CLINICAL EVALUATION OF COMPOUNDS OF MICROBIAL ORIGIN

H.- P. Kuemmerle

Tokyo Medical College, Japan ;
P.O.Box 89, D-8173 Bad Heilbrunn, F.R.G.

The clinical trial of a new compound of microbial origin is based on the principles of rational methodology. Clinical chemotherapy has developed its own laws and rules that are determined by the particularities of infectious diseases. Three essential factors must be considered in this set of problems : the microorganism, the patient, and the physician (drug?). The development of a new antibiotic follows the general principles of clinical pharmacology which are valid for all groups of substances. Clinical trials consist of three or four phases. Phase I can be subdivided into Ia and Ib, and Phase II into IIa and IIb. In Phase I the pharmacodynamic and pharmacokinetic features of a substance, which have been determined in preclinical research, must be objectified and verified. Generally, about 10-20 subjects suffice for a population of volunteers and patients; however, this varies according to country.

The prerequisites for a decision on the therapeutic efficacy of a substnace are very careful planning and unproblematic performance of the clinical trials in Phase IIa. Only under such conditions can the side-effects be clearly recorded according to frequency and severity. As a rule, at the end of Phase IIa clarity is obtained on the importance of the new substance in comparison with other existing substances for the range of indications. This comparative assessment also determines the further course of the clinical trials. If, for example, the new substance shows significant advantages for therapy, it will be tested on a larger number of patients in Phase IIb. At that

time proof of therapeutic advantages over other substances with similar indications will be sought in double-blind and cross-over studies. The overall general experience with a well-designed, well-conducted clinical trial consisting of a small patient population is frequently superior to that gained with trials involving large numbers of patients, especially multicentre trials. Likewise a well-designed, well-conducted open clinical study can be more valuable than double-blind studies. Such comparative trials should employ the commonly used chemotherapeutic agent for each indication as a standard of comparison for new drugs.

The term "antibiotic" in clinicopharmacological or pharmacological therapy is understood to cover all biosynthetic, semi- and fully synthetic antibiotics and their derivatives. Both experimental and applied clinical pharmacology differentiate between two types of antibiotic activity : bacteriostatic and bactericidal activity. The type of activity is determined by the effect that can be expected in the organism at certain concentrations after a defined duration of action. Other mechanisms of action play an essential role at the various sites of activity (cell wall, cytoplasmic membrane, protein synthesis, synthesis of nucleic acids). The phenomenon of persistence plays a larger role in in vivo studies than in vitro.

The phenomenon of bacterial resistance is a relative concept. Statements about resistance can be made only in connection with an active antibacterial substance and its dosage. The relevant parameters include potency in vitro, minimal inhibitory concentration and concentration of antibiotic at the site of action. If the minimal inhibitory concentration in vitro is higher than the serum or tissue concentrations achievable in vivo, the microorganism can be said to be resistant. Three types of resistance must be differentiated : natural, primary, and secondary. Secondary resistance can be developed in several ways, all of which are dependent on the antibiotic and its mechanism of action, for example, the streptomycin type (one-step mutation) and the penicillin type (multiple-step mutation).

Although the transfer of resistance plays an important role today, it cannot be treated in detail here for reasons of time limitation.

Nowadays it is necessary to differentiate between protein binding and protein inactivation. Protein binding is usually measured as the binding of active substances to serum proteins, primarily serum albumin. Consequently, albumin binding would be a more appropriate term. A high serum binding is generally considered negative. Tissue proteins can differ from albumin by their amino acid sequence, tertiary structure and other types and capacity of binding. For these reasons the therapeutic efficacy of a drug in the tissue cannot be sufficiently assessed on the basis of protein binding and inactivation of serum albumin. For example, high serum binding of a drug, which is usually reversible, could be considered positive because of its transport function in the tissue. However, it is not yet possible to decide whether the protein binding factor of a substance to serum protein is positive or negative, for no correlation has been made with therapeutic relevance. Nevertheless, extremely high values of protein binding, > 95%, must be considered disadvantageous.

