Drug Synergy – Mechanisms and Methods of Analysis

Hans-Georg Breitinger
The German University in Cairo,
Egypt

1. Introduction

The term synergy is derived from the Greek *syn-ergos*, "working together". Synergies have been described in many settings and situations of life, including mechanics, technical systems, human social life, and many more. In all cases, synergy describes the fact that a system, i.e. the combination and interaction of two or more agents or forces is such that the combined effect is greater than the sum of their individual effects.

This definition implies that there are three possible ways of such an "interaction of agents or forces": these forces could simply add up, not affecting each other (no interaction), their combination could produce a greater than expected result (synergy), or the combination could lead to a result that is less than the sum of the individual effects. This "negative" summation is called antagonism.

Interactions of biologically active agents are an important aspect of pharmacology and biomedicine. In this context, interaction describes the biological activity that results from the presence of several drugs at the same time. Such situations occur in numerous clinical situations:

- combinations of cytotoxic drugs in the treatment of cancer and infections require lower doses of each drug to obtain better therapeutic effects with less side-effect toxicity.
- combinations of antibiotics likewise combine better efficiency with fewer side effects and reduced development of resistance.
- many serious clinical situations require administration of several drugs simply because of multiple therapeutic indications. Although in such a case drug combinations are not formulated to look for synergies, the interactions of these drugs need to be assessed.
- the effect of one drug may be augmented by another drug that does not produce such an effect on its own

In all these cases, multiple drugs are administered, and will show some form of interaction, synergistic, antagonistic, or none. Methods to determine and quantify drug interactions are thus an essential tool in pharmacology. Historically, extracts from plants, animals, or even soils were the first classified pharmaceuticals. These were complex mixtures rather than single agents, and some ingredients may have interacted with others. Over the years of development of pharmacy, isolation, synthesis and marketing of single drugs became the accepted standard. Whether a complex mixture or a combination of drugs is used, the biological interaction of all active substances should be known. Synergy may be observed in simple systems – two drugs that only act on one target protein can show synergism. In such a case we can study the interaction of the drugs mechanistically and determine why and

how several drugs can reinforce each other (or why they do not). Synergy may also be observed in complex settings, such as patients receiving multiple medications. Usually, more than one biological target (protein, pathway, or even organ) are involved in such cases, and single mechanistic descriptions are not appropriate. Additional parameters to consider are drug absorption, tissue distribution, and clearance. It may be expected that many drugs interfere with metabolism of other drugs. Thus, a substance B that slows down clearance of an active drug A, say by blocking metabolizing enzymes or excretion, may lead to a higher effective concentration of A that remains in the body for a longer time. As a result, one would notice a greater effect of drug A when given together with B, although the two drugs have completely different modes of action. While certainly the combination of these two drugs would have a "combined effect is greater than the sum of their individual effects", their combination is synergistic in practical application, but not by the strict definition.

2. Basic models and mechanisms – Synergy on a molecular level

2.1 A simple reaction scheme for enzyme inhibition

Drug interaction and synergy has been intensively studied for more than 100 years, and some of the numerous concepts will be briefly introduced in this chapter. The simplest model cases will be presented, leading to a molecular definition of drug synergy.

Let us assume a simple enzyme following the laws of mass action and Michaelis-Menten kinetics. In the simplest case, this enzyme has an active site, where substrate is being converted into product, and possesses one or several specific binding sites for inhibitors (Fig. 1A). A competitive inhibitor by definition binds to the active site of the enzyme, displacing the substrate. Thus, a mixture of two purely competitive inhibitors will only ever target the active site. This is known as mutually exclusive binding. If only the simplest mechanistic case is considered, one would not expect a second competitive inhibitor to have any notable effect on the first one, other than raising the total amount of inhibitory molecules.
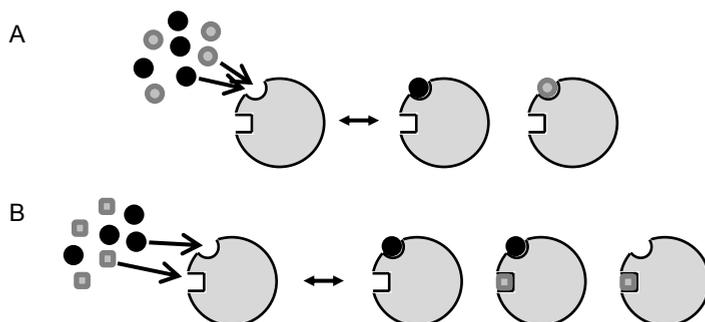

Fig. 1. Schematic representation of inhibition mechanisms (A) Competitive inhibition. Inhibitor (open circles) binds to the active site of the target protein. The agonist (solid circles) binds to the same site. By definition of competitive inhibition, all competitive inhibitors bind to the same (active) site. Thus, binding of two competitive inhibitors must be mutually exclusive, and they cannot act synergistically on the same target protein. (B) Non-competitive inhibition. Inhibitor (open squares) binds to a site different from the active site of the target molecule. In pure non-competitive inhibition agonist binding is not affected by the inhibitor. Inhibition is due to conversion of the target protein into an inactive state.

In case of non-competitive inhibition, the inhibitor binds to a location on the enzyme different from the active site. Assuming that bound inhibitor converts the enzyme into an inactive (non product-forming) state, presence of a non-competitive inhibitor simply lowers the amount of active enzyme molecules (Fig. 1B). There are states, where both substrate and inhibitor are bound to the enzyme. The effect of several non-competitive inhibitors applied together raises the question if synergy can be observed in such a simple system. If two non-competitive inhibitors bind to the same site on their target enzyme, this inhibitory site can either be occupied by inhibitor A or B, but not by both inhibitors at the same time (Fig. 2). This would be a case of mutually exclusive binding of two inhibitors. If one inhibitor is present, and the second one added, one may observe indeed a greater extend of inhibition, but this would only be due to larger amounts of inhibitory molecules being present. At all times, we could predict the total amount of inhibition by summations. In the simplest molecular case, two inhibitors targeting the same site would produce an additive effect only.
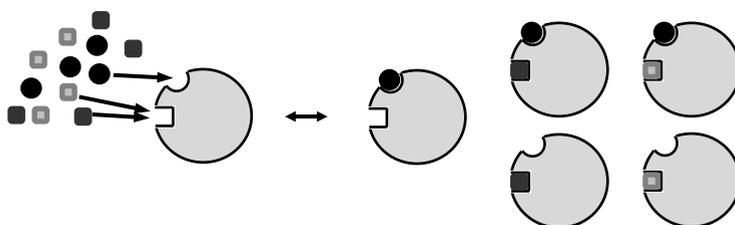

Fig. 2. Reaction scheme for two non-competitive inhibitors targeting the same site. Two non-competitive inhibitors (squares) bind to the same site on the target protein. In this case their binding is mutually exclusive. Presence of the second inhibitor increases the total amount of inhibitor causing in increased inhibitory effect. This increase is only due to simple additivity, and not synergy.

If, however, two different binding sites for non-competitive inhibitors exist on an enzyme, two inhibitors may bind simultaneously (Fig. 3). Inspection of the reaction schemes (Fig. 2, 3) shows that if two inhibitors have specific, independent sites on the enzyme, we will observe states where the enzyme indeed has two inhibitors bound (Fig. 3). These states cannot exist if both inhibitors bind to the same site (Fig. 2). Thus, if two inhibitors are able to bind simultaneously, we have a case of "mutually non-exclusive" binding. Here, presence of the second inhibitor will not only give an additive effect (increase of the number of inhibitory molecules), but will generate additional inhibited states of the enzyme. Therefore, on a molecular level we would expect a superadditive effect of two such inhibitors.

