3. Monitoring of antimicrobial chemotherapy

The efficacy of treatment can be checked either by testing the bactericidal activity of the patient's serum against the offending pathogen or by periodical monitoring of the antibiotic concentrations in the serum. Several authors advocate the former test as a mean of ensuring optimal efficacy in the treatment of endocarditis and septicemia[16,26]. The major disadvantage of this method is the lack of a standardized technique. Furthermore only the treatment of culture-positive infections can be assessed.

Periodical measurements of serum concentrations are of greater practical importance, particularly in patients receiving aminoglycosides. In vitro and in vivo studies demonstrate a quantitative relationship between aminoglycoside concentrations in the blood and their clinical efficacy[1,5,11,13,20,27]. By contrast, the relationship between administered doses of aminoglycosides and attainable serum concentrations is fairly unpredictable[4,12,14,17,24,29]. Therefore it appears mandatory to monitor aminoglycoside serum concentrations in critical situations such as life-threatening infections or in patients with unstable renal function. Determinations of serum levels of non-aminoglycoside antibiotics may be useful in dialysis patients or to test compliance in unreliable patients with tuberculosis.

In conclusion, continuous education of physicians – provided by clinical microbiologists – is essential to ensure optimal antimicrobial chemotherapy. The importance of determining the suitability of specimens submitted to the microbiological laboratories and the need for greater efficiency in the service facilities are discussed as examples of how the microbiologist can best utilize his knowledge of infectious diseases and the pharmacology of antibiotics to the benefit of the patients.

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Scope and limitations of experimental chemotherapy*

by O. Zak

Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd, CH-4002 Basel (Switzerland)

The beginnings of experimental chemotherapy, which might best be defined as 'the treatment of animals with simulated human infections', date back to the turn of the century[15,40]. Since then, it has evolved to become an indispensable tool for research into the treatment of infectious diseases and for antibiotic research in particular. This is clearly evident from the annual crop of publications on the subject. Of 519

papers presented at the 18th I.C.A.A.C. in 1978, for example, 18% contained data originating from animal experiments.

The present paper is an attempt to classify and critically review the models used in experimental chemotherapy, to identify the main factors influencing the results, and to estimate the clinical relevance of these results, by reference to some selected examples.

According to their purpose and nature, the models used in experimental chemotherapy can be divided into 4 main categories: Basic antimicrobial screening models; ex vivo models; monoparametric models; discriminative models.

Basic antimicrobial screening models

The principal characteristics of - or criteria for - the models in this category, which corresponds to the 'Type-I-infections' of O'Grady[43], are:

- 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.
- Results readily amenable to evaluation, preferably based on one single 'all-or-nothing' parameter (e.g. survival or death).

Examples of models that meet the above requirements are: systemic infection in mice[8,38,65] (the most commonly used in vivo test in antibiotic research), rats or rabbits; the thigh-lesion test[45,56]; intra-abdominal sepsis due to large-bowel flora[3,64]; meningitis in mice[69]; and pneumonia in mice[32,72].

Full technical details of these models can be found in the pertinent literature. Briefly, they consist in infecting the animal (i.p., i.v., i.m. in systemic infections, intracerebrally in meningitis, intranasally in pneumonia or i.m. for thigh lesions) with a standardized dose of the respective organism and treating it, once or repeatedly, with various doses of antibiotics, the first dose usually being administered very shortly after or even simultaneously with the infection. At the end of the experiment, generally 5–10 days after infection, the survival rates, or the degree of thigh swelling is recorded, and the activity of the drugs is calculated for example by probit analysis[35], in terms of the doses affording protection from death in 50% of the animals, or inhibiting thigh swelling by 50% (ED_{50}, PD_{50}, CD_{50} in mg/kg, etc.). Provided the experimental systems are sufficiently well standardized, they are an extremely useful means of recognizing and comparing the antimicrobial efficacy, the tolerability and the toxicity of drugs, or drug combinations, in vivo, and can even indicate whether a drug is more likely to be active by the oral route or by injection[38].

The relevance of the results obtained in these models to clinical chemotherapy is, however, limited by several inherent drawbacks. The few examples given below illustrate the importance of these shortcomings. Further drawbacks are mentioned in the excellent reviews published by Bergeron[5], Barza[6], and Miller[38].

1. The rapidly fatal course usually observed in systemic infections in mice[9] or rabbits[73], in which up to 10^7 organisms per ml are detected in the blood a few hours after infection, is not generally a characteristic of human disease.
2. Since treatment is administered more or less at the time of infection, the bacteria are exposed to the antibiotics from the very onset of the infection, or even before the real pathogenic process has begun. The spread of the organisms in the body and the activation of the body's defence mechanisms occur in the presence of the antibiotics. The effects produced are therefore, in a sence, prophylactic rather than therapeutic.
3. The models are highly sensitive to the size of the infective dose. It has repeatedly been shown that an increase in the challenge dose by 1–2 log units can render antibiotic ineffective, even if it is virtually unaffected by the size of the inoculum used in vitro[10,61].
4. The pharmacokinetics and the metabolism of a drug in animals can be quite dissimilar to those found in man[5,6]. The data on the binding of some cephalosporins to serum proteins of various origins shown in table 1 indicate only one of many possible differences that can have an important bearing on the outcome of treatment.
5. The efficacy of antimicrobials in mice depends to a great extent on the 'low-resistance period', which also varies in its duration from one bacterial genus to another[51,52].
6. The results of chemotherapy are also dependent on the strain and the source of the animal: For example, striking and unpredictable differences were found between the responses to various antibiotics shown by MF_2 mice and by individual breeds of NMRI mice (table 2).
7. Differences between the responses to therapy in infected male and female mice have also been described[20].