The antibacterial spectra of activity of the individual antibiotics cannot be interpreted as a list of indications, much less a timetable. They provide primarily negative, disqualifying information and permit only suggestions about which substances may be inactive in individual cases. Their value is in general limited by the different susceptibilities of various strains of individual species and by the ratios of primary resistance.

Clinical case histories alone are not a criterium for the usefulness or even superiority of a new antibiotic or active combination, since ethical viewpoints are most often opposed to a stringent trial. The clinical efficacy is only the keystone of a detailed experimental or clinicopharmacological investigation. Essential and confir-

124

Thai J. Pharmacol.
Vol.8 No.2 May-Aug. 1986

med principles for the assessment of new antibiotic substances include microbiological and pharmacokinetic investigations.

The antibacterial activity of new drugs or drug combinations should always be tested in comparison with the most active substances existing. Since the findings of in vitro tests depend primarily on the methods used, statements of a relative nature are thus more suitable.

The various tests used can be subdivided into microbiological and non-microbiological methods. Whereas the microbiological methods test the activity of a chemotherapeutic drug or antibiotic against a growing organism, non-microbiological methods are based on physical or chemical changes. The microbiological methods can be subdivided into diffusion test, dilution test, turbidity test, and urease test.

Although they are not as precise as the non-microbiological methods, their accuracy can be improved by increasing the number of parallel tests performed. The non-microbiological methods are based on chemical, physical or immunological principles. They can be applied without any important disadvantage when concentrations of antimicrobial agents with a known metabolic stability are to be measured. Thus, antimicrobial agents have to first be investigated to a certain extent to exclude these disadvantages as much as possible from the very start. Recently, there has been an increase in the use of non-microbiological methods, which entail use of radioactive isotopes (radioimmunoassays and radioenzyme assays).

Animal trials are still the most reliable basis for evaluating the efficacy of a chemotherapeutic agent. Statements that a drug has better or poorer activity than another drug on the basis of animal studies cannot be extrapolated to human patients, for there are considerable differences between humans and various animal species, e.g. , course of infection, site of infection under experimental conditions, and metabolism. To ensure that the first application of an antimicrobial agent in humans is safe, animal experiments are conducted to de-

termine acute, subacute and chronic toxicity, embryotoxicity and tera-
togenesis. The investigation of a drug for carcinogenicity and muta-
genesis in animal experiments poses special problems when the results
are extrapolated to humans. These problems can and should be judged by
only a few specialists. Such investigations should only be performed
when there is sufficient that such effects could occur. In principle,
however, the drug registration and licensing requirements of the indi-
vidual countries decide here.

Pharmacokinetic investigations of a new antibiotic are nowa-
days an inseparable part of our clinicopharmacological experience and a
new drug's characterization. The goal of such investigations is not
only to gain more knowledge about the pharmacokinetic processes in the
human organism and to determine the parameters of absorption, distribu-
tion, and excretion and their most important constants. First of all,
the goal is to ascertain an optimal dosage. Such dosage studies must be
performed on both healthy volunteers and patients. Armed with knowledge
of pharmacokinetics, the physician can actively manage and control each
antibiotic, even in difficult and complex situations in the hospital and
the practice when the underlying conditions for the course of certain
pharmacokinetic processes and their resulting concentration steps are
altered by infections or other pathological developments. Applied cli-
nical pharmacology rests on the following three pillars :

1. The pharmacological efficacy of a drug correlates with
its plasma concentration-time data or urine concentration-time; the
corresponding blood, plasma, serum or urine specimens have been collec-
ted at the precise interval of sampling required.

2. A method for determining the concentration of drug and/
or metabolites in biological fluid is available which is sensitive, se-
lective, reproducible, unproblematical, and applicable.

