doses, can

strongly affect the outcome and often preclude accurate comparisons between agents; and (v) at least when mice are used, careful standardization of the animals is required, because strain (and even supplier), sex, and age all appear to affect susceptibility to infection, which, in turn, influences the efficacies of antimicrobial agents. Despite these limitations, these basic screening models give rough indications of the potential of a new anti-infective agent with respect to efficacy, optimal routes of administration, and toxicity; indeed, it is on the basis of such results that decisions regarding the viabilities of new antibiotics are often made.

**Ex vivo models.** *Ex vivo* models use a foreign body, usually implanted subcutaneously, which is subsequently infected with bacteria prior to treatment of the animal. These bodies, or the fluid accumulated in them, can be sampled and a variety of measurements can be made by using this material *ex vivo*. Implanted fibrin clots or dialysis sacks constitute bodies that permit the diffusion of the antibiotic into the site of infection but that restrict the entry of cellular and humoral components of the host defense systems. Implantation of a porous, hollow device, usually subcutaneously, results in encapsulation by granulomatous or fibrous tissue and the cavity being filled with an exudatelike fluid. Such an implant permits both diffusion of antibiotics and the entry of phagocytes and antibodies and thus affords an opportunity to examine the effectiveness of antibiotics together with host defenses. Such models may be valuable for determining the capacities of antibiotics to penetrate a specific site of infection, the rate of killing of bacteria, and the effects of the anti-infective agent on the physiology and morphology of bacteria, as well as whether selection of resistance can occur.

**Monoparametric models.** The broad classification of monoparametric models refers to those in which a single indicator of antibiotic effectiveness is measured, as opposed to the assessment of an ultimate therapeutic cure. Determinations of bacterial counts in tissues or of the concentrations of antibiotics in tissues (6) are typical examples. Although these models can be similar to the basic screening models, they are usually more complicated experimentally and, therefore, are used at a later stage in the evaluation of a compound and should be exploited to assess likely indications for antibiotics, for example, the penetration and killing effects of antimicrobial agents in soft tissues. Evaluation of the capacities of antimicrobial agents to sterilize infected tissues (for example, murine *Candida albicans* kidney infections) is particularly critical for the development of agents effective against chronic infections. Given the pressures to reduce the use of experimental animals, a judicious choice of models should facilitate multiple comparisons of efficacy by using a single animal; therefore, whenever possible, monoparametric models should give way to polyparametric models.

**Discriminative models.** Discriminative models are the most technically complicated models and are designed to mimic the initiation and progress of infection in humans. In these models, multiple parameters of efficacy are measured to ascertain whether an antimicrobial agent is suitable for the treatment of a particular indication in humans. Such models are also used to test the validity of new therapeutic strategies, such as novel drug combinations, dosage regimens, and adjunctive therapies with anti-inflammatory or immunostimulatory agents, etc. Although many criteria for an ideal discriminative model have been described (21), it is hardly possible to meet all of them. Despite that, experimental

studies with discriminative models have delineated effective therapeutic strategies now adopted clinically, for example, (i) the combination of rifampin with vancomycin or isoazolyl penicillin for the treatment of osteomyelitis (32, 47, 48), (ii) the combination of  $\beta$ -lactam and aminoglycoside antibiotics for the treatment of endocarditis (12, 17, 39), and (iii) adjunctive dexamethasone therapy for bacterial meningitis (18, 23–26, 37, 38). Tunkel and Scheld (40) have provided a comprehensive review of the contributions made by animal models to the therapy of meningitis and endocarditis in humans.

#### PHARMACOKINETIC PARAMETERS IN ANIMAL MODELS

Perhaps the major drawback in the evaluation of anti-infective agents in animal models is the pharmacokinetic dissimilarities between humans and animals. The most prominent feature is that small animals eliminate compounds faster (4, 11, 30). However, if these differences are realized, either they can be exploited in studies designed to discern principles of antibiotic therapy or the model can be modified to present pharmacokinetics that mimic those in humans.

Comparison of the pharmacokinetic profiles of drugs determined in both humans and animals has led to the development of procedures for predicting pharmacokinetics in humans on the basis of data obtained from animals (4, 11, 30, 36) and should also lead to the elucidation of structure-activity relationships. Given the recent trend among clinicians to prefer antibiotics with long half-lives in serum (19), the elaboration of reliable predictors of pharmacokinetics will be useful in the selection of antibiotics for further development. However, considering recent proposals that the pharmacokinetics of free antibiotic in serum (antibiotic not bound to serum proteins) afford a more realistic indicator of efficacy in humans (13, 14), such scaling protocols must be viewed with caution, owing to the sometimes dramatic differences in the serum protein binding of antibiotics between animals and humans (49).

Repeated fractional dosing (10, 15, 16, 22, 27, 35, 44) or continuous infusion (29, 41) to obtain serum drug concentrations or pharmacokinetics that mimic those in humans have been used to override the faster elimination of antibiotics in animals; however, the advantages of these approaches are limited insofar as they presuppose a knowledge of the pharmacokinetic profiles in humans. They are still useful for the evaluation of compounds with unknown pharmacokinetics when the drugs are administered in a regimen similar to the regimen used for the administration of standard compounds. Nevertheless, the exploitation of faster elimination profiles has made it possible to establish therapeutic principles by using animal models. Fractional dosing of  $\beta$ -lactams (and erythromycin), which prolongs the levels in serum, is superior to single bolus dosing, which results in a sharp peak of antibiotic in serum, because the time during which concentrations in serum are above the MIC appears to be a key factor governing the efficacies of  $\beta$ -lactams (and erythromycin) *in vivo* (10, 15, 16, 27, 44). By contrast, bolus dosing of aminoglycosides is superior to fractional dosing, because the *in vivo* efficacy of this class of antibiotics is influenced by the peak concentrations in serum and log[area under the concentration-time curve] pharmacokinetic parameters (10, 15, 16, 27, 44).

Lastly, such pharmacokinetics-related differences between classes of antibiotics emphasize the need for careful comparison between different groups of agents. Because the

maximal efficacies of  $\beta$ -lactams are associated with prolonged supra-MICs in serum, and the maximal efficacies of aminoglycosides are associated with the peak level and total exposure to the drug (the area under the concentration-time curve), the dosage scheme used in several basic screening models (one or two doses soon after infection) will bias the results such that the potential efficacies of aminoglycosides are overestimated and the efficacies of  $\beta$ -lactams are underestimated (27, 44). Clearly, in the comparative evaluation of anti-infective agents, careful consideration of pharmacokinetic differences and the establishment of optimal dosing strategies appear to be of critical importance for obtaining reliable results.

#### CONCLUSIONS

The evaluation of antimicrobial agents in animals has been, and will continue to be, a necessary part of the development of new agents and optimal therapeutic strategies. Although guidelines for the assessment of toxicity are available, inherent limitations of animal models of infectious diseases have so far precluded the formulation of guidelines for testing the efficacies of antimicrobial agents. Each class of models has its drawbacks, but if the limitations are realized and the questions proposed are ones that the model can answer, the data obtained can be reliably interpreted.

As knowledge of the efficacies of antibiotics in both animals and humans grows, it can be applied to develop improved models of infectious disease, inspire greater confidence in the prediction of the efficacies of new antibiotics or therapeutic regimens. The availability of such models will help to resolve the two diametrically opposed ethical problems facing experimenters who use animals for the evaluation of antimicrobial agents: the need to reduce the number of animals used and the need to ensure more reliable preclinical evaluation of the efficacies of antimicrobial agents.

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