PROCEEDINGS OF THE EUROPEAN SOCIETY FOR THE STUDY OF DRUG TOXICITY

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TOXICOLOGICAL PROBLEMS OF DRUG COMBINATIONS

EXCERPTA MEDICA

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TOXICOLOGICAL PROBLEMS OF DRUG COMBINATIONS

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GENERAL INTRODUCTION TO THE SYMPOSIUM ON

TOXICOLOGICAL PROBLEMS OF DRUG COMBINATIONS

M. KRAMER

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Combined drug therapy plays an important role in the treatment of all diseases. This holds true not only for the great number of fixed combinations which are commercially available but also and even more for the fact that very often several different drugs are administered simultaneously. If one looks into a department of internal medicine of a hospital one will hardly find a patient who receives less than three different drugs while many receive more than five. The situation becomes still more complex by the various formulations and excipients which may alter the release and the absorption of various constituents in different ways. In many instances the administration of more than one drug is necessary and unavoidable for successful treatment, but it is no secret that multiple drug therapy is not always based upon scientific reasoning.

Drug interaction and particularly drug antagonism has always been a fascinating problem to pharmacologists. Also the complexity of the basic processes of antagonistic effects has been recognized for some time. A. G. Clark who played a leading part in this field summarized his opinion on the problems of antagonistic action in the statement: 'Imperfect knowledge appears to be the most probable reason for any apparent simplicity in processes of drug antagonism'.

Thinking of the quantitative evaluation of combined drug effect one has to recall an eminent scientist who devoted a considerable part of his life to this particular problem: Walter Siegfried Loewe who continued to work actively in his laboratory until the last years of his life.

He started his studies on the effects of drug combinations at Dorpat (1912–1928) together with Muischnik and later mainly with Kaer. During the Dorpat period a total number of 11 communications with the title '*Über Kombinationswirkungen*' appeared in *Naunyn-Schmiedeberg's Archiv fur experimentelle Pathologie und Pharmakologie*. Loewe continued these studies later in Salt Lake City. For the demonstration of the combined effect of two drugs Loewe introduced the term isoboles, that is lines connecting those pairs of doses which are equieffective in regard to an adequately selected endpoint of a combined effect. The family of isoboles for various endpoints of the same effect is presented in a graded isobologram and graded isobolograms for all pertinent effects of a combination when assembled in one graph form multiple isobolograms. The figure shows a multiple isobologram of combined phenobarbital-metrazol effects. Just considering the isobole of the LD_{50}: the value for metrazol alone is about 100 mg/kg, for phenobarbital alone 250 mg/kg but for the combination the most favourable value is about 400 mg/kg of metrazol in combination with 200 mg/kg of phenobarbital. The toxic action of metrazol is therefore strongly antagonized by phenobarbital. Using those two dimensional multiple isobolograms, such as those depicting equieffectiveness in regard to the various effects of the combinations of two single drugs, it is possible to analyse their interactions quantitatively. If the combination contains three constituents a three-dimensional approach might be used in order to evaluate the various interactions. The study of the mutual influence of more than three constituents is almost impossible.

Loewe was certainly aware of the fact that his approach had his limitations and did not

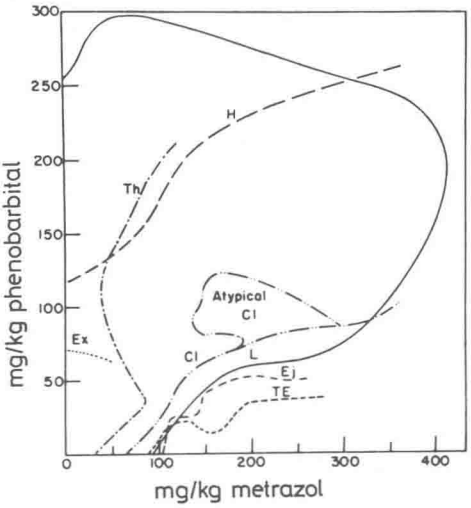

Fig. 1. Survey of multiple isobologram of combined phenobarbital-metrazol effects. Isoboles are those of median effective doses for threshold seizure (Th), generalized clonic seizure (Cl), tonic extensor seizure (TE), ejaculation (Ej), prehypnotic excitation (Ex), hypnotic (H) and lethal effect (L).

give much information about the biochemical or biophysical mechanisms involved. During the presentation of his paper on antagonisms and antagonists at the 20th International Physiological Congress in Brussels in 1956 he stated 'If drug effect designates the resulting alteration of a physiological function and drug action the underlying alteration of the conditions of that function, antagonism is primarily a problem of combined drug *effect*. The 'Why' of a phenomenon cannot be studied without adequate knowledge of the 'What'; the phenomenon itself'.