It should be noted that the considerations above are made following some basic assumptions, namely that binding of an inhibitor will convert the enzyme to an inactive state, binding of substrate and inhibitor is reversible, and binding of any compound is fully independent from all other compounds. Thus, the equilibrium binding constant for inhibitor A is the same whether A binds to the unliganded enzyme, or to the enzyme that has substrate and/or another inhibitor bound. Given these assumptions, the mechanisms for activation (Fig. 4A), and non-competitive inhibition (Fig. 4B,C) show the different states in which an enzyme exists in the presence of two non-competitive inhibitors that bind to the same site (Fig. 4B), or to different sites of the enzyme (Fig. 4B).
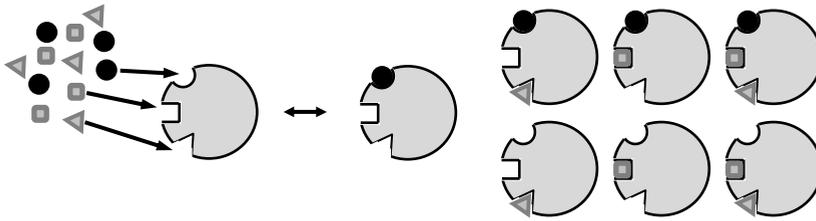
Fig. 3. Reaction scheme for two mutually non-exclusive non-competitive inhibitors. Non-competitive inhibition by two inhibitors (squares, triangles) binding to different sites on the target protein. Here, bindign of one inhibitor does not prevent binding of the other. Note that presence of the second inhibitor creates new inactive states of the target protein that are not possible if only one inhibitor is present. This applies even in the simplest theoretical case, where binding affinities of agonist and inhibitors are completely independent of each other. Thus, in the presence of two inhibitors of the same target protein that follow the rule of Bliss independence, i.e. mutually non-exclusive binding, synergy must be a necessary consequence.
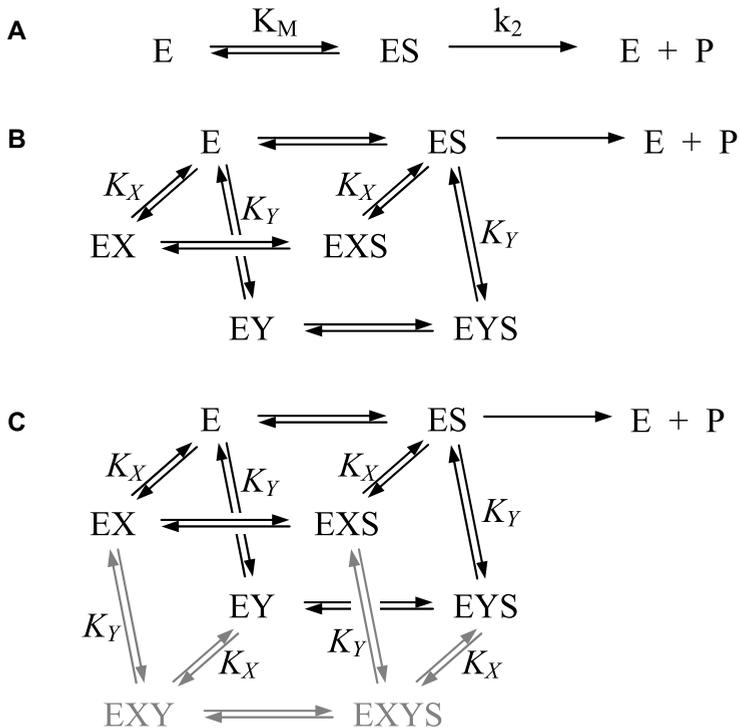

Fig. 4. Mechanism of catalysis and non-competitive inhibition of a Michaelis-Menten enzyme. (A) Mechanism for the activation of an enzyme following Michaelis-Menten kinetics. E = enzyme, S = substrate, P = product, K_M = Michaelis constant, k_2 = rate of product formation from ES. A simplified MM kinetic scheme is used, assuming no backward reaction EP $\rightarrow$ ES. (B) Inhibition of a Michaelis-Menten enzyme by two non-competitive inhibitors X and Y, which are mutually exclusive (e.g. binding to the same site). It is assumed that enzyme with

bound inhibitor is completely inactive, ie it does not form product. Either inhibitor X or Y can bind at any given time. Presence of the second inhibitor can only exert an additive effect but is not synergistic. (C) Inhibition of a Michaelis-Menten enzyme by two non-competitive inhibitors X and Y, which are mutually non-exclusive, i.e. binding to different sites on the enzyme. Here, both inhibitors may bind simultaneously, giving rise to synergistic inhibition.

2.2 Michaelis-Menten enzymes

In this section, a simple derivation of enzyme inhibition by one or two non-competitive inhibitors is given. To illustrate the consequences of mutually exclusive vs. non-exclusive binding, the simplest mechanisms are used.

From the mechanism of a Michaelis-Menten-type enzyme (Fig. 4A) and the law of mass action, we find:

$$K_M = \frac{[E][S]}{[ES]} \quad \rightarrow \quad [ES] = \frac{[E][S]}{K_M} \tag{1}$$

The enzyme E can only exist as free enzyme E, or enzyme-substrate-complex ES. The total enzyme concentration is $[E_{tot}]$, K_M is the Michaelis constant. Only ES can form product, the maximum rate of product formation is V_{max}.

$$[E_{tot}] = [E] + [ES] \tag{2}$$

$$V_{max} = k_2 [E_{tot}] \tag{3}$$

V_0, the actual rate of product formation at a given concentration of substrate depends on the fraction of ES that is present in the equilibrium. Thus V_0 can be expressed in terms of "ocupancy", or f_{ES}, the fraction of enzyme present in the enzyme-substrate complex ES.

$$f_{ES} = \frac{[ES]}{[E] + [ES]} \tag{4}$$

$$V_0 = k_2 [ES] \quad \rightarrow \quad V_0 = V_{max} \frac{[ES]}{[E_{tot}]} \quad , \quad V_0 = V_{max} \frac{[ES]}{[E] + [ES]} = V_{max} f_{ES} \tag{5}$$

Note that this equation converts readily to the common form of the Michaelis-Menten equation, if the definition of K_M (equation 1) is substituted into equation 5.

$$V_0 = V_{max} \frac{1}{1 + \frac{K_M}{S}} \tag{6}$$

In the presence of a single non-competitive inhibitor, additional enzyme species are possible (EX, EXS in Fig. 4B). By definition of an inhibitor, these do not lead to any product formation. Then f_{ES} becomes

$$f_{ES,X} = \frac{[ES]}{[E] + [ES] + [EX] + [EXS]} \tag{7}$$

The rate of product formation in presence of one non-competitive inhibitor is

$$V_{0,X} = V_{\max} \frac{1}{\left(1 + \dfrac{K_M}{S}\right)\left(1 + \dfrac{X}{K_X}\right)} \tag{8}$$

For two mutually exclusive inhibitors X and Y (Fig. 4B), one obtains:

$$V_{0,X,Y} = V_{\max} \frac{1}{\left(1 + \dfrac{K_M}{S}\right)\left(1 + \dfrac{X}{K_X} + \dfrac{Y}{K_Y}\right)} \tag{9}$$

And for two mutually non-exclusive inhibitors (Fig. 4C), the rate equation is

$$V_{0,X,Y} = V_{\max} \frac{1}{\left(1 + \dfrac{K_M}{S}\right)\left(1 + \dfrac{X}{K_X}\right)\left(1 + \dfrac{Y}{K_Y}\right)} \tag{10}$$

There is a simple technique to determine the type of enzyme inhibition by two inhibitors, and whether their action on the enzyme is synergistic. To this end, the ratio of the initial rates in the absence (control, V_0), and in the presence of inhibitor ($V_{0,X}$) is measured. S_0 is the control signal, S_X is the signal obtained in the presence of inhibitor.

$$\frac{S_0}{S_X} = \frac{V_0}{V_{0,X}} = \frac{V_{\max} \dfrac{1}{\left(1 + \dfrac{K_M}{S}\right)}}{V_{\max} \dfrac{1}{\left(1 + \dfrac{K_M}{S}\right)\left(1 + \dfrac{X}{K_X}\right)}} = \left(1 + \frac{X}{K_X}\right) \tag{11}$$

