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Animal models in the pharmacokinetic/pharmacodynamic evaluation of antimicrobial agents

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ABSTRACT

Animal infection models in the pharmacokinetic/pharmacodynamic (PK/PD) evaluation of antimicrobial therapy serve an important role in preclinical assessments of new antibiotics, dosing optimization for those that are clinically approved, and setting or confirming susceptibility breakpoints. The goal of animal model studies is to mimic the infectious diseases seen in humans to allow for robust PK/PD studies to find the optimal drug exposures that lead to therapeutic success. The PK/PD index and target drug exposures obtained in validated animal infection models are critical components in optimizing dosing regimen design in order to maximize efficacy while minimize the cost and duration of clinical trials. This review outlines the key components in animal infection models which have been used extensively in antibiotic discovery and development including PK/PD analyses.

1. Introduction

The pharmacology of antimicrobial therapy can be divided into two distinct components. The first of these components is pharmacokinetics (PK), which examine how the body handle drugs, including absorption, distribution, metabolism and elimination, the other component is pharmacodynamics (PD), which examine the relationship between drug PK, a measure of in vitro potency (usually the minimum inhibitory concentration [MIC]), and the treatment outcome (usually efficacy or sometimes drug toxicity). The time course of antimicrobial activity is a reflection of the interrelationship between PK and PD. PK/PD relationships are vital in facilitating the translation of microbiological activity into clinical situations and ensuring that antibiotics achieve a successful outcome. A large number of studies have indicated that antibiotics can be divided into two major groups (Fig. 1): those that exhibit concentration-dependent killing and prolonged persistent effects (e.g. aminoglycosides, fluoroquinolones), for which the area under the concentration-time curve (AUC) and peak concentration in relation to the MIC of the organism causing the infections (AUC/MIC and Cmax/MIC, respectively) are the major PK/PD indices correlating with efficacy; the other group is those antibiotics that exhibit time-dependent killing and minimal-to-moderate persistent effects (e.g. Beta-lactam and macrolide classes), the time (expressed as a percentage of the dosing interval) that drug concentration exceed the MIC (%T > MIC) is the major parameter determining efficacy. To identify the PK/PD indices most closely associated with efficacy, dose-fractionation studies are used. In such studies, the same total drug exposure is administered using different dosing intervals, for instance, a dose might be delivered as 100 mg once daily or in 4 equally divided doses throughout the day, regardless of dosing interval, each regimen would have identical AUC_{0-24}/MIC values, but different $\%T_{>MIC}$ and C_{max}/MIC values. However in clinical trials, usually only 1 dose and 1 doing interval are studied, making discrimination of the PK/PD linked measured difficult, therefore, we usually rely on animal infection models to determine the PK/PD index (also called PK/PD parameter) and target (i.e. the magnitudes of exposure required to gain certain PD endpoints, e.g. stasis or 1 log killing of pathogens in animals, or 90% chance of clinical effectiveness) that is linked to efficacy. Importantly, available PK/PD data derived from infected patients have shown remarkable concordance between the PK/PD in patients and from animal data.[1] This means that, in many circumstances, we can translate the PK/PD profile from animal models to effective treatment regimens in humans.

Infections caused by antibiotic-resistant bacteria have increased rapidly and new antimicrobial agents are urgently needed. However, the paucity of new antibiotics in the drug

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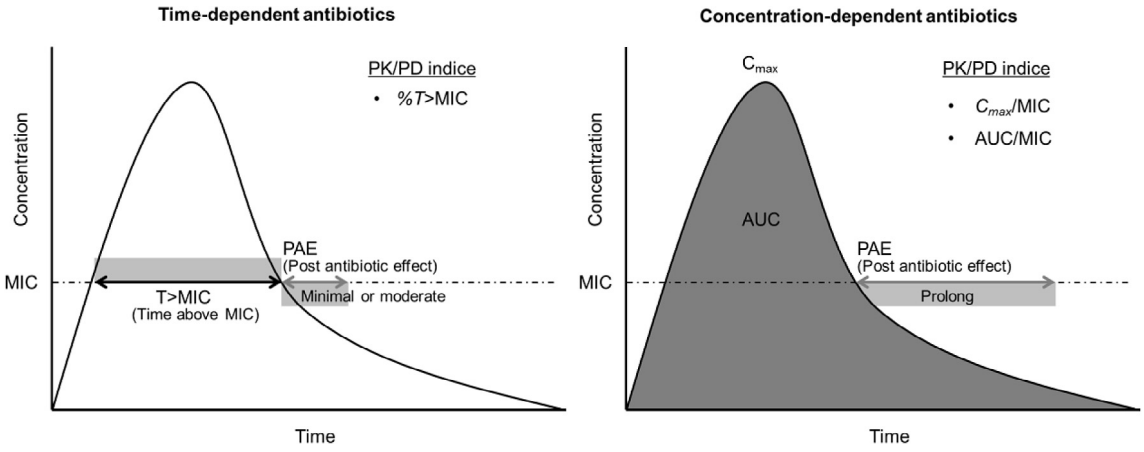

Fig. 1. Principal PK/PD characteristics of antimicrobial drugs.

discovery pipeline is presenting a significant unmet global need.[2] In antibiotic discovery and development, PK/PD evaluation in animal infection models play an essential role in designing the optimal dosing regimen and planning clinical trials, both of which are extremely costly. Identification of PK/PD relationships using animal models in an early discovery stage can lower the attrition rate and provide a tool to enable rational go or no-go decision making. Additionally, for drugs developed to ameliorate or prevent serious or life-threatening infections, when human efficacy studies are not ethical and clinical trials are not feasible, animal models are especially important, FDA may grant marketing approval based on adequate and well-controlled animal efficacy studies.[3]

For these animal models, there are several variables that are taken into consideration. These can include host-specific variables such as the animal species, route of infection, infection site, immune status, end organ/tissue sampling and optimal endpoint measure. Pathogen-specific variables include the genus/species, inoculum size, virulence, and drug-susceptibility. Finally, therapeutic variables include route of drug administration, timing of therapy, dose level, frequency of administration, penetration to the site of infection, metabolism and/or elimination, and duration of therapy. This list of variables may seem challenging; however, carefully controlled animal model studies are the cornerstone of PK/PD therapeutic evaluations that lead to dosing regimen optimization, limiting drug-related toxicity, guiding therapeutic drug monitoring, and setting of drug susceptibility breakpoints. The aim of this paper is to outline these key factors in animal PK/PD models.

2. Pharmacokinetic considerations

Pharmacokinetic (PK) measurements are necessary to ensure an anti-infective agent will be present at sufficient concentrations and microbiologically active at a given site of infection in a mammalian host. The PK characteristics, such as area under the drug concentration curve (AUC) or elimination half-life, of some antibiotics can vary significantly according to the route of administration, formulation, animal species, age, body condition, gender, and physiological status, all of which contribute to differences in drug efficacy.[4] Because of this, the PK properties of antimicrobial agents need to be determined in each animal species used in pre-clinical studies.

2.1. Protein binding

Most drugs bind to proteins such as albumin, α-globulin, β-globulin, and γ-globulin, or other biological materials such as α_1-acid glycoprotein, lipoproteins, and erythrocytes.[5] Thus, free, unbound drug concentration in plasma decreases as the degree of binding to these compounds increases. There are numerous studies that have demonstrated that only the free (unbound) fraction of drug is available for pharmacological activity.[6,7] The presence of infection in animals could potentially impact the free fraction. A representative example is a study which azithromycin tissue concentrations were compared in uninfected and infected tissue in a rat thigh infection model. Greater free drug exposures were observed in the infected animal tissues in comparison to the uninfected animal tissues.[8] Also, the mean azithromycin AUC in the inflammatory blisters was more than 2 times higher than that in the noninflammatory blisters, while the respective AUCs in serum were not significantly different.[9] This may be due to chemotactic drug delivery in infected tissues, as phagocytes migrate to the infection site and increase local drug concentrations by releasing azithromycin from their lysosomes.[10] It has also been shown that the local pH values in the interstitial space fluid can be reduced, due to the metabolic activities of infiltrating neutrophils (anaerobic glycolysis) and infecting pathogens (e.g., production of short-chain fatty acids[11]), which result in a further increase in local interstitial fluid concentrations. The degree of protein binding can vary highly between different species as well, and therefore it is recommended that protein binding be determined in each species of animal used in pre-clinical trials and consideration of whether the infected status might impact this level. The impact of changes in protein binding is especially significant for highly protein bound and high clearance antibiotics, like ertapenem (85–95% protein binding). If the protein binding changes from 90% to 95%, then the free concentration would be assumed to decrease twofold. Conversely, it is possible that severe hypoalbuminemia could result in an increased unbound concentration that is then more rapidly eliminated by supranormal renal clearance. These changes would eventually result in significant lower concentration of ertapenem (0.1 mg/L) than the target concentration (1 mg/L).[12,13] Therefore, protein binding and the changes in binding conditions should be taken into consideration when designing dosing regimens and interpreting PK/PD study results.

2.2. Tissue site

An important consideration in PK studies is which compartment or tissue site is relevant for drug concentration and PK parameter determination. Traditionally, blood stream (i.e. whole blood, serum or plasma) measurements of drug concentration have been utilized; however, there are situations in which

tissue-specific drug concentration measurement is necessary and potentially more informative. This is especially true for sites of infection that are considered sequestered (e.g. brain, CSF, urine, eye, placenta) or for pathogens that are primarily intracellular in nature. Currently, where this occurs most commonly is for drugs being considered for the treatment of pulmonary infections (i.e. pneumonia) which can include measurement of drug concentrations in the epithelial lining fluid (ELF). However, the penetration of drugs into ELF sometimes can sometimes be different in animals and humans. For example, the importance of early examination of tissue penetration in humans and in relevant animal species is shown by the penetration of ceftobiprole into ELF in murine pneumonia. By comparing the AUC_{ELF}:AUC_{serum} of ceftobiprole (0.69) and ceftazidime (0.201), a dose of 1.5 g/day of ceftobiprole was chosen for a phase 3 clinical trial to compare efficacy with ceftazidime (6 g/day) in patients, however the AUC_{ELF}:AUC_{serum} of ceftobiprole in humans was later found to be only 0.153. With comparable ELF penetration and in vitro potency of ceftobiprole and ceftazidime, one can easily predict that the dosage of ceftobiprole would be insufficient.[14] Additionally, penetration to sites of infection can be preferentially different leading to better efficacy based on the specific site of infection. An example of this important finding is the revelation of PD targets that are approximately one half in lung versus thigh infection models for an investigational oxazolidinone based on preferential penetration into ELF.[15,16]

A concern in tissue-specific PK is in the processing of samples for PK measurement. The most common and convenient method of processing tissue samples for drug concentration measurement is tissue homogenization.[7,17] However, tissues have two distinct fluid components consisting of the interstitial and intracellular compartments. When homogenized, these two compartments are irrevocably mixed. Since the intracellular compartment is usually of larger volume, drugs that concentrate more in the interstitial compartment will appear to be much lower in total concentration than drugs that accumulate in the intracellular compartment, this approach underestimates concentrations of drugs that equilibrate predominantly in the interstitial fluid (e.g., beta-lactams and aminoglycosides) and overestimates the concentrations of those that accumulate mostly within cells (e.g., fluoroquinolones and macrolides).[18] The importance of this varies depending upon the pathogen. Most bacterial and fungal pathogens produce disease predominantly in the extracellular space. However, some pathogens, such as *Legionella* spp, reside exclusively or primarily in the intracellular compartment where intracellular drug concentrations should be a more accurate predictor of efficacy.