Today the knowledge about the manifold possibilities by which drugs may interact has considerably increased and there are now also methods for the study of interactions available. This was the reason apart from the general importance of the problem for holding a symposium devoted especially to the toxicological implications of drug interaction.

One important point is the mutual inhibition or acceleration of the metabolic breakdown which will be introduced by C. T. Dollery. More recently interactions on non-specific binding sites of the plasma albumins have become known which are particularly significant in toxicology. Dicumarol and its derivatives can be displaced from their albumin binding sites by phenylbutazone, oxyphenbutazone, indomethacin or clofibrate and bleeding may occur which has been even fatal in some cases. Methotrexate, a competitive inhibitor of folate reductase is bound to plasma albumin and can be displaced by various acidic drugs, including sulphonamides and salicylates thus enhancing the toxicity of methotrexate.

Most of the studies carried out so far have been done in animals. Comparatively few well documented data are available from clinical pharmacological studies in man. There is at present much rumour about drug combinations and one of the goals of our symposium is to boil it down to the facts. We would like to get an answer to the question: Is there a general risk in combined drug therapy or is this risk limited to a few well defined instances? I do hope that at the end of the symposium we will know more about what is clinically really relevant. Such a complex situation as drug interaction certainly requires an appropriate and careful consideration.

I. MODIFICATION OF THE METABOLISM OF A DRUG

MODIFICATION OF THE METABOLISM OF A DRUG

Introduction

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The action of drugs in the body is terminated by uptake into tissues, by excretion in urine or bile, or by metabolism. For the majority of drugs metabolism is the most important route of inactivation although by no means all metabolites are inactive. Metabolism is important because most drugs are lipid soluble substances that are able to diffuse back across the renal tubule as the urine is concentrated so that renal clearance is low. A drug evenly distributed in the body water of an adult man with a volume of distribution of 50 litres and cleared at the urine flow rate of 1 ml/min would have a half life of 577 hours. Conjugation or oxidation usually gives rise to more water soluble derivatives that are pharmacologically inactive and can be excreted in the urine.

The pharmacological action of a wide range of drugs is related to their steady state plasma concentration. Familiar examples include diphenylhydantoin, propranolol, lignocaine and barbiturates. During prolonged therapy drugs are often administered at regularly spaced intervals with the aim of achieving a consistent effect that is maintained throughout the period between doses (Fig. 1A). For this to be achieved the dosage interval must usually be shorter than the half-life. An example is shown in Figure 1B. It is important to appreciate that the

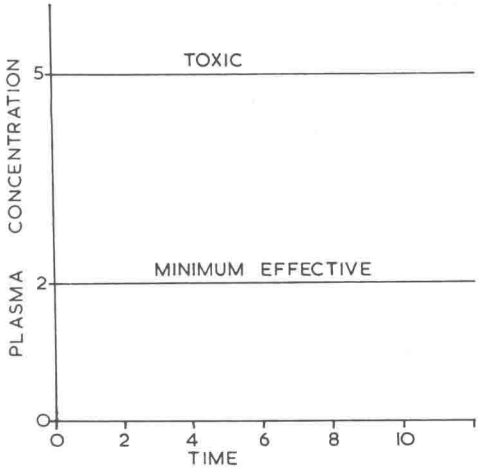

Fig. 1A. In this single compartment model the minimum effective plasma concentration has been set at two units and the toxic level at five units.

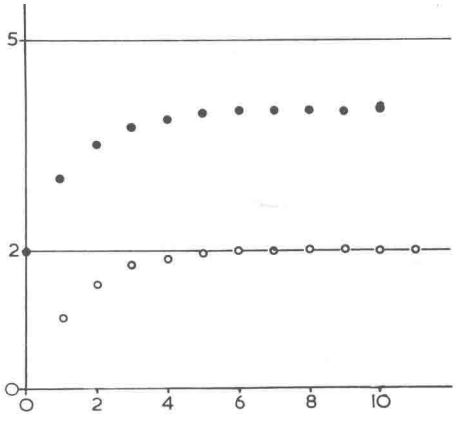

Fig. 1B. A drug dose has been chosen that when administered at the same intervals as the half-life gives a peak concentration (•—•) in the therapeutic range and a minimum level (o—o) immediately before the next dose which is just effective.

