Interactions between drugs and chemicals in industrial societies.
G.L. Plaa, P. du Souich, S. Erill, editors.

ANIMAL MODELS FOR THE STUDY OF DRUG INTERACTIONS

G. ZBINDEN
Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, Schwerzenbach, Switzerland

MAGNITUDE OF THE PROBLEM.

The use of fixed-ratio drug combinations and polypharmaceutical drug therapy are common occurrences in medical practice. For example, a survey of 38 patients on methicillin therapy at the Johns Hopkins Hospital in Baltimore showed that 6 to 32 additional drugs were administered to these subjects (1). It is, thus, not surprising that almost 10% of the adverse drug reactions observed in clinic patients were clearly due to interactions of two or more therapeutic agents (2). Moreover, it is probable that many additional instances of toxic responses caused by drug interactions are not recognized, because intake of multiple drugs through self-medication, use of over-the-counter preparations and prescriptions obtained by consulting more than one physician, is often unknown.

REGULATORY TESTING REQUIREMENTS

For fixed combination products, most regulatory agencies require toxicity testing which is comparable to that conventionally done with single new drugs, using the components of the combination in the ratio proposed for use in human therapy. While this approach is convenient, it has many shortcomings due to a disregard for species-specific pharmacokinetic characteristics of the components, and a neglect of functional disturbances that are particularly frequent and important consequences of drug interactions (3).

In medical practice, ad hoc combinations of two or more drugs are often prescribed. For this reason, regulatory agencies recommend that single new drugs are also investigated for potential adverse effects due to interactions with therapeutic agents likely to be co-administered. The most popular experimental technique is the determination of the median lethal dose (LD_{50}) of the new drug

with and without simultaneous administration of the compounds expected to be used in ad hoc combinations (4-5). For example, the preclinical safety program of the H_2 blocker cimetidine included combined LD_{50} determinations with as many as 29 additional drugs (6). Since this experimental approach also disregards the pharmacokinetic characteristics of the components and uses mortality as end-point, rather than morbidity, its reliability is questioned, and the need for better experimental techniques is now generally acknowledged.

MECHANISMS OF ADVERSE DRUG INTERACTIONS

Clinical experience shows that most undesirable or toxic reactions observed with drug combinations are identical to those known to occur with one or the other of the components. In many instances the toxic reactions are due to pharmacokinetic interferences or disturbances of metabolic disposition of drugs used in combination (7)(Table I). As a consequence, the inherent toxic effects of the components of the combination are enhanced. In some cases, the drug interaction involves an inhibition of absorption or an accelerated elimination of one component. The clinical consequence of such interactions may be a loss of therapeutic efficacy.

Another type of drug interactions is due to the activity of drugs at receptor sites and effects on intracellular processes. The most frequent occurrence is the additive action of substances that have an affinity to identical receptors, e.g. enhancement of central nervous system depression with combinations of sedative drugs and possibly also ethanol. Other mechanisms are listed in Table I.

In rare instances, the toxic effects of drug combinations are quite unlike any of the adverse signs and symptoms known to occur with the components. As an example, unexpected kidney failure of patients treated with tetracyclines following anesthesia with methoxyflurane may be mentioned (8).

TABLE I.

MECHANISMS OF ADVERSE DRUG INTERACTIONS

Pharmacokinetic and Metabolic Interferences
Displacement from carrier (e.g. albumin binding) sites
Competition for renal tubular excretion
Interference with pH-dependent renal excretion
Inhibition of gastrointestinal absorption
Stimulation or inhibition of enzymatic metabolism
Inhibition of uptake at target sites
Interactions at Tissue Levels
Additive effects (components act on identical receptor)
Supraadditive effects (components have same effect but through different mechanisms)
Synergism (one component having no effect of its own increases toxicity of the other)
Inhibition of repair mechanisms

EXPERIMENTAL PROCEDURES FOR THE DETECTION OF ADVERSE DRUG INTERACTIONS

A realistic approach to animal testing for drug interactions is based on the observation that most adverse effects are due to enhancement of the intrinsic toxicity of the components of the combination product. Therefore, the experiments should determine whether the pharmacological and toxicological effects are enhanced or attenuated, or whether the biological profiles of the active ingredients are altered when more than one drug is administered at the same time (9). In some cases, such observations can be made in the course of routine single- and repeated-dose toxicity studies conducted with the drug combinations. This is possible mainly for adverse effects that can be demonstrated by conventional

clinical biochemistry, hematology and pathomorphological techniques. An example is shown in Figure 1.