Several approaches have been used to determine the tissue-specific distribution, for example imaging techniques including single photon emission computed tomography (SPECT), positron emission tomography (PET) and magnetic resonance spectroscopy (MRS), however these techniques fail to differentiate protein bound from unbound drug and fail to identify the exact compartment of the compound within the extracellular fluid or the cells.[19] Microdialysis (MD) is a technique suitable to determine a drug's free concentration in almost any tissue (brain tissue,[20] lung tissue,[21] soft tissue,[22] bile,[23] eye[24] etc.) and has been frequently used in both experimental and clinical PK studies.[19,25] Although MD is currently not required in drug development, it has been recommended by the FDA for the assessment of bioavailability and bioequivalence of topically applied generic drugs.[26]

2.3. Effect of animal species on antibiotic pharmacokinetics

The host animal species can have dramatic effects on the PK of a drug. Smaller mammals such as mice, rats and rabbits often exhibit more rapid metabolism and elimination, and therefore half-lives in these models can be considerably shorter than in larger mammals such as humans.[18,27,28] The impact of route of administration on drug PK can also vary according to animal species and different drugs, for example the $t_{1/2}$ of rifampicin in rats following intravenous administration was 4.7 h, which is shorter than the $t_{1/2}$ (9.3 h) following oral administration, but the same increase in half-life was not evident in mice.[29] However, some drugs are not affected by the route of administration and animal species, for instance, the PK parameters of ceftaroline administered through the intramuscular route in diverse animal species were similar to those observed when the drug was administered intravenously.[30] Finally, even the strain of animal can affect the PK. For example, BALB/c mice and DBA/2 mice display markedly different serum drug concentrations of itraconazole over time.[31]

2.4. Effect of infection on antibiotic pharmacokinetics

The infection process can have a marked effect on the PK of a drug. This could be related to the infection causing changes in the penetration of antibiotics in the physiologic characteristics of the tissue due to the inflammatory process initiated by the host or a change in drug clearance. Infections in lung, bone and central nervous system have been found to alter tissue site penetration, while infections of the bloodstream have been shown to expand the V_d (volume of distribution) and enhance drug clearance.[32] For example, a fourfold increase in cerebrospinal fluid vancomycin levels was reported in animals with meningitis versus healthy controls.[33] Additionally, lung infection was found to produce large discordance between the blood and pulmonary profiles of tedizolid and linezolid.[34] The translatability of pre-clinical animal model PK to patients therefore usually includes both uninfected and infected animal PK to determine if the disease state significantly alters drug PK.

2.5. Effect of animal age

Age can have also exhibit profound effects on drug PK in many mammalian species. However, the clinical applicability of using age-related PK in an animal model and correlating it to age-related PK in a human is limited. For example, plasma PK of five beta-lactam antibiotics are markedly different in neonatal versus adult rats.[35] For example some studies have demonstrated that neonatal rats absorb beta-lactams better than adults following oral administration, and the clearance of these drugs is reduced.[36] However, there is no corollary study in humans (i.e. neonates) to determine if these differences are clinically relevant. When differences do occur in the animal model, for example ertapenem pharmacokinetics change in relation to age,[37] it can provide the stimulus to study the PK in different age groups in humans. However, when age-related differences do not occur in the animal model, it doesn't necessarily indicate that there are not significant clinical differences in drug PK in different aged humans. With this caveat aside, there are examples of age-related changes in antimicrobial PK in animal models.[38–41] In general, drug concentrations are higher in aged animals compared to young animals for a given dose. For example, aged rats (22–24 months) had higher concentrations (C_{max} and AUC) and prolonged elimination rates ($T_{1/2}$) for gentamicin compared to younger rats (2–3 months).[38] Differential rates of metabolism and elimination most likely account for these differences, which are often clinically relevant in humans as well.

2.6. Strategies to mimic human PK in animal models

The two most common strategies to attempt to mimic human PK in an animal model where there is rapid metabolism or clearance of the drug is to either directly alter the clearance/metabolism or provide a means of very rapid drug replenishment by frequent

or continuous dosing systems. Impairment in renal clearance of a drug can result in slower elimination, which is relevant if this is the major clearance organ (e.g. cephalexin[42]). In mice, this has been accomplished by a single subcutaneous injection of uranyl nitrate (10 mg/kg) 3 days prior to animal infection.[43] Uranyl nitrate produces acute tubular necrosis and subsequent stable but decreased renal glomerulofiltration for a maximal duration of 7 days.[44,45] For example, Craig and colleagues administered uranyl nitrate to mice receiving amikacin and demonstrated an increased half-life, peak concentration, and AUC for each dose when compared to non-renally impaired mice.[45] The resultant PK parameters and concentration-time curves more appropriately simulated human amikacin PK. Antimicrobial agents actively secreted by renal tubular cells can be competitively blocked by other compounds that utilize the same excretion process. An example of this is probenicid, a weak organic acid which blocks the secretion of penicillin and other cephalosporins.[46] A variety of renal impairment mechanisms have also been reported for rats.[42] This includes proximal tubular necrosis induced by cisplatin (one dose at 5 mg/kg IP), papillary necrosis induced by 2-bromoethylamine hydrobromide (one dose at 75 mg/kg IV), glomerulonephritis induced by sodium aurothiomalate (six weekly injections of 0.05 mg/kg IV), and anti-rabbit antibodies to rat glomerular basement membrane (single IV injection).

Continuous dosing of antimicrobials has been utilized to counteract the effect of rapid antimicrobial clearance in small rodents. There are a number of systems that have been utilized including tissue cage infusion,[47] infusion pumps,[48–52] and more recently sophisticated computer programmable pumps.[53] These systems work best from an efficacy standpoint for time-dependent drugs in which the time above MIC is the driving pharmacodynamic index.

3. Immune suppression in the animal model

Animal models of anti-infective therapy often utilize immune suppression. There are several reasons for this model design. First, an un-confounded evaluation of antimicrobial effect can be performed if the immune system is removed or significantly inhibited from affecting the outcome. Therefore, one will get a more robust drug-effect evaluation by removing confounders that will artificially enhance antimicrobial efficacy. Secondly, many animals are inherently resistant to microbes that are pathogens in humans and immune suppression is required to mimic disease in patients. The effects of immune suppression have been explored in a number of studies. As might be expected, in general there is a reduction in the amount of drug necessary to achieve similar microbiological outcome (i.e. net stasis or 1-log kill) in non-neutropenic compared to neutropenic anti-bacterial models.[54] This reduction can be as much as 2- to 4-fold lower but appears to vary dependent upon the drug class and microorganism. A common approach to induce neutropenia is to administer cyclophosphamide (150 mg/kg) 4 days before bacterial inoculation followed by a second dose (100 mg/kg) 1 day before the inoculation. This regimen has been proved to produce profound neutropenia, which could persist for 4 days, with complete resolution by 7 days.[55] Different cyclophosphamide dosing regimens have also been used to study the impact of various degrees of immunosuppression or temporary neutropenia on the PK/PD target.[56]

4. Common animal infection models for antimicrobial PK/PD study

Various different animal models have been used for experimental antibacterial PK/PD study. A description of the most commonly used models is provided in this review. In general, mice and rats are the preferred experimental animals because of their low cost and ease of handling. Virulent bacterial strains are used to develop infections. A high inoculum, immunocompromised animals,[57] and/or adjuvants[58,59] (like mucin or formalin[60]) may be required to produce progressive infection. The time to initiation of antibacterial therapy is dependent on many factors, for example the virulence of the pathogen, the inoculum size, the inherent generation time for the microbial species, and the status of the host immune state,[61] and can vary from 2 to 24 h after initial inoculation.

4.1. Thigh infection model

The rodent thigh lesion model was originally described by Selbie and Simon in 1952[62] and continues to be the work horse for animal model PK/PD antimicrobial efficacy studies. This model is commonly employed in the development of new antimicrobial agents and has been shown to be helpful for predicting efficacy for a number of human applications (e.g. pneumonia, skin and soft tissue infection, intra-abdominal infections and septicemia).[1,63] The model is also attractive as use of two thighs per animal (two biological replicates) limits the total needed for each experiment. Briefly, the model involves intra-muscular injection of an inoculum (10^5–10^8 CFU in a volume of 0.1 mL of broth) into the dorsal thighs of anesthetized mice. Mice are then treated with an antimicrobial agent at 1–2 h after the thigh inoculation for a defined period, euthanized at study endpoint, and CFU enumerated from each thigh. In order to make the data most meaningful, zero-hour control mice are optimal to determine the viable burden at the start of therapy. This allows one to determine whether infectious burden increased, decreased, or remained stable over time. Untreated controls are also necessary to assess fitness of each bacterial strain in the animal model. Most studies utilize a neutropenic mouse model, but immunocompetent animals can also be used to study the impact of leukocytes.[54,64] An experimental thigh infection model has also been described for rats.[65] While CFU determination of pathogen abundance is most commonly performed, novel techniques such as resonance imaging,[66] luminescent bacteria,[65,67] radiolabeled bacteriophages,[68] quantitative PCR, and antigen/antibody testing have been developed for certain pathogens as a means to monitor infectious burden in animals.

4.2. Acute bacterial pneumonia models

Murine models of acute pneumonia are increasingly incorporated into drug development and PK/PD studies for drugs intended for this infection site. The reliance upon these lung infection models has stemmed from the recognition of differential penetration of antimicrobials to the site of bronchopneumonia, the epithelial lining fluid, ELF. Many contemporary lung infection models have been described, including a recent thorough review of murine models to mimic human pneumonia.[69] Important considerations in the models include host immune dysfunction, organism pathogenicity in mice, route of infection, inoculum size, experimental duration, and endpoint (e.g. mortality, organism burden, etc.). Similar to the thigh model, mice are commonly rendered neutropenic.