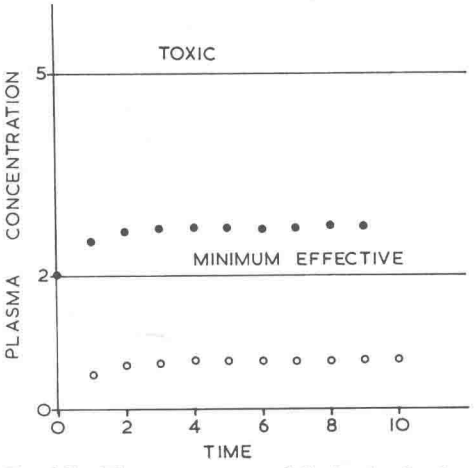

Fig. 1C. The rate constant of elimination has been doubled ('enzyme induction') compared with 1B but the dose and dose interval are unchanged. Most of the time the plasma concentration is below the minimum effective level.

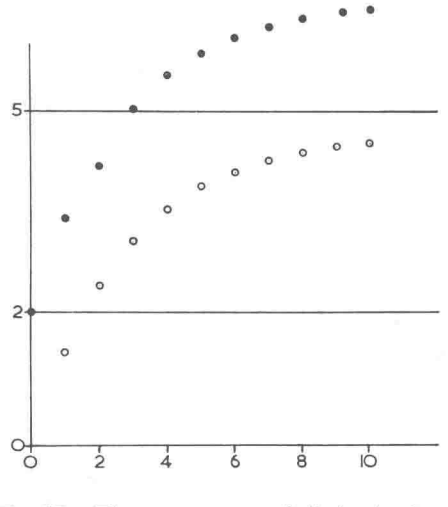

Fig. 1D. The rate constant of elimination has been halved compared with 1B ('enzyme inhibition') but dose and dose interval are unchanged. Plasma concentration now rises progressively into the toxic range.

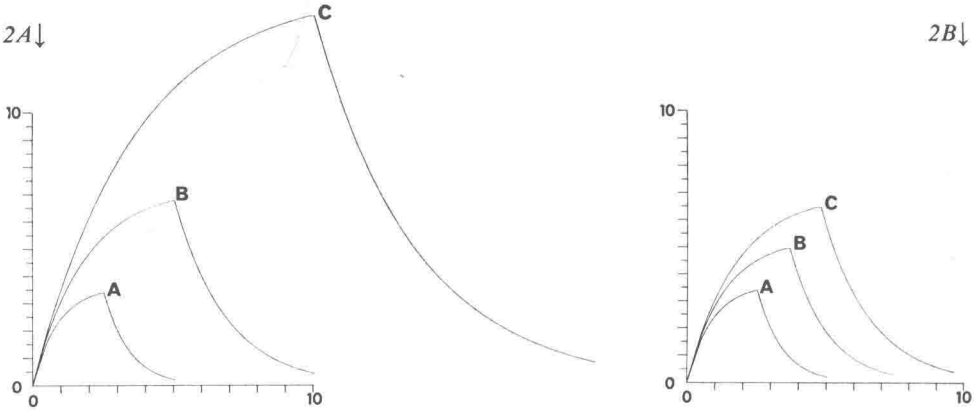

Fig. 2A. In this single compartment model the drug is infused continuously for four half-lives and then stopped. The rate constant of elimination in curve A has been set so that 91% is due to metabolism and 9% due to renal excretion. In curve B the metabolic rate constant has been reduced to one half of the value in A and in curve C it has been reduced to one quarter of the value in A. Note the very much higher concentration reached due to 'inhibition of metabolism' and the longer period of time required to approximate this to a steady-state level.

Fig. 2B. In this example the overall rate constant of elimination in curve A is the same as in curve A in Figure 2A. However, this is now made up of 45% due to renal excretion and 55% to metabolism. Halving the metabolic rate constant in curve B and halving it again in curve C has much less effect on the steady-state concentration because the drug is eliminated to a substantial extent unchanged by the kidney.

rate of metabolism controls both the duration of action of a dose but, under these conditions, also sets the steady-state level. If the dosage interval is much greater than the half-life only the duration of action of each dose will be altered.

When induction or inhibition of drug metabolizing enzymes alters the half-life the situation is altered. In the example in Figure 1C the half-life has been halved so that it is equal to half the dosage interval and as a result the steady-state plasma concentration has been reduced so that much of the time it is at an ineffective level. If the contrary change is brought about by doubling the half-life so that it is twice the dosage interval, the drug concentration rises towards a new steady state and the drug would have to be discontinued before reaching it because it reaches a toxic level (Fig. 1D).

