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Evaluation of synergism or antagonism for the combined action of antiviral agents

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Summary

The criteria used for the evaluation of synergism or antagonism for the combined action of antiviral agents are critically reviewed. The isobole method is the only generally applicable approach which does not require any assumptions about the shape of dose-response curves. A microcomputer-based isobole method is proposed which constructs response surfaces and a complete set of isoboles in the dose range under study from a relatively small number of experiments. The objective of this work is to take a step towards standardization of definitions, terminology, and methods for the evaluation of synergism or antagonism in antiviral drug combination experiments.

Synergism; Antagonism; Antiviral combination experiment; Isobole method; Mathematical modeling

Introduction

A promising approach for increasing the effectiveness of antiviral therapy is to seek agents which, in combination, exhibit an effect greater than expected from the effects of single agents. Especially with the advent of recombinant proteins, this area of research has been rapidly expanding (Hall and Duncan, 1988). Further progress in this field requires the profound evaluation of possible interactions between the agents used in combination. Upon inspection of the relevant literature, it was seen that different criteria are used for the prediction of the effect to be

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expected and thus for the evaluation of synergism and antagonism. To the best of our knowledge, the following five approaches have been primarily applied: (1) comparison of the combined action with the most effective single constituent (Hilfenhaus et al., 1987; Fattal-German et al., 1988; Cho et al., 1976), (2) multiplication of effects (Eppstein and Marsh, 1984; Connell et al., 1985; Veckenstedt et al., 1987), (3) summation of effects (Pancheva-Golovinska, 1975; Fleischmann et al., 1979; Mestan et al., 1988; Hughes et al., 1988), (4) the median-effect principle (Johnson et al., 1989; Lin et al., 1989), (5) the isobole method (Ahmad and Tyrrell, 1986; Huggins et al., 1984; Kirsi et al., 1984).[1] Note that summation of logarithms corresponds to multiplication of the original data. Throughout this paper these five approaches will be referred to as criteria (1) to (5).

From the work of Berenbaum (1981, 1985), it is now well known that the application of criteria (1) to (4) is only appropriate if special requirements in regard to the shapes of the dose-response relations are met. The only generally applicable procedure is the isobole method. In the relevant literature on the combined action of antivirals, one can find a substantial number of cases where various criteria are applied in an incorrect manner. Furthermore, due to the application of incorrect criteria, experimental designs are frequently used which do not permit the evaluation of the type of interaction at all. This unsatisfactory situation is probably due to both unawareness of the limits of applicability of the criteria (1) to (4), and to the fact that the construction of isoboles requires experimental data for various dose combinations at equi-effective levels. This is, for certain types of experiments, a laborious task. There is an urgent need for standardization in the examination of combined antivirals (Hall and Duncan, 1988; Moellering, 1980).

In this paper a short discussion of the reasons for the restricted applicability of criteria (1) to (4) is presented. This discussion is mainly based on the work of Berenbaum (1981, 1985). Some of the same arguments can also be found in the article by Hall and Duncan (1988) of which we became aware upon finalization of this paper. In addition, a microcomputer-based version of the isobole method is proposed which does not require experiments at equi-effective levels. The application of this method is illustrated for both an in vivo and an in vitro experiment. Finally, the problem of an appropriate experimental design is addressed. The basic objective of this work is to take a step towards standardization of definitions, terminology, and methods for the evaluation of synergism or antagonism for the combined action of antiviral agents.

Criteria for the evaluation of synergism and antagonism

The following discussion refers to experiments for which only the doses and the effects of agents used in combination, and alone, have to be known. This does not

[1]The papers cited represent typical examples. A rather comprehensive compilation of the literature on the combined action of antivirals is given by Hall and Duncan (1988).

require any information on the underlying mechanism of interaction. Unless otherwise stated, it is assumed that only two agents are combined. Currently, the main body of experimental work in antiviral combination experiments is performed with two agents (Hall and Duncan, 1988). Furthermore, it is important to note that a combination of agents always means a combination of particular doses of these agents. Different dose combinations of the same agents may exhibit different types of interaction. Three types of interaction are recognized:

(a) zero interaction, in which the effect of a combination is what is expected from the effects of the single agents;
(b) positive interaction or synergism, in which the response of the combination is greater than expected;
(c) negative interaction or antagonism, in which the response is lower than expected.

Whereas there is widespread confusion in the terminology used in other fields such as cancer chemotherapy, this is not true for antiviral research. Besides the terms synergism and antagonism, potentiation is primarily used as a synonym for synergism, and zero interaction is usually referred to as additivism or summation. The last terms may be misleading if the mathematical operation of addition is implied. Following the proposal of Berenbaum (1985), it is thus recommended to apply the terms synergism, antagonism, and zero interaction. The definitions given so far require a procedure for the evaluation of the effects of the agents combined and of the effects of the single agents in the case of zero interaction. The methods primarily applied in antiviral combination experiments were already mentioned in the introduction.

Before discussing these procedures in more detail it should be precisely defined what is meant by zero interaction. We adopt the view that the 'interaction' of an agent with itself is by definition a zero interaction. In other words, the 'combination' of two amounts of the same agent can never show synergism or antagonism. Hence, all methods which lead to other results should be discarded.

