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Analysis of drug interactions in combined drug therapy by reflection method[1]

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KEY WORDS drug synergism; drug antagonism; drug combinations; combination drug therapy; drug dose-response relationship; biometry; drug interactions

ABSTRACT

AIM: To set up a new method to analyze multidrug interaction in combined drug therapy. METHODS: Based on a dose-effect curve of the combined drugs and the equieffective test, a new mathematical model was set as $Q = (E_o - E_t)/L$ ($-1 < Q < 1$ addition, $Q \leq 1$ antagonism, $Q \geq 1$ synergism) where E_o = an observed value (or its fitted value) of combined effect, E_t = an expected value of combined effect, and L = an equieffective cutoff between E_o and E_t, decided by the equieffective criterion of a special field, the number of data points, and the experimental error. The different types of experimental data were analyzed by the new model. RESULTS: This reflection method could deal with data in combined drug therapy, unnecessary to distinguish between independent and similar action, or exclusive and non-exclusive case among drugs. The number of drugs for combination did not need to be limited. But the experimental data should be enough to fit a dose-effect curve of drugs in combination. If every dose-effect curve of drugs for combination was made, a series of Q values was obtained from all levels of dose-effect for a systematic analysis. To large animal or human experiment, the points of dose-effect of each drug used alone could be reduced to even 1 point. The results of analysis took the criterion of a special field and laboratory error into account in this method. CONCLUSION: The reflection method is an effective method for analysis of multidrug interaction in combined drug therapy.

INTRODUCTION

Addition is a basic concept in analysis of multidrug effects, based on two types of interaction[1,2]: (1) similar action, in which drugs were supposed to have the same mechanisms of actions, to act at the same sites and to behave like different doses of the same drug in combination, and (2) independent action, in which drugs were supposed to act at different sites with different mechanisms of action. Other classifications gave some terms of similar meaning, such as similar and dissimilar interaction[3], exclusive and non-exclusive case[4], *etc*. However, to experiment *in vivo*, the two actions may coexist. For example, at low dose it indicates independent action and at high dose it maybe similar action, or in contrast, especially the effects of drugs expressed at the same organs. It will become more complicated when feedback, tolerance or hypersensitivity, *etc*, happened in body. So it is difficult to distinguish between similar and independent action, and to give a definition of addition. Some methods based on above classifications can not be used to deal with data *in vivo* effectively and precisely. Our previous method, the parameter method[5], can analyze this type of data, but it asks the maximal effect (E_{max}) in combination to be examined precisely and to be of practical significance, which is often difficult for experiment *in vivo* and for clinical trial in combined drug therapy.

It can be inferred that an interaction, whether it is similar or independent, or coexistent, can be indicated from the dose-effect curve of combined drugs. In this study, we try to take the dose-effect curve as a criterion irrespective of the types of interaction, and propose a more general method to analyze multidrug interaction in combined drug therapy from the change regularity of the

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curve.

SETTING UP THE METHOD

Formula $Q = (E_o - E_t)/L$ ($-1 < Q < 1$ addition, $Q \leq -1$ antagonism, $Q \geq 1$ synergism) (1)

where E_o is a combined effect or its fitted value, and E_t is an expected value of the combined effect. L is an equieffective cutoff[5] between E_o and E_t, calculated by

$$L = | E_t \cdot W - s_{\bar{x}-\omega} \cdot T | \quad (2)$$

where W, an equieffective criterion decided by a special field, generaly equals 0.05 - 0.1 in an experiment *in vivo* according to new drug biological statistics. T is a value of one-sided $t_{0.05}$, and its degree of freedom (f) = $N - n - 1$, where n is the number of combined drugs ($i = 1, 2 \cdots n$) and N is the sum of dose points in single drug and in combined drugs. $s_{\bar{x}-\omega}$ is a common standard error of E_o and E_t, calculated by

$$s_{\bar{x}-\omega} = \sqrt{s_{\bar{x}-o}^2 + s_{\bar{x}-t}^2 + \sum_{i=1}^{n} s_{\bar{x}-i}^2} \quad (3)$$

where $s_{\bar{x}-o}$, $s_{\bar{x}-t}$, and $s_{\bar{x}-i}$ is the standard error of E_o, E_t, and E_i respectively. To repeated and discontinued dose-effect data which can not be fitted as a curve, the standard error of observed effects is calculated by Equation 4. To continuous dose-effect data, the standard error of a fitted effect is calculated by Equation 5, and to discontinued and unrepeated observed effect or effect rate(single point), its standard error ($s_{\bar{x}-i}$) equals 0.

$$s_{\bar{x}-x} = \sqrt{\frac{\sum_{j=1}^{R} E_j^2 - (\sum_{j=1}^{R} E_j)^2/R}{R \cdot (R-1)}} \quad (4)$$

where R = number of repeated effects (E_j) at a dose.

$$s_{\bar{x}-x} = \sqrt{\frac{\sum_{j=1}^{M} (E_j - \hat{E}_j)^2}{M-2} \left(1 + \frac{1}{M} + \frac{(D_x - \bar{D})^2}{\sum_{j=1}^{M} (D_j - \bar{D})^2}\right)} \quad (5)$$

where E_j = a fitted value of observed effect (E_j), $\bar{D}$ = the mean dose, and M = the number of dose points for fitting a dose-effect curve of separate drug or combined drugs.

Principle and steps

1 The dose-effect curve of drug A and drug B in combination is made at a fixed proportion (P) (Fig 1).

2 D_a, a common dose of A, produces an effect E_a and D_b, a common dose of B, produces an effect E_b (the proportion of D_a and D_b is P) (Fig 1).

3 E_a reflects to the curve, and $D_{a'}$, equieffective dose of A and B in combination, is obtained, and $D_{b'}$ (obtained is the same as $D_{a'}$) (Fig 1).

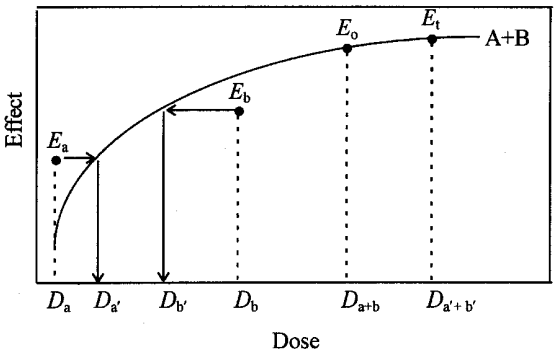