Thus, a straight-line curve is obtained when S_0/S_X is plotted against [X], the (varied) concentration of inhibitor X. The slope of this line (Fig. 6) gives the inhibition constant K_X. This plot is linear over the entire range of inhibitor concentration.
In the case of two mutually exclusive inhibitors, the ratio becomes

$$\frac{S_0}{S_{X,Y}} = \frac{V_0}{V_{0,X,Y}} = \frac{V_{\max} \dfrac{1}{\left(1 + \dfrac{K_M}{S}\right)}}{V_{\max} \dfrac{1}{\left(1 + \dfrac{K_M}{S}\right)\left(1 + \dfrac{X}{K_X} + \dfrac{Y}{K_Y}\right)}} = \left(1 + \frac{X}{K_X} + \frac{Y}{K_Y}\right) \tag{12}$$

Presence of the second inhibitor only results in an additional term (Y/K_Y) that shifts the $S_0/S_{X,Y}$ curve upwards. This term indicates additivity of the two inhibitors, but inhibitory potency (slope of the curve) is not altered.
For two mutually non-exclusive inhibitors, the ratio is

$$\frac{S_0}{S_{X,Y}} = \frac{V_0}{V_{0,X,Y}} = \frac{V_{max}\dfrac{1}{\left(1+\dfrac{K_M}{S}\right)}}{V_{max}\dfrac{1}{\left(1+\dfrac{K_M}{S}\right)\left(1+\dfrac{X}{K_X}\right)\left(1+\dfrac{Y}{K_Y}\right)}} = \left(1+\frac{X}{K_X}\right)\left(1+\frac{Y}{K_Y}\right) \qquad (13)$$

The difference between mutually exclusive and non-exclusive inhibitors can directly be seen from an experiment where the concentration of inhibitor X is held constant, and only [Y] is varied. Equation 13 can be rearranged to:

$$\frac{S_0}{S_{X,Y}} = 1 + \frac{X}{K_X} + \frac{Y}{K_Y}\left(1+\frac{X}{K_X}\right) \qquad (14)$$

Compared to the case of mutually exclusive inhibitors, the curve of $S_0/S_{X,Y}$. Y is shifted upwards by a constant concentration of X, and the slope of the curve also increases by a factor of $(1+X/K_X)$.

The ratio method shown here applies to the simplest case of synergistic action of drugs, two substances binding to the same target. It requires some basic kinetic data to be collected and gives a simple linear graph that can be quickly inspected for a qualitative result whether two substances act on the same or on different sites on an enzyme, and thus whether these two substances can be synergistic on their target or not. It should be noted that by taking the ratios, the control signal (uninhibited case, i.e. the largest signal) is divided by a signal that becomes progressively smaller and thus carries a higher error. It is needed to detect whether two curves have the same slope (mutually exclusive binding, additive effect), or different slopes (mutually non-exclusive binding, synergy). This difference has to be clearly demonstrated from experiment and data analysis, requiring data of sufficient quality to make this distinction.

The technique provides two important pieces of information:

1. The value of K_X, the inhibition constant, is unchanged if two inhibitors are only additive, and is decreased (~ higher inhibitory potency) in the presence of the second inhibitor. Therefore, we have a clear, mechanism-derived definition of synergy on the molecular level.
2. Conversely, the method allows to determine whether two inhibitors bind to the same, or to different sites on an enzyme. This may be an important result for drug development, and is obtained without need of structural data. (Note: strictly speaking, the result only tells whether binding of two inhibitors is mutually exclusive or non-exclusive)

The method has originally been presented for ligand-gated ion channels by Karpen and Hess (Karpen, Aoshima et al. 1982; Karpen and Hess 1986), and subsequently been used for the study of action of multiple inhibitors on ion channels (Karpen, Aoshima et al. 1982; Karpen and Hess 1986; Breitinger, Geetha et al. 2001; Raafat, Breitinger et al. 2010).

The basic mechanism presented here is by far not sufficient to describe multimeric enzymes, enzynmes requiring cofactors, and various modes of inhibition. Enzymes may form multimers, binding of one inhibitor may affect binding of other others, and binding sites my overlap. More complex mechanisms of inhibition of Michaelis-Menten enzymes have been discussed, including those of several inhibitors acting on a single enzyme (Palatini 1983). Action of several inhibitors as well as antagonistic interaction of enzyme inhibitors have

been studied (Asante-Appiah and Chan 1996; Schenker and Baici 2009), and a major development in drug interaction analysis was the detailed mathematical treatment of enzyme kinetics and inhibition by Chou and Talalay (Chou 1976; Chou and Talalay 1977; Chou and Talalay 1981; Chou 2006; Chou 2010), covering the mechanistic Michaelis-Menten approach as well as logistic approaches.

2.3 Ligand-gated ion channel receptors

Ligand-gated ion channels are principal mediators of rapid synaptic transmission between nerve cells and in the neuromuscular junction. Compared to Michaelis-Menten type enzymes, their mechanism of activation is more complex, requiring an additional transition (Hess 1993; Colquhoun 1998). First step of ion channel activation is binding of the activating ligand (a neurotransmitter), which is governed by the principle of mass action (Hess 1993; Colquhoun 1998). Usually, more than one ligand molecule is required; depending on receptor type, models with two or three ligands binding prior to efficient channel opening have been discussed. Ligand binding induces an conformational change, where the receptor protein converts from the closed to an open ion-conducting state (Fig. 5A) (Hess 1993;
Colquhoun 1998). Only the passing ions generate an electric signal and this signal can be recorded using patch-clamp techniques. Similar to the ES complex in enzymes, only the liganded receptor can undergo the opening transition. The mechanisms of non-competitive inhibition by two inhibitors binding to the same (Fig. 5B), or different (Fig. 5C) sites have been given. A similar derivation to the one for MM-enzymes can then be made.

The signal in this case is not a rate of product formation, but an ionic current, namely the rate of ion translocation through the open channel. Assuming a constant transmembrane voltage, and only one conducting state (ie only one channel size, in reality several conductance levels have been observed for each ion channel receptor).

The observed signal S_L would then be:

$$S_L = I_L = n_{Ch}J_{ion} \qquad (15)$$

where I_L is the observd current, n_{Ch} is the number of open channels, and J_{ion} is the ion translocation rate. The maximum current signal would be observed if all ion channel were open at the same time. F_{open}, the fraction of open channels, would then be equal to 1 (a theoretical value only).

$$S_{max} = I_{max}F_{open} \qquad (16)$$

Assuming that only receptors with two bound ligands can undergo the opening transition (Fig. 5A), we can define the fraction of open channels as

$$F_{open} = \frac{\overline{[RL_2(open)]}}{[R] + 2[RL] + [RL_2] + \overline{[RL_2(open)]}} \qquad (17)$$

Using the law of mass action, we can define

$$K_D = 2\frac{[R][L]}{[RL]} \qquad (18)$$

$$K_D = \frac{[RL][L]}{[RL_2]} \tag{19}$$

$$\phi = \frac{[RL_2]}{[RL_2(open)]} \tag{20}$$

we can then obtain

$$S_0 = I_{max}F_{open} = I_{max}\frac{1}{\left(\dfrac{K_D}{L}+1\right)^2 \phi + 1} \tag{21}$$