The problems associated with the multiplication and the summation of effects

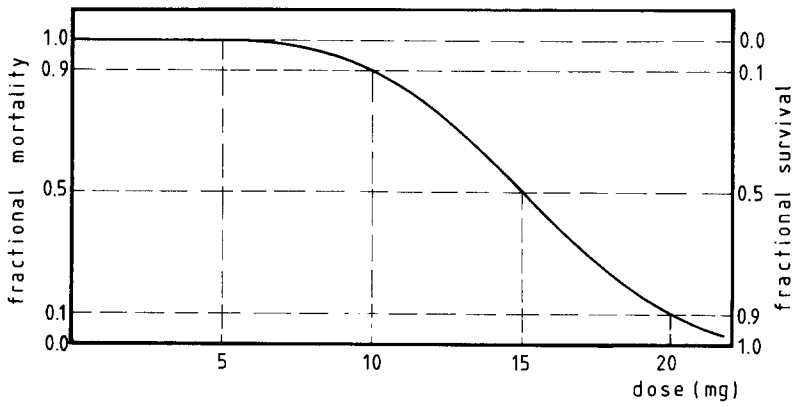

Fig. 1. Dose-response curve for fractional survival or mortality.

will be illustrated by the following simple example (Berenbaum, 1981). Let us assume that two equal amounts (10 mg) of the same substance with the dose-response curve shown in Fig. 1 are used in a 'combination' experiment. According to Fig. 1, 10 mg of the agent reduces the fractional mortality from 100 to 90%. In the 'combination' experiment where 2 × 10 mg (20 mg) are administered, the effect-multiplication criterion predicts a fractional mortality of 81% (0.9 × 0.9 = 0.81) for zero interaction. A fractional mortality of, for example 10%, would thus be classified as highly synergistic. However, the dose-response curve shows that 20 mg yields a fractional mortality of 10%, which corresponds to the real case of zero interaction. The same difficulty is connected with the effect-summation criterion. Before discussing this approach, one problem in comparing the criteria (2) and (3) has to be pointed out. For response values between 0 and 1, multiplication yields lower, and summation higher, effect values as compared to the effect of the constituents. Hence, proper dose-response relations must be used. For survival experiments this is easily done by either using fractional survival, or mortality. For the application of the effect-multiplication criterion fractional mortality was used, see Fig. 1. For the effect-summation criterion, fractional survival has to be invoked. This method predicts 20% fractional survival (0.1 + 0.1) and this corresponds to 80% fractional mortality, which is almost the same value as predicted by the effect-multiplication criterion. Of course, since the real response for zero interaction corresponds to 90% survival (10% mortality) this is also an incorrect result. For smaller values of the fractional mortality the difference in the effects predicted by effect-multiplication and effect-summation is more marked. For example, a fractional mortality of 0.6 for both agents gives, according to effect-multiplication, a value of 0.36. On the other hand, effect-summation leads to a fractional mortality of 0.2.

The inadequacy of effect-summation is especially obvious if data on fractional survival add up to values larger than 1. Berenbaum (1981) has pointed out that applying effect-multiplication or effect-summation to dose-response curves of the type shown in Fig. 1 is equivalent to saying that a combination of an agent with itself is more effective than the agent on its own. This is hardly logical. Effect-multiplication can only be adopted if all agents have exponential dose-response curves while effect-summation requires linear dose-response curves. In all other cases (other types of dose-response curves, different dose-response curves for different agents, or unknown dose-response curves) the application of these criteria is not justified.

A comparison of the effect of the combined agents with the effect of the most effective constituent [criterion (1)], is only appropriate if one agent does not exhibit a response when used alone, but affects the action of other agents when used in combination. In this case, the evaluation of synergism or antagonism is simply done by comparing the effect of the combination to the effect of the effective agent. One should, however, realize that it does not suffice to know that a single dose of an agent has no effect. One has to be sure that this agent has no effect over a larger dose range. This argument may also be illustrated by means of the dose-response curve shown in Fig. 1: 5 mg of the agent does not exert an effect on fractional

mortality. If, in a 'combination' experiment, 5+10 mg are used, according to criterion (1) one would have to compare the effect of 10 mg (90% mortality) to the effect of the combination. 50% mortality would thus be classified as synergistic. In fact, however, 50% mortality corresponds exactly to the case of zero interaction. Hence, criterion (1) can only be adopted if there is no effect over a dose range larger than that used in combination. It is, of course, an important experimental fact, with possibly far-reaching therapeutical implications, if a combination produces an effect larger than either of the agents alone. This fact alone, however, does not allow any conclusion on the type of interaction. Even though the absolute effect value of the combination is higher than the effect values of the constituents, there may be a zero, synergistic, or antagonistic interaction. Information on the type of interaction is, however, an essential requirement for further studies on its origin. It thus makes no sense to adopt criterion (1) as an 'operational definition' as opposed to using a 'mathematical definition' [criterion (5)] (Moellering, 1980).