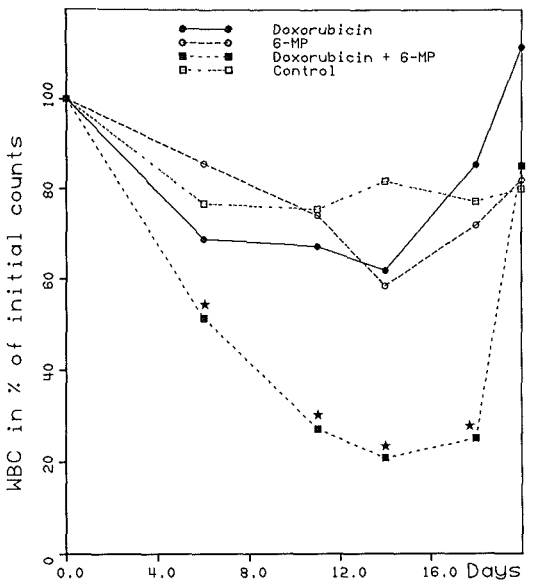

Fig. 1.
White cell counts in groups of 10 male Sprague Dawley rats (SIV 50) treated with 1 mg/kg doxorubicin i.p. and 50 mg/kg 6-mercaptopurin p.o. alone or in combination on days 2-6 and 9-13. Data are given as % of initial counts.
* = statistically significant difference (p 0.02, U-test of Mann and Whitney, 2-tailed) compared to controls and groups treated with single drugs.

In some cases, the dosing schedule has to be adjusted to the pharmacodynamic and pharmacokinetic characteristics of the components of the drug combination. An example is the interaction between various drugs and coumarin derivatives. Hemorrhagic complications caused by enhancement of the anticoagulant action of these substances by concomitant drug therapy are among the most frequent and serious adverse drug interactions (10). That standard acute toxicity studies are inadequate to detect such hazards was shown with cimetidine whose LD_{50} was not altered when it was administered to rats in combination with warfarin (6). However, when the dosing schedule was modified to account for the kinetics of the

pharmacodynamic effect of the coumarin derivative on prothrombin synthesis, the enhancement of the warfarin-induced coagulopathy by cimetidine could be readily demonstrated (Table II).

TABLE II

INTERACTIONS OF CIMETIDINE AND WARFARIN

Cimetidine mg/kg/day	Warfarin mg/kg	Prothrombin time (sec)	Stypven time (sec)	PTT (sec)
2x200	-	15.1±0.6	16.3±2.5	26.3±2.8
-	1x0.4	26.7±4.5	35.9±6.2	48.5±4.5
2x200	1x0.4	40.7±4.9*	40.4±4.6	59.4±7.1*

Groups of 6 male Sprague Dawley rats (SIV 50, weight 200 - 300 g) were treated orally. Warfarin Na was administered 1 h after the first dose of 4th day. (Mean ± 1 standard deviation).
* = statistically significant difference between cimetidine and warfarine combination and groups treated either with cimetidine or warfarin, p 0.05, U-test of Mann and Whitney, 2-tailed

SELECTION OF THE APPROPRIATE TOXICOLOGICAL END-POINTS

For the evaluation of adverse effects due to drug interactions, it is often not sufficient to use the conventional measurements of hematological parameters, clinical biochemistry and terminal pathomorphology. In many cases, pharmacological assay methods that are not part of the toxicological routine, must be adapted for the purpose of detecting undesirable properties of drug combinations. As an example, the hazard of hemorrhagic complications due to interaction of dicoumarol anticoagulants and aspirin is mentioned. As shown in Table III, this adverse effect is not explained by an enhancement of the anticoagulant effect of the dicoumarol derivative, as it was observed with cimetidine (Table II).

TABLE III

INTERACTION OF ASPIRIN AND WARFARIN

Aspirin mg/kg/day	Warfarin mg/kg	Prothrombin time (sec)	Stypven time (sec)	PTT (sec)
2x25	-	15.8±1.0	14.0±1.3	25.2±5.9
-	1x0.4	28.1±3.4	29.6±4.1	37.6±10.3
2x25	1x0.4	23.0±2.0	23.8±5.1	36.1±11.0

		Bleeding time (sec)
1)-	-	328±74
-	1x0.2	650±446
1x25	-	476±325
1x25	1x0.2	1207±532*