A

$$R \xrightleftharpoons{K_D} RL \xrightleftharpoons{K_D} RL_2 \xrightleftharpoons{\Phi} \overline{RL_2}\textbf{(open)}$$
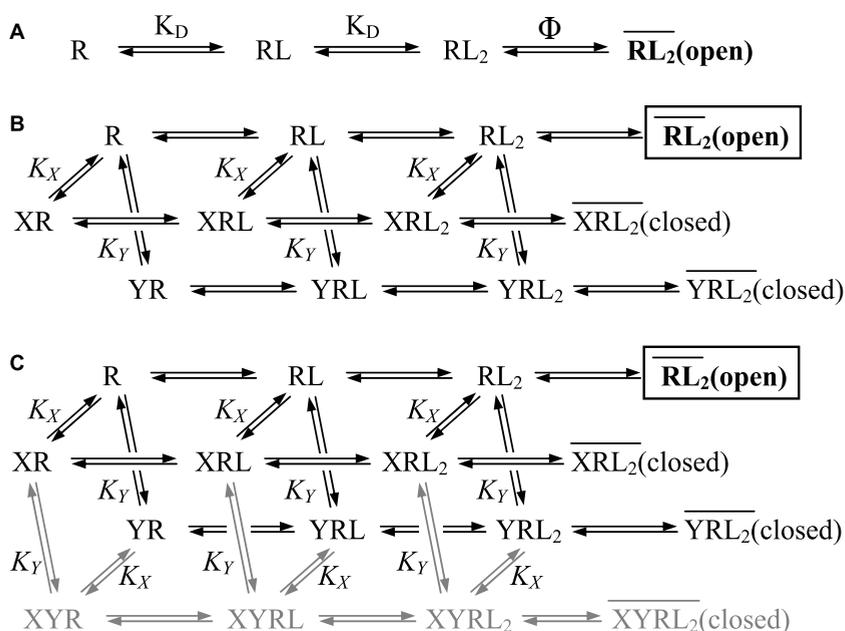
B

C

Fig. 5. Mechanisms of activation and non-competitive inhibition of ion channel receptors (A) Minimum mechanism for the activation of a ligand-gated ion channel. Note that the channel-opening reaction comprises two elementary steps, ligand binding (dissociation constant K_D) and conformational change to the open state (open-close equilibrium Φ). R = receptor, L = activating ligand. In this example binding of two ligand molecules is needed prior to channel opening. (B) Inhibition of an ion channel receptor by two non-competitive inhibitors X and Y, which are mutually exclusive (e.g. binding to the same site). Either inhibitor X or Y can bind at any given time. Presence of the second inhibitor can only exert an additive effect but is not synergistic. (C) Inhibition of an ion channel receptor by two non-competitive inhibitors X and Y, which are mutually non-exclusive, targeting different sites on the receptor. Synergism is then observed as a necessary consequence of two mutually non-exclusive inhibitors.

In the presence of one non-competitive inhibitor X, we obtain the following equation for the signal S_X:

$$S_X = I_{max} \frac{1}{\left[\left(\frac{K_D}{L}+1\right)^2 \phi + 1\right]\left(1+\frac{X}{K_X}\right)} \tag{22}$$

where K_X is the inhibition constant, L is the concentration of activating ligand, and X the concentration of inhibitor. One can now readily compute the ratios of control current signal to signal in presence of inhibitor:

$$\frac{S_0}{S_X} = \frac{I_{max}\dfrac{1}{\left[\left(\frac{K_D}{L}+1\right)^2 \phi + 1\right]}}{I_{max}\dfrac{1}{\left[\left(\frac{K_D}{L}+1\right)^2 \phi + 1\right]\left(1+\frac{X}{K_X}\right)}} = 1+\frac{X}{K_X} \tag{23}$$

In case of two inhibitors binding to the same site (mutually exclusive), the ratio again becomes

$$\frac{S_0}{S_{X,Y}} = 1+\frac{X}{K_X}+\frac{Y}{K_Y} \tag{24}$$

For two non-exclusive inhibitors, targeting different sites on the recpetor, this ratio then is

$$\frac{S_0}{S_{X,Y}} = 1+\frac{X}{K_X}+\frac{Y}{K_Y}\left(1+\frac{X}{K_X}\right) \tag{25}$$

Equations 23 – 25 are identical to equations 11-14.

Similar to the treatment of Michaelis-Menten enzymes, we obtain again a system of linear equations that describes the action of one or two inhibitors of ion channel receptors. If the concentration of inhibitor X is held constant, and the concentration of the second inhibitor, Y, is varied, the ratio $S_0 / S_{X,Y}$ is shifted up by a constant amount X/K_X but the slope $(1/K_Y)$ is unchanged. The slope of the ratio curve represents the inhibitory potency, and the constant upward shift is due to the additive effect of two mutually exclusive inhibitors.

In the presence of two mutually non-exclusive inhibitors, the slope (ie inhibitory potency) is increased by a factor of $(1 + X/K_X)$. Thus, if the mechanism underlying this analysis were followed, the "amount of synergy" could be calculated as $1 + X/K_X$. Often, quality of the data does not permit this quantitation, although the qualitative demonstration of synergy (increased inhibitory potency of drug A in the presence of drug B) is statistically safe. Thus, by taking the ratios of control and inhibited signals, we arrive at an equation that becomes mechanism-independent and corresponds to the principal equations used to describe drug interactions. The ratio method results in a simple graph that describes the type of joint action of two inhibitors on a common enzyme, neurotransmitter receptor, or general target protein (Fig. 6).
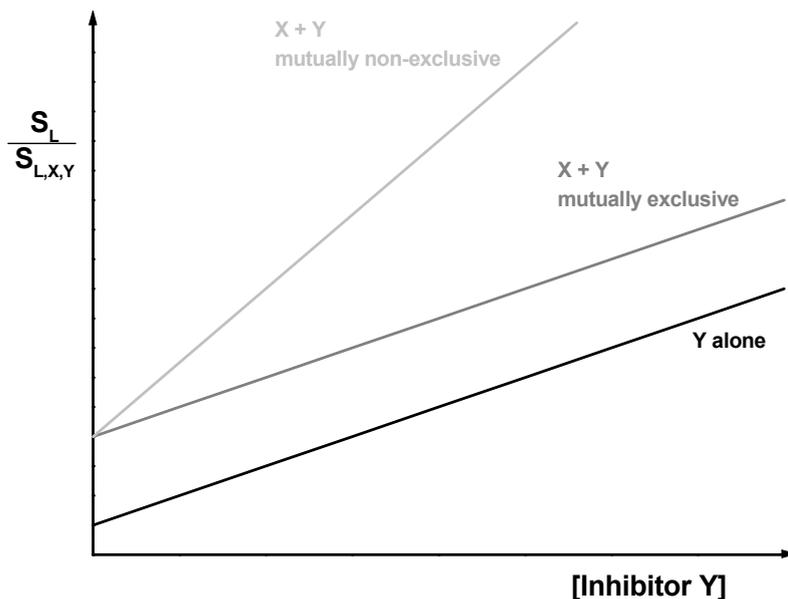
Fig. 6. Ratio method graph. Graph of signal ratio $S_0/S_{X,Y}$ vs inhibitor concentration for the case of one inhibitor (black curve), two mutually exclusive inhibitors (gray curve), and two mutually non-exclusive inhibitors (light gray curve). In case of mutually non-exclusive binding the inhibitory potency of inhibitor Y is increased in the presence of inhibitor X, as indicated by the lower value of K_Y computed from the slope of the inhibition ratio curve. Note that the formalism described here becomes mechanism-independent and applies to Michaelis-Menten type enzymes as well as to more complex mechanisms of ion channel receptor inhibition.

So far, a simple description of the action of two inhibitors on a common target has been derived. The mechanisms were based upon (i) a common binding site for two inhibitors, leading to mutually exclusive binding (Fig. 2), or (ii) two independent binding sites, leading to mutually non-exclusive binding (Fig. 3), Indeed, these simple models underlie (i) the principle of Loewe additivity (Loewe 1953; Berenbaum 1989), also referred to "similar", or "homodynamic" action of drugs. Here, the expectation value for zero interaction is just additivity. Independent inhibitor sites (Fig. 3), in contrast, correspond to Bliss independence, "dissimilar", "heterodynamic", or "independent" action of drugs (Bliss 1939; Berenbaum 1989). The combined effect of two such drugs will be more than additive, fulfilling the basic criterion of synergy. It has been recognized that these are the two limiting mechanisms for drug interaction (Bliss 1939; Finney 1942; Plackett and Hewlett 1948), and indeed both models are being used in the literature as zero interaction reference (Greco, Bravo et al. 1995).