According to Chou and Talalay (1984) sigmoid dose-response curves can be described by the so-called median-effect equation, eq. (1).

$$f_a(D) = (D/D_m)^m/[1+(D/D_m)^m] \tag{1}$$

In eq. (1) D is the dose, f_a the response (fraction affected), and D_m corresponds to the dose required to produce the median effect (LD_{50}, ED_{50}, ...). The coefficient m governs the sigmoidicity of the dose-response curve. Chou and Talalay (1984) examined the effects of combinations of agents, classifying them as mutually exclusive and mutually non-exclusive. These notions originate from the fact that the median-effect equation was derived invoking the law of mass action for enzyme inhibitors or other agents. They defined a so-called interaction index CI_{MEP}

$$CI_{MEP} = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2} + \frac{\alpha(D)_1\,(D)_2}{(D_x)_1\,(D_x)_2} \tag{2}$$

where the subscript MEP refers to median-effect principle, $(D_x)_1$ and $(D_x)_2$ are the doses of agents one or two required to produce x percent effect alone, $(D)_1$ and $(D)_2$ are the doses used in combination, and $\alpha=0$ for mutually exclusive agents and $\alpha=1$ for mutually non-exclusive agents [criterion (4)]. CI_{MEP} values that are smaller than, equal to, or greater than 1 are claimed to represent synergism, zero interaction, or antagonism. Recently, a computer software for this approach has been published (Chou and Chou, 1986). Several investigators have used this program for assessing the effect of combined antiviral agents (Johnson et al., 1989; Lin et al., 1989). As can be seen below eq. (2) is for mutually exclusive agents ($\alpha=0$) identical with the isobole equation. In this case CI_{MEP} represents a correct measure of the extent of interaction. On the other hand, for mutually non-exclusive agents ($\alpha=1$) Berenbaum has shown that such combinations are necessarily interactive (Berenbaum, 1988). Furthermore, an incorrectness in the derivation of

the equations was claimed for this case (Giessner, 1988). In any case eq. (2) with $\alpha=0$ is correct. Provided the dose-response curves for the single agents can be described by the median-effect equation, eq. (1), this equation can be used to determine the doses $(D_x)_1$ and $(D_x)_2$ for any effect levels without having experimental data for these levels. If the experimental dose-response relations for single agents cannot be described by eq. (1) the computer analysis is usually not performed, see for example the paper by Johnson et al. (1989). Therefore, it is important to note that the isobole equation is correct for any types of dose-response curves. A further drawback of the computer software for the median-effect principle is that the parameters for the median-effect equation are determined for the linearized version of this equation. This excludes response values of 0% or 100% and is also at odds with current developments in the field of parameter estimation. Nonlinear parameter estimation can be easily performed by computers, and thus any bias in the parameters caused by linearization can be avoided. Nevertheless, fitting mathematical functions to experimental data may substantially facilitate the evaluation of synergism or antagonism. The microcomputer-based isobole method described in this paper relies on the same idea. However, contrary to the approach of Chou and Talalay (1984, 1986) more flexible mathematical functions are used which are appropriate for all types of dose-response curves. Furthermore, mathematical modelling is not restricted to the dose-response curves of single agents but extends to the complete response surface.

The isobole method (Loewe, 1953) [criterion (5)], is a well-known procedure for the evaluation of synergism or antagonism. It requires experimental data for the agents used alone and in different dose combinations at equi-effective levels. If the various dose combinations for any equi-effective level are plotted in the manner shown in Fig. 2, a combination is said to show zero interaction if the data points are on the straight line connecting the doses of the single agents. Points below this line correspond to synergistic interactions and points above the straight line indicate antagonism. This behavior can be expressed in terms of the isobole eq. (1).

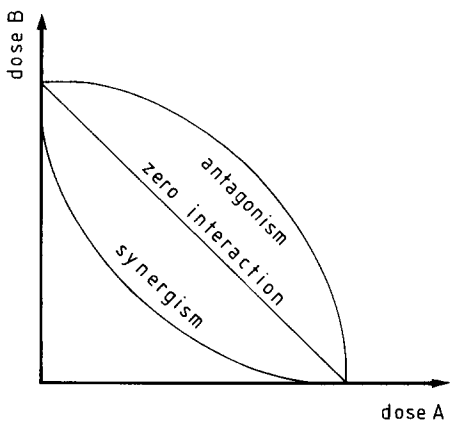

Fig. 2. Isoboles for synergistic, antagonistic, or zero interaction.

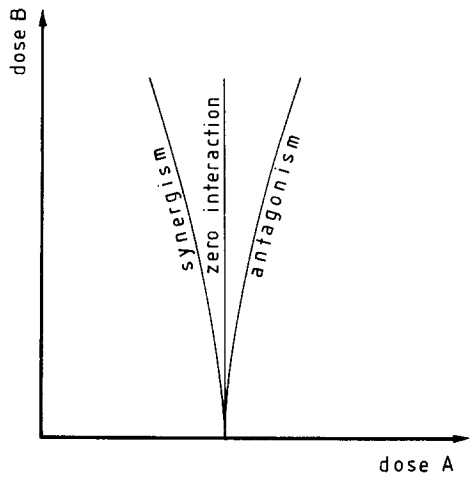

Fig. 3. Isoboles for synergistic, antagonistic, or zero interaction if substance A does not exhibit an effect.