The route of infection for production of pneumonia includes aerosolization of the inoculum with subsequent inhalation, intranasal instillation with ensuing aspiration, injection into the trachea via percutaneous puncture with a fine needle, or direct instillation into the lungs by tracheal intubation.[69] In general, the animals are anesthetized and inoculated with approximately 10–50 μL (mice and rats) or 0.5 mL (rabbits) of a bacterial suspension (10^5–10^8 CFU). Experiment duration can vary depending on pathogenicity of the infecting organism, but usually doesn't need to be prolonged more than 24–48 h for bacterial pathogens to produce death in

untreated control mice. Finally, determination of organism burden is most commonly performed by quantitative culture techniques (CFU determination). The aforementioned pneumonia model techniques are now increasingly utilized for hospital-/health care-acquired pneumonia pathogens. Representative examples include *Acinetobacter*,[70–79] MRSA,[16,59,80–86] *Pseudomonas aeruginosa*,[87–95] and *Klebsiella pneumoniae*.[96–103]

4.3. Chronic bacterial pneumonia models

Chronic pneumonia usually occurs in the setting of pre-existing lung conditions such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF). These lung diseases produce intermittent obstruction and variable loss of immune function with subsequent risk for chronic or recurrent infection. These pre-existing conditions do not exist in outbred rodents and bacteria commonly involved in these infections are rapidly cleared from the airways in rodents.[104–106] Therefore, strategies to mimic periodic obstruction and prevent bacterial clearance have been developed. To date the most common method used to achieve airway obstruction is the preparation the bacterial inoculum in agarose or alginate beads.[105,107] This method has been employed successfully to yield a persistent *P. aeruginosa* infection in rats for up to 35 days.[105] Genetically modified mouse models have been developed to study disease pathogenesis but to our knowledge have not been utilized for antimicrobial PK/PD investigations.

The pathogen of choice for most chronic pneumonia studies is *P. aeruginosa*. This is clinically applicable as it is not only the most common isolate found in colonization and infection in COPD and CF patients, but is also particularly difficult to treat and has resistance selection is common.[108] Murine models have also been adapted to investigate the impact of antimicrobial therapy in chronic *P. aeruginosa* pneumonia.[109–113] Notably, no single drug treatment has been found to eradicate the infecting organism in this chronic model. For example, Macia and colleagues examined ciprofloxacin and tobramycin monotherapy and combination therapy in a murine model of chronic pneumonia.[110] After exposure to ciprofloxacin, infection with a hypermutable isolate of *P. aeruginosa* resulted in a rapid increase in drug resistant subpopulations. This effect was not observed with tobramycin monotherapy. Finally, the combination of the two drugs appeared synergistic against the hypermutable isolate. Thus this study design appears useful for investigation of PK/PD dosing strategies to prevent the emergence of resistance. Inhalational drug administration is an additional area of investigation garnering more interest for chronic pneumonia.[109,114–118] The advantage of this route of administration method is directly targeting antimicrobial therapy to the site of infection as well as limiting systemic toxicity that can be problematic for certain antimicrobial agents.

4.4. Skin and soft tissue infection models

Animal models have become standard tools for studying external traumatic wound infections and testing new antimicrobial strategies despite of advances in the infections care and management. Numerous skin and soft tissue infection models have been described, including models for wounds, skin abrasion, burns, and abscesses, and a variety of foreign body materials and skin traumas have been used to promote the infection.[119]

To produce a deep infection, the bacteria are commonly injected subcutaneously, using microbeads as abscess promoters.[120] For studying superficial infections the tape-stripping method can be applied.[121] The fur and most of the epidermal layer are removed using elastic adhesive bandage, and the bacteria are added directly on the damaged skin area. It is common that hairless or shaved mice are used in these models. The main factors which determine the severity of the traumatic wound infection model include bacterial inoculum, size of the wound and immune-competence of the animals. The end-point for these models usually includes histopathological examination of sections of lesions and counting of viable bacteria recovered from the inoculation sites to determine the inoculation producing 50% probability of infection (ID50).[122] Methods that utilize in vivo imaging to noninvasively and longitudinally monitor the bacterial burden[123] and real-time monitoring of bacterial infections in vivo using bioluminescent bacteria have also been described.[124] Each of these models should allow PK/PD analysis but thus far have not been commonly used for this purpose.

4.5. Septicemia models

Sepsis is a serious clinical problem involving complex mechanisms and animal models of sepsis have played a major role for in vivo efficacy of numerous antibiotics.[125,126] There have been various animal models of sepsis utilizing different paradigms. Endotoxin, bacterial infusion, cecal ligation and puncture, and colon ascendens stent peritonitis models are the commonly practiced methods at present. However for the assessment of efficacy of novel antibiotics, the most commonly used model is the mouse septicemia model due to the simplicity of the endpoint analysis. The model involves rendering the animal neutropenic and then the animal is infected by an intraperitoneal injection of 0.1–0.5 mL of a log-phase bacterial suspension, sometimes 5% mucin is injected along with the bacteria to produce septicemia.[125] Endpoints following antimicrobial therapy can include mortality (% survival) and bacterial load (CFU) in the blood. This mouse sepsis model is less time consuming and labor intensive due to no requirement of tissue homogenization compared to the thigh infection model.[122]

4.6. Meningitis models

Rabbits are typically used to study experimental meningitis and the model was described originally by Dacey and Sande in 1974[127] and was used for the assessment of efficacy of numerous antibiotics.[128–138] In this experimental infection model, animals are placed in the stereotactic frame and anesthetized intramuscularly with 35 mg/kg of ketamine and 5 mg/kg of xylazine. Meningitis is induced using an intracisternal injection of 0.25 mL of an endotoxin-free saline suspension containing 10^4–10^6 CFU/mL of inoculum. Antibiotic treatment is commonly started 14–18 h after inoculation. An indwelling spinal needle in the cisterna magna and an arterial catheter enable serial CSF and serum sampling to determine bacterial counts. The use of rabbits instead of smaller rodents is to the larger size of these animals which allows for device maintenance and repeated CSF sampling. However, rat meningitis models were have been developed, and may also be useful for assessment of antibiotic therapy.[139]

4.7. Urinary tract infections models

Models of urinary tract infections (UTI) are commonly used to assess antimicrobial efficacy given it is one of the most common infectious diseases of human. Rodents (i.e. mice and rats) are the most common animal model utilized and there are few important factors one needs to consider. First, not all bacteria are inherently pathogenic in the rodent urinary system and therefore in some studies manipulation (i.e. obstruction or direct instillation into renal parenchyma) is necessary. Secondly, vesicoureteral reflux is a naturally occurring phenomenon in rodents due to the lack of ureterovesical valves.[140] In its simplest form, UTI can be induced in rodents by instillation of normally pathogenic microorganisms,

such as 50 μL of *E. coli* (usually 10^7–10^9 CFU), into the bladder by urethral catheterization and clamping the catheter or urethra for a short period of time to prevent immediate inoculation expulsion and promote infection.[141–144] As long as the bacterial strain has necessary virulence factors (i.e. type 1 or type P fimbriae), a UTI with high bacterial counts in the bladder and kidney will ensue over a period of 1–8 days. The endpoints are usually bacterial cultures of bladder and kidney homogenates. Additional parameters monitored may include morbidity and blood cultures, while homogenates of liver and spleen can also be taken to monitor dissemination of the infection outside the urinary tract. This model has been successfully employed to study the antimicrobial efficacy and PK/PD relationships of antimicrobial therapy.[142,145–147]

Urinary obstruction models provide a framework to study pyelonephritis and UTI with organisms that are not intrinsically pathogenic to the rodent urinary systems.[148,149] This model is more technically demanding as it usually requires animal surgery to directly inoculate the bladder with the pathogen and then ligate one ureter to cause an obstructed infection. Antimicrobial efficacy has also been examined using this model although given the complexity there is less robust pharmacodynamic analyses than the unobstructed model.[150–152] Direct instillation of organism into the parenchyma (i.e. poles) of one or both kidneys has also been demonstrated as a means to study antimicrobial efficacy in the urinary system but is also technically demanding.[153–155] A final mechanism that has been utilized is hematogenous seeding of the urinary system to produce UTI.[156,157] Normally, no manipulations of the animals are required, but strain screening for ability to reproducibly colonization kidneys is important. Recent examples of this model have include study with *Enterococcus*, *Staphylococcus*, and *Klebsiella.*[158–161]

4.8. Animal models of infectious endocarditis

Animal models of endocarditis have been utilized for decades to examine optimal antimicrobial therapy for this common life-threatening infection. Additionally, they are perhaps the area of animal model evaluation that has garnered the most translatable clinical applicability.[162–168] The most common animal species utilized are rabbits, although rodent models have also been developed. The infection model itself usually consists of canalization of the right carotid artery with a polyethylene intravenous catheter and advancing it across the aortic valve. This results in the development of sterile vegetations on the aortic valve which can then be colonized/infected with a bacterial inoculum through the catheter. Antibiotic treatment starts 1–2 days after the inoculation.[61] Endpoints in this model include CFU/vegetation and morbidity; blood samples are also collected to test for sepsis and relapse of infection following treatment. This model remains a very important tool in optimizing antimicrobial therapies for endocarditis, especially combination therapy with aminoglycosides and novel combinations for aminoglycoside-resistant Enterococcal endocarditis and methicillin-resistant *Staphylococcus aureus* endocarditis.[169–178]

4.9. Animal models of intraperitoneal infection

Peritoneal infections can be produced by one of two mechanisms: direct inoculation of organism into the peritoneal cavity or by cecal perforation as the result of ligation and puncture (CLP). The former mechanism was initially performed by surgically placing a gelatin incased inoculum into the peritoneal cavity.[179–181] However, more recently this has been simplified by direct inoculation of the inoculum by syringe into the peritoneal cavity to produce an infectious peritonitis.[180–189] To enhance pathogenicity talcum (magnesium hydropolysilicate) or mucin are often utilized in inoculum preparation and serves as a foreign body irritant to promote infection while having no effect on antimicrobial therapy. In this model, a 0.25–1.0 mL saline suspension of organisms (10^4–10^8 CFU) is injected; drug treatment is initiated 1–6 h after inoculation depending on the time for bacteria to reach a suitable start bacterial count; blood and peritoneal fluid are collected and used for CFU determination.[58,190] The CLP model is another surgical technique to mimic secondary bacterial peritonitis.[191,192] In this model, the animal is anesthetized and the abdominal cavity is aseptically entered whereby the cecum is identified, ligated, and perforated with a needle before closing the abdomen.