It is intuitive, and favoured by this author to view the concept of Loewe additivity as the zero interaction reference, and noting the superadditive response from Bliss independence as synergism. This definition is widely accepted (Segel 1975; Chou and Talalay 1977; Berenbaum 1989). Furthermore, it allows for a very intuitive definition of zero interaction, proposed by Loewe: if drug A and B are the same, B being a dilution of A. Naturally, action of "both" drugs would be similar, and thus we have a perfect model of additivity.

However, what happens if we already know that drug A and B have completely different modes of action? Two drugs could be targeting different enzymes in a biochemical pathway. Of such a combination of drugs – having dissimilar action – we would expect superadditive behaviour. Can we call this synergy, or is it just expected from the mechanism and is now our zero reference? Arguments can be found for either view, and both models (and many more) are thus used and debated in the literature.

Once we move to more complicated systems, mechanism-based analysis is no longer feasible, and more general descriptions of drug interaction are needed. However, they all relate to the basic models of additivity and independence that were described above.

Equation 25 can be rearranged into the form

$$\frac{S_0}{S_{X,Y}} = 1 + \frac{X}{K_X} + \frac{Y}{K_Y} + \frac{X}{K_X}\frac{Y}{K_Y} \tag{26}$$

This equation is similar to a general equation that describes describing the joint action of two drugs on a specific target or biochemical process, presented by Greco et al. (Greco, Bravo et al. 1995).

$$1 = \frac{D_1}{ID_{X,1}} + \frac{D_2}{ID_{X,2}} + f\left(\frac{D_1}{ID_{X,1}}, \frac{D_2}{ID_{X,2}}, \alpha, p\right) \tag{27}$$

Here, D_1 and D_2 are concentrations of drug 1 and 2 in a mixture; $ID_{X,1}$ and $ID_{X,2}$ are the concentrations that produce a certain effect (corresponding to EC_{50}, or IC_{50} values); α is the synergism/antagonism parameter and p represents additional parameter(s) describing the "interaction" (joint action) of the two drugs.

The models and derivations given above are indeed the simplest approach to synergism between drugs. At this time, we do not even have a complete description of the action of every drug. It has been pointed out that under physiological conditions, it is expected that indeed presence of a drug will always result in an altered state of metabolism and thereby affect other drugs (Gessner 1974). In many patients multiple drug regimes have to be given, and the metabolism of a critically ill person may differ from a healthy "control" volunteer. Taken together, medical reality is not sufficiently described by simplified models. However, as shown above, even from simple model cases we can understand mechanisms of synergy and can derive mechanism-independent formalisms to determine the type of joint action of drug combinations.

In biomedical modelling, an alternative approach is the use of a mechanism-free description of activity, such as enzyme activity, ion channel function, the throughput of an entire biochemical pathway, or even cell survival in toxicity assays. The most common approach is the use of logistic equations that simply connect concentration of an effector (agonist or inhibitor) to the measured effect (enzyme activity, product formation, cell survival). The most comonly used formalism is that of the Hill equation.

$$E_0 = E_{\max}\frac{1}{1 + \left(\frac{EC_{50}}{L}\right)^n} \tag{28}$$

Here, E_0 is the observed effect, E_{max} is the maximum signal, EC_{50} is the concentration of ligand L that produces 50 % of the maximum response, and n is a coefficient defining the steepness of the dose-response curve. The similarity to Michaelis-Menten type enzyme kinetics is obvious, yet the logistic formalism is not based on any mechanism. Indeed, complex clinical situations require use of mechanism-free models to analyze drug interactions (Chou 1976; Berenbaum 1978; Berenbaum 1980; Chou and Talalay 1981; Berenbaum 1989; Tallarida 1992; Greco, Bravo et al. 1995).

In the following section some principles and formalisms for the analysis of drug synergism are briefly reviewed. An exhaustive review of all concepts is outside the scope of this text, readers are directed to several excellent, comprehensive reviews (Berenbaum 1989; Greco, Bravo et al. 1995; Tallarida 2001; Chou 2002; Toews and Bylund 2005; Chou 2006; Tallarida 2006; Bijnsdorp, Giovannetti et al. 2011).

3. Mechanisms and techniques of synergy testing in complex biomedical settings

An example, modified from Berenbaum (Berenbaum 1989) is that of a woodcutter, able to cut 10 trees in a day. He is joined by a second woodcutter, also able to cut down 10 trees in a day. Together, they manage to cut 15 trees in one day. How do we describe this situation?

One approach is that cutter A achieves 10 trees per day, our expectation value. Addition of cutter B results in 15 trees being cut, so there is synergy. Such an approach has been proposed e.g. by Gaddum, who only considered the effect of one agent and whether it was affected by another one being added (Gaddum 1940). This formalism is not used widely, as it obviously assigns synergism to the effects of several drugs too readily.

Conversely, one would say that with two cutters, each able to cut 10 trees per day, the expectation value is 20 trees/day. If only 15 are achieved, they are antagonising each other. This is the application of additivity, and clearly, the combined effect is sub-additive, 20 trees would be just additive, and more than 20 would mean synergy.

Mechanistically, one might argue that if cutter A works on a tree, then cutter B would not work on the same tree. Their action would be mutually exclusive, and the additive result would be expected. If, however, they are willing to work at the same tree together, they will be able to cut this tree in a much shorter time. In this case, they would be able to cut more than 20 trees in a day and their action would be mutually non-exclusive, leading to synergy.

As stated above, pure mechanistic analysis is not sufficient (and not possible) for most clinical cases, so a general, mechanism-free analysis of drug interaction is needed. Berenbaum (Berenbaum 1989) has pointed out the similarity to non-parametric statistical tests that do not require information about the meaning of the values, or the distribution of populations from where the values originate. The equivalent in dose-response analysis is a logistic equation, that just describes a dose-response curve without any requirement of a mechanism. In such a setting, one would just define the desired outcome (enzyme inhibition, cell death, reduction of virus titer, ...), and then measure the effect achieved by varying doses of each drug alone, and in combination.

The mechanisms shown above illustrate just the simplest mechanistic model. In real life, the situation is more complicated, as mechanisms of enzyme or receptor acitivity are more complex. Furthermore medical intervention is not only directed at single proteins, but at entire pathways or controlling structures, such as transcription factors, that initiate or control biochemical processes. Some therapies, such as cancer chemotherapy even aim at

cell destruction, i.e. they interfere with a complete living organism. In most of these situations, mechanisms of action are not known, or are too complex to work with. The additional problem is that with increasing complexity of the biological system, one finds an increasing paucity of experimental data. Even a simple dose-response curve, traditionally recorded with seven sensibly spaced concentration points, carries a significant error. By the rule of parsimony, one has to choose the simplest possible mechanism to describe experimental data. Thus, research is confronted with the dilemma of either oversimplification, or overinterpretation of results – a working compromise between these two extremes is needed. The pertinent models and methods have been extensively analyzed and reviewed in two excellent papers by Berenbaum (Berenbaum 1989), and Greco et al. (Greco, Bravo et al. 1995).