$$\Sigma_i\ (x_i/X_i) = I = \begin{matrix} <1\ \text{(synergism)} \\ 1\ \text{(zero interaction)} \\ >1\ \text{(antagonism)} \end{matrix} \qquad (3)$$

The quantities x_i are the doses of the individual agents i in a combination, and the X_is are the doses of the agents that individually would produce the same magnitude of effect as the combination. I is the index of interaction. Eq. (3) is valid for any number of agents while, Fig. 2, of course, is restricted to only two substances. Berenbaum (1985) has shown that eq. (1) holds irrespective of the shape of the dose-response curves. In view of this result the median-effect principle for mutually exclusive agents [criterion (4)] is nothing more than the isobole approach. The isobole method can also be applied if an agent, when used alone, does not show an effect. In this case, the X_i for this agent is assumed to be infinite, and the isobole for zero interaction is now a straight line parallel to the dose axis of the agent which exhibits no effect (Fig. 3). As shown in this Figure, synergism is indicated in this case by a deviation in the direction of the dose axis, and antagonism by a deviation away from the dose axis. For many combination experiments the shapes of the dose-response curves are either not known, or do not correspond to the types mentioned above. Therefore, the isobole approach is the only generally applicable method.

Several measures have been proposed for the quantification of synergism and antagonism. Except for the index of interaction I and the combination index according to Chou and Talalay (1984) CI_{MEP}, which were already mentioned, we are aware of the FIC index (FIC, fractional inhibitory concentration) (Elion et al., 1954), the combination index CI (Spector et al., 1982), and the index DI (degree of interaction) (Rada and Hanusovska, 1987). The FIC index is calculated according to eq. (4).

$$FIC = (MIC_A^{comb}/MIC_A^{alone}) + (MIC_B^{comb}/MIC_B^{alone}) \quad (4)$$

MIC_A^{comb} and MIC_A^{alone} stand for the minimal inhibitory concentrations of agent A when used in combination or alone. Eq. (4) is nothing more than the isobole equation for the effect produced by the minimal inhibitory concentration. The interpretation of the numerical values of the FIC index is thus the same as for the interaction index I. The FIC index is a correct measure for the evaluation of synergism and antagonism in combination experiments. The combination index CI, proposed by Spector et al. (1982), is calculated according to eq. (5).

$$CI = \ln[(E_{Ax}E_B)/(E_{AB}xC) \quad (5)$$

E_A and E_B are the effects of agents A and B, when used alone, E_{AB} is the corresponding combined effect, and C is the effect of the control. It is claimed that values of $CI>0$ indicate synergism, values of $CI<0$ antagonism and that $CI=0$ corresponds to zero interaction. As can easily be seen from eq. (3), the CI is based on the effect-multiplication criterion. The combination index CI is thus of no general applicability and can only be used for exponential dose-response curves.

Rada and Hanusovska (1987) proposed a plaque inhibition assay for the rapid detection of synergism. In this assay, the diameter of the inhibitory zone of two substances A and B, is compared to the zone diameter produced by substance A alone, where in the combination experiment, substance B is used at subinhibitory concentration. The index DI is then calculated according to eq. (6):

$$DI = D_{AB} / D_A \quad (6)$$

where D_{AB} and D_A are the zone diameters of the combination experiment and of a substance alone. This method of evaluation is obviously based on criterion (1). As already noted, it does not suffice to know that a single dose of substance B has no effect. If it can, however, be ensured that substance B shows no effect over a larger dose range, then the index DI is a correct measure of the interaction of agents. This could easily be checked by using one and the same substance in a 'combination' experiment and then comparing the results to the one obtained with this substance alone.

The inhibition quotient (Surjono and Wigand, 1981) and a method originally used by Valeriote and Lin (1975) occasionally applied in antiviral research, see for example Park et al. (1984), are nothing more than applications of the effect-multiplication criterion and thus subject to the same restrictions.

A microcomputer-based isobole method

As already noted, the application of the isobole method can be a laborious task for certain types of experiments. The amount of work increases further if one is not only interested in the isobole for one effect level, but rather in a more com-

