Animal Models as Predictors of the Safety and Efficacy of Antibiotics

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As opposed to the testing of safety, the testing of the efficacy of antibiotics in animals is not specified in any directives or guidelines and not explicitly required by regulatory authorities. There exists, however, no doubt that in the evaluation of new compounds testing of both safety and efficacy forms an essential link between in vitro tests and clinical trials. It is inconceivable that clinicians would be prepared to conduct a trial in patients without evidence of the efficacy of the antibiotic in question in an appropriate animal model of infection. Both the models for testing safety and those for testing efficacy suffer from a number of shortcomings. If investigators are aware of these deficiencies and take them into account when interpreting the results, the predictive value of the models can be significantly enhanced.

Two events in the history of antibiotics** appear sufficient to establish the predictive value of data on safety and efficacy derived from experiments in animal models. The first was the surprising finding that the dyestuff prontosil, injected in concentrations between 0.01 and 1 %, was well tolerated, and, although devoid of antimicrobial activity in vitro, protected mice from death due to pneumococcal infection (1). The other was the advent of penicillin. It was only at the suggestion of Chain et al. (2) that penicillin was tested for efficacy against gram-positive bacterial infections in mice and found to possess unique potential as a systemic therapeutic agent, far beyond the role of a local antiseptic or diagnostic tool assigned to it by Fleming (3). These results in mice have been fully corroborated in clinical trials: both the sulfonamide and penicillin proved safe and effective against a variety of infectious diseases in man.

Following these discoveries, the testing of antibiotics in vivo has come to be recognized as an indispensable link between the demonstration of activity in vitro and clinical trials in patients, and the development of a new antibiotic has been almost inconceivable without conclusive proof of its safety and effectiveness in laboratory animals. Hundreds of models of different infectious diseases in a variety of animal species have been established and used in the evaluation of many thousands of antibiotics (4). The wider the experience accumulated, however, the more it becomes evident that the predictive value of results obtained in animals is not as easy to determine as has generally been supposed on the strength of the findings made with prontosil and penicillin. Even the best animal model has shortcomings and pitfalls which may greatly limit the relevance of results, unless the investigator is aware of them (5-10).

In the present paper, the sections on the ethical aspects of experimentation on laboratory animals and on testing for safety in animals are confined to a brief review of the directives of the regulatory authorities and some general comments. Greater emphasis is placed on the testing for efficacy, for which virtually no official directives exist. Using selected examples, the merits and pitfalls of the various models of infection for testing antibiotics are briefly discussed, and their predictive value and contribution to clinical medicine indicated. Finally, a few recommendations are given that may help increase the acceptance of results of in vivo tests by the drug regulatory authorities.

** For the sake of convenience, the term 'antibiotic' is used to refer to all potentially effective antimicrobial agents, whether obtained by fermentation or by chemical synthesis.

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Ethical Aspects of Using Animals

The attitude of the public towards animal experiments is often severely critical, if not hostile, so that even in a short review like this it would be almost unthinkable not to take a stand on the ethics of experimentation on animals.

The use of animals in the search for possible treatments is an integral part of medical research aimed at improving human health and welfare (11). Nowadays, the principle of reverence for life demands that experiments on animals should be restricted to a minimum, without however denying human beings the fulfillment of their own claims to security (12).

In 1983 the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences published guidelines that are intended to serve as a self-imposed code of conduct for all scientists exercising their professions in the country (13). The underlying principles can be summed up as follows:

- No experiments on animals should be performed without deliberation and without need.
- No animal should be made to suffer needlessly.
- If the infliction of pain is inevitable, it must be fully justified by the expected gain in knowledge.
- Each individual scientist bears full responsibility for his own actions.

In 1986 the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (14) was signed by the Council of Europe. Briefly, the member states agreed that, as set forth in the preamble, "man has a moral obligation to respect all animals and to have due consideration for their capacity for suffering and memory. In his quest for knowledge, health and safety, man has a need to use animals if there is a reasonable expectation that the result will be to the overall benefit of man." Furthermore, the member states resolved to limit the use of animals for experimental and scientific purposes and to strive to replace their use wherever practical. Finally, they seek to ensure that experiments attended by pain, suffering, distress or lasting harm are performed only if there is compelling reason, and even then kept to a minimum.

Animal Models as Predictors of Safety

The pharmaceutical industry undoubtedly bears the prime responsibility for ensuring the safety of new antibiotics. Since the mid-1950s, safety testing procedures have been extended, and refined. It is regrettable, however, that it took a disaster of the magnitude of the thalidomide affair to awaken the authorities to the need to impose more stringent demands for safety testing, especially in laboratory animals.

Today the scientist responsible for evaluating the safety and toxicity of an antibiotic is assailed with official requirements, guidelines and recommendations. In drawing up a test protocol, the scientist has to take account of Part B of the Directive of the Commission of the European Communities of November 18, 1987 (15), as well as Section 4 of the OECD Guidelines for the testing of chemicals. Descriptions of individual methods to be used, whether for short-term, long-term or genetic toxicology studies, have been published regularly by the OECD since 1981 (16). All investigations also have to be in conformity with Good Laboratory Practice, i.e., meet the requirements of the Nonclinical Laboratory Practice Regulations (11).

Despite the detailed program for the testing of safety in animals demanded by the authorities, there are still reactions to new antibiotics in man that cannot be foreseen, such as hypersensitivity reactions, discomfort, certain neurological disorders or rarely occurring effects.

Inherent differences between man and animals, or certain species of animals, can greatly reduce the predictive value of data obtained from tests with antibiotics. Thus, metabolic differences can be expressed in differences in the pharmacokinetic profile, and anatomical differences can lead to faulty conclusions. For example, the size of the S3 segment of the proximal renal tubules of the rat compared to that of the much less developed human S3 segment renders findings of proximal tubular damage in the rat largely irrelevant to man (17). Another problem is created by differences in the intestinal flora of man and certain animal species. For instance, the predominantly gram-positive bacterial population of the gut of the rabbit or guinea-pig makes performance of long-term studies with broad-spectrum antibiotics in these animals impossible as this would inevitably lead to selection of gram-negative enterobacteria and/or Clostridium difficile, and lethal septicaemia (18). Further limitations of safety testing in animals are similar to those encountered in tests of efficacy and will be described in the next section.

The merits of safety or toxicity testing in animals, however, far outweigh the shortcomings. These tests are invaluable as a reasonably reliable means of predicting toxic effects in target organs, or of estimating the maximum tolerated dose. Again, further details will be mentioned in connection with tests of efficacy.