Some of the main concepts are just briefly described:

- Median effect analysis
- Interaction index, isobole method and combination index
- Response surface analysis

3.1 Median effect analysis

Chou et al. derived the median effect equation which follows from a detailed derivation of MM enzyme mechanisms (Chou 1976; Chou and Talalay 1977; Chou and Talalay 1981; Chou 2006).

$$\frac{d}{M} = \frac{E_d}{1 - E_d} \tag{29}$$

where d is the dose of a drug, E_d the effect caused by this amount of drug, M the median (dose causing 50 % effect, i.e. EC_{50} or IC_{50}). Indeed, such an equation can be derived by rearrangement of the Michaelis-Menten equation (6):

$$\frac{[S]}{K_M} = \frac{\dfrac{V_0}{V_{max}}}{1 - \dfrac{V_0}{V_{max}}} \tag{30}$$

Here, [S] is the substrate concentration that gives the observed V_0, K_M is the Michaelis constant, and V_0/V_{max} is the effect caused by [S], expressed here as the fractional velocity. The median effect equation has been proposed as a central, unified equation from which the basic equation sets of Henderson-Hasselbalch, Scatchard, Hill, and Michaelis-Menten can be derived (Chou 2006; Chou 2010). The median effect equation has been derived from MM-type enzymes from mathematical analysis. It can be extended to multiple-site systems in the form (Chou 2006)

$$\frac{E_d}{1 - E_d} = \left(\frac{d}{M}\right)^n \tag{31}$$

where n is the constant giving the slope of the dose-response curve. Note that n has often been equated with the number of binding sites, but this is an oversimplification that should be avoided since it is not valid in most cases. The value of n may be a measure of the degree of cooperativity between binding sites, but nothing more.

The equation can also be expressed in the form

$$\frac{f_A}{f_{UA}} = \left(\frac{d}{M}\right)^n \tag{32}$$

where f_A and f_{UA} are the fractions of affected and unaffected enzyme, respectively. The importance of the median effect equation is that it is composed of ratios of effects (E_d, ($1-E_d$), or f_A and f_{UA}) and of the dose ratio (actual dose d, median dose M). Although derived from mechanistic analysis, the median effect equation cancels out mechanism-specific constants, and just links dose and effect in dimensionless ratios. This makes it a very versatile tool for the analysis of complex systems. The median effect equation can be linearized by taking logarithms on either side, giving the Hill plot (see Berenbaum 1989) which is a straight line for the plot of $\log(f_A/f_{UA})$ vs. $\log d$.

$$\log\left[\frac{f_A}{f_{UA}}\right] = n\left(\log d - \log M\right) \tag{33}$$

Thus the median effect equations can be seen as an extremely useful rearranged form of dose-response curves, linking ratios of drug doses to ratios of observed effects. The median equation will work with both, mechanism-based (eg Michaelis-Menten), and effect-based (eg logistic) equations, and provides a dimensionless measure for drug effects. The technique has been extensively tested and derived from mechanistic as well as purely mathematical considerations. The group of T.C. Chou have pioneered this field and developed software packages (CompuSyn ands CalcuSyn) that allow reliable testing of drug interaction parameters (Chou 2002; Chou 2006; Chou 2010). Well-founded in theory, the technique has found widespread use (Chou 2002; Chou 2006; Chou 2010; Bijnsdorp, Giovannetti et al. 2011), and the initital paper by Chou and Talalay (Chou and Talalay 1984) has been intensely cited and discussed.

3.2 Interaction index, isobole method and combination index

The interaction of two or more drugs to produce a combined effect can be described by the interaction index I (Berenbaum 1977).

$$I = \frac{D_1}{ID_{X,1}} + \frac{D_2}{ID_{X,2}} \tag{34}$$

or written in terms of the median equations above

$$I = \frac{d_1}{M_1} + \frac{d_2}{M_2} \tag{35}$$

where, D_1, D_2 d_1 and d_2 are concentrations of drug 1 and 2 that produce a certain effect if applied together; $ID_{X,1}$, $ID_{X,2}$, M_1 and M_2 are the concentrations that produce the same effect when given alone. For instance, if we want 50 % inhibition, then equation 34 would be:

$$I = \frac{D_1}{IC_{50,1}} + \frac{D_2}{IC_{50,2}} \tag{36}$$

Here, $IC_{50,1}$ and $IC_{50,2}$ are the IC_{50} values of drug 1 and drug 2 alone. D_1 and D_2 are the doses of drug 1 and 2, respectively, that also produce 50 % inhibition when given together. The interaction index, proposed by Berenbaum (Berenbaum 1977), should be constant in case of zero interaction. The method has been extended by Berenbaum (Berenbaum 1985) and developed into a general method based on analysis of each drug alone and then simulating the combined action of both drugs based on Loewe additivity (see also Greco, Bravo et al. 1995).

The interaction index underlies one of the most widely used graphical representations of drug synergism and antagonism, the isobologram. Isoboles were first used by Fraser in 1870 (Fraser 1870; Fraser 1872) as simple, intuitive illustration without mathematical derivation. Here, the doses of drugs A and B give abscissa and ordinate, respectively, and the effect of drug combinations is plotted as graph (Fig. 7). In the example (Fig. 7), the effect plotted is for 50 % inhibition of an enzyme. The effects of each drug alone (i.e. IC_{50}) can be read from the axes. The isobologram shows an effect, such as IC_{50} (IC_{10} or IC_{80}, whatever effect is of interest) and which drug concentration is needed to achieve this effect.
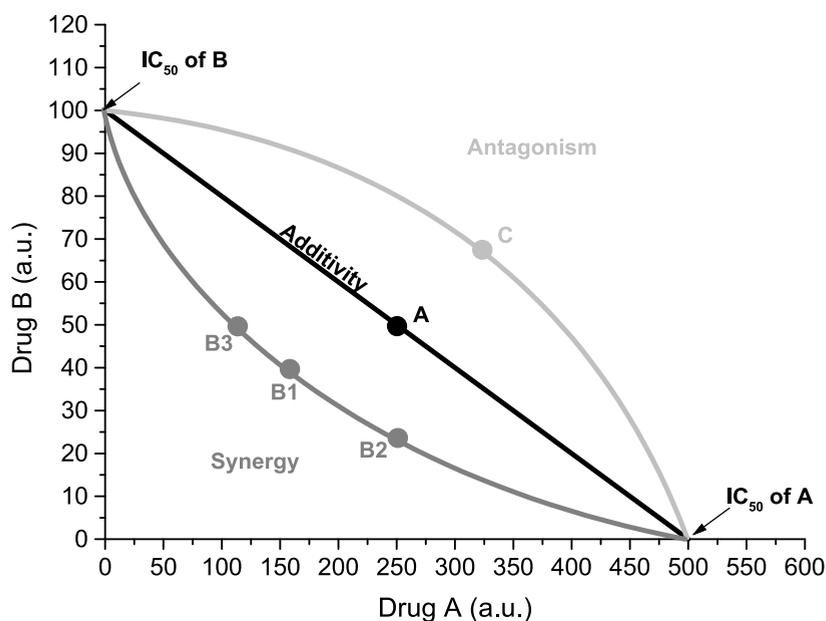

Fig. 7. Isobologram. Abscissa and ordinate units are the concentrations of drugs A and B. The solid black line connects concentrations that produce the same effect on the target protein, enzyme, or system. In this example, the IC_{50} line is given. In the simulation, drug A has an IC_{50} (concentration giving 50 % inhibition) of 500 a.u. (arbitrary units), IC_{50} of drug B is 100 a.u. From additivity (black line), the combination of 250 a.u. of A with 50 a.u. of B should also give 50 % inhibition (point A). If, 50 % inhibition are achieved at lower concentrations of the two drugs (e.g. 150 a.u. of A and 40 a.u. of B, point B1), the drugs would show synergism. If the observed inhibition by the combination was less than 50 %, drug A and B would interact in an antagonistic way (point C). Model lines of synergism (gray line) and antagonism (light gray line) are drawn. Note that in case of synergy between two drugs, the IC_{50} curve would not be a straight line but an upward concave (gray line), in case of antagonism a downward concave (light gray line). In practical application, one

would determine IC_{50} of one drug in the presence of a constant concentration of the other. IC_{50} would be found with the combination of 250 a.u. of A and 24 a.u. of B (point B2), or 110 a.u. of A and 50 a.u. of B (point B3).

