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Commentary

A search for interaction among combinations of drugs of abuse and the use of isobolographic analysis

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SUMMARY

What is known and Objective: **Individuals who abuse drugs usually use more than one substance. Toxic consequences of single and multi-drug use are well documented in the Treatment Episodes Data Set that lists drug combinations that result in hospital admissions. Using this list as a guide, we focused our attention on combinations that result in the most hospital admissions and searched the PubMed database with the objective of determining the number of such publications and, in particular, those that used the term *synergism* in their titles or abstracts.**

Comment: **Using the search criteria produced an extensive list of published articles. However, a further intersection of the search terms with the term *isobole* revealed a surprisingly small number of literature reports.**

What is new and Conclusion: **Because the method of isoboles is the most common quantitative method for distinguishing between drug synergism and simple additivity, the small number of investigations that actually employed this quantification suggests that the term *synergism* is not properly documented in describing the toxicity among abused substances. The possible reasons for this lack of quantification may be related to a misunderstanding of the modelling equations. To help rectify this possible hurdle to understanding and clinical utility, the theory and modelling are discussed here.**

WHAT IS KNOWN AND OBJECTIVE

It is well known that alcohol, cocaine, opioids, marijuana and various stimulants are prominent among substances that are frequently abused. These substances have been extensively studied, and the results of that effort are represented in a vast body of publications. It is also well known that drug abusers do not usually confine their usage to a single drug. In that regard, information from the Treatment Episode Data Set (TEDS) is revealing in that it provides drug combination data that resulted in hospital admissions due to drug abuse toxicity (Table 1). The TEDS data are useful and emphasize the need for even more information on drug–drug interactions among the classes of abused drugs; of specific importance are those reactions that are synergistic (i.e. greater than additive).

Therefore, it is of interest to ask whether drug synergism has been rigorously determined for pairs of abused substances. Addressing that question is the main aim of this communication, which also summarizes the theory that answers the question. Our use of the TEDS database guided us in the selection of drug combinations of interest for our further data mining of the substances that are of special interest. Specifically, we examined the PubMed database to locate and count publications that include synergistic interactions for these widely abused combinations of drugs.

We included the search term *synergism* as a key word that describes supra-additive interactions between drugs. Specifically, synergism refers to effects of the drug combination (either toxic or beneficial) that are numerically greater than the combination effect that is suggested (predicted) by the potency/efficacy profiles of the individual drugs. The profile is determined from each drug's individual dose–effect relation, where the 'effect' is meant to be some common effect (therapeutic or toxic) that is produced by each drug. The most common method for assessing synergism is the *isobolographic* method introduced and popularized by Loewe.[1–3] In this method, one first identifies the particular effect of interest that is common to each drug, then obtains the dose-effect curve of each drug and then derives a graph in Cartesian coordinates that plots dose pairs that are expected to yield the specified effect at some specified magnitude (often 50% of the maximum effect). This derived curve is termed the additive 'isobole' and is convenient for visualization of additivity or non-additivity of effects of drug combinations. Because each drug individually produces the specified effect, it is reasoned that the presence of one drug (let it be designated 'A') reduces the dose of the second drug (designated 'B') needed in the production of the specified effect. Therefore, the graph of the dose of drug B against the dose of drug A is a monotone decreasing curve that may be linear or nonlinear. This curve is termed the additive *isobole* (See Fig. 1). As all points on the curve (drug-dose pairs) are expected to produce the specified effect level, experimentally determined points that graph below the curve mean that lesser quantities of the drugs are needed to produce the effect, thereby indicating a synergistic interaction. Equivalently, synergism is indicated when a point

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(dose pair) on the isobole gives an effect that is greater than the specified effect for that isobole. A point on the graph that plots above the isobole indicates a subadditive interaction (antagonism). This graphical approach, which is highly dependent on the shape of the dose-effect curves of the individual drugs, has been extensively examined and expanded. As the appearance of Loewe's early works (*op. cit.*), there have appeared many theoretical and procedural details that are contained in more recent works and reviews.[4–11] The aim in all of these is the distinction between synergism and simple additivity or subadditivity. The terms *synergism* and *isobole* are therefore key words in our data mining procedure that is described below.