prehensive picture for different effect levels. The dose-response relationship for two agents is described by a three-dimensional response surface. The basic idea of the approach to be presented is to fit a mathematical function describing the response surface to the experimental data. After having done this, the isoboles can easily be calculated for any effect levels. The advantage of this method lies in the fact that a relatively small number of experiments yields the complete set of isoboles. An approach of this type was, for example, invoked in cancer combination chemotherapy using a logistic function (Carter et al., 1983). One serious disadvantage of the procedure of Carter, is that the result is more or less dependent on the type of mathematical function used. For example, a logistic function presupposes that the response surface shows one maximum. Different types of response surfaces may require different mathematical functions. A further difficulty develops with the large number of parameters involved (5 in the logistic case for two agents) as compared to the usually small number of dose combinations studied. According to our view, a more powerful method is to fit so-called spline functions piecewise to the experimental data (Shoup, 1984). Due to the piecewise fitting this approach is appropriate for all types of response surfaces and for all types of dose-response curves for the single agents. Microcomputer programs which perform the fitting procedure and the calculation of isoboles are now widely distributed and amenable to routine application. Any software capable of producing 3D graphics and contour plots can be applied.[1] The mathematical background of this procedure will not be discussed here. The contour plots generated correspond directly to the isobolograms. Due to the interactive features of these programs the construction of response surfaces and contour plots is very easy and needs almost no further explanation. In addition to a file with the experimental data (a number of lines which contain the doses of agents 1 and 2 and the corresponding response) and options which control the kind of representation (for example the number of lines for the grid of the response surface, angles defining the position of an observer looking at the response surface, effect levels for which the isoboles are to be calculated, etc.), the only further information which a user has to provide is the so-called smoothing factor. A value of 1 means no smoothing at all, and smaller values correspond to increased smoothing. To demonstrate the power and possible drawbacks of this procedure, applications to two data sets are shown in Figs. 4 to 11. It should be noted that the data were taken directly from the literature. Therefore, the experimental design was not aimed at the construction of response surfaces or isoboles. Figs. 4 to 7 refer to the combined action of tumor necrosis factor (TNF) and interferon-γ (IFN-γ) on the yield of vesicular stomatitis virus in an in vitro assay (Hughes et al., 1988). The actual response shown is the logarithmic reduction of the virus yield. In this case, the experimental design used is adequate for the construction of response surfaces. Fig. 4 shows the response surface without any smoothing. On the dose axis, the dose-response curves for the single agents can be seen. The advantage of data representation without smoothing is that all

[1]We used the programs QGRID, SURF, and TOPO distributed by Golden Software, 1984.

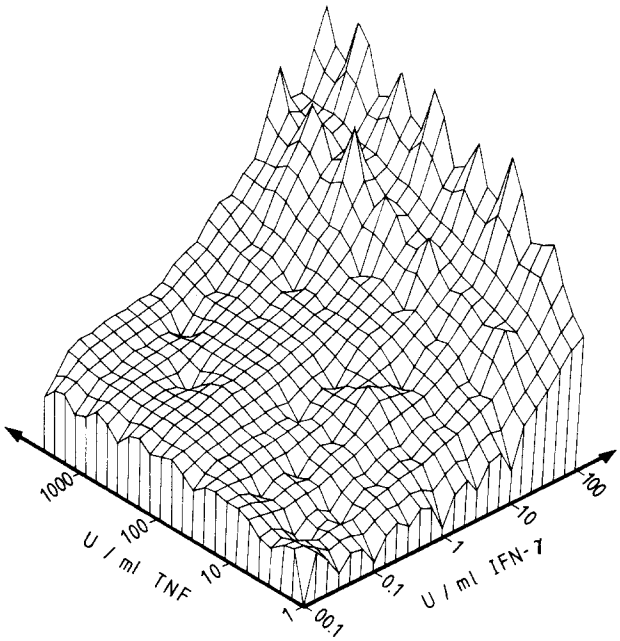

Fig. 4. Response surface for the combined action of the tumor necrosis factor (TNF) and interferon-γ (IFN-γ) on the inhibition of vesicular stomatitis replication in HEp-2 cells. (Experimental data according to Hughes et al. (1988), Fig. 1; smoothing factor = 1 (no smoothing); response: logarithmic reduction of virus yield; experimental design: 1, 3, 10, 30, 100, 300, 1000, 3000 units/ml TNF and 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 units/ml IFN-γ.)

experimental data are shown with their correct response value. One drawback is, however, that the interpolation procedure leads to maxima and minima between the dose combinations studied. These may be artifacts. For example, for IFN-γ doses of 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 100 units/ml were investigated. The maxima on the dose axis for IFN-γ are certainly artifacts. The same is true for the minima between the several peaks for 100 units/ml of IFN-γ and the dose range covered for TNF. These artifacts can be at least partially removed by a slight smoothing, see Fig. 6. One should, however, realize that the response values are slightly changed by the smoothing procedure. This can be seen by comparing the isobolograms in Figs. 5 and 7. Since both representations have advantages and disadvantages, it is recommended to use both. The interpretation of isobolograms can be simply done by invoking eq. (3) and Figs. 2 and 3. Since the doses in Figs. 5 and 7 are plotted on a logarithmic scale, isoboles corresponding to zero interaction show downward concavity (see, for example, Ahmad and Tyrrell, 1986). If the isoboles meet both, or at least one dose axis, the interaction index I according to eq. (3) can be calculated from the isobologram (Fig. 7). One should note that in the following discussion most dose values are calculated numbers. This means that not all digits given are significant. For the 0.8-isobole and a dose combination of 0.1 and 4.16 the isobole equation yields