Equations 34 – 36 define straight lines for two drugs that do not show any interaction (synergism or antagonism). Two drugs showing aditivity wold be expected to fall on the additivity line (Fig. 7). If the two drugs act synergistically, lower concentrations would be needed in the mixture to achieve the same effect. Their combination graph would be an upward concave (gray line in Fig. 7), following the unequality

$$\frac{D_1}{ID_{X,1}} + \frac{D_2}{ID_{X,2}} < 1 \tag{37}$$

Conversely, two antagonistic drugs would require higher doses in combination to achieve the same effect, and the resulting isobole would be an upward convex line (red line in Fig. 7), of the general (un)equation

$$\frac{D_1}{ID_{X,1}} + \frac{D_2}{ID_{X,2}} > 1 \tag{38}$$

Representing a form of median effect equationry, isoboles have become a useful tool to present complex modes of drug interaction. An excellent review by Greco et al (Greco, Bravo et al. 1995) derives isoboles as 2-D sections through three-dimensional plots of drug action data. Depending on the shapes of the dose-response curves of both drugs, isoboles do not need to be linear (Greco, Bravo et al. 1995). Also, drug combinations may be biphasic, showing concentration ranges of synergy and ranges of antagonism (Berenbaum 1989).

Equations 34 and 35 apply to the case of Loewe additivity, where the two drugs do not show synergy or antagonism. For drugs showing any type of interaction, equation 34 was extended to define a combination index (CI), indicating type and amount of interaction between two (or more) drugs with respect to the experimantal parameter being studied (Chou and Talalay 1983).

$$CI = \frac{D_1}{ID_{X,1}} + \frac{D_2}{ID_{X,2}} = \begin{array}{l} > \ 1 \ \text{for antagonism} \\ \ \ 1 \ \text{for additivity} \\ < \ 1 \ \text{for synergy} \end{array} \tag{39}$$

The CI can take values between 1 and infinity for antagonism, and runs between 0 and 1 for synergy. Chou and Chou (1988) have introduced the dose reduction index DRI (Chou and Chou 1988), which is based on the interpretation of the Combination index equation (39). Assuming that two drugs show synergy, one expects that a lower dose of each is needed to achieve the same effect. This lower concentration (D_1 and D_2 in equations 34-39) can be related to the median (IC_{50} in equation 36), to give the dose reduction index DRI.

$$CI = \frac{1}{(DRI)_1} + \frac{1}{(DRI)_2} \tag{40}$$

Both, combination index CI and DRI can be used to plot drug combination data for visualization of synergy or antagonism (Fig. 8, see (Chou 2006)).
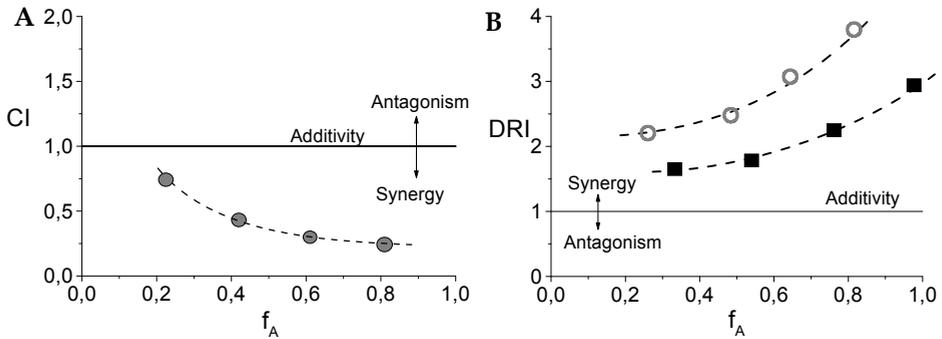
Fig. 8. Visualization of drug interaction data. (A) CI-f_A plot: The combination index CI is plotted versus f_A, the fraction of affected enzyme or biological function. (B) DRI plot: the dose reduction index DRI is plotted against f_A. See text for definitions of terms.

3.3 Response surface analysis

Response surfaces can be calculated and are a way to represent effects of drug combinations as a contour plot where drug concentrations are plotted as a horizontal x-y- plane, and the effect is plotted on the z axis. Isoboles can be seen as 2D sections through response surfaces, and the method allows graphical analysis of drug interaction data, albeit at requirement of quite some mathematical and computational effort. From the dose-response data of each drug alone, the expected response surface based on the zero interaction reference of choice, is plotted. Then actual drug combination data are entered into the plot, and similar to isobole analysis, deviations from the reference surface indicate synergism or antagonism. The technique has been applied to synergism studies (Tallarida, Stone et al. 1999), and its general use reviewed and commented in great detail (Berenbaum 1989; Greco, Bravo et al. 1995; Tallarida 2001).

3.4 Practical limitations

There is a need for a definition of synergy, antagonism, and the zero case (neither one nor the other). Sometimes, specific problems are discussed and authors feel compelled to use a unique treatment of the data. Pharmacologists, stasticians, clinicians, and representatives from other fields have different views and concepts. In various major reviews, 13 models to treat drug combination data have been proposed. The author would not encourage decisions as to right or wrong. Each model may be appropriate for a given situation, and not applicable to others. However, all models discussing synergy can be traced back to only two types of the "zero" (no interaction) case as discussed before

- Loewe Additivity
  Both drugs exert an effect but are mutually exclusive, either one or the other can be active at a given time. This corresponds to a common site of interaction in the simplest mechanistic case (Fig. 2).
- Bliss Independence
  Both drugs are mutually non-exclusive, both can be active at the same time. In the simplest case, each drug has a specific, independent interaction site (Fig. 3).

Indeed all models refer to these two basic cases. Obviously, both have a different expecation of joint action of two drugs. Loewe additivity is best described by equation 34, and any deviation from this may be considered as synergy. In case of Bliss independencs, one would expect both drugs to act independently, and therefore the zero case already includes a more than additive effect of both drugs. The author sees two problems with this definition: (i) two purely additive drugs would have to be called antagonistic, including the sham combination of a drug with itself. (ii) If two mutually non-exclusive drugs already produce a superadditive effect, and we do not yet call this synergy, how do we define "true" synergy? In terms of isoboles, the baseline (no synergy, no antagonism) is already curved, in the ratio method (Fig. 6), the slope of the inhibition curve is increased already for the zero case, and one calculates a CI of less than one. Thus, to identify synergy, one has to select a gradual increase. It may be fairly easy to identify a deviation from a straight line (isobole), but for the CI a deviation from <1 to <<1 is expected. In the ration method, the steepness of th slope may be hard to compute, as the ratio $S_X/S_{control}$ is toe be calculated from small numerical values and thus carries a large error.

Thus the definition of Loewe additivity is preferred by this author as the definition of no synergy. As shown in Fig.s 2 and 3, it can clearly be defined in mechanistic term. Dose-response analysis also follows the definition. Addtivity correctly describes the purest control experiment, sham mixtures of the same drug, and it follows the general definition of synergy, where a combination produces more thant the sum of the individual components.

It should be noted, however, that in many clinical applications, drug combinations are used that target two completely different target proteins, or pathways. In radiotherapy, the combination of radiation and drugs work together, and in combination lower doses of either are required compared to a single treatment. No baseline of Loewe additivity can be proposed for such a combination. Likewise, combinations of drugs that target completely different cellular pathways may work synergistically towards cell killing even though the two drugs are not mutually exclusive in their activity. Obviously, there is no single methodology that is appropriate for all biomedical situations.

An additional problem in interpreting drug combination data is the quality of the measured data. Biological systems invariably carry experimental error, and thus borderline cases are almost impossible to assign. For example, a combination index is calculated to be 0.9 – is this a real deviation from unity (and thus synergy), or is it experimental error?