5. Antimicrobial PK/PD modeling

In antibiotic development, PK/PD indices are intended to normalize the drug exposure relative to the in vitro susceptibilityof the respective pathogen.[193] Once the optimal PK/PD index and target is identified and validated for a new compound, it can be used to optimize the dosing regimen and determination of preliminary susceptibility breakpoints.

Practically, there are a number of key experimental elements in antimicrobial PK/PD studies. First, one must determine the dose range to study for both pharmacokinetic and pharmacodynamic studies in the animal model. This is often based on pilot studies in animals and on the in vitro exposures that have been identified that yield bacterial stasis or killing (i.e. in vitro MIC testing). The dose range selected often includes 4–6 total doses that vary by 2- to 4-fold from dose to dose. Thus the complete dose range can attempt to cover a low dose with little or no effect to a high dose which produces maximal effect. The next step is measuring pharmacokinetics in the animals. An important consideration is to select multiple doses and time points to measure PK and optimally the doses selected should encompass the dose range studied to account for possible nonlinear PK that can be observed with higher dose levels. Pharmacokinetic measurement requires the collection of serum or plasma from three or more animals per time point after administration of a pre-determined dose of drug. Drug levels can then be determined by bio-assay or chemical analysis such as liquid chromatography-mass spectrometry (LC–MS), the latter being much more frequently used in modern PK/PD studies. Site specific PK can be measured as well for a number of infection models. For example, bronchial alveolar lavage (BAL) fluid can be obtained from groups of animals at multiple time points after drug administration identical to the time points above for serum or plasma and the BAL fluid can be similarly analyzed for drug concentration. These PK studies form the basis of calculating key pharmacokinetic measures for PK/PD studies including maximum drug concentration (Cmax), area under the drug concentration curve over 24 h (AUC_{0-24}), and elimination half-life ($T_{1/2}$), which is instrumental in calculating time above MIC.

Another key component in PK/PD study design is to identify a group of study organisms that can grow readily in the animal model and have variable susceptibility (i.e. MIC) to the investigational drug. To this end, it is crucial that the study utilizes standard strains of bacteria (i.e. ATCC isolates), clinical isolates that are quite susceptible to the investigational drug, and isolates with varying drug resistance to the investigational drug. PK/PD studies that lack robust clinical applicability are often the result of the use of a single isolate, lack of clinical isolates, and/or lack of MIC variation. In our experience, the use of a minimum of three isolates is often necessary; however, the PK/PD data fit and estimates generated are often much more robust and clinically applicable with more isolates examined. Additionally, one must confirm that the group of organisms selected can grow properly and cause disease in the animal model (i.e. fitness studies). This is relatively straight forward to

perform by infecting animals with the organism and measuring burden over time in untreated animals. Organisms that do not grow over time in the animal model are likely not useful for PK/PD studies. As mentioned in the above paragraphs, numerous strategies exist to optimize the fitness of organisms in the animal models.

Once the dose range, PK studies, and group of organisms are identified one can move forward with PD studies to determine the optimal PK/PD index and PK/PD target. There are three PK/PD indices that are clinically relevant and impact dosing regimen design and these include: 1) The peak drug level over MIC ratio (C_{max}/MIC), 2) 24-h area under the drug concentration-time curve over MIC ratio (24-h AUC/MIC), and 3) Percentage of time over 24 h in which drug concentrations exceed the MIC (T > MIC). Identification of the optimal PK/PD index is usually determined using dose-escalation time-kill studies and dose fractionation studies, the latter being a key component as described below. Time-kill studies examine the effect of single, escalating drug doses on the extent of killing and antimicrobial activity over time. This study design allows one to determine whether increasing drug exposures lead to enhanced activity (i.e. concentration-dependence) and whether there are microbiological effects noted after drug concentrations fall below the MIC, known as post-antibiotic effects (PAE). For some drugs, there are effects that persist following exposure and sometimes the effects are prolonged after the drug concentration has fallen below the MIC (e.g. fluoroquinolones, aminoglycosides, and triazoles). It is important to note that this experimental design can give insights into the relative importance of concentration-effect and PAEs, which will impact the determination of the best PK/PD index that predicts efficacy and resultant dosing regimen design; however, by itself it does not allow for a true PK/PD index determination. The reason for this is the dosing interval does not vary (all animals receive single-escalating doses and effect noted at various time points) and as one escalates the dose administered all three of the PK/PD indices will increase in concert. Thus one cannot reliably determine which of the three indices may be the most predictive of efficacy given the interdependence of the three indices as the doses increase. Dose-fractionation study design addresses this dilemma by reducing the interdependence of the three PK/PD indices. In dose-fractionation design, the total drug dose administered over a given time period (e.g. 24 h) is the same but fractionated into different dosing intervals. For example, one could take a 40 mg/kg dose and administer the whole dose once (Q24 h) to a group of infected animals, administer 20 mg/kg twice (Q12 h) to a group of infected animals, administer 10 mg/kg four times (Q6 h) to a group of infected animals, and finally 5 mg/kg eight times (Q3 h) to a group of infected animals. In doing this, the total drug exposure is the same in every group and thus $AUC_{0\text{-}24}$ will be nearly identical in all groups; however, the Cmax will be much higher in the Q24 h group and the T > MIC will be much higher in the Q3 h group. The result is three PK/PD indices have been clearly separated as one changes dose and dosing interval in the dose-fractionation study. As with the PK studies, it is usually best to study multiple doses in the fractionation design that include the dosing range being studied to encompass the full range of drug effect. One can often predict the PK/PD index predictive of efficacy based on visual examination of the dose-response curves from these dose-fractionation studies. If the efficacy was much more pronounced in the infrequently administered dosing regimens, Cmax/MIC is likely most predictive. If efficacy was much more pronounced in the shorter dosing intervals, T > MIC is likely most predictive. Finally, if the outcome was similar with each dosing interval, AUC/MIC is likely most predictive. Mathematically, the PK/PD index determination is performed using the E_{max} model or Hill equation to examine the relationship between each PK/PD index and effect.[194] The fit of the relationship is typically determined using a statistical assessment of variation such as the coefficient of variation (R^2).

A final component of PK/PD studies is the examination of the drug exposure to MIC ratio (based on the optimal PK/PD index identified) target for a particular outcome of interest for multiple organisms to generate an overall robust PK/PD target. In essence, the question is what drug exposure magnitude indexed to MIC results in an outcome of interest. Outcomes can include net stasis; 1-log, or 2-log kill. The outcome measure ideally should be correlated with clinical evidence of which measure predicts optimal outcome in patients, but this is often lacking and continues to be an area of active PK/PD research to optimize translatability of pre-clinical PK/PD studies to clinical use.

6. Conclusion

Developing safe and effective dosing regimens is a significant challenge in antibiotic development, which can be achieved by the integration of PK and PD information in preclinical experimental models. Hence, accurate and predictive animal infection PK/PD models are an extremely powerful tool which can streamline the drug development process and optimize therapeutic effect. In this review, we summarized the factors that can affect animal model PK/PD studies of antimicrobial agents and the general approach to design robust PK/PD studies. We believe carefully controlled animal model studies will continue to make a significant contribution to the development for new antibiotics and dosing regimen optimization for current antibiotics.

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References

1. Ambrose Paul G, Bhavnani SM, Rubino Christopher M, et al. Pharmacokinetics-pharmacodynamics of antimicrobial therapy it's not just for mice anymore. *Clin Infect Dis*. 2007;44:79–86.
2. Boucher HW, Talbot GH, Benjamin Jr DK, et al. Infectious Diseases Society of A: 10 × '20 progress–development of new drugs active against gram-negative bacilli: an update from the Infectious Diseases Society of America. *Clin Infect Dis*. 2013;56:1685–1694.
3. Services DoHaH: Product Development Under the Animal Rule Guidance for Industry. In. Edited by Administration FaD; 2015.
4. Lopez-Cadenas C, Sierra-Vega M, Garcia-Vieitez JJ, Diez-Liebana MJ, Sahagun-Prieto A, Fernandez-Martinez N. Enrofloxacin: pharmacokinetics and metabolism in domestic animal species. *Curr Drug Metab*. 2013;14:1042–1058.
5. Dasgupta A. Usefulness of monitoring free (unbound) concentrations of therapeutic drugs in patient management. *Clin Chim Acta*. 2007;377:1–13.
6. Schmidt S, Barbour A, Sahre M, Rand KH, Derendorf H. PK/PD: new insights for antibacterial and antiviral applications. *Curr Opin Pharmacol*. 2008;8:549–556.
7. Mouton JW, Theuretzbacher U, Craig WA, Tulkens PM, Derendorf H, Cars O. Tissue concentrations: do we ever learn? *J Antimicrob Chemother*. 2008;61:235–237.
8. Gonzalez D, Schmidt S, Derendorf H. Importance of relating efficacy measures to unbound drug concentrations for anti-infective agents. *Clin Microbiol Rev*. 2013;26:274–288.
9. Ballow CH, Amsden GW, Highet VS, Forrest A. Pharmacokinetics of oral azithromycin in serum, urine, polymorphonuclear leucocytes and inflammatory vs non-inflammatory skin blisters in healthy volunteers. *Clin Drug Investig*. 1998;15:159–167.
10. Schentag JJ, Ballow CH. Tissue-directed pharmacokinetics. *Am J Med*. 1991;91:5S–11S.
11. Lardner A. The effects of extracellular pH on immune function. *J Leukoc Biol*. 2001;69:522–530.
12. Burkhardt O, Kumar V, Katterwe D, et al. Ertapenem in critically ill patients with early-onset ventilator-associated pneumonia: pharmacokinetics with special consideration of free-drug concentration. *J Antimicrob Chemother*. 2007;59:277–284.
13. Roberts JA, Pea F, Lipman J. The clinical relevance of plasma protein binding changes. *Clin Pharmacokinet*. 2013;52:1–8.
14. Ambrose PG, Bhavnani SM, Ellis-Grosse EJ, Drusano GL. Pharmacokinetic-pharmacodynamic considerations in the design of hospital-acquired or