Ex vivo models

This category comprises models of 2 different types. In the 1st, bacteria are injected into fibrin clots[4,5,12], or dialysis sacks[39], shortly after the implantation of these vehicles into animals, and antibiotics are then administered. In this way, the microorganisms can be exposed to the antibiotics in the absence of immunoglobulins and leucocytes[5].

In the 2nd type of model, small pieces of perforated

Table 1. Binding of selected cephalosporins to serum proteins*

Antibiotic	Concentration tested (μg/ml)	Bound to serum protein (%) of Human	Mouse	Rat	Rabbit	Dog
Cephacetrile	12.5	27±7**	41±1	45±29	48±21	18± 5
	50	22±6	43±3	50±26	46±18	20±10
Cefazolin	12.5	85±2	14±5	63± 3	90± 4	23± 4
	50	81±1	14±3	56± 2	91± 4	23± 3
Cefoxitin	12.5	48±4	19±4	27± 3	34± 5	28± 7
	50	47±7	5±3	30± 4	26±11	29± 7

* Determined microbiologically after 4 h equilibrium dialysis against phosphate buffer pH 7.4 at 37 °C in a Dianorm apparatus[66].
** Mean of 4–11 experiments ± SD.

Table 2. Efficacy of cephacetrile, cephaloridine and rifampicin against systemic infection due to *Staphylococcus aureus* 10B

Strain of mice and breed	ED_{50} (mg/kg) Cephacetrile	Cephaloridine	Rifampicin
MF_2-Tif (Switzerland)	5.5 (3.9–7.7)*	0.08 (0.05–0.12)	0.05 (0.04–0.08)
NMRI-Tif (Switzerland)	320 (180–570)	0.9 (0.6–1.4)	0.07 (0.05–0.09)
NMRI-IVA (Germany)	20 (11–36)	n.e.	n.e.
NMRI-Han (Germany)	60 (40–90)	0.5 (0.4–0.7)	0.04 (0.02–0.07)

Challenge dose 3×10^7 organisms per mouse i.p. Antibiotics administered s.c. to 10 mice per dose level immediately after infection. ED_{50}'s calculated from survival rates on day 5 of infection. * Mean ED_{50} and 95% confidence limits (in brackets) calculated from 2–5 experiments. n.e., Not examined.

rubber or plastic tubing, small spiral springs[2], or – in larger animals – perforated plastic balls[1] are implanted, mostly s.c. They are infected by injection, after they have been surrounded by granulation tissue and filled with 'tissue' fluid, which also contains serum proteins[47] and polymorphonuclears, macrophages, mononuclear cells and lymphocytes[50]. The animals are then treated with single antibiotics or combinations. Depending on the experimental design, the foreign bodies, or samples of the fluid are examined and their antibiotic concentration, bacterial counts, etc., determined.

Models of both categories can yield valuable information on the ability of antibiotics to penetrate to the specific site of infection as well as on alterations in bacterial populations, resistance patterns, or the morphology of microorganisms due to changing levels of the antibiotics.

The 2nd type of ex vivo model may well be of clinical relevance to some special human infections, such as abscesses[50]; however, in small rodents, most of the above-mentioned drawbacks inherent in the screening models also apply to the ex vivo experimental systems.

Monoparametric models

A considerable number of the models applied in experimental chemotherapy are technically too complex for use in a large-scale screening program; yet, on the other hand, they do not meet the requirements for discriminative models, and therefore constitute a category per se. The basis of these models is again the infection of animals and their treatment with antimicrobials; but, instead of – or even in addition to – waiting for the appearance of therapeutic effects, examinations are made during the experiments and, for preference limited to 1 single parameter.

The determination of the bacterial counts in the blood or tissues during or after treatment, for instance, can frequently serve to demonstrate the potency of a drug to kill the organisms in vivo[16,28,34,69]. Models that permit the antibiotic concentrations reached in the blood or tissues of infected animals[59] to be monitored also belong in this category. Modified screening models, to take another example, have proved suitable for the study of the morphological response of bacteria to antibiotics during treatment[10], or for assessing the efficacy of antibiotic sub-MICs against systemic infections in mice[10,70]. The rabbit model developed for investigating the effects of sub-MICs of antibiotics on the outcome of infection[36,73] and the model of systemic infections in immunosuppressed rodents[31,72] can likewise be included in this category.