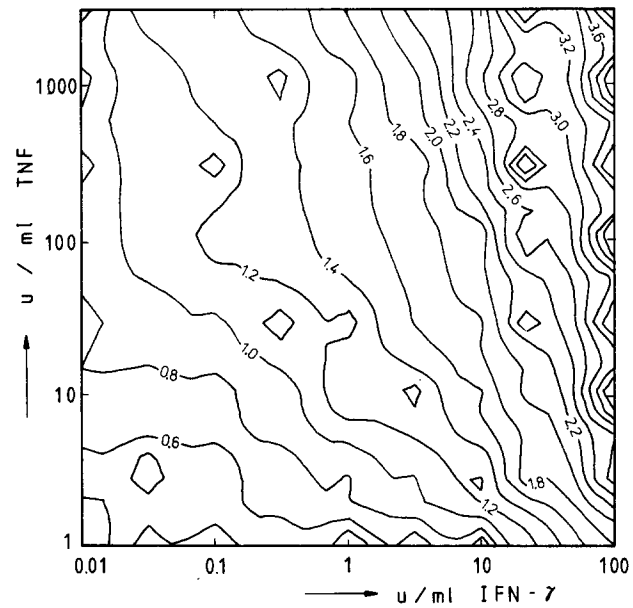

Fig. 5. Isobologram for the response surface shown in Fig. 4.

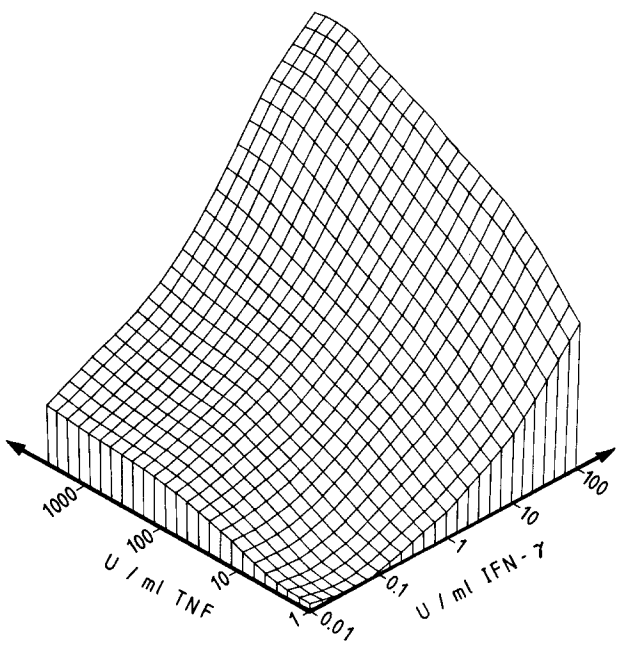

Fig. 6. Response surface for the combined action of the tumor necrosis factor (TNF) and interferon-γ (IFN-γ) on the inhibition of vesicular stomatitis replication in HEp-2 cells (data as shown in Fig. 4, but smoothing factor = 0.99).

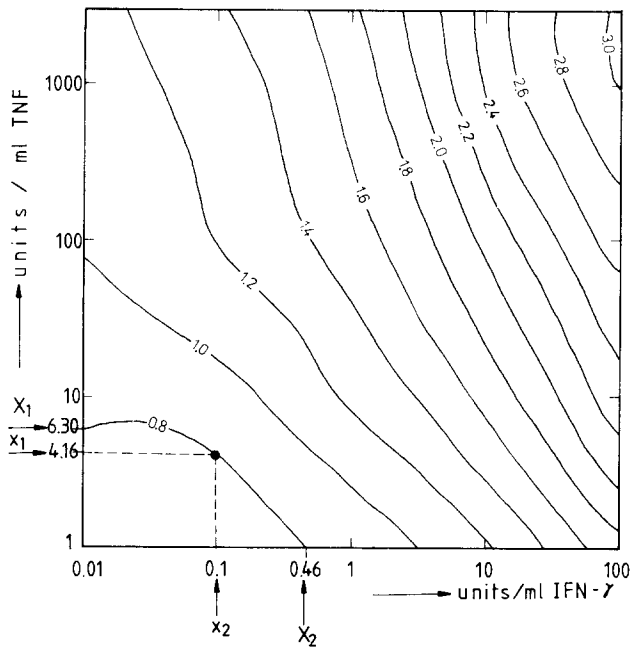

Fig. 7. Isobologram for the response surface shown in Fig. 6; note that all isoboles shown represent synergism (the full circle represents the dose combination referred to in the text and the quantities x_i and X_i are the doses used for the calculation of the index of interaction I, eq. (7).

$$I = (0.1/0.46) + (4.16/6.30) = 0.88 \quad (7)$$

The dose data used are indicated in Fig. 7. The value of I=0.88 indicates slight synergism. Isoboles which do not meet the dose axes in the dose range studied and which show higher response values than the other isoboles that meet the axis, also indicate synergism. The conclusion which can be drawn from the isobolograms shown in Fig. 5 or 7 is that, except for a very small region at low doses which may possibly show zero interaction, the complete remaining response surface can be classified as synergistic. In Figs. 8 to 11 the combined effect of acyclovir (ACV) and interferon-α (IFN-α) on the survival of mice infected with herpes simplex virus type 1 is shown (Connell et al., 1985). As for most in vivo experiments, the experimental design is not as appropriate as for in vitro experiments. One should thus realize that response surfaces and the isobolograms reflect both experimental facts and possible inadequacies of the design used. For example, in Fig. 8, the two maxima on the dose-response curve of IFN-α would certainly disappear if experimental data with these doses were available. Further, the valley between 12500 and 25000 units of IFN-α for an ACV dose of 100 mg/kg may also be due to lacking experimental data. On the other hand, it is felt that the graphical representation of response surfaces provides a good impression of the adequacy of the experimental design and can lead to proposals for further experiments. As in the