ventilator-associated bacterial pneumonia studies: look before you leap! *Clin Infect Dis*. 2010;51(suppl 1):S103–S110.
15. Louie A, Liu W, Kulawy R, Drusano GL. In vivo pharmacodynamics of torezolid phosphate (TR-701), a new oxazolidinone antibiotic, against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* strains in a mouse thigh infection model. *Antimicrob Agents Chemother*. 2011;55:3453–3460.
16. Lepak AJ, Marchillo K, Pichereau S, Craig WA, Andes DR. Comparative pharmacodynamics of the new oxazolidinone tedizolid phosphate and linezolid in a neutropenic murine *Staphylococcus aureus* pneumonia model. *Antimicrob Agents Chemother*. 2012;56:5916–5922.
17. Redington J, Ebert SC, Craig WA. Role of antimicrobial pharmacokinetics and pharmacodynamics in surgical prophylaxis. *Rev Infect Dis*. 1991;13(suppl 10): S790–S799.
18. Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. *Int J Antimicrob Agents*. 2002;19:261–268.
19. Muller M, dela Pena A, Derendorf H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. *Antimicrob Agents Chemother*. 2004;48:1441–1453.
20. Tsai TH, Chen YF, Chen KC, Shum AY, Chen CF. Concurrent quantification and pharmacokinetic analysis of cefotaxime in rat blood and brain by microdialysis and microbore liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 2000;738:75–81.
21. Eisenberg EJ, Conzentino P, Eickhoff WM, Cundy KC. Pharmacokinetic measurement of drugs in lung epithelial lining fluid by microdialysis: aminoglycoside antibiotics in rat bronchi. *J Pharmacol Toxicol Methods*. 1993;29:93–98.
22. Kovar A, Dalla Costa T, Derendorf H. Comparison of plasma and free tissue levels of ceftriaxone in rats by microdialysis. *J Pharm Sci*. 1997;86:52–56.
23. Tsai TH. Pharmacokinetics of pefloxacin and its interaction with cyclosporin A, a P-glycoprotein modulator, in rat blood, brain and bile, using simultaneous microdialysis. *Br J Pharmacol*. 2001;132:1310–1316.
24. Waga J, Ehinger B. Intravitreal concentrations of some drugs administered with microdialysis. *Acta Ophthalmol Scand*. 1997;75:36–40.
25. Muller M. Microdialysis in clinical drug delivery studies. *Adv Drug Deliv Rev*. 2000;45:255–269.
26. Chaurasia CS, Muller M, Bashaw ED, et al. AAPS-FDA workshop white paper: microdialysis principles, application and regulatory perspectives. *Pharm Res*. 2007;24:1014–1025.
27. Gerber AU, Brugger HP, Feller C, Stritzko T, Stalder B. Antibiotic therapy of infections due to *Pseudomonas aeruginosa* in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. *J Infect Dis*. 1986;153:90–97.
28. Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis*. 1988;158:831–847.
29. Bruzzese T, Rimaroli C, Bonabello A, Mozzi G, Ajay S, Cooverj ND. Pharmacokinetics and tissue distribution of rifametane, a new 3-azinomethyl-rifamycin derivative, in several animal species. *Arzneimittelforschung*. 2000;50:60–71.
30. Grau S, Sorli L, Luque S. Pharmacokinetics and pharmacodynamics of ceftaroline. *Enferm Infecc Microbiol Clin*. 2014;32(suppl 2):15–20.
31. MacCallum DM, Odds FC. Influence of grapefruit juice on itraconazole plasma levels in mice and guinea pigs. *J Antimicrob Chemother*. 2002;50:219–224.
32. Onufrak NJ, Forrest A, Gonzalez D. Pharmacokinetic and pharmacodynamic principles of anti-infective dosing. *Clin Ther*. 2016.
33. Krontz DP, Strausbaugh LJ. Effect of meningitis and probenecid on the penetration of vancomycin into cerebrospinal fluid in rabbits. *Antimicrob Agents Chemother*. 1980;18:882–886.
34. Keel RA, Crandon JL, Nicolau DP. Pharmacokinetics and pulmonary disposition of tedizolid and linezolid in a murine pneumonia model under variable conditions. *Antimicrob Agents Chemother*. 2012;56:3420–3422.
35. Morita E, Mizuno N, Nishikata M, Miyake K. Comparison of the pharmacokinetics of five beta-lactam antibiotics between neonatal and adult rats. *Dev Pharmacol Ther*. 1990;14:223–230.
36. Morita E, Mizuno N, Nishikata M, Takahashi K. Effect of gastrointestinal maturation on absorption of beta-lactam antibiotics. *J Pharm Sci*. 1992;81:337–340.
37. Boulamery A, Marsot A, Bruguerolle B, Simon N. Population pharmacokinetics of ertapenem in juvenile and old rats. *Fundam Clin Pharmacol*. 2014;28:144–150.
38. Tanira MO, Ali BH, Bashir AK. Effect of endotoxin on gentamicin pharmacokinetics in old and young adult rats. *Life Sci*. 1997;60:413–424.
39. Thadepalli H, Chuah SK, Reddy U, et al. Efficacy of trovafloxacin for treatment of experimental Bacteroides infection in young and senescent mice. *Antimicrob Agents Chemother*. 1997;41:1933–1936.
40. Cusack BJ, Young SP, Vestal RE, Olson RD. Age-related pharmacokinetics of daunorubicin and daunorubicinol following intravenous bolus daunorubicin administration in the rat. *Cancer Chemother Pharmacol*. 1997;39:505–512.
41. McMartin DN, Engel SG. Effect of aging on gentamicin nephrotoxicity and pharmacokinetics in rats. *Res Commun Chem Pathol Pharmacol*. 1982;38:193–207.
42. Maiza A, Daley-Yates PT. Variability in the renal clearance of cephalexin in experimental renal failure. *J Pharmacokinet Biopharm*. 1993;21:19–30.
43. Giacomini KM, Roberts SM, Levy G. Evaluation of methods for producing renal dysfunction in rats. *J Pharm Sci*. 1981;70:117–121.
44. Andes D, Craig WA. In vivo activities of amoxicillin and amoxicillin-clavulanate against Streptococcus pneumoniae: application to breakpoint determinations. *Antimicrob Agents Chemother*. 1998;42:2375–2379.
45. Craig WA, Redington J, Ebert SC. Pharmacodynamics of amikacin in vitro and in mouse thigh and lung infections. *J Antimicrob Chemother*. 1991;27:29–40.
46. Nierenberg DW. Drug inhibition of penicillin tubular secretion: concordance between in vitro and clinical findings. *J Pharmacol Exp Ther*. 1987;240:712–716.
47. Thonus I, de Lange-Macdaniel A, Otte C, Michel M. Tissue cage infusion: a technique for the acheivement of prolonged steady state in experimental animals. *J Pharmacol Methods*. 1979;2:63–69.
48. Astry CL, Nelson S, Karam GH, Summer WR. Interactions of clindamycin with antibacterial defenses of the lung. *Am Rev Respir Dis*. 1987;135:1015–1019.
49. Naziri W, Cheadle WG, Trachtenberg LS, Montgomery WD, Polk Jr HC. Second place winner of the Conrad Jobst Award in the gold medal paper competition. Increased antibiotic effectiveness in a model of surgical infection through continuous infusion. *Am Surgeon*. 1995;61:11–15.
50. Thauvin C, Eliopoulos GM, Willey S, Wennersten C, Moellering Jr RC. Continuous-infusion ampicillin therapy of enterococcal endocarditis in rats. *Antimicrob Agents Chemother*. 1987;31:139–143.
51. Tran Ba Huy P, Meulemans A, Wassef M, Manuel C, Sterkers O, Amiel C. Gentamicin persistence in rat endolymph and perilymph after a two-day constant infusion. *Antimicrob Agents Chemother*. 1983;23:344–346.
52. Roosendaal R, Bakker-Woudenberg IA, van den Berghe-van Raffe M, Vink-van den Berg JC, Michel BM. Impact of the dosage schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in *Klebsiella pneumoniae* pneumonia and septicemia in leukopenic rats. *Eur J Clin Microbiol Infect Dis*. 1989;8:878–887.
53. Robaux MA, Dube L, Caillon J, et al. In vivo efficacy of continuous infusion versus intermittent dosing of ceftazidime alone or in combination with amikacin relative to human kinetic profiles in a Pseudomonas aeruginosa rabbit endocarditis model. *J Antimicrob Chemother*. 2001;47:617–622.
54. Craig WA, Andes DR, Stamstad T. In vivo pharmacodynamics of new lipopeptide MX-2401. *Antimicrob Agents Chemother*. 2010;54:5092–5098.
55. Zuluaga AF, Salazar BE, Rodriguez CA, Zapata AX, Agudelo M, Vesga O. Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. *BMC Infect Dis*. 2006;6:55.
56. Guo B, Abdelraouf K, Ledesma KR, Chang KT, Nikolaou M, Tam VH. Quantitative impact of neutrophils on bacterial clearance in a murine pneumonia model. *Antimicrob Agents Chemother*. 2011;55:4601–4605.
57. Azoulay-Dupuis E, Bedos JP, Mohler J, et al. Activity of gemifloxacin against quinolone-resistant *Streptococcus pneumoniae* strains in vitro and in a mouse pneumonia model. *Antimicrob Agents Chemother*. 2005;49:1046–1054.
58. Knudsen JD, Frimodt-Moller N, Espersen F. Experimental *Streptococcus pneumoniae* infection in mice for studying correlation of in vitro and in vivo activities of penicillin against pneumococci with various susceptibilities to penicillin. *Antimicrob Agents Chemother*. 1995;39:1253–1258.
59. Laohavaleeson S, Tessier PR, Nicolau DP. Pharmacodynamic characterization of ceftobiprole in experimental pneumonia caused by phenotypically diverse *Staphylococcus aureus* strains. *Antimicrob Agents Chemother*. 2008;52:2389–2394.
60. Miyazaki S, Nunoya T, Matsumoto T, Tateda K, Yamaguchi K. New murine model of bronchopneumonia due to cell-bound Haemophilus influenzae. *J Infect Dis*. 1997;175:205–209.
61. Nielsen EI, Friberg LE. Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs. *Pharmacol Rev*. 2013;65:1053–1090.
62. Selbie FR, Simon RD. Virulence to mice of Staphylococcus pyogenes: its measurement and its relation to certain in vitro properties. *Br J Exp Pathol*. 1952;33:315–326.
63. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis*. 1998;26:1–10. quiz 11-12.
64. Andes D, Craig WA. Pharmacodynamics of a new cephalosporin, PPI-0903 (TAK-599), active against methicillin-resistant *Staphylococcus aureus* in murine thigh and lung infection models: identification of an in vivo pharmacokinetic-pharmacodynamic target. *Antimicrob Agents Chemother*. 2006;50:1376–1383.
65. Zhang H, Kalkeri G, Mani N, Grossman TH. Development and validation of a multi-dose neutropenic rat thigh infection model using real-time monitoring of *Staphylococcus aureus* growth in vivo. *Vivo*. 2008;22:667–672.
66. Marzola P, Nicolato E, Di Modugno E, et al. Comparison between MRI, microbiology and histology in evaluation of antibiotics in a murine model of thigh infection. *MAGMA*. 1999;9:21–28.
67. Rocchetta HL, Boylan CJ, Foley JW, et al. Validation of a noninvasive, real-time imaging technology using bioluminescent *Escherichia coli* in the neutropenic mouse thigh model of infection. *Antimicrob Agents Chemother*. 2001;45:129–137.
68. Rusckowski M, Gupta S, Liu G, Dou S, Hnatowich DJ. Investigations of a (99m) Tc-labeled bacteriophage as a potential infection-specific imaging agent. *J Nucl Med*. 2004;45:1201–1208.
69. Mizgerd JP, Skerrett SJ. Animal models of human pneumonia. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L387–L398.
70. Mutlu Yilmaz E, Sunbul M, Aksoy A, Yilmaz H, Guney AK, Guvenc T. Efficacy of tigecycline/colistin combination in a pneumonia model caused by extensively drug-resistant *Acinetobacter baumannii*. *Int J Antimicrob Agents*. 2012;40:332–336.
71. Song JY, Cheong HJ, Lee J, Sung AK, Kim WJ. Efficacy of monotherapy and combined antibiotic therapy for carbapenem-resistant *Acinetobacter baumannii*

