

About the evaluation of drug combination

Zheng-jun JIN¹

Department of Pharmacology, Shanghai Second Medical University, Shanghai 200025, China

The article: “Two useful methods for evaluating antihypertensive drugs in conscious freely moving rats” by Su *et al*^[1] represents two aspects of typical advance in pharmacology research methods. The first one is the use of conscious freely moving hypertensive rats in hypotensive drug experiments: it includes the measurement of animal’s blood pressure (BP) by intra-arterial catheterization and delivering BP direct data, which are both instantaneous and averageable over a defined time. Needless to say, this method requires a sophistication beyond any usual indirect BP measurement (by tail, for example). Rats, being conscious, preserve their internal reflexes, which, under anesthesia, would be totally or partially inhibited. From this point of view, their BP data, contain much more information than any usual BP measuring method. A real-time BP analysis software should be created in order to make full use of the rich information of the data.

The second aspect is the application of the modified Bürgi formula, whose concept is universally accepted in pharmacology, namely: $q = \text{observed value} / \text{expected value}$ ^[2]. If the expected value represents the “addition of drug combination”, $q=1$ would mean simple addition; $q>1$, synergism or potentiation, $q<1$, antagonism. However, in practical application, the q never equals exactly 1; so a tolerance of ± 0.15 was proposed as an upper and lower limit^[3]. The major modification to the formula was the replacement of the original denominator by the Sum of Probability of Independent events: $P_A + P_B - P_A \times P_B$, A and B denoting two independent events. The response percentage would be considered as probability^[4].

The “Sum of Probability for Independent Events”

is universally recognized^[5] in scientific sphere, be it macro- or microscopic. Pharmacologists, especially those show interest in the study of mechanism of action, would like to have mechanism-specific “addition formula”. However, in the search of new drugs, the inner mechanism of action is usually unknown. In the pursuit of the best combination, one is interested in the selection of the combination showing the highest efficacy or the least toxicity. The magnitude of the benefit is much less important than the ranking of the combined effects. The mathematical formula is only a tool. As a tool, it will be useful as long as it fulfills the task it is assigned to. From this point of view, the Probability Sum of Independent Events is useful in terms of sensitivity: the parameter q derived from Bürgi’s original idea, provides a good estimation of the ranking. The q parameter thus derived is mechanism-free: it will deliver a true ranking, independent of its mechanism of action. It enjoys a good reproducibility and shows a sound relation with common sense^[1,3].

When using this formula, one does not need to change the dose of the drugs involved. In FDA statement about drug combination, the dose of the combined drugs should not be changed. This statement is really noteworthy, for the slope of dose-response curves of every drug is different. Cutting the dose in half, does not imply that the effect is reduced to half of the original effect. Recently, 46 dose-response formulas based on Bliss method have been analyzed (Data kindly provided by Shanghai Bureau of Biological Standardization and Institute of New Drug Toxicology). The end-point was 50 % mortality. Tab 1 showed the new situation (computational issues) at halving the LD_{50} .

To our surprise, only one case of the 46 happened at half of 50 % mortality (25 %). The majority would happen around 5 % (38/46). This mortality distribution table shows clearly the danger of changing the dose of the drugs involved in the combination. The experience of our lab (made during the year 1956-1957)^[3] con-

¹ Correspondence to Prof Zheng-jun JIN, the former Vice Editor-in-Chief of Acta Pharmacologica Sinica.

E-mail qmeing@sh163.net

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Tab 1. The relationship between mortality and frequency.

Mortality/%	Frequency
0	2
5	38
10	5
15	0
25	1
30	0
35	0
40	0
45	0
50	0

firmed once again the truth of the table: halving the LD₅₀ of the tartar emetic led to a zero mortality, but not 25 % effect as many authors stated. Of course, one would expect a much less steeper slope in the efficacy experiment. However, the mathematical principle remains the same: there is an individual slope for every individual drug. In the combination of two drugs A and B, halving the dose of each drug in the combination would produce a misleading result.

The probability sum formula, using the unchanged dose effect, does not suffer from the drastic and most of time unpredictable variation of the effect. It uses the actual effect of both single groups A and B (the best estimation of single effects).

The design of the experiment being straightforward, is easily carried out, and the computation is within every one's reach. The formula, with its design, is very cost effective.

A final word to say, but not the last, is the optimal choice of the effect level: theoretically, an effect near 40 %–60 % would be adequate, allowing equal opportunity to stimulatory and inhibitory effects. A choice of higher effect level would be good for inhibitory drugs, and vice versa.

Some questions or critics:

1) Drug effects may be related, not independent.

The goal being making a rank of different combinations, any method allowing a sound and impartial ordering, may be used. The relation between drugs is difficult to assess, and may be deferred after the ranking. The ranking, per se, is rather mechanism-free.

2) The transformation of quantitative data into quantal data, is rather arbitrary.

Even with an arbitrary line of success or failure, the ranking is not affected, since the same level is ap-

plied to each individual datum. Besides, arbitrary judgement is universally accepted even in Olympiads, so long as an equal opportunity and impartiality are practiced. In every special field, only those engaged in the actual animal (or human) experiments are most qualified to define the demarcation line. The demarcation should be based on empirical basis, depending on many variables. A demarcation level, based on researcher's experience, is adequate in absence of any objective demarcation criterion. On the other hand, changing the demarcation level would only change the relative percentage, and not the ranking, which is only an order of placement according to the magnitude of percentages.

3) The parameter q versus the significance test.

Nowadays, scientists, especially biologists, are using significance tests quite frequently. We have compared the Fisher Exact P test with the q , and found that, a significant P ($P < 0.05$), goes far beyond the synergism, and requires a strong potentiation. Therefore, the significance test is not applicable in case of addition. On the other hand, a ranking does not need a significant difference. A minute difference of few milliseconds will make an Olympic champion in free style 100 m swimming, while that difference would be considered as a pure chance, hence not significant at 0.05 level. We therefore conclude that a ranking problem does not need a significance, while a high reproducibility is much more needed. We may repeat the experiment several times. If the ranking remains the same, that would prove the formula enjoys a high reproducibility, which is the case in the article of Su *et al*.

4) The direct measurement of BP in conscious rats, coupled with the modified formula of combination, endowed the article with an attractive progress and efficiency.

REFERENCES

- 1 Su DF, Xu LP, Miao CY, Xie HH, Shen FM, Jiang YY. Two useful methods for evaluating antihypertensive drugs in conscious freely moving rats. *Acta Pharmacol Sin* 2004; 25: 148-51.
- 2 Jin ZJ. Addition in drug combination. *Acta Pharmacol Sin* 1980; 1: 70-6.
- 3 Zhang XW, Jin ZJ. The Probability sum and the Isobole in drug combination, a comparison. *Advances in pharmacology*. Beijing: Chinese Society of Pharmacology; 1982. p 44-56.
- 4 Jin ZJ. The probability sum method in drug combination. *Acta Univ Sec Shanghai* 1981; 1: 15-6.
- 5 Sachs L. *Applied statistics*. New York: Springer-Verlag; 1982. p 42.