Our search utilized 38 group substance names (GSNs) as listed in Table 2. Some substance names included a wild card symbol to facilitate matching to various lexical forms of the same name. Not all GSNs are disjoint; that is, some were constructed as the unions of several other

Group substance names for which the PubMed query results were small. We initially performed searches for each of the 38 GSNs. For each of these, a query was designed to return the PubMed identifier among all entries in that database that contain at least one of the substance names in either the title or abstract. Thus, our first search strategy counted all papers that include the substance name in the title or abstract. We proceeded by taking into account the co-occurrences of substances, for example, in GSN #1, we used 'buprenorphine *or* buprenex'. A further strategy identified papers that include pairs of substances from the different groups, and an additional strategy counted papers with pairs of substance names (from different groups) and either the term 'isobole' or 'synergy' or 'drug synergism'. To further filter the search process, we searched for pairs (from different groups) and 'isobole' or 'synergy'. The most refined final search included pairs of substance names and the term 'isobole'.

COMMENT

We see from Table 1 that combinations of alcohol with marijuana and alcohol with cocaine account for the greatest percentage of hospital admissions. Also notable are combinations of cocaine and marijuana, opioids and cocaine, and various CNS stimulants with alcohol. This group of five pairs, therefore, guided the main targets of further exploration in regard to reports of synergism. (It is interesting to note that tranquilizers and other sedative hypnotics are not a large number.) Table 2 shows the 38 search terms that were used in a list that was derived from broad Drug Enforcement Administration (DEA) categories consisting of *narcotics, CNS depressants, stimulants, hallucinogens, cannabis, anabolic steroids, inhalants* and *alcohol*. A search of the PubMed database revealed that there are 447 074 published papers that include in their title or abstracts one or more names represented in this list of 38. Among this large number of reports, the greatest number of publications was for category #38 alcohol (194 241), #30 testosterone (59 429), #17 benzodiazepines (41 120), #8 morphine (36 866), #18 cocaine (25 397) and #29 marijuana (22 094). Among the total of 447 074 papers, there were 77 385 that included the term *synergy* or *synergism* in the title or abstract and 1265 papers that included the term *isobole*. When the search focused on publications that contain *at least two substance names* in title or abstract, we found 59 957 publications. Of these, there were only 481 that contained either or both the terms i*sobole* or *synergy* (*synergism*). When confined to just the single term *isobole*, with at least two substances, the number of publications dropped to 59. When the list of 38 is viewed against the five drug combinations that resulted in the most hospital admissions (shown in Table 1), we get the numbers of publications[12–16] shown in Table 3 that deals with these toxic combinations of interest. Here, we see a rather large number of publications involving alcohol with the several groups indicated. Yet, among all of these, our search showed that the term *isobole* or *isobolographic analysis* is mentioned in only three publications, specifically each with cocaine. A somewhat similar result was found among the cocaine/marijuana publications. Here, there was only one paper, and there was only one paper dealing with cocaine and opioids. These few are referenced in the Table 3.

WHAT IS NEW AND CONCLUSION

Our data mining effort was extensive and it revealed a very large number of publications, both clinical and preclinical, that deal with the major drugs of abuse. In our total database, more than 77 000 of these publications made reference to synergism. This is a term that is very frequently associated with the toxicity of drug combinations. While there is no doubt that certain drug combinations can be dangerous, whether the interaction is synergistic or simply additive. Yet, it is important to look more closely into the use of the term synergism, a word with a very specific quantitative meaning. This term should be used only if the drug combination has been subjected to a quantitative analysis that distinguishes between the observed combination effect and the effect that is expected from the individual drug potencies. In most cases, and certainly among the drug groups detected here, an analysis of synergism almost always uses isobolographic methods. Other methods of analysis, such as response surface analysis,[5,17] have also been used, but are less common. In that regard, our data mining effort shows a drastic drop to only 59 publications that include the word *isobole* in the publication's title or abstract. Of course, it is possible that some of the papers that concluded synergism used quantification, but just did not use the term isobole. Therefore, no definitive conclusion can be drawn. Yet, the omission in the abstract of the method (isobole or other) that led to a conclusion of synergism seems unlikely if a quantitative method was used in the analysis. This point is reinforced by the magnitude of the drop: over 77 000 papers mentioning synergism, but only 59 publications mentioning isobole.