3. Computers and programs are available for deriving the
concentration-time curve of the drug in blood or its accumulation in
urine, and from these values the pharmacokinetic parameters are calcu-

126

Thai J. Pharmacol.
Vol.8 No.2 May-Aug. 1986

lated. These parameters are combined with the patient's clinical history to determine drug dose, dose interval, etc. Besides pharmacokinetics, biotransformation and bioavailability are of major importance; they include the different routes of administration.

In general, the pharmacokinetics of antibiotics conforms with the natural laws of other drugs, as regards absorption, distribution and excretion. Complicated pharmacokinetic analyses are not always the prerequisite for determining bioavailability. It is completely sufficient for basic information to calculate the area under the plasma concentration curve,the peak serum concentration and the time in which the blood concentration is reached, and the cumulative elimination of the drug in urine. The absolute bioavailability is obtained by comparing the levels found after administration of the drug by the route to be tested with the levels obtained after intravenous administration of the same drug dosage (the bioavailability of the latter is 100%). The relative bioavailability is obtained by comparing the test substance with the standard drug.

Large interindividual variations can occur during absorption processes. These are especially evident when semi-synthetic penicillins are given. Likewise the same substances produced by different firms can have various bioavailabilities. Considerable differences in concentration have also in part been established for these products. Blood or plasma concentrations and the influence that absorption, diffusion and elimination processes have on them provide important parameters for applying antibiotics. These concentrations can be considered therapeutically effective if they clearly exceed the minimal inhibitory concentrations of the drug for the microorganism causing the infection. However, serum peak concentrations, which are usually reached temporarily, must not be overvalued. The average concentrations that are reached are more important. The blood or plasma levels of a substance can serve as an evaluation standard only when combined with an at least approximate knowledge of the substance's diffusion. The determination of the tissue concentration of a drug is subject to considerable methodologic errors,

since it is generally based on blood or urine concentrations of the test substances.

The so-called volume of distribution is defined as a fictitious volume of the organism, i.e., at the moment of distribution equilibrium (homogeneous distribution of the antibiotic between tissue and blood) the same concentration is reached in the total body as in the blood plasma. The volume of distribution constant is dependent on the physicochemical properties of the antibiotic, especially its degree of serum protein binding. Determination of the absolute volume of distribution not only gives a concrete idea of the antibiotic in the human organism, but it is also an important pharmacokinetic constant with other pharmacokinetic parameters can be calculated. For example, according to the one-compartment model of pharmacokinetics, the substance is distributed in only one volume. We will not go into further detail here.

Other important criteria for evaluating an antibiotic are the type and amount of its elimination from the human organism. Elimination is termed the irreversible excretion of an antibiotic from a specific volume, either from the total organism or from one of its parts, the so-called compartments. This process is reflected first of all in the blood or plasma levels, although the antibotic is eliminated from individual compartments or from the total human organism. Depending on the type of excretion we can differentiate three different types of drug : renal elimination only (e.g.,gentamicin, cephalosporins), extrarenal elimination only, e.g.via metabolism (chloramphenicol, rifampicin), partially renal, partially extrarenal elimination. Renal excretion can take place by means of tubular or glomerular filtration, frequently by both mechanisms. Therefore, substances that are primarily eliminated renally must be applied in reduced doses in order to avoid accumulation in patients with renal function disturbances. Since the kidneys are the main organ of elimination for most antimicrobial substances, renal insufficiency has a significant influence on the kinetics of these substances. It poses both the danger of overdosage (if dosage is not reduced) and un-

128

Thai J. Pharmacol.
Vol.8 No.2 May-Aug. 1986

derdosage (if dosage is too small).