foregoing example, slight smoothing partially removes these artifacts (Fig. 10). The isobolograms in Figs. 9 and 11 are very similar. Contrary to the isobologram shown in Fig. 7, in the case of Fig. 11, regions of zero interaction, antagonism, and synergism can be identified. For low doses of both agents, up to a response of 0.48, the interaction is definitely antagonistic, see Fig. 2. The isobole at 0.60 circumscribes a synergistic region. The interpretation of the isobole at a fractional survival of 0.56 is slightly more involved. It meets the dose axis for IFN-α at 15 630 units. A dose combination of 50 mg/kg ACV and 8568 units IFN-α is definitely synergistic because in this case the equi-effective ACV dose for zero interaction is 111 mg/kg. This is the dose where a straight line through the dose combinations 0 mg/kg ACV + 15 630 units IFN-α and 50 mg/kg ACV + 8568 units IFN-α for the effect level of 56% fractional survival meets the ACV axis. A dose combination of 400 mg/kg ACV and 7444 units IFN-α gives, according to eq. (3), for the case of zero interaction

$$(7444/15630) + (400/X_{ACV}) = 1 \tag{8}$$

The value of X_{ACV} is 770 mg/kg. This means that the dose combination of 400 mg/kg ACV and 7444 units IFN-α is synergistic if the 0.56-isobole meets the ACV axis at a dose higher than 770 mg/kg. This includes the case where the 0.56-isobole does not meet this axis at all. If, however, the isobole meets the axis at a dose lower than 770 mg/kg, the dose combination is antagonistic. If an isobologram contains both antagonistic and synergistic regions, there is definitely also a range (or line)

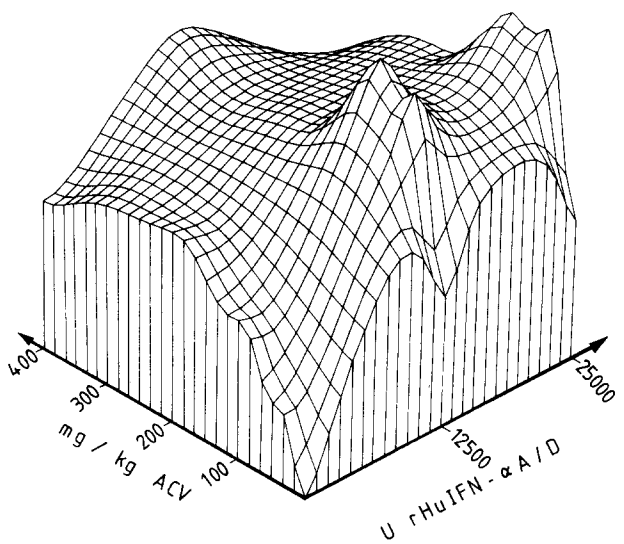

Fig. 8. Response surface for the combined action of acyclovir (ACV) and interferon-α (IFN-α) on fractional survival of mice infected with herpes simplex virus type 1. (Experimental data after Connell et al. (1985), Table 1; smoothing factor = 1 (no smoothing); response: fractional survival; experimental design: 0, 50, 100, 200, 400 mg/kg ACV and 0, 12 500 and 25 000 units rHuIFN-αAD.)

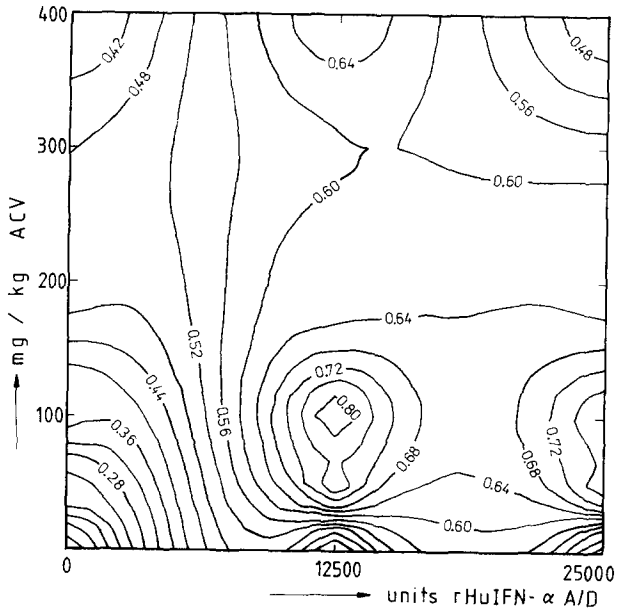

Fig. 9. Isobologram for the response surface shown in Fig. 8.