All these models are, in general, very helpful in differentiating the distinctive properties of various antibiotics or in furnishing data on those effects of antibiotics in vivo which can hardly, if at all, be investigated in human beings.

Discriminative models

The essence of the discriminative model is that it simulates human infection as closely as possible.

Models of this type should help to discriminate the potential therapeutic effects of drugs, or drug combinations, and to delimit the indications in which they might be effective in man. They are also frequently developed and used to investigate interactions between drug, host, and parasite from a point of view

other than the purely therapeutic one (e.g. to see what happens to the bacteria, the tissues, or the drug during treatment of a special infection, how the immune system reacts, etc.). The ideal model of this category should exhibit the features summarized in table 3.

Several models appear to satisfy these criteria fairly well. The wide variety of indications covered is illustrated by the selected examples briefly mentioned below. Fuller descriptions of the models themselves are given in the pertinent literature.

Right- or left-sided endocarditis, in rabbits[18,19,26,54] or rats[67], induced by temporary catheterization of the heart and subsequent i.v. infection with various bacteria or *Candida* sp. is probably the model that most closely simulates the corresponding human disease, as regards the pathogenesis, course, and outcome of the infection.

Models of pneumonia due to *Pseudomonas aeruginosa* in immunosuppressed dogs[13], keratitis in guinea-pigs[14,33,72], burn-wound infection[33], *Candida* vaginitis in rats[55], otitis media[30] and osteomyelitis in rabbits[41,42] are further examples of well-established and accepted discriminative models.

Experimental urinary-tract infection (U.T.I.), especially in the rat, is one of the most widely used models. A fair number of techniques of inducing U.T.I., including those outlined below, have been described and already reviewed[23,29,49]. Although the initial phase of many of them entails an artificial preconditioning of the animal[49], the characteristics of the infection induced are very similar to those seen in man.

Reproducible, acute to subacute, rarely chronic, ascending pyelonephritis has been induced simply by injecting the appropriate bacteria into the bladder[63], by inserting a piece of nylon thread into the bladder[48], or fixing a surgical clip in the bladder wall[31] prior to such injections. Further techniques include the insertion of an infected steel cylinder into the bladder[62]; transurethral infection after temporary ligation of the ureter[44]; i.v. injection after preconditioning with carageenan[72]; intramedullar injection of bacteria[17,7], a technique that can be used to infect each of the kidneys with a different organism[74]; and infection after preconditioning with oestradiol[11], or spontaneous infection due to catheterization[71].

Table 3. Features of the ideal discriminative animal model (modified from Harter and Petersdorf[24])

Technique of infection	Simple
Causative organism route of entry spread in the body Tissue involvement	Identical to situation in man
Course and duration of disease	Predictable Reproducible Amenable to analysis
Susceptibility to chemotherapy	Measurable Reproducible

A simple, highly reproducible and reliable method of inducing pyelonephritis in rats has been developed in our laboratories by modifying a technique described by Heptinstall[25]. A mixture of bacteria and Urographin® is instilled transurethrally under X-ray control. The distension of the renal pelvis obviates the need for preconditioning. If the inoculum reaches the pelves of both kidneys, bilateral pyelonephritis regularly develops and, depending on the strain of bacteria employed can persist for up to 3 months[68]. If the inoculum reaches only one pelvis, there is an 80% probability that unilateral infection will develop.

Experimental meningitis induced in rabbits and dogs by injection of bacteria into the CSF is another widely used model[21,24,46,54,57,58]. It has repeatedly been observed that the antibiotic concentrations reached in the CSF and the therapeutic response achieved in the rabbit model are consistent with clinical observations[46,57]. There seem, however, to be differences in the pathological processes in the rabbit and in man. In meningitis due to *E. coli* and enterococci, we have found that untreated controls die from septicaemia rather than from the original disease. *Pseudomonas* infection, on the other hand, does not show the tendency to become generalized, the primary cause of death being severe meningitis[75].

Investigations using discriminative models have undoubtedly made a significant contribution to our knowledge of the respective diseases and of the therapeutic possibilities. In the latter regard, it should be borne in mind that even in the best discriminative model, the outcome of therapy can be influenced by species-related and possibly human-unrelated course of infection, drug pharmacokinetics, drug metabolism, immunological status, and 'side effects' of therapy, such as the penicillin-like toxicity of β-lactams in rabbits, guinea-pigs, hamsters, etc.

To sum up: it cannot be denied that our knowledge of the treatment of human infections has been greatly enriched by experimental models. Experimental chemotherapy affords means – perhaps the only one so far available – of estimating the efficacy and tolerability of a drug before its use in man, and of finding new approaches to the treatment of infections.

The technical merits of experimental chemotherapy are no less important: the properties of a drug can be investigated in groups of animals large enough to bear statistical analysis; provided the system is a good one, the tests are replicable and the results they yield reproducible; and, lastly, individual effects can be studied separately, by varying the parameters.

The real value of any animal model used in experimental chemotherapy, however, depends much more upon our ability to interpret the results obtained

and to realize the merits and limitations of the test system than upon the excellence of the model itself. In this respect, a touch of scepticism is by no means out of place.

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