Hemodialysis and peritoneal dialysis result in a very diffe-
rent loss of activity of the various antimicrobial agents. The effect
of dialysis on the drug pharmacokinetics in the organism depends on
several factors, the most important of which is the passage of the drug
or antibiotic through the dialysis membranes. This itself, however, is
highly dependent on the degree of serum protein binding of the indivi-
dual substance. Moreover, the properties of the dialysis machine also
play a very important role. Several different methods of calculating
these factors are given in the relevant literature. For example, the
half-life of the antibiotic can be derived from a number of serum level
assays in the different procedures of dialysis, and then this is used to
select the optimal dosage. As a general principle, the patient must be
monitored very carefully in the hospital, since the renal function can
change very rapidly. This rule also applies to many other life-threa-
tening conditions that cause a temporary renal insufficiency.

Besides the kidneys, elimination also involves the liver,
biotransformation and catabolism, and all other processes that can lead
to permanent tissue retainment of the drug. Most chemotherapeutic agents
are metabolized and degraded in the organism by oxidation, reduction,
hydrolysis and conjugation. How much drug is excreted unchanged by kid-
neys, bile, faeces and, less so, by lungs and skin varies greatly depen-
ding on the individual active substances. It can amount to < 1 - >70%
of the administered dose. Most of the metabolic and catabolic reactions
are inactivating processes; only a very few cases, compounds that are
inactive in vitro are initially activated in the organism. Examples of
the latter include chloramphenicol esters and several N-acetyl-sulfona-
mides. The pathways of metabolism determine the duration of drug action,
the processes of diffusion and elimination, and also the occurrence of
toxic side-effects and allergic reactions. The incidence of the latter
is especially high in premature and newborn infants, patients with renal
and liver diseases, and elderly patients, as well as those with anomalies
of fermentation.

The amount of parenterally administered drug that is excreted in the faeces indicates its concentration in the bile. It also indicates when precautionary measures are required in patients with liver function disturbances. In contrast, the amount of orally administered drug eliminated in the faeces is primarily dependent on the intestinal adsorption quotient of the active substance. The interindividual variation here is often considerable. Important serum or elimination half-life values for determining appropriate dosage can be derived from the excretory conditions of a drug. Besides the pharmacokinetic analysis of the time course of changes in drug blood levels, the changes in urine concentrations are also important. These values provide information on the renal elimination of the antibiotic, as well as on the total character of the excretion process. The most important and best known pharmacokinetic parameter is the so-called biologic half-life, $t_{\frac{1}{2}}$; it is considered the basic constant. The biologic half-life is specific for each substance. It is a variable which under certain circumstances is dependent on the serum protein binding and fluctuates expecially in patients with renal function disturbances.

Clearance methods play a great role in the evaluation of drug elimination. They give information on aspects of quantity and rate of elimination and also on the routes of elimination. Pharmacokinetics is concerned primarily with the so-called total clearance, involving renal and extrarenal clearance. Both are closely related. The extrarenal clearance is the difference between the total and renal clearance. It is the parameter for determining the extrarenal elimination and probably also for the biotransformation of the antibiotic. The so-called clearance ratio is determined from the renal clearance of the investigated antibiotic and the renal clearance of endogenous creatinine. If their ratio is equal to 1, the antibiotic is eliminated only by glomerular filtration. If the quotient is larger than 1, the elimination can be assumed to involve tubular secretion (e.g., in a number of penicillins and cephalosporins). Only the unbound portion of antibiotics with strong protein binding are filtered in the glomeruli. To calculate renal clea-

rance, the bound portion has to be subtracted from the total plasma con-
centration. The excretion ratio obtained characterizes the actual me-
chanism of renal elimination.

These values are very important for the practical applica-
tion of antibiotics, for they have furthered our understanding of the
distribution of antibiotics between the adrenal cortex and the renal
medulla. In cases where the infection is localized in the kidneys, this
could influence the choice of the antibiotic. It is also essential to
know that whereas antibiotics with extensive protein binding cannot be
excreted by glomerular filtration, they can be eliminated by tubular
secretion.