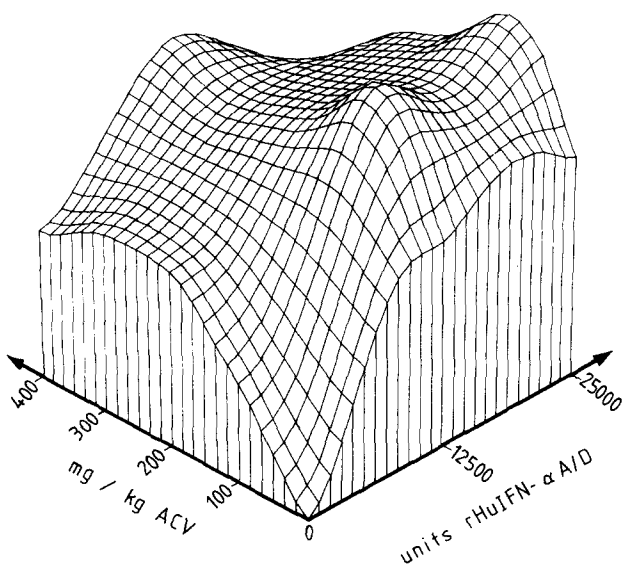

Fig. 10. Response surface for the combined action of acyclovir (ACV) and interferon-α (IFN-α) on the fractional survival of mice infected with herpes simplex virus type 1 (data as shown in Fig. 8, but smoothing factor = 0.99).

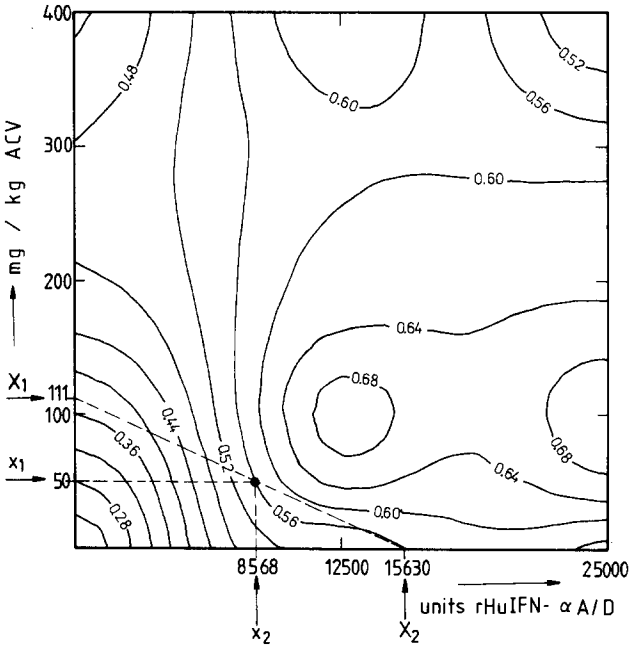

Fig. 11. Isobologram for the data shown in Fig. 10; note that at low doses up to a response value of 0.52 the isoboles are antagonistic and that the isobole at 0.6 circumscribes a synergistic region; the line of zero interaction is between the isoboles with response values of 0.48 and 0.52. (The full circle represents one of the dose combinations referred to in the text and the quantities x_i and X_i are the corresponding doses.)

of zero interaction. In Fig. 11 this line is probably between the 0.48 and the 0.52 isoboles. The authors have identified the dose combinations at 12500/200, 25000/400, and 25000/200 as antagonistic, where the first number indicates the IFN-α dose and the second one the ACV dose. All other dose combinations have been classified as synergistic (criterion of effect-multiplication). While the isobologram also shows definite antagonism for the combination 25000/400, the interpretation for the two other cases is at least questionable.

It should be noted that for closed isobole loops the interaction index I is generally 0, while the graphical representation of isoboles can still differentiate between different effect levels; see, for example the isoboles for effect levels between 2 and 3 in Fig. 7, or the isoboles for 0.64 or 0.68 in Fig. 11. The main advantage of the method presented, however, does not lie in the evaluation of the type of interaction for single dose combinations, but rather in the comprehensive view of the whole response surface with its different possible types of interactions for different dose ranges. This may lead to suggestions for the investigation of further dose combinations and to new insights concerning the origin of antagonism or synergism in the system under study. This method can be applied to any type of combination experiments, be it in vitro (plaque assay, direct determination of virus yield), or in vivo.

Experimental design

A design often used is to study the effects of two agents at certain doses, and then to perform the combination experiment with the same doses. It follows from the arguments presented above that it is principally impossible to determine the type of interaction from this design.

Using the microcomputer-based isobole method, equi-distant doses, either on a linear or a logarithmic scale over the whole dose range to be studied, should be chosen. For in vivo experiments, a rather general pattern displayed by dose-response curves is that with increasing dose the percentage of survival first increases, then reaches a maximum and finally declines due to the toxicity associated with the antiviral agents. It is recommended to include the toxicity region.

From Fig. 2 one can see that possible deviations from zero interaction are greatest at about the midpoint of the isobole. Therefore a possible interaction between two agents is most efficiently detected using a dose combination made up of 1/2 of the equi-effective doses for each of the agents. Provided the complete dose-response curves for the single agents are known, and the isoboles display the simple pattern shown in Fig. 2, the type of interaction can be detected by means of only one combination experiment. One should use dose combination corresponding to zero interaction for a certain effect level, and if the effect produced by the combination exceeds the effect expected for zero interaction, the interaction is synergistic.

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