EDITORIAL II

SYNERGY AND ISOBOLES

When two drugs with similar properties are administered simultaneously, their effects are often predictable from consideration of their relative potencies. This applies, for example, to combinations of most inhalation anaesthetics: when two agents are given together, their relative potencies (when expressed as MAC values) are usually additive [1]. In other instances, the effect of two related drugs is greater than the response anticipated by simple summation. The presence of supra-additive effects is sometimes referred to as synergism (literally, "working together"). Unfortunately, synergism has more than one meaning, and its precise definition is controversial; in the past, it has been used in at least seven different senses [2], and the concepts associated with it may be the source of considerable confusion and ambiguity [3].

In recent years, several authors have described the occurrence of synergy between the hypnotic effects of midazolam and other commonly used anaesthetic drugs (e.g. methohexitone, thiopentone and propofol) [4-6]. In these studies, synergy was analysed and defined by the use of isoboles (graphs showing equieffective combinations of drugs). Each axis on the graph corresponds to a drug, for which individual ED₆₀ values for hypnosis have been determined previously by probit analysis of the dose-response relationship; the loss of response to command was invariably used as an index of hypnotic activity. The individual ED₆₀ values for hypnosis of the agents are plotted on the two co-ordinates of the graph, and are joined by a straight line between the axes (which is considered to define a purely additive interaction).

As combinations of midazolam and methohexitone, thiopentone or propofol produced effects that were below the additive line (or produced a curve that was concave upwards), it was suggested that synergism was present. When isoboles are used to demonstrate synergy, the word clearly implies "supra-additivity"; it was therefore considered that combinations of midazolam with thiopentone, methohexitone or propofol had supra-additive hypnotic effects [4-6].

The use of isoboles has an extremely long and controversial history; they were used first by Fraser to illustrate the antagonism between phystostigmine and atropine, more than 120 years ago [7, 8], and were used subsequently by Loewe to analyse synergic reactions between drugs [9, 10]. In the past, the analysis of interactions between drugs by isoboles has often been treated with considerable suspicion and circumspection [11, 12]. The method is usually regarded as rather empirical and intuitive, and appears to take little or no account of any differences in the dose-response relationships of the two drugs. However, the real problem with isoboles is concerned with their interpretation. According to their proponents, isoboles can be used to demonstrate synergism, summation and antagonism, without reference to the mechanism of action of the drugs concerned [13]. Synergism and antagonism are simply separated by the line of additivity, and pharmacodynamic factors are considered to be of little importance. A different interpretation has been advanced by Chou and Talalay [3, 14], using a kinetic approach based on the theoretical interpretation of inhibition studies in Michaelis-Menten and Hill-type enzyme systems. According to this view, isoboles can be used to demonstrate synergism when the effects of drugs are "mutually exclusive"—that is, when they act at (or are bound by) the same receptor or enzyme site. In contrast, combinations of drugs that act at different sites ("mutually non-exclusive") cannot be analysed by isoboles, as agents with purely additive effects may give rise to isoboles that are interpreted incorrectly as synergic [3].

This concept can be expressed in a mathematical form. When two mutually non-exclusive drugs have additive properties, it can be shown that [3, 14]:

\[
\frac{D_1}{(ED_{60})_1} + \frac{D_2}{(ED_{60})_2} + \frac{D_1 \cdot D_2}{(ED_{60})_1 \cdot (ED_{60})_2} = 1
\]

where \(D_1\) and \(D_2\) = doses of the two drugs which, when given together, are equi-effective with \((ED_{60})_1\) and \((ED_{60})_2\). Consequently:

\[
\frac{D_1}{(ED_{60})_1} + \frac{D_2}{(ED_{60})_2} < 1
\]

This relationship defines an isobole that demonstrates supra-additive effects [13], although the two drugs, by definition, only produce summation.

Consequently, problems of interpretation may arise when combinations of mutually non-exclusive drugs are analysed by isoboles. These difficulties may well apply to combinations of benzodiazepines and i.v. anaesthetics, as their dose-response relationships suggest that the drugs are mutually non-exclusive. In addition, it is generally accepted that both benzodiazepines and i.v. anaesthetics act on the GABA_A receptor–chloride channel complex, which is present at many post-synaptic sites in the CNS [15]. In its natural state, the GABA_A receptor (in common with the nicotinic cholinergic receptor) consists of five distinct subunits. Barbiturates, propofol and steroid anaesthetics act on the β-subunits, at a site that is closely related to the chloride channel [15-19]; in contrast, benzodiazepines are bound by the α-subunits and indirectly facilitate inhibitory transmission [20]. In addition, the benzodiazepine antagonist flumazenil does not attenuate the response to i.v. anaesthetics [18]. For these reasons, midazolam and i.v. anaesthetics appear to be mutually non-exclusive drugs, and the validity of using isoboles to analyse interactions between them is questionable.
Nevertheless, these investigations [4–6] suggest that combinations of benzodiazepines and i.v. anaesthetics produce hypnosis in smaller doses than anticipated from studies in which each drug is given alone. In addition, they have opened the long-standing and controversial problems associated with the interpretation of isoboles. Are they just a convenient method of demonstrating the effects of different drugs in combination, so that the smallest dose combination that is consistent with the required effect can be defined? Or can they be used to define synergism, summation and antagonism, irrespective of whether they have a rational or logical explanation at a cellular level? These are important and controversial questions, which deserve careful consideration. However, most of the evidence suggests that isoboles may not be the best or the most appropriate way to investigate synergetic reactions between anaesthetic drugs with dissimilar mechanisms of action.

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REFERENCES