The pharmacokinetic principles of antibiotics are generally
calculated on the basis of a single application. However, in actual
practice, antibiotics are administered either repeatedly at short inter-
vals or where especially indicated over a longer period. This was the
occasion for intensive clarification of pharmacokinetic laws of drugs
given on a regular basis. Such knowledge is the basis for selecting
optimal antibiotic doses. Repeated administrations can easily lead to
accumulation of the antibiotic, during which the blood or plasma levels
alternate between minimal and maximal concentrations. After a certain
interval, which is equal to about five times the $t_{\frac{1}{2}}$ of the antibiotic,
a steady state arises, which is termed a plateau concentration, i.e. ,
the amount of drug absorbed is equal to the amount eliminated. It is
necessary to determine the mean serum concentration for certain bacteri-
cidal antibiotics which have a short biologic half-life (most penicil-
lins and some cephalosporins).

Pharmacokinetics also allows the individualization of dos-
age. This is absolutely necessary in patients with kidney and liver
function disturbances, pregnant women, newborns and infants, and geria-
tric patients, and also during the course of a disease. Administration
of a normal dose in such cases can increase the risk of pharmacotherapy
or even result in failure of therapy. Optimal dosage regimens must be

established for these patients in order to guarantee a maximal efficacy
at a minimal risk. This can be exemplified in cases of renal insuffi-
ciency; here the individual half-life of a drug or antibiotic can be
estimated from the patient's creatinine clearance.

Pharmacokinetic trials are to be sure the most important but
not the sole prerequisite for a rational and economical antibiotic the-
rapy in hospital and practice. Together with the results of in vitro
microbiological investigations and the preclinical data and clinical
experience obtained from animal models of infections, they provide the
criteria to evaluate the therapeutic value of a new antibiotic. In
practice, pharmacokinetic results cannot be evaluated unconditionally,
for they have to do with biological variables. Often the concrete in-
terpretation is very difficult and occasionally even very questionable.
Nevertheless, the dosage regimens that have been derived from pharmaco-
kinetic calculations and confirmed by them have proven successful in
both hospital and practice, especially in the case of renal insufficiency.

Pharmacokinetics must also be understood dynamically. Single
steps of a dynamic process, e.g., blood or serum levels or serum protein
binding, can be deceptive and confusing when presented in isolation, out-
side the total context. A critical and objective evaluation is only pos-
sible when all principles are considered together.

Microbiological studies of the normal or pathologically
altered flora should be conducted at the same time as the clinicopharma-
cological investigations, in order to determine the range of antibacte-
rial activity of a new antibiotic. The clinicopharmacological trials
are brought to a close in Phase IIa with the first therapeutic applica-
tions in the relevant range of indications. At this point the new drug's
profile of activity and potency and the characterization of its activity
have been sufficiently clarified.

Side-effects (allergic, toxic, and biological) are inves-
tigated and carefully registered according to the following well-known
procedures. In most cases it is not possible to differentiate between

purely allergic, toxic or biological reactions. Generally, all of these reactions have a summation effect. The individual drug response and sensibility permit a multitude of disease forms to develop.

When viewed within the whole picture, primarily toxic reactions to antimicrobial agents are quite minimal. For example, polyvalent antibiotics are known to strongly activate the mucous membranes. Under particular conditions (administration on empty stomach, long retainment in intestines, enteral excretory pathway) toxic reactions of the mucous membranes can occur. Depending on the individual drug response of the organism, the underlying disease, etc., these reactions can take on clinical forms ranging from minor to severe. Regular antibiotic administration is by no means the absolute prerequisite for allergic reactions to arise or to be induced. If other drugs administered earlier have a similar chemical structure to that of the new antibiotic, they can serve as the inducing agent. The same role can be played by dietary fiber, excipients, contamination, etc. An allergic predisposition can be created by these factors, but they alone are not the decisive factors inducing allergic reactions. Usually, several factors are involved. The abuse of antibiotics in therapy, their use in agriculture and animal husbandry, in food preservatives, etc., have also played a significant role in the increased rate of sensitization to them.