pneumonia in an immunosuppressed mouse model. *Int J Antimicrob Agents*. 2009;33:33–39.
72. Dudhani RV, Turnidge JD, Nation RL, Li J. FAUC/MIC is the most predictive pharmacokinetic/pharmacodynamic index of colistin against *Acinetobacter baumannii* in murine thigh and lung infection models. *J Antimicrob Chemother*. 2010;65:1984–1990.
73. Harris G, Kuo Lee R, Lam CK, et al. A mouse model of *Acinetobacter baumannii*-associated pneumonia using a clinically isolated hypervirulent strain. *Antimicrob Agents Chemother*. 2013;57:3601–3613.
74. Koomanachai P, Kim A, Nicolau DP. Pharmacodynamic evaluation of tigecycline against *Acinetobacter baumannii* in a murine pneumonia model. *J Antimicrob Chemother*. 2009;63:982–987.
75. Pachon-Ibanez ME, Docobo-Perez F, Jimenez-Mejias ME, et al. Efficacy of rifampin, in monotherapy and in combinations, in an experimental murine pneumonia model caused by panresistant *Acinetobacter baumannii* strains. *Eur J Clin Microbiol Infect Dis*. 2011;30:895–901.
76. Pachon-Ibanez ME, Docobo-Perez F, Lopez-Rojas R, et al. Efficacy of rifampin and its combinations with imipenem, sulbactam, and colistin in experimental models of infection caused by imipenem-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2010;54:1165–1172.
77. Pichardo C, Pachon-Ibanez ME, Docobo-Perez F, et al. Efficacy of tigecycline vs. imipenem in the treatment of experimental *Acinetobacter baumannii* murine pneumonia. *Eur J Clin Microbiol Infect Dis*. 2010;29:527–531.
78. Tang HJ, Chuang YC, Ko WC, et al. Comparative evaluation of intratracheal colistimethate sodium, imipenem, and meropenem in BALB/c mice with carbapenem-resistant *Acinetobacter baumannii* pneumonia. *Int J Infect Dis: IJID*. 2012;16:e34–e40.
79. Yuan Z, Ledesma KR, Singh R, Hou J, Prince RA, Tam VH. Quantitative assessment of combination antimicrobial therapy against multidrug-resistant bacteria in a murine pneumonia model. *J Infect Dis*. 2010;201:889–897.
80. Bhalodi AA, Crandon JL, Biek D, Nicolau DP. Efficacy of ceftaroline fosamil in a staphylococcal murine pneumonia model. *Antimicrob Agents Chemother*. 2012;56:6160–6165.
81. Crandon JL, Kuti JL, Nicolau DP. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant *Staphylococcus aureus* with a range of vancomycin MICs in a murine pneumonia model. *Antimicrob Agents Chemother*. 2010;54:5115–5119.
82. Docobo-Perez F, Lopez-Rojas R, Dominguez-Herrera J, et al. Efficacy of linezolid versus a pharmacodynamically optimized vancomycin therapy in an experimental pneumonia model caused by methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother*. 2012;67:1961–1967.
83. Karau MJ, Tilahun AY, Schmidt SM, Clark CR, Patel R, Rajagopalan G. Linezolid is superior to vancomycin in experimental pneumonia caused by Superantigen-Producing *Staphylococcus aureus* in HLA class II transgenic mice. *Antimicrob Agents Chemother*. 2012;56:5401–5405.
84. Koomanachai P, Crandon JL, Banevicius MA, Peng L, Nicolau DP. Pharmacodynamic profile of tigecycline against methicillin-resistant *Staphylococcus aureus* in an experimental pneumonia model. *Antimicrob Agents Chemother*. 2009;53:5060–5063.
85. Reyes N, Skinner R, Kaniga K, et al. Efficacy of telavancin (TD-6424), a rapidly bactericidal lipoglycopeptide with multiple mechanisms of action, in a murine model of pneumonia induced by methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2005;49:4344–4346.
86. Tessier PR, Keel RA, Hagihara M, Crandon JL, Nicolau DP. Comparative in vivo efficacies of epithelial lining fluid exposures of tedizolid, linezolid, and vancomycin for methicillin-resistant *Staphylococcus aureus* in a mouse pneumonia model. *Antimicrob Agents Chemother*. 2012;56:2342–2346.
87. Aoki N, Tateda K, Kikuchi Y, et al. Efficacy of colistin combination therapy in a mouse model of pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. 2009;63:534–542.
88. Bretonniere C, Jacqueline C, Caillon J, et al. Efficacy of doripenem in the treatment of *Pseudomonas aeruginosa* experimental pneumonia versus imipenem and meropenem. *J Antimicrob Chemother*. 2010;65:2423–2427.
89. Crandon JL, Nicolau DP. Human simulated studies of aztreonam and aztreonam-avibactam to evaluate activity against challenging gram-negative organisms, including metallo-beta-lactamase producers. *Antimicrob Agents Chemother*. 2013;57:3299–3306.
90. Crandon JL, Schuck VJ, Banevicius MA, et al. Comparative in vitro and in vivo efficacies of human simulated doses of ceftazidime and ceftazidime-avibactam against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2012;56:6137–6146.
91. Hagihara M, Crandon JL, Urban CM, Nicolau DP. KPC presence in *Pseudomonas aeruginosa* has minimal impact on the in vivo efficacy of carbapenem therapy. *Antimicrob Agents Chemother*. 2013;57:1086–1088.
92. Jacqueline C, Roquilly A, Desessard C, et al. Efficacy of ceftolozane in a murine model of *Pseudomonas aeruginosa* acute pneumonia: in vivo antimicrobial activity and impact on host inflammatory response. *J Antimicrob Chemother*. 2013;68:177–183.
93. Louie A, Fregeau C, Liu W, Kulawy R, Drusano GL. Pharmacodynamics of levofloxacin in a murine pneumonia model of *Pseudomonas aeruginosa* infection: determination of epithelial lining fluid targets. *Antimicrob Agents Chemother*. 2009;53:3325–3330.
94. Morinaga Y, Yanagihara K, Nakamura S, et al. In vivo efficacy and pharmacokinetics of tomopenem (CS-023), a novel carbapenem, against *Pseudomonas aeruginosa* in a murine chronic respiratory tract infection model. *J Antimicrob Chemother*. 2008;62:1326–1331.
95. Sabet M, Miller CE, Nolan TG, Senekeo-Effenberger K, Dudley MN, Griffith DC. Efficacy of aerosol MP-376, a levofloxacin inhalation solution, in models of mouse lung infection due to *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2009;53:3923–3928.
96. Bakker-Woudenberg IA, ten Kate MT, Goessens WH, Mouton JW. Effect of treatment duration on pharmacokinetic/pharmacodynamic indices correlating with therapeutic efficacy of ceftazidime in experimental *Klebsiella pneumoniae* lung infection. *Antimicrob Agents Chemother*. 2006;50:2919–2925.
97. Docobo-Perez F, Nordmann P, Dominguez-Herrera J, et al. Efficacies of colistin and tigecycline in mice with experimental pneumonia due to NDM-1-producing strains of *Klebsiella pneumoniae* and *Escherichia coli*. *Int J Antimicrob Agents*. 2012;39:251–254.
98. Goessens WH, Mouton JW, Ten Kate MT, Sorgel F, Kinzig M, Bakker-Woudenberg IA. The therapeutic effect of tigecycline, unlike that of Ceftazidime, is not influenced by whether the *Klebsiella pneumoniae* strain produces extended-spectrum beta-lactamases in experimental pneumonia in rats. *Antimicrob Agents Chemother*. 2013;57:643–646.
99. Hilliard JJ, Melton JL, Hall L, et al. Comparative effects of carbapenems on bacterial load and host immune response in a *Klebsiella pneumoniae* murine pneumonia model. *Antimicrob Agents Chemother*. 2011;55:836–844.
100. Hirsch EB, Guo B, Chang KT, et al. Assessment of Antimicrobial combinations for *Klebsiella pneumoniae* carbapenemase-producing K. pneumoniae. *J Infect Dis*. 2013;207:786–793.
101. Padilla E, Alonso D, Domenech-Sanchez A, et al. Effect of porins and plasmid-mediated AmpC beta-lactamases on the efficacy of beta-lactams in rat pneumonia caused by *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2006;50:2258–2260.
102. Pichardo C, del Carmen Conejo M, Bernabeu-Wittel M, et al. Activity of cefepime and carbapenems in experimental pneumonia caused by porin-deficient *Klebsiella pneumoniae* producing FOX-5 beta-lactamase. *Clin Microbiol Infect*. 2005;11:31–38.
103. Pichardo C, Rodriguez-Martinez JM, Pachon-Ibanez ME, et al. Efficacy of cefepime and imipenem in experimental murine pneumonia caused by porin-deficient *Klebsiella pneumoniae* producing CMY-2 beta-Lactamase. *Antimicrob Agents Chemother*. 2005;49:3311–3316.
104. Hansen EJ, Toews GB. Animal models for the study of noninvasive Haemophilus influenzae disease: pulmonary clearance systems. *J Infect Dis*. 1992;165(suppl 1):S185–S187.
105. O'Reilly T. Relevance of animal models for chronic bacterial airway infections in humans. *Am J Respir Crit Care Med*. 1995;151:2101–2107. discussion 2107-2108.
106. Toews GB, Gross GN, Pierce AK. The relationship of inoculum size to lung bacterial clearance and phagocytic cell response in mice. *Am Rev Respir Dis*. 1979;120:559–566.
107. Johansen HK, Hoiby N. *Rat Model of Chronic Pseudomonas aeruginosa*. San Diego: Academic Press; 1999.
108. Schiff JB, Small GJ, Pennington JE. Comparative activities of ciprofloxacin, ticarcillin, and tobramycin against experimental *Pseudomonas aeruginosa* pneumonia. *Antimicrob Agents Chemother*. 1984;26:1–4.
109. Hraiech S, Bregeon F, Brunel JM, et al. Antibacterial efficacy of inhaled squalamine in a rat model of chronic *Pseudomonas aeruginosa* pneumonia. *J Antimicrob Chemother*. 2012;67:2452–2458.
110. Macia MD, Borrell N, Segura M, Gomez C, Perez JL, Oliver A. Efficacy and potential for resistance selection of antipseudomonal treatments in a mouse model of lung infection by hypermutable *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2006;50:975–983.
111. Moser C, Van Gennip M, Bjarnsholt T, et al. Novel experimental *Pseudomonas aeruginosa* lung infection model mimicking long-term host-pathogen interactions in cystic fibrosis. *APMIS: Acta Pathol Microbiol Immunol Scand*. 2009;117:95–107.
112. Song Z, Wu H, Mygind P, et al. Effects of intratracheal administration of novispirin G10 on a rat model of mucoid *Pseudomonas aeruginosa* lung infection. *Antimicrob Agents Chemother*. 2005;49:3868–3874.
113. van Gennip M, Moser C, Christensen LD, et al. Augmented effect of early antibiotic treatment in mice with experimental lung infections due to sequentially adapted mucoid strains of *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. 2009;64:1241–1250.
114. Beaulac C, Sachetelli S, Lagace J. Aerosolization of low phase transition temperature liposomal tobramycin as a dry powder in an animal model of chronic pulmonary infection caused by *Pseudomonas aeruginosa*. *J Drug Target*. 1999;7:33–41.
115. Cannon CL, Hogue LA, Vajravelu RK, et al. In vitro and murine efficacy and toxicity studies of nebulized SCC1, a methylated caffeine-silver(I) complex, for treatment of pulmonary infections. *Antimicrob Agents Chemother*. 2009;53:3285–3293.
116. Ferrari F, Lu Q, Girardi C, et al. Nebulized ceftazidime in experimental pneumonia caused by partially resistant *Pseudomonas aeruginosa*. *Intensive Care Med*. 2009;35:1792–1800.
117. Goldstein I, Wallet F, Nicolas-Robin A, Ferrari F, Marquette CH, Rouby JJ. Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. *Am J Respir Crit Care Med*. 2002;166:1375–1381.
118. Lu Q, Girardi C, Zhang M, et al. Nebulized and intravenous colistin in experimental pneumonia caused by *Pseudomonas aeruginosa*. *Intensive Care Med*. 2010;36:1147–1155.