Drug combinations can be very useful in therapy and can be quite important in the production of toxic reactions. Synergistic interactions are especially important in these cases of toxic reactions and also because this finding is often a first step in an understanding of mechanism, a fact well illustrated in the review by Tallarida and Raffa,[11] that describes the basis of the various methods used in quantitating drug–drug interactions. The rather modest use of the term isobole (or employment of other quantitative methods) among the authors who use the term synergism may be due to some confusion that has surrounded the isobole method of analysis. The source of this confusion may be due to the different views of its originator, Loewe, and a subsequent analysis by Berenbaum[4] that are summarized in the latter's extensive review. Loewe was clear in suggesting that the additive isobole

Table 1. Treatment Episode Data Set (TEDS). Below are substance abuse combinations by selected primary substance of abuse: TEDS number and percent distribution.

Primary substance	Secondary and tertiary substances	Number	Percent of all admissions
All admissions		1 882 584	100.0
Alcohol		807 939	42.9
No other substance	N/A	444 781	23.6
1 other substance	Marijuana	117 272	6.2
	Cocaine	87 674	4.7
	Opiates	17 556	0.9
	Stimulants	12 256	0.7
	Other	12 027	0.6
2 other substances	Cocaine and marijuana	53 499	2.8
	Marijuana and stimulants	16 047	0.9
	Cocaine and opiates	16 777	0.9
	Marijuana and opiates	6285	0.3
	Cocaine and stimulants	4400	0.2
	Stimulants and opiates	1222	0.1
	Marijuana and other	9460	0.5
	Cocaine and other	4755	0.3
	Opiates and other	2875	0.2
	Stimulants and other	1053	0.1
Cocaine		241 699	12.8
No other substance	N/A	71 123	3.8
1 other substance	Alcohol	70 520	3.7
	Marijuana	23 074	1.2
	Opiates	6502	0.3
	Stimulants	1980	0.1
	Other	2362	0.1
2 other substances	Alcohol and marijuana	44 874	2.4
	Opiates and alcohol	6774	0.4
	Stimulants and alcohol	2587	0.1
	Opiates and marijuana	2748	0.1
	Stimulants and marijuana	2065	0.1
	Opiates and stimulants	498	*
	Alcohol and other	3349	0.2
	Marijuana and other	2116	0.1
	opiates and other	844	*
	Stimulants and other	283	*
Opiates		331 272	17.6
No other substance	N/A	141 565	7.5
1 other substance	Cocaine	54 426	2.9
	Alcohol	33 576	1.8
	Marijuana	14 277	0.8
	Stimulants	3370	0.2
	Other	10 950	0.6
2 other substances	Cocaine and alcohol	30 630	1.6
	Cocaine and marijuana	12 733	0.7
	Alcohol and Marijuana	10 741	0.6
	Cocaine and stimulants	2871	0.2
	Stimulants and alcohol	1743	0.1

Table 1 (*continued*)