Groups of 7 or 8 male Sprague-Dawley rats (SIV 50) were treated orally with 2x25 mg/kg aspirin per day for 4 days. One hour after the first dose of the 4th day, 0.4 mg/kg warfarin Na was administered by gavage. Blood coagulation studies were performed 20 hours after warfarin treatment.
Groups of 10 rats as above were treated as follows: 0.2 ml/kg water orally. 0.2 mg/kg warfarin Na orally. 25 mg/kg aspirin orally. 0.2 mg/kg warfarin Na orally, 18 h later 25 mg/kg aspirin orally. Bleeding time was measured in methoxyfluran anesthesia 20 hours after warfarin and 2 hours after aspirin administration by the tail transsection technique (11).
* = statistically significant difference compared to all other groups. p 0.01, U-test of Mann and Whitney, 2-tailed.
1) water-treated control

From this observation it is concluded that standard coagulation studies do not permit an assessment of the dangerous drug interaction between oral anticoagulants and non-steroidal antiinflammatory agents. However, a marked prolongation of bleeding time can be observed in animals treated with a combination of warfarin and aspirin (11). It is probable that the adverse effect of the combination is due to a disturbance of platelet function.

In developing models for the detection and evaluation of adverse drug interactions, one can often make a selection from several toxicological end-points. The preferred testing technique must then be determined through validation studies with reference substances. The following example illustrates how two completely different experimental models can be used to assess an adverse drug interaction.

In order to assess the potential hazards of an anti-asthmatic combination product containing a beta-adrenergic agonist and a methylxanthin such as aminophylline, the known cardiotoxic manifestation of beta-adrenergic agonists, i.e. the induction of myocardial infarctions in rodents after one or two administrations, can be used (12). The results of such an experiment demonstrating a dramatic enhancement of the cardiotoxic effect of isoproterenol by aminophylline, are shown in Table IV.

TABLE IV

ENHANCEMENT OF MYOCARDIAL NECROSIS INDUCED BY ISOPROTERENOL BY PRETREATMENT WITH AMINOPHYLLINE

Pretreatment mg/kg in 2 min	Severity of myocardial necrosis (number of rats)				
	0	1+	2+	3+	4+
0.9% NaCl	–	3	3	–	–
aminophylline, 30	–	–	2	3	–
aminophylline, 60	–	–	–	3	2

Groups of 5 to 6 male Sprague-Dawley rats (SIV 50, weight approximately 250g) were anesthetized with nitrous oxide-ether and infused with 30 or 60 mg/kg aminophylline or 0.9% NaCl over a period of 2 minutes into the subclavial vein. Eight minutes later, D,L-isoproterenol, 0.05 mg/kg/minute, was infused intravenously for 5 minutes. Rats were killed after 24 hours, hearts were prepared for histopathological analysis, and the degree of myocardial necrosis was assessed by blind evaluation. A rating scale from 0 (no myocardial lesion) to 4+ (extensive myocardial necrosis) was applied.

The interaction can also be investigated by measuring functional changes, e.g. the electrocardiogram following infusion of the beta-adrenergic agonist in animals with and without pre-treatment with aminophylline. This was done in the experiment described in Figure 2. In rats pretreated with aminophylline, the isoproterenol-induced changes, mainly disappearance of T-wave, occurred earlier. In addition, in these animals bradycardia and prolongation of the PR interval developed. The probable mechanism of the interaction and its clinical relevance were discussed in a previous paper (13).

In some instances potential adverse interactions can be predicted from the known pharmacological and toxicological properties of the components of the drug combination. In such cases, an effort must be made to measure those parameters that are expected to be enhanced, inhibited or otherwise altered by the simultaneous administration of more than one drug. In practice, pharmacologists usually deal with acute interaction studies using their specially designed model systems. Toxicologists, on the other hand, are more concerned with drug interactions resulting from cumulative effects after prolonged exposure. The following examples of cardiovascular interaction studies illustrate these two approaches.

In an effort to determine the interactions of tricyclic antidepressants with a variety of other drugs, Nymark and Rasmussen (14) concentrated on the most relevant toxic effect, cardiac arrhythmia. Amitriptyline was infused into rabbits, and enhancement of the cardiac arrhythmias was investigated by electrocardiography. Several agents, e.g. dihydroergotamine, dichloroisopropylnoradrenaline, noradrenaline, and ajmaline were found to enhance the arrhythmogenic action of the tricyclic antidepressant.