119. Dai T, Kharkwal GB, Tanaka M, Huang YY, Bil de Arce VJ, Hamblin MR. Animal models of external traumatic wound infections. *Virulence*. 2011;2:296–315.
120. Bunce C, Wheeler L, Reed G, Musser J, Barg N. Murine model of cutaneous infection with gram-positive cocci. *Infect Immun*. 1992;60:2636–2640.
121. Kugelberg E, Norstrom T, Petersen TK, Duvold T, Andersson DI, Hughes D. Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrob Agents Chemother*. 2005;49:3435–3441.
122. Velkov T, Bergen PJ, Lora-Tamayo J, Landersdorfer CB, Li J. PK/PD models in antibacterial development. *Curr Opin Microbiol*. 2013;16:573–579.
123. Cho JS, Zussman J, Donegan NP, et al. Noninvasive in vivo imaging to evaluate immune responses and antimicrobial therapy against *Staphylococcus aureus* and USA300 MRSA skin infections. *J Invest Dermatol*. 2011;131:907–915.
124. Kuklin NA, Pancari GD, Tobery TW, et al. Real-time monitoring of bacterial infection in vivo: development of bioluminescent staphylococcal foreign-body and deep-thigh-wound mouse infection models. *Antimicrob Agents Chemother*. 2003;47:2740–2748.
125. Reyes N, Aggen JB, Kostrub CF. In vivo efficacy of the novel aminoglycoside ACHN-490 in murine infection models. *Antimicrob Agents Chemother*. 2011;55:1728–1733.
126. Brunetti J, Falciani C, Roscia G, et al. In vitro and in vivo efficacy, toxicity, bio-distribution and resistance selection of a novel antibacterial drug candidate. *Sci Rep*. 2016;6:26077.
127. Dacey RG, Sande MA. Effect of probenecid on cerebrospinal fluid concentrations of penicillin and cephalosporin derivatives. *Antimicrob Agents Chemother*. 1974;6:437–441.
128. Vivas M, Force E, Garrigos C, et al. Experimental study of the efficacy of daptomycin for the treatment of cephalosporin-resistant pneumococcal meningitis. *J Antimicrob Chemother*. 2014;69:3020–3026.
129. Cabellos C, Garrigos C, Taberner F, Force E, Pachon-Ibanez ME. Experimental study of the efficacy of linezolid alone and in combinations against experimental meningitis due to *Staphylococcus aureus* strains with decreased susceptibility to beta-lactams and glycopeptides. *J Infect Chemother*. 2014;20:563–568.
130. Suntur BM, Yurtseven T, Sipahi OR, Buke C, Buke M. Rifampicin+ceftriaxone versus vancomycin+ceftriaxone in the treatment of penicillin- and cephalosporin-resistant pneumococcal meningitis in an experimental rabbit model. *Int J Antimicrob Agents*. 2005;26:258–260.
131. Rodriguez-Cerrato V, Ghaffar F, Saavedra J, et al. BMS-284756 in experimental cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother*. 2001;45:3098–3103.
132. Ahmed A, Jafri H, Lutsar I, et al. Pharmacodynamics of vancomycin for the treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother*. 1999;43:876–881.
133. Paris MM, Hickey SM, Trujillo M, Shelton S, McCracken Jr GH. Evaluation of CP-99,219, a new fluoroquinolone, for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother*. 1995;39:1243–1246.
134. Stucki A, Acosta F, Cottagnoud M, Cottagnoud P. Efficacy of ceftaroline fosamil against *Escherichia coli* and *Klebsiella pneumoniae* strains in a rabbit meningitis model. *Antimicrob Agents Chemother*. 2013;57:5808–5810.
135. Cottagnoud P, Cottagnoud M, Acosta F, Stucki A. Efficacy of ceftaroline fosamil against penicillin-sensitive and -resistant *Streptococcus pneumoniae* in an experimental rabbit meningitis model. *Antimicrob Agents Chemother*. 2013;57:4653–4655.
136. Stucki A, Cottagnoud M, Acosta F, Egerman U, Laeuffer JM, Cottagnoud P. Efficacy of doripenem against *Escherichia coli* and *Klebsiella pneumoniae* in experimental meningitis. *J Antimicrob Chemother*. 2012;67:661–665.
137. Bardak-Ozcem S, Turhan T, Sipahi OR, et al. Daptomycin versus vancomycin in treatment of methicillin-resistant *Staphylococcus aureus* meningitis in an experimental rabbit model. *Antimicrob Agents Chemother*. 2013;57:1556–1558.
138. Calik S, Turhan T, Yurtseven T, Sipahi OR, Buke C. Vancomycin versus linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis in an experimental rabbit model. *Med Sci Monit*. 2012;18:SC5–SC8.
139. Liu XJ, Zhang XL, Han QZ. Establishment of rat pneumococcal meningitis models: a histopathological analysis. *Int J Clin Exp Pathol*. 2015;8:2242–2248.
140. Roberts JA. Vesicoureteral reflux and pyelonephritis in the monkey: a review. *J Urol*. 1992;148:1721–1725.
141. Hagberg L, Engberg I, Freter R, Lam J, Olling S, Svanborg Eden C. Ascending, unobstructed urinary tract infection in mice caused by pyelonephritogenic *Escherichia coli* of human origin. *Infect Immun*. 1983;40:273–283.
142. Hvidberg H, Struve C, Krogfelt KA, Christensen N, Rasmussen SN, Frimodt-Moller N. Development of a long-term ascending urinary tract infection mouse model for antibiotic treatment studies. *Antimicrob Agents Chemother*. 2000;44:156–163.
143. Johnson JR, Berggren T, Manivel JC. Histopathologic-microbiologic correlates of invasiveness in a mouse model of ascending unobstructed urinary tract infection. *J Infect Dis*. 1992;165:299–305.
144. Johnson JR, Brown JJ. Defining inoculation conditions for the mouse model of ascending urinary tract infection that avoid immediate vesicoureteral reflux yet produce renal and bladder infection. *J Infect Dis*. 1996;173:746–749.
145. Kerrn MB, Frimodt-Moller N, Espersen F. Effects of sulfamethizole and amdinocillin against *Escherichia coli* strains (with various susceptibilities) in an ascending urinary tract infection mouse model. *Antimicrob Agents Chemother*. 2003;47:1002–1009.
146. Frimodt-Moller N. Correlation between pharmacokinetic/pharmacodynamic parameters and efficacy for antibiotics in the treatment of urinary tract infection. *Int J Antimicrob Agents*. 2002;19:546–553.
147. Tsuchimori N, Yamazaki T, Okonogi K. Therapeutic effects of cefozopran against experimental mixed urinary tract infection with *Enterococcus faecalis* and *Pseudomonas aeruginosa* in mice. *J Antimicrob Chemother*. 1997;39:423–425.
148. Glauser MP, Ransley P, Bille J. Urinary tract infections, pyelonephritic scars, and chemotherapy. *Experimental Models in Antimicrobial Chemotherapy*, Vol. 1. London: Academic Press; 1986.
149. Brooks SJ, Lyons JM, Braude AI. Immunization against retrograde pyelonephritis. I. Production of an experimental model of severe ascending *Escherichia coli* pyelonephritis without bacteremia in rats. *Am J Pathol*. 1974;74:345–358.
150. Glauser MP. Bonard: Treatment of experimental ascending *Escherichia coli* pyelonephritis with ceftriaxone alone and in combination with gentamicin. *Chemotherapy*. 1982;28:410–416.
151. Glauser MP, Lyons JM, Braude AI. Prevention of pyelonephritis due to *Escherichia coli* in rats with gentamicin stored in kidney tissue. *J Infect Dis*. 1979;139:172–177.
152. Glauser MP, Lyons JM, Braude AI. Synergism of ampicillin and gentamicin against obstructive pyelonephritis due to *Escherichia coli* in rats. *J Infect Dis*. 1979;139:133–140.
153. Bergeron MG, Marois Y. Benefit from high intrarenal levels of gentamicin in the treatment of *E. coli* pyelonephritis. *Kidney Int*. 1986;30:481–487.
154. LeBrun M, Grenier L, Gourde P, Bergeron MG, Labrecque G, Beauchamp D. Effectiveness and toxicity of gentamicin in an experimental model of pyelonephritis: effect of the time of administration. *Antimicrob Agents Chemother*. 1999;43:1020–1026.
155. Miller TE, Findon G, Rainer SP, Gavin JB. The pathobiology of subclinical pyelonephritis–an experimental evaluation. *Kidney Int*. 1992;41:1356–1365.
156. Frosco MB, Melton JL, Stewart FP, Kulwich BA, Licata L, Barrett JF. In vivo efficacies of levofloxacin and ciprofloxacin in acute murine hematogenous pyelonephritis induced by methicillin-susceptible and-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother*. 1996;40:2529–2534.
157. Kaijser B, Larsson P. Experimental acute pyelonephritis caused by enterobacteria in animals. A review. *J Urol*. 1982;127:786–790.
158. Fu KP, Foleno BD, Lafredo SC, LoCoco JM, Isaacson DM. In vitro and in vivo antibacterial activities of FK037, a novel parenteral broad-spectrum cephalosporin. *Antimicrob Agents Chemother*. 1993;37:301–307.
159. Griffith DC, Harford L, Williams R, Lee VJ, Dudley MN. In vivo antibacterial activity of RWJ-54428, a new cephalosporin with activity against gram-positive bacteria. *Antimicrob Agents Chemother*. 2003;47:43–47.
160. Montgomerie JZ, Schick DG. Treatment of enterococcal pyelonephritis with trovafloxacin and rifampin: in vitro-in vivo contrast. *Antimicrob Agents Chemother*. 1998;42:188–189.
161. Sapico FL, Ginunas VJ, Montgomerie JZ, Canawati HN. Cefpirome, alone and in combination with gentamicin for enterococcal pyelonephritis in the rodent model. *Diagn Microbiol Infect Dis*. 1991;14:297–300.
162. Bayer AS, Greenberg DP, Yih J. Correlates of therapeutic efficacy in experimental methicillin-resistant *Staphylococcus aureus* endocarditis. *Chemotherapy*. 1988;34:46–55.
163. Baddour LM. Virulence factors among gram-positive bacteria in experimental endocarditis. *Infect Immun*. 1994;62:2143–2148.
164. Carbon C. Experimental endocarditis: a review of its relevance to human endocarditis. *J Antimicrob Chemother*. 1993;31:71–85.
165. Eliopoulos GM, Thauvin-Eliopoulos C, Moellering Jr RC. Contribution of animal models in the search for effective therapy for endocarditis due to enterococci with high-level resistance to gentamicin. *Clin Infect Dis*. 1992;15:58–62.
166. Gutschik E. The Enterococcus endocarditis model in experimental animals and its relevance to human infection. *J Antimicrob Chemother*. 1993;31:87–95.
167. Lefort A, Fantin B. Rabbit model of bacterial endocarditis. In: Zak O, Sande MA, eds. *Handbook of Animal Models of Infection*. San Diego: Academic Press; 1999:611–618.
168. Santoro J, Levison ME. Rat model of experimental endocarditis. *Infect Immun*. 1978;19:915–918.
169. Dube L, Caillon J, Jacqueline C, Bugnon D, Potel G, Asseray N. The optimal aminoglycoside and its dosage for the treatment of severe *Enterococcus faecalis* infection. An experimental study in the rabbit endocarditis model. *Eur J Clin Microbiol Infect Dis*. 2012;31:2545–2547.
170. Batard E, Jacqueline C, Boutoille D, et al. Combination of quinupristin-dalfopristin and gentamicin against methicillin-resistant *Staphylococcus aureus*: experimental rabbit endocarditis study. *Antimicrob Agents Chemother*. 2002;46:2174–2178.
171. Jacqueline C, Asseray N, Batard E, et al. In vivo efficacy of linezolid in combination with gentamicin for the treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents*. 2004;24:393–396.
172. Miro JM, Garcia-de-la-Maria C, Armero Y, et al. Addition of gentamicin or rifampin does not enhance the effectiveness of daptomycin in treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2009;53:4172–4177.
173. Navas D, Caillon J, Gras-Le Guen C, et al. Comparison of in vivo intrinsic activity of cefepime and imipenem in a *Pseudomonas aeruginosa* rabbit