Primary substance	Secondary and tertiary substances	Number	Percent of all admissions
	Stimulants and marijuana	1265	0.1
	Cocaine and other alcohol	5145	0.3
	Marijuana and other	4777	0.3
	Other	2657	0.1
	Stimulants and other	546	*
Marijuana		283 527	15.1
No other substance	N/A	99 870	5.3
1 other substance	Alcohol	99 531	5.3
	Cocaine	11 046	0.6
	Stimulants	9 959	0.5
	Opiates	2280	0.1
	Other	5752	0.3
2 other substances	Alcohol and cocaine	18 817	
	Alcohol and stimulants	13 561	0.7
	Stimulants and cocaine	2339	0.1
	Alcohol and opiates	3169	0.2
	Cocaine and opiates	1386	0.1
	Stimulants and opiates	523	*
	Alcohol and other	11 022	0.6
	Cocaine and other	1926	0.1
	Stimulants and other	1445	0.1
	Opiates and other	901	*
Stimulants		126 063	6.7
No other substance	N/A	37 773	2.0
1 other substance	Alcohol	19 137	1.0
	Marijuana	21 707	1.2
	Cocaine	3052	0.2
	Opiates	1396	0.1
	Other	1625	0.1
2 other substances	Marijuana and alcohol	25 135	1.3
	Cocaine and alcohol	4233	0.2
	Cocaine and marijuana	4314	0.2
	Opiates and alcohol	1280	0.1
	Marijuana and opiates	1333	0.1
	Cocaine and opiates	763	*
	Marijuana and other	2032	0.1
	Alcohol and other	1444	0.1
	Cocaine and other	519	*
	Opiates and other	320	*
Other		92 084	4.9

The Treatment Episode Data Set (TEDS) is maintained by the Office of Applied Studies, Substance Abuse and Mental Health Services Administration (SAMHSA). The TEDS system includes records for some 1.5 million substance abuse treatment admissions annually.
*Less than 0.1%.

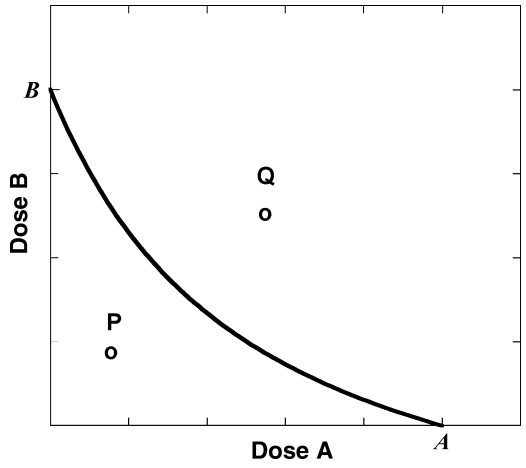

Fig. 1. The smooth curve is an isobole, a monotone decreasing curve of dose pairs of drugs A and B that individually produce the same effect and which are used in combination to produce a specified effect level (usually 50% of the maximum). The curve shows that the presence of the second drug reduces the needed dose of the first when both are present, and there is no interaction between the two drugs, a situation termed 'additive'. The intercepts *A* and *B* denote the individual doses needed to achieve the effect. The shape of the isobole (curvature) depends on the potency ratio of the two substances. Regardless of its shape, the isobole allows an assessment of synergism if the actual dose combination gives the effect with lesser quantities, such as point P, which falls below the curve. In contrast, an actual dose pair above the isobole, such as point Q, indicates a subadditive interaction.

could be nonlinear, but he justified his view with a rather loose mathematical treatment that had cumbersome notation. Berenbaum rejected Loewe's reservations by providing an argument that included a sham demonstration that is described subsequently. In short, Berenbaum took the linear equation $x/A + y/B = 1$ to be the *definition* of an additive interaction for a dose pair (x,y) with individual potencies of A and B, respectively. For example, if the specified effect level is 50% of the maximum, then A = ED50 of drug A and B = ED50 of drug B. However, this is not the definition; it is, instead, a consequence of the fact that two agonist drugs have a constant relative potency as we now show. In other words, dose x for drug A and dose y for drug B give a ratio $R = A/B$ (ratio of ED50's) that is the same for all equally effective x,y pairs. In this case, any tested dose x of drug A has a dose B-equivalent that is x/R. From this it follows that the dose of drug B alone that gave the specified effect (denoted B) could be achieved by adding the actual y and the equivalent, that is, $y + x/R = B$. On rearrangement this becomes

$$x/A + y/B = 1 \quad (1)$$

If, however, the potency ratio R is not a constant, then Eq. (1) does not hold and the additive isobole will be generally nonlinear. Berenbaum came to the conclusion that this linear relation *defines* the isobole, but his approach used only one agent and a dilution of that agent, which he considered as the two 'drugs'. But that situation does not constitute a proof because the diluted drug and the actual drug will necessarily have a constant potency ratio, a situation in which the isobole is clearly linear as shown above. If,