The tricyclic antidepressant drugs also have a cardiodepressant action thought to be due to quinidine-like membrane stabilizing properties (15-16). Since these drugs accumulate in the heart on chronic administration, repeated-dose toxicity studies are appropriate to detect potential adverse interaction with drugs having similar properties, such as quinidine. In experiments in rats, it was indeed possible to demonstrate a gradually developing additive effect of quini-

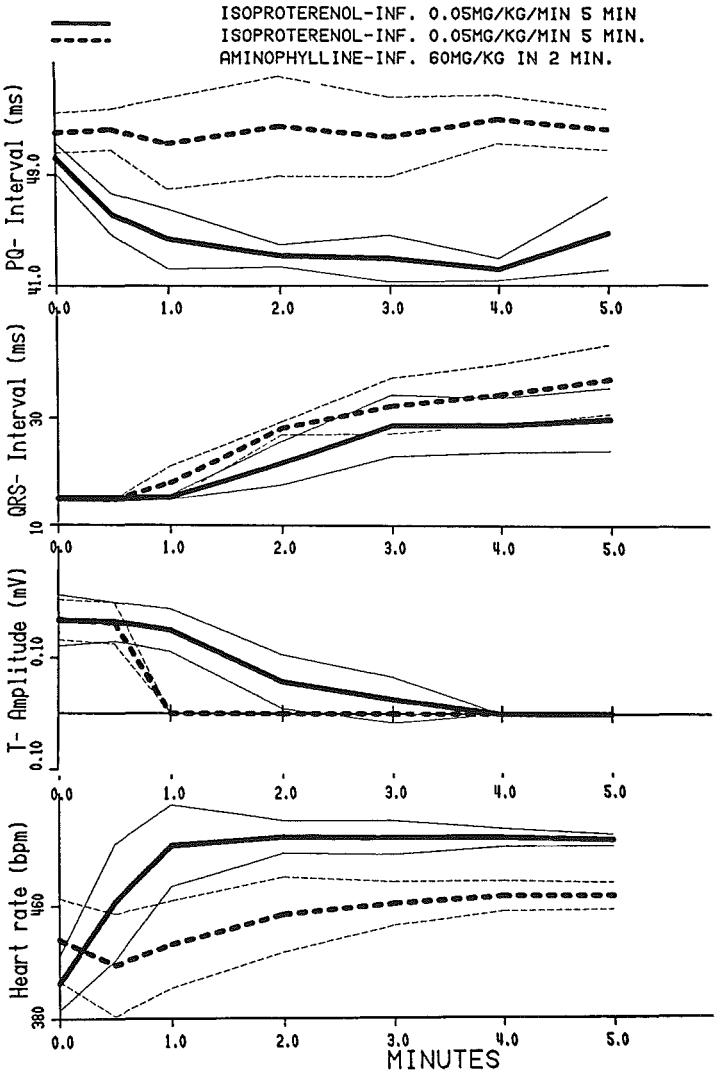

Fig. 2
Graphical representation of electrocardiographic changes observed in the same rats as described in TABLE IV. The heavy lines are the means of the groups, the fine lines indicate the significance limits (graphical t-test). Pretreatment with 60 mg/kg aminophylline inhibited tachycardia and shortening of PQ-interval caused by a 5 minute infusion of isoproterenol (0.05 mg/min). Disappearance of the T-wave and widening of the QRS- interval occurred much earlier in aminophylline-pretreated animals. The lower dose of aminophylline (30 mg/kg) had the same effects.

dine and the tricyclic antidepressant maprotiline on pace maker function, atrioventricular and intraventricular impulse conduction (17)

DETECTION OF PHARMACOKINETIC AND METABOLIC INTERACTIONS

As demonstrated in Table I, many adverse effects of drug combinations are explained by disturbances of absorption, distribution, protein binding and elimination of one component caused by the simultaneous administration of a second drug. Furthermore, some drugs interfere with the metabolism of another component of the combination product. It is evident that the best method to detect such interactions is the measurement of the relevant metabolic and pharmacokinetic parameters of the components singly and in combination.

The major question still debated is whether it is reasonable or even necessary to conduct such investigations in laboratory animals or whether it would not be more relevant to use human subjects. Some valuable information can certainly be obtained from studies in animals. However, the pharmacokinetic behavior of drugs, even binding to carrier proteins, and particularly also the metabolic disposition of the chemicals, often differ greatly between various species of laboratory animals and between animals and man. For this reason, the major part of the studies concerned with the detection of pharmacokinetic and metabolic drug interactions should be performed in humans. This is all the more important, as marked differences in pharmacokinetic and metabolic characteristics exist in different human populations and even among individuals.