endocarditis model: effect of combination with tobramycin simulating human serum pharmacokinetics. *J Antimicrob Chemother*. 2004;54:767–771.
174. Gavalda J, Torres C, Tenorio C, et al. Efficacy of ampicillin plus ceftriaxone in treatment of experimental endocarditis due to *Enterococcus faecalis* strains highly resistant to aminoglycosides. *Antimicrob Agents Chemother*. 1999;43:639–646.
175. Gavalda J, Onrubia PL, Gomez MT, et al. Efficacy of ampicillin combined with ceftriaxone and gentamicin in the treatment of experimental endocarditis due to *Enterococcus faecalis* with no high-level resistance to aminoglycosides. *J Antimicrob Chemother*. 2003;52:514–517.
176. Yang SJ, Xiong YQ, Boyle-Vavra S, Daum R, Jones T, Bayer AS. Daptomycin-oxacillin combinations in treatment of experimental endocarditis caused by daptomycin-nonsusceptible strains of methicillin-resistant *Staphylococcus aureus* with evolving oxacillin susceptibility (the "seesaw effect"). *Antimicrob Agents Chemother*. 2010;54:3161–3169.
177. Tsaganos T, Skiadas I, Koutoukas P, et al. Efficacy and pharmacodynamics of linezolid, alone and in combination with rifampicin, in an experimental model of methicillin-resistant *Staphylococcus aureus* endocarditis. *J Antimicrob Chemother*. 2008;62:381–383.
178. Jacqueline C, Caillon J, Grossi O, et al. In vitro and in vivo assessment of linezolid combined with ertapenem: a highly synergistic combination against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2006;50:2547–2549.
179. Weinstein WM, Onderdonk AB, Bartlett JG, Louie TJ, Gorbach SL. Antimicrobial therapy of experimental intraabdominal sepsis. *J Infect Dis*. 1975;132:282–286.
180. Frimodt-Moller N, Knudsen JD. The mouse peritonitis/sepsis model. In: Zak O, Sande MA, eds. *Handbook of Animal Models of Infection*. San Diego: Academic Press; 1999:127–136.
181. Frimodt-Moller N. The mouse peritonitis model: present and future use. *J Antimicrob Chemother*. 1993;31:55–60.
182. Michea-Hamzehpour M, Pechere JC, Marchou B, Auckenthaler R. Combination therapy: a way to limit emergence of resistance? *Am J Med*. 1986;80:138–142.
183. Michea-Hamzehpour M, Auckenthaler R, Regamey P, Pechere JC. Resistance occurring after fluoroquinolone therapy of experimental *Pseudomonas aeruginosa* peritonitis. *Antimicrob Agents Chemother*. 1987;31:1803–1808.
184. Pechere JC, Marchou B, Michea-Hamzehpour M, Auckenthaler R. Emergence of resistance after therapy with antibiotics used alone or combined in a murine model. *J Antimicrob Chemother*. 1986;17:11–18.
185. Pechere JC, Vladoianu IR. Development of resistance during ceftazidime and cefepime therapy in a murine peritonitis model. *J Antimicrob Chemother*. 1992;29:563–573.
186. Marchou B, Michea-Hamzehpour M, Lucain C, Pechere JC. Development of beta-lactam-resistant *Enterobacter cloacae* in mice. *J Infect Dis*. 1987;156:369–373.
187. Lucain C, Regamey P, Bellido F, Pechere JC. Resistance emerging after pefloxacin therapy of experimental *Enterobacter cloacae* peritonitis. *Antimicrob Agents Chemother*. 1989;33:937–943.
188. Mimoz O, Gregoire N, Poirel L, Marliat M, Couet W, Nordmann P. Broad-spectrum beta-lactam antibiotics for treating experimental peritonitis in mice due to *Klebsiella pneumoniae* producing the carbapenemase OXA-48. *Antimicrob Agents Chemother*. 2012;56:2759–2760.
189. Vimont S, Aubert D, Mazoit JX, Poirel L, Nordmann P. Broad-spectrum beta-lactams for treating experimental peritonitis in mice due to *Escherichia coli* producing plasmid-encoded cephalosporinases. *J Antimicrob Chemother*. 2007;60:1045–1050.
190. Woodnutt G, Berry V, Mizen L. Simulation of human serum pharmacokinetics of cefazolin, piperacillin, and BRL 42715 in rats and efficacy against experimental intraperitoneal infections. *Antimicrob Agents Chemother*. 1992;36:1427–1431.
191. Shrotri M, Peyton JC, Cheadle WG. Mouse peritonitis using ceccal ligation and puncture. In: Zak O, Sande MA, eds. *Handbook of Animal Models of Infection*. San Diego: Academic Press; 1999:173–181.
192. Dupont H, Montravers P. Rat polymicrobial peritonitis infection model. In: Zak MA, Sande MA, eds. *Handbook of Animal Models of Infection*. San Diego: Academic Press; 1999:189–194.
193. Schmidt S, Schuck E, Kumar V, Burkhardt O, Derendorf H. Integration of pharmacokinetic/pharmacodynamic modeling and simulation in the development of new anti-infective agents - minimum inhibitory concentration versus time-kill curves. *Expert Opin Drug Discov*. 2007;2:849–860.
194. Mouton JW, Dudley MN, Cars O, Derendorf H, Drusano GL. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. *J Antimicrob Chemother*. 2005;55:601–607.