The problem of nosocomial infections, i.e., infections that patients contact during hospitalization, is as old as the existence of hospitals. Whereas earlier hospital fires, surgical erysipelas, stomatitis, puerperal fever, tetanus, etc., were especially feared, nowadays infections with staphylccocci, gram negative bacteria, and fungus such as Candida cause the majority of hospital infections. The special additional feature of the "modern form" of hospital infection in the age of chemotherapy is the increased number of infections due to agents that are resistant to individual antibiotics. This poses special therapeutic problems, especially since the microorganisms frequently develop multiple resistance to various active substances. The cause of hospital infection is complex. It is difficult to combat, because numerous

factors are involved. An essential, perhaps the most decisive, role is played by all factors that negatively influence the equilibrium between the causative agent and the patient, i.e., that reduce the patient's resistance and immune defenses.

Acute worsening and life-threatening complications can occur in infectious diseases caused by microorganisms that produce endotoxins, especially when massive loading doses of antibiotics are given at the onset of therapy. These reactions are observed with not only bactericidal but also with bacteriostatic antibiotics. Their cause is most often ascribed to the effect of endotoxins released during bacterial disintegration or bacteriolysis. Occasionally, single or additional allergic reactions are assumed to be involved, for example, Herxheimer's reaction and therapeutic shock.

The increasing consumption of drugs has been accompanied by increased disturbances resulting from interactions between drugs administered simultaneously. In many cases the course of reactions has been clarified. However, this does not mean that simple guidelines can now be set up and followed so as to avoid possible life-threatening intoxications due to the prescription of several drugs, which is frequently necessary. Since enzyme mechanisms frequently play a decisive role in such situations, data from animal experiments on this complex topic should be interpreted as suggestions, at the most, and should not be extrapolated to humans. With this in mind, the following explanations are meant to encourage the testing of undesired reactions appearing during the course of chemotherapeutic measures so as to determine whether there is interference between active substances.

Pharmacokinetic data on the individual drugs can be used as starting points for estimating the influence of multiple-drug administration on the organism. The mechanisms involved in drug interactions are given in the following general breakdown :

1. Pharmacokinetic interaction : drugs can influence the absorption, distribution, biotransformation or excretion of other drugs.

134

Thai J. Pharmacol.
Vol.8 No.2 May-Aug. 1986

2. Pharmacologic or pharmacodynamic interaction : two drugs can exercise an additive or synergistic pharmacologic influence that leads to undesired effects. However, two drugs can also have an antagonistic pharmacologic effect. In both cases the action of the drugs on the same or different receptors can play a role.

3. Various interactions : this group includes drug interactions that cannot be assigned to either of the preceding categories. An example is provided by the sulfonamides. Although otherwise insoluble, they require an acidic urine for elimination but crystalluria can develop if the pH is too low.

Finally, a few brief remarks should address the effects of antibiotics on laboratory values. The different possibilities of interaction can be determined and must be strictly and clearly differentiated from each other : the presence of an antibiotic can falsify the methods of detection, and the antibiotic can change clinicochemical parameters. It is practical from a critical and objective standpoint not to describe the effect of antibiotics on laboratory values as drug interactions. They are not interactions, but first of all the consequence of unspecific chemical methods of detection, and secondly, a part of the activity profile of the antibiotic or drug.

In conclusion, the clinicopharmacological evaluation of a new antibiotic involves a multitude of trial criteria, which permit an interpretation of its clinical relevance and usefulness. In the last analysis, the clinical significance of a new antibiotic depends on the decision to assign the drug to a certain category. This rests on the personal experience of the clinical pharmacologist examining the drug as well as on the indispensible cooperation of the specialist disciplines involved.

(Lecture given in Pharmacokinetics and Clinical Pharmacology of Cardiovascular and Renal Drugs and Poisons Workshop at ASEAN Training Centre for Primary Health Care Development, Mahidol University, Salaya Campus, Thailand.)