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PHARMACOLOGICAL METHODS IN TOXICOLOGY

G. ZBINDEN
F. GROSS

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1. PREFACE

It is a widely held belief that information on potential adverse effects of new drugs is primarily found in the pages of the toxicologists' reports. However useful, or even indispensable, they may be, toxicological studies cover or detect only part of a drug's undesirable features and cannot recognize or predict many other problems that may develop once the new agent is in clinical use. In this book we deal mainly with disturbances of organ functions, which often are undesired companions of the beneficial actions of drugs. These adverse reactions manifest themselves in a great variety of symptoms, ranging from common complaints, such as dizziness, headache, gastrointestinal upset and palpitations, to serious, even disabling dysfunctions of the cardiovascular system, behavioral disorders, neurological and neuroendocrine abnormalities.

Most of these functional disturbances are difficult to recognize in conventional toxicological studies, which are mainly designed to detect changes in biochemical parameters and organ structure. It is for this reason that we turn to the pharmacologists, whose experimental techniques are especially adapted to studying and measuring organ functions. Are these techniques equally well suited for the demonstration of adverse functional effects of drugs? What is their predictive value for reactions occurring in man, and in what way might they contribute to an increase in drug safety? A large number of pharmacologists have enthusiastically responded to our call for help. They have met frequently and have discussed with their toxicological and clinical colleagues the possibilities they can offer for the use of pharmacological methods in preclinical safety evaluation of new drugs. In the fall of 1977, they presented their findings at an International Workshop, which was attended by many representatives of academic, governmental, and industrial research laboratories. In the light of the lively discussions they had with many specialists in the field, they have revised their reports, papers and proposals, and have prepared them for publication in this book. We owe them thanks for their effort and dedication.

The present volume, which contains most of the contributions made at the workshop, the essential parts of the discussions, as well as proposals of how to proceed in the future, has both the advantages and the shortcomings of a book reporting on a scientific meeting. It does not offer the comprehensiveness of a textbook; the contributions may cover one or the other field very well, but leave a substantial gap in others; incoherencies are unavoidable and even contradictions may have crept in. On the other hand, it points out actual problems, gives up-to-date views of various intensely discussed questions and information about practical experience with special techniques and methods. It was clear to all participants of the Workshop that our knowledge and experience of the use of pharmacological techniques in toxicology was fragmentary. True, in certain areas, we were able to build on an impressive body of knowledge, and tentative guidelines for the most effective and practical approaches were readily developed. In other fields, however, pharmacological testing procedures have rarely, if ever, been tried to answer toxicological questions. In these areas the participants had to limit themselves to mere listing of potentially useful procedures, and many went home with the firm intention to try to validate some of these speculations in their own laboratories. It is our hope that such approaches will mark a new beginning which will bear fruit at a future workshop of this kind.

The organizers of the Workshop have used the camel as an emblem of the event. Many of the participants have offered interesting and witty speculations about the meaning of this choice. What, in fact, we intended, was to remind our visitors of the old saying that "a camel is a committee's design of a racehorse", a contraption consisting of many excellent parts, but useless for the intended purpose. With this

comparison we hoped to make the point that the toxicological testing procedures should not be dictated once and for all by a committee of experts, but should evolve from the continuing scientific endeavors of those who are intimately concerned with new drug development and drug safety.

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May 1978

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2. GENERAL CONCEPTS

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1. HISTORICAL BACKGROUND

Disaster struck the young, ambitious drug industry in 1937, when an elixir of sulfanilamide containing 72 per cent diethylene glycol reached the American market and, in only a few weeks, killed over 100 people. At this time it became clear that never again should a new drug be given to patients before extensive toxicological studies in animals were performed. An urgent request went to the scientific community asking for the development of methodology by which new chemical substances could be screened for dangerous toxic properties.

Quite naturally, the pharmacologists who had a vested interest in a smooth functioning of the new drug development process, were the first to heed the call. Their foremost priority was to detect serious organ damage, such as nephrotoxicity, which was the cause of death of the patients receiving the ill-fated elixir of sulfanilamide (Geiling and Cannon, 1948). To reach this limited goal they chose a medical approach. It consisted of exposure of animals to single and repeated treatments with the test compounds, and endeavored to detect organ damage by means of gross observation, and of hematological, gross pathological and histopathological investigations. These studies were later supplemented by an ever growing number of tests provided by clinical chemistry (Barnes and Denz, 1954; Lehman *et al.*, 1955; Zbinden, 1963).

Pharmacologists were soon joined by clinical and surgical pathologists, veterinarians and pharmacists who lent their skills to the new branch of biology which was now called toxicology. Together they developed a comprehensive testing system which was still based on the original medical concept, i.e. an omnibus procedure in which drug administration and toxic reaction monitoring closely resembled that used in human patients. Further significant additions to the system included drug administration to pregnant animals for the study of adverse effects on reproduction and fetal development, life-time experiments for the detection of late toxic reactions, such as tumor induction, and the introduction of pharmacokinetic investigations, in an effort to deal with the problem of species differences.