Table 2. Drugs of abuse

	Term(s)	PubMed search count[a] (Title and/or Abstract, unless stated otherwise)
1	Buprenorphine, buprenex	3210
2	Butorphanol, stadol	1029
3	Codeine	3409
4	Fentanyl, duragesic	12 089
5	Hydrocodone, vicodin	397
6	Hydromorphone, dilaudid	801
7	Methadone, dolophine	8715
8	Morphine, astramorph	36 866
9	Oxycodone, oxycontin	1154
10	Propoxyphene, darvon	828
11	Mephobarbital, mebaral	81
12	Pentobarbital, nembutal	13 000
13	Diazepam, valium	16 838
14	Chlordiazepoxide, librium	2919
15	Alprazolam, xanax	1752
16	Lorazepam, ativan, temesta	2712
17	Benzodiazepine[a], diazepam, valium, chlordiazepoxide, librium, alprazolam, xanax, lorazepam, ativan, temesta	41 120
18	Cocaine	25 397
19	Phentermine	407
20	Diethylpropion	194
21	Methamphetamine, dextroamphetamine	6579
22	Methylphenidate, dexmethylphenidate, ritalin, adderall	4300
23	Caffeine	19 365
24	Ketamine	10 380
25	mdma, methylenedioxyamphetamine	2709
26	Lsd, lysergic acid	4137
27	Mescaline, trimethoxyphenethylamine, phenethylamine	590
28	Psilocybin	321
29	Tetrahydrocannabinol, thc, cannab[a], marijuana	22 094
30	Testosterone, dihydrotestosterone	59 429
31	Nitrous oxide	11 539
32	Alkyl nitrites	46
33	Amyl nitrite	428
34	Butyl nitrite	60
35	Isopropyl nitrite	9
36	Isobutyl nitrite	79
37	Inhalant[a], nitrous oxide, alkyl nitrites, amyl nitrite, butyl nitrite, isopropyl nitrite, isobutyl nitrite	14 969
38	Alcohol	194 241

U.S. Department of Justice Drug Enforcement Administration.
aIn the above the column total is 524 193. When corrected for duplicates the sum is 447 074 as reported in the text.

Table 3. Number of publications of the named drug and combinations using isoboles

Combination	Relevant publications	Isobolar analysis
Alcohol with marijuana (#38 with #29)	4326 of 11 589 (on alcohol and other drugs)	0
Alcohol with cocaine (#38 with #18)	3245 of 11 589 (on alcohol and other drugs)	3[12–14]
Alcohol + other stimulants (#38 with #19–25)	1691 of 11 589 (on alcohol and other drugs)	0
Cocaine with marijuana (#18 with #29)	2435 of 7516 (cocaine and other drugs)	1[15]
Cocaine with opioids (#18 with #3–9)	2015 of 7516 (cocaine and other drugs)	1[16]

however, the shapes of the individual dose-effect curves differ, (e.g. if the drugs have different *E*max values) then the potency ratio is not constant, and it is easy to show that the isobole is not linear.[8–11] Hence, the Berenbaum assertion did not include the more general case of non-parallel dose-effect curves and therefore Loewe's original assertion that isoboles can be nonlinear is correct. The fact that isoboles can be (and often are) nonlinear should not detract from their usage in quantitating drug combinations because the nonlinear isobole represents no major mathematical challenge. An interaction model or method that starts with the linear form above as the *definition* of additivity will be very restricted in its use. Further, the confusion resulting from Berenbaum's rejection of Loewe's general case might explain, in part, the relatively small use of isoboles included among the many publications detected in our search.

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