Even with the best data, however, analysis of joint action of two drugs has another inherent problem. Two different drugs may have different dose-response characteristics. In this case, changes in effective concentrations may suggest synergy where there is none. A principal illustration of this problem is given in Fig. 9 (adapted from Chou (Chou 2006; Chou 2010)), showing that the same relative concentration change can produce quite different effects (Fig. 9 A,B). Even for a single compound, there is a marked difference whether one investigates concentrations below or around EC_{50}, or near saturation. Addition of the same drug in the concentration range around EC_{50} (ie the steepest part of the dose-response curve) gives rise to a strong increase in signal which may be misinterpreted as synergy. The shapes of isobolograms for drugs with different dose-response characteristics, and the complications resulting from this fact have been extensively studied (Berenbaum 1989; Greco, Bravo et al. 1995; Tallarida 2001; Chou 2006).
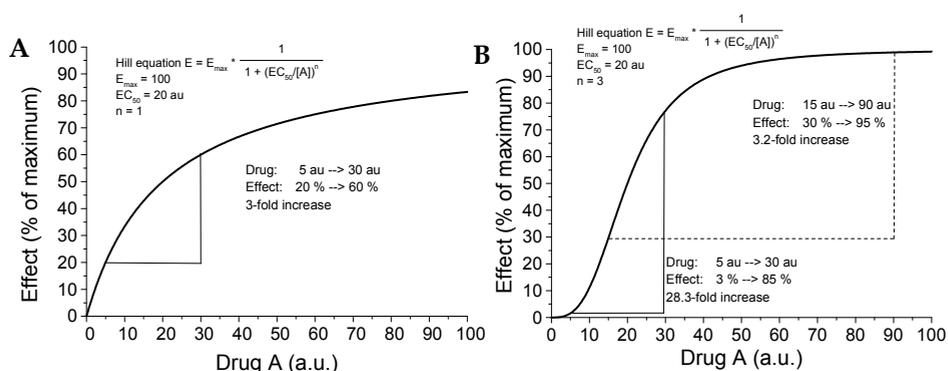
Fig. 9. Dose-effect curves of different shape. Both curves were simulated using the Hill equation and the parameters E_{max} = 100 % , EC_{50} = 20 au (arbitrary units). (A) Curve simulated for n =1 (hyperbolic curve). (B) Simulated for n = 3 (sigmoidal curve). Note the difference in curve shape, and the different effect of a change of concentration of agonist A from 5 to 30 au. In the hyperbolic case, the effect increases 3-fold, in the sigmoidal case, the increase is 28.3-fold. Effects of changes in agonist concentration are different depending on the response range where they happen. Sigmoidal dose-response curves are steepest around the median (panel B). In the example, a 6-fold raise in concentration (from 5 to 30 au) will cause a 28.3-fold increase in the observed effect. Raising the concentration from 15 to 90 au, the effect only increases 3.2-fold. Thus, if presence of a second drug B increases cooperativity of drug A, or if drug B shifts the relevant dose-response range of A towards the median by purely additive (non-synergistic) means, one would observe a higher increase in effect than expected from addition and wrongly interpret this as synergy.

4. Borderlines of synergism – Potentiation, coalism, inertism, metabolic interference

From the simplest models presented here to advanced discussions, the situations of synergy could be traced back to the simple principles of additivity vs. independence. In all those cases, both drugs were having the same effect alone or in combination. The only difference was the magnitude of the effects. Synergism can also occur with combinations of drugs or methods that have completely different modes of action. In cancer therapy a combination of radiation and cytostatica is often used. Combination of substances and environmental conditions (heat, pH, radiation) have indeed been analyzed for synergy (Johnson, Eyring et al. 1945). There are cases of one drug having no activity, but augmenting the activity of another, as observed for antinociception by acetaminophen in combination with phentolamine (Raffa, Stone et al. 2001). The extreme case would be the combination of two drugs that have no effect alone, but are effective in combination. On the othe hand, self-synergy of paracetamol has been described by Tallarida et al., who showed that the drug binds to targets in different locations and thus facilitates its own activity (Raffa, Stone et al. 2000). An interesting approach is an attempt to predict drug synergism from gene microarray data (Jin, Zhao et al. 2011).

Effect of drug combination	Both drugs have same effect individually	Only one drug is effective individually	None of the two drugs has an effect individually
Greater than zero reference	Synergy, Synergism	Synergism (potentiation)	Coalism
Equal to zero reference	Additiviy / Independence	Inertism	Inertism
Smaller than zero reference	Antagonism	Antagonism	

Table 1. The terminology of the combined action of drugs (after Greco, Bravo et al. 1995)

Another effect leading to apparent synergy or antagonism is the effect some drug may have on uptake, metabolism and clearance of other drugs. Depending on the route of administration, metabolism by first liver pass must be considered, including one of the most critical steps of drug biotransformation, namely oxygenation (thus hydrophilization) by cytochrome P450, an oxygenase that catalyzes oxygenations of substrates using NADPH and oxygen (O_2). This oxygenation R–H $\rightarrow$ R–OH is a crucial step in metabolism and eventual clearance of drugs and pharmaceuticals from the body. To date, 56 subtypes of cytochrome P450 are found in humans, some of which are critical in metabolism of endogenous substances such as medical drugs. Substances interfering with cytochrome P450 may, therefore, have an impact on drug clearance and thus on the actual concentration of a certain drug in the body (Flockhart 1995; Flockhart and Oesterheld 2000; Shin, Park et al. 2002; Takada, Arefayene et al. 2004).

Resource	Internet address	Comment
Drug interactions checker	http://www.drugs.com/drug_interactions.html	Tool to query compounds that interact with a given drug
Medscape drug interaction checker	http://reference.medscape.com/drug-interactionchecker	Tool to report on interactions between two drugs
Cytochrome P450 drug interaction table	http://medicine.iupui.edu/clinpharm/ddis/	List of drugs metabolized by cyt P450 isoforms (Flockhart 2007)
Grapefruit juice/citrus fruit juice interactions	http://www.mayoclinic.com/health/food-and-nutrition/AN00413	Short list of drugs that interact with dietary citrus fruits
Private resources	http://www.environmentaldiseases.com/article-drug-interactions.html	Website discussing case individual studies of interfering drugs

Table 2. Internet tools for drug interactions in clinical settings

There are numerous internet tools that list known drug interactions. A brief list of some such resources is given in table 2. Thus, some practical aspects have been covered, although synergisms and other interactions of drugs are not yet given enough weight in approval or recommendations of drug use. This is particularly relevant for the less well-defined field of herbal remedies. Their interaction with anticancer agents has been studied (Sparreboom, Cox et al. 2004), but our knowledge in this area is still far from comprehensive.

To date, the study of drug interaction in the biomedical field is widespread and must include the following aspects:
- mechanism of action of a single drug
- mechanisms of action of two (or more) drugs acting on the same physiological target
- interaction of two drugs through side effects, secondary targets, etc
- effects on metabolism of the primary drug
- top-down observations of the performance of drug combinations in patients

Going down this list it becomes clear that pure mechanistic studies – although essential – are not sufficient to cover all aspects of drug interaction. Clinical observation is the – equally essential – other end of the spectrum and the gap between these two positions is indeed narrowing.

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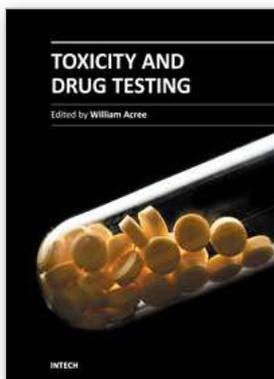
Toxicity and Drug Testing
Edited by Prof. Bill Acree

Modern drug design and testing involves experimental in vivo and in vitro measurement of the drug candidate's ADMET (adsorption, distribution, metabolism, elimination and toxicity) properties in the early stages of drug discovery. Only a small percentage of the proposed drug candidates receive government approval and reach the market place. Unfavorable pharmacokinetic properties, poor bioavailability and efficacy, low solubility, adverse side effects and toxicity concerns account for many of the drug failures encountered in the pharmaceutical industry. Authors from several countries have contributed chapters detailing regulatory policies, pharmaceutical concerns and clinical practices in their respective countries with the expectation that the open exchange of scientific results and ideas presented in this book will lead to improved pharmaceutical products.

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