It is impossible to judge the effectiveness of the enormous efforts toxicologists have made in the last 40 years. There are no statistics giving us the number of deadly compounds which they have recognized and prevented from being administered to human subjects. We know even less about agents which they may have unjustifiably kept from being introduced into clinical practice. There are, of course, a number of chemicals which caused toxic reactions in man and which were not spotted by the toxicologists. The great majority of these, however, caused adverse effects which were either specific for man (e.g. hypersensitivity reactions), or were never intended to be recognized by the toxicological testing program. Keeping this in mind, it is apparent that the original purpose of the toxicological testing system, i.e. the recognition of poisonous drugs and the prevention of their use in man, was, to a large extent, achieved. However, the toxicological methodology, as it has evolved over the last 30–40 years, has not yet reached a satisfactory level of scientific sophistication and cost-effectiveness. It is quite possible that a more directed approach to drug toxicology which attempts to measure the action of drugs on single biological events, may provide us with more scientific and more economical means to assess the harmful qualities of new chemicals. This possibility is our first and most important justification to consider the introduction of pharmacological methods into toxicology.

The adverse drug reactions which the standard toxicological test procedures do not aspire to recognize, include most of the functional side-effects. Clinical experience indicates, however, that these are much more frequent than the toxic reactions due to morphological and biochemical lesions. For example, of the forty-five most frequently observed adverse reactions to drugs observed in over 10,000 patients treated with seventy-seven different drugs or drug combinations and reported in eighty-six unselected drug evaluation papers, forty-two were disturbances of physiological functions (Zbinden, 1966a). True, many of these side-effects were purely subjective, readily reversible, and thus of minor importance. Others, however, greatly upset the patients' wellbeing, discouraged them from adhering to the prescribed therapy, or were prodromal symptoms of more severe lesions. Finally, it must be recognized that functional side-effects can be dangerous and may lead to serious complications, even death. It is, thus, highly desirable that the toxicological evaluation of new drugs includes experimental procedures which can detect disturbances of physiological functions. It is obvious that these will be based on techniques developed for the assessment of pharmacodynamic drug actions.

2. PRACTICAL PROCEDURES

For the use of pharmacological techniques in toxicological test programs three possibilities must be considered: the first includes all acute pharmacological experiments which measure specific effects of drugs on selected functional parameters. In these procedures the test compounds may be investigated *in vitro* using isolated cells, tissues and organs. They may also be tested in animals using various invasive techniques. The animals are usally sacrificed after the acute experiments. The second approach involves the application of repeated pharmacological measurements in chronically treated animals. It is clear that for such studies only noninvasive techniques can be used. The third possibility is a combination of the two previous approaches: it also uses *in vitro* techniques, e.g. isolated organs or invasive *in vivo* methods but works with animals at the end of a chronic drug treatment. In these studies the animals cannot serve as their own controls. Thus, only procedures with small inter-individual variations are suited for such investigations.

All three approaches have, of course, been used by pharmacologists and toxicologists in the past. For example, clinical data sheets summarizing the experimental background of new drug candidates contain a variety of acute pharmacological experiments which are not strictly related to the primary therapeutic action of the compounds. However, only rarely is an effort made to derive meaningful toxicological information from such data. The situation is somewhat better with regard to the repeated use of noninvasive pharmacological techniques in the course of chronic toxicity experiments. Measurements of blood pressure, body temperature and locomotor activity, recordings of the electrocardiogram, evaluations of the sensory, neuromuscular and pulmonary functions, gastrointestinal motility, appetite and behavior have been tried repeatedly for this purpose. However, none of these techniques is as yet routinely used by toxicologists, and we are still far away from a standardization of the experimental methods.

Pharmacological studies using animals at the end of a chronic toxicity experiment are only rarely tried. One of the reasons is the fear that the tissues may be altered by these procedures, and would thus be lost for histopathological evaluation. It was also mentioned already that the lack of pretreatment measurements reduced the usefulness of such experiments. Nevertheless, terminal pharmacological testing of chronically treated animals offers a good opportunity for the introduction of many acute pharmacological techniques into chronic toxicity experiments.