CONCLUSIONS

Interactions of drugs given simultaneously as fixed combination products or in the course of a multi-drug therapy can lead to serious adverse reactions. Fortunately, since the majority of undesirable interactions can be be predicted from the known properties of the components, or can be recognized in appropriate animal experiments and clinical pharmacological studies, the hazard to the patients is largely preventable.

Only a limited number of adverse drug interactions can be recognized in standard toxicological experiments conducted with the combination products. Therefore, more directed investigations aimed at identifying the potential of one drug to alter the pharmacological and toxicological profile of another agent, and to interfere with its pharmacokinetics and metabolic fate must be performed.

ACKNOWLEDGMENTS

Cardiovascular interaction experiments mentioned in this paper were supported by a grant from the Swiss National Science Foundation. I thank Mrs. H. Spichiger, Dr. K. Fent and Mr. Ch. Fellmann for technical assistance.

REFERENCES

1. Seidl LG, Thornton GF, Cluff LE (1965) Amer J Publ Health 55:1170-1175
2. Boston Collaborative Drug Surveillance Program (1972) JAMA 220:1238-1239
3. Zbinden G (1984) Proc. 9th Internat Congr Pharmacol London Vol I. The Mac Millan Press Ltd pp 43-49
4. Chen G, Ensor CR (1953) J Lab Clin Med 41:78-83
5. Chen G, Ensor CR (1954) Arch Int Pharmacodyn Ther 100:234-248
6. Brimblecombe RW, Leslie GB (1984) In: Laurence DR, McLean AEM, Weatherall M (eds) Safety of New Drugs. Laboratory Predictions and Clinical Performance, Academic Press London, pp 65-91
7. Kabins SA (1972) JAMA 219:206-212
8. Kuzucu EY (1970) JAMA 211:1162-1164
9. Zbinden G (1976) Progress in Toxicology, Special Topics, Vol 2. Springer Verlag, Berlin
10. Koch-Weser J (1977) In: Williams JT, Saunders CA (eds) Clinical Cardiology, Vol 3. Grune and Stratton, New York, pp 563-570
11. Tamborini P (1986) Interaktion zwischen Warfarin und Aspirin (Acetylsalicylsäure) bei Ratten. Diss Med Univ Zurich
12. Whitehurst VE, Joseph X, Hohman JR, Pledger G, Balazs T (1983) J Am Coll Toxicol 2:147-153
13. Zbinden G (1986) Schweiz Rundschau Med 75:324-327
14. Nymark M, Rasmussen J (1966) Acta pharmacol toxicol 24:148-156
15. Langslet A, Johansen WG, Ryg M, Skomedal T, Oxy I (1971) Eur J Pharmacol 14. 333-339
16. Callahan M (1979) JACEP 8:413-425
17. Zbinden G, Spichiger H (1982) Arch Toxicol 51:43-51

Discussion - Animal models for the study of drug interactions

G.L. Plaa

I see that the terminology used in Europe is not exactly the same as that in North America. I think that we should all go back to the original statistical terminology. In this terminology one could talk about interaction when two drugs produce an effect that is greater or lesser than the expected additive effect. "Supraadditive" and "infraadditive" seem to be better than terms like as "potentiation", "synergism" or "antagonism".

G. Zbinden

The problem is that there is no international agreement on this and that the terminology used somehow reflects the local pharmacological teaching. I very specifically avoided the term potentiation since it leads to confusion. I believe that additive effects, supraaditive effects and synergism are enough to describe these interactions.

G.L. Plaa

I agree with you that to routinely study potential pharmacokinetic interactions in laboratory animals does not make much sense, in view of the large interspecies differences. However, in the case of compounds, such as those that act on the myocardium, the oral hypoglycemics, etc, where interactions could be life threatening, some in vivo or even in vitro studies may be worth undertaking.

G. Zbinden

Of course, there are excellent acute models that can be used in these cases. However, when considering possible interactions with environmental agents, chronic studies are needed.

N.I. Redmond

Could you comment on what you feel would be a general requirement, in Europe and the USA or Canada, for preclinical toxicity testing of a fixed dose drug combination?

G. Zbinden

If you have a new drug which is a combination of known products then you have to do essentially the same studies as you do for a single drug. However, I have had mixed experiences with carcinogenicity. I have been trying to explain to regulatory agencies that if a compound A is not a carcinogen and another compound B is not a carcinogen, putting them in the same tablet does not make them carcinogenic either. Sometimes this point of view has been accepted, but some other it has not. The most important thing, in my opinion, is to do clinical studies aimed at eliciting a possible pharmacokinetic interaction in man, and short term studies - one to two months - to see if the toxicological spectrum of either of the components is enhanced or diminished.