The changes are less striking if appreciable excretion takes place via the kidneys under normal conditions. The conditions set in Figure 2A are that a drug is mainly eliminated by metabolism. The first curve shows the concentration reached during a prolonged infusion (A) and the second when the metabolic rate constant is halved (B) and then halved again (C). In the second example (Fig. 2B) renal excretion contributes about 45% of the initial elimination of the drug. Reducing the metabolic rate constant to half (B) and a quarter (C) of its initial value has much less effect than it has when renal elimination is responsible for only about 9% of the normal excretion.

Changes in routes of metabolism

The effect of changes in the rate of oxidative drug metabolism upon toxicity may be exerted by changes in the route of metabolism as well as alterations in the steady state plasma concentration. The direction and magnitude of these changes depends upon whether it is the parent drug, a metabolite or a reactive intermediate that is the toxic substance.

Evidence is accumulating that points to an important role in drug toxicity for reactive intermediates, especially epoxides formed in the process of hydroxylation. These substances have a very short life but can form co-valent bonds to proteins within the cell. The liver toxicity of brombenzene is enhanced by induction of oxidative metabolism and decreased by inhibition with SKF 525A. Brombenzene causes centrilobular degeneration in the liver and autoradiographic studies have shown that the label persists in the area where the cells are necrotic (Brodie *et al.*, 1971).

Inhibition of metabolism can have a devastating effect if the pharmacologically active substance is normally degraded in the gut wall or liver during or immediately after absorption. A familiar example is the enhanced tyramine response after a monoamine oxidase inhibitor but the same situation could arise with other substances such as the catecholamines that are normally conjugated in the gut wall if a drug or natural product was administered that impaired this defence.

Our ability to avoid such disasters can only be improved by increasing our knowledge of the mechanisms.

REFERENCE

BRODIE, B. B., CHO, A. K., KRISHNA, G. and REID, W. D. (1971): Drug metabolism in man: past, present and future. *Proc. N.Y. Acad. Sci.*, *179*, 11.

THE INDUCTION OF THE MICROSOMAL OXIDASE (CYT. P-450)

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To protect the organism against intoxications is one of the main functions of the liver. Significant amounts of foreign compounds altering, if not to say, polluting man's normal environment invade our body as smoke particles and toxic gases, as dyes, preservatives and disinfectants, and last but not least as drugs. Drug usage particularly of sedatives and stimulants has changed completely; formerly employed as drugs for therapeutic purposes, they have now to be regarded as mass-products consumed in a similar manner as food.

All these and many other compounds foreign to the organism should be regarded as drugs which, broadly defined, are chemical agents interfering with biochemical reactions in living cells. They are excreted by the kidney in unchanged form only very slowly, if they are lipid soluble. If taken daily they accumulate in the organism and would achieve a dangerous level if the liver had no capacity to convert lipid-soluble drugs into more water-soluble compounds.

Fortunately, the endoplasmic reticulum in liver cells prevents the accumulation of drugs. Enzymes are attached to the lipid layers of the membranes catalyzing the metabolism of drugs to more water soluble compounds (Brodie *et al.*, 1958). A great variety of lipid-soluble drugs increase their own metabolism and the metabolism of other compounds not related pharmacologically or chemically by inducing the drug-hydroxylating enzyme system in the endoplasmic reticulum of the liver. This nonspecific phenomenon, which has been observed in all mammalian species so far as investigated, can be viewed as an adaptive process which protects the organism against an overload of foreign compounds (Remmer, 1964).

I. THE TIME COURSE OF INDUCTION

The increase and decrease of the oxidation rate after a single administration of an inducing agent is accompanied by a rise and fall of cytochrome P-450 in the liver cells, indicating that induction is due to a real augmentation of the hydroxylating enzyme (Fig. 1). The time course of induction gives further evidence for the involvement of cyt. P-450 in hydroxylations of foreign compounds (Remmer, 1964).

Substances, such as insecticides, which are converted very slowly to more water soluble, excretable metabolites, act as inducers as long as a sufficient level is maintained in the liver (Fig. 2). It is very well known that a considerable part of DDT is converted to DDE. Its inducing capacity is comparable with that of DDT, but it is retained even longer than DDT in the organism. After a single injection of DDT which induces the formation of the same amount of cyt. P-450 as a single dose of 80 mg/kg phenobarbital, the increase is considerably delayed. The maximum is achieved after 6–7 days compared with 1–2 days after application of phenobarbital. The reason for the much slower new formation of cyt. P-450 is not quite clear; it is even possible that not DDT but a conversion product is the real inducing agent. But this has to be elucidated.

If a shorter acting drug is administered continuously or a longer acting one once or twice daily a similar time course of induction takes place. After 3–4 days a plateau is achieved indicating a new steady state level.