3. THE SELECTION OF TEST

The toxicologist who is shopping for pharmacological test procedures finds himself confronted with hundreds of tempting choices, ranging from a simple manipulation of

an animal to check its righting reflex, to a highly computerized cardivascular test battery. He will soon find out, however, that few, if any, of these test procedures were originally designed with the toxicologist's problems in mind. On the contrary, many tests are strictly pharmacological tools, optimized to measure a very selective action of one particular type of drug. To this end interfering factors are rigidly eliminated. The drugs must be administered in a special way to assure maximal concentration where it matters. Thus, pharmacologists do not hesitate to use intracerebral, intracoronary, intracarotid or at least the intravenous route of administration even with compounds intended to be taken orally by the patient. Regulatory mechanisms are eliminated by surgical interventions such as vagotomy, sympathectomy and pithing. Dragging out whole organs and observing them strictly under *in vitro* conditions is a legitimate act in pharmacological circles. Whenever it is convenient, receptors are sensitized or blocked by means of an astonishingly varied supply of standard compounds. All this is done in an effort to detect and pinpoint even the most trifling pharmacological characteristics of the test compounds. The pharmacologist is, of course, well aware that findings obtained in such experiments are by no means indicative that similar actions would occur under different conditions. His main objective, at this stage of the investigation, is an analysis of the pharmacological spectrum and the establishment of appropriate dose–effect relationships. And nobody tells him which leads he must pursue and which aspects he dares to neglect.

For the toxicologist life is, unfortunately, not so simple. Any hint of a hazardous effect, even if it is based on a most artificial test procedure, must be taken seriously. It must be reported to governmental regulatory agencies, and must be brought to the attention of the clinical pharmacologist and his informed and consenting patient. And then, of course, it must be investigated further, in an effort to find a plausible explanation of its mechanism. No wonder that he hesitates to step on the treacherous ice of pharmacological drug testing.

The distinction between irrelevant results, false positive data and toxicologically relevant observations is one of our most difficult tasks. In the past, we have often been content with a faithful listing of the experimental observations, and have left it to the clinicians to draw the necessary toxicological conclusions. It is important, however, that pharmacologists be kept informed about the adverse reactions occurring in patients. Only by careful and continued comparisons of the pharmacological test results with the observations in man, will he learn to make toxicological predictions from his laboratory data.

4. THE FALSE NEGATIVE RESULTS

In the preceding paragraphs we have discussed the toxicological problems which result from the often very high sensitivity of pharmacological test procedures. But just as bad, if not worse, are pharmacological tests which fail to detect a toxicologically significant drug action. Many pharmacological methods were developed to demonstrate the action of standard drugs. They are thus quite specific for a certain chemical class of agents and not necessarily suited to demonstrate similar actions of a different class of drugs. For example, the continuous avoidance procedure, a behavioral test in which rats or monkeys continuously postpone an aversive foot shock by pressing a lever, is a very sensitive test for the demonstration of barbiturate-like sedation and amphetamine-like stimulation (Heise and Boff, 1962). It does not, however, measure the sedative and stimulating effects of all other types of drugs. For example, phenindamine and diphenhydramine, two antihistamines with sedative qualities in man, elicited a response indistinguishable from that of amphetamine (Zbinden, 1966b). Likewise, many neuropharmacological test procedures by which minute doses of psychopharmacological agents can easily be detected, are poorly suited for the demonstration of agents causing serious central and peripheral neurotoxicity (Alder and Zbinden, 1973). This is particularly true for test procedures for which the animals must be subjected to at a rigid training process.

5. EMBARAS DE RICHESSE

It was pointed out earlier that there are numerous pharmacological test procedures which could be incorporated into toxicological evaluations of new drugs. Most of them, however, have not been systematically studied for this purpose. Before they are recommended for use in the toxicology laboratory they must be evaluated, using a large number of standard drugs whose clinical side-effects are well known. It should, at the same time, also be recognized that a pharmacological property can be measured by a variety of tests. In such cases, not much is gained from using more than one method.

In designing the strategy for the selection of pharmacological tests in toxicology, one should be aware that the usefulness of an experimental procedure is not a function of the cost and complexity of the equipment necessary to obtain the results. One should always try to start out with a simple, rapid and inexpensive procedure which can give an overall indication whether or not it is worthwhile to use more sophisticated, more complicated and costlier methods. One should also have the courage not to use a test procedure, even if it is very dear to one's heart, if the preliminary observations indicate that there is nothing to be gained from it.

Toxicologists should always remember that a pharmacological test should never be done just because:

(a) it is available,

(b) it has always been done, and

(c) it is done by others.

And before they perform an experiment they should:

(a) be reasonably sure that it will answer a specific toxicological question,

(b) know that the results can be interpreted based on experiments with standard drugs, and

(c) be convinced that there are no simpler means to have their question answered.

During the 4 days of the International Workshop on the Use of Pharmacological Methods in Safety-Evaluation of New Drugs we were, indeed, confronted with an embaras de richesse. We saw many beautiful test methods, elegant equipment and clever experimental procedures. And as we now meet them again, let us not forget the fact that there is no limit to what one can do with (or to) a drug in a well equipped and well financed laboratory. But unlike others, toxicologists must not climb a mountain because it is there, and they must not do an experiment just for the fun of it. Each of us must critically review the approaches which eminent specialists from all over the world have opened to us. Let us try their tests, and let us keep those which help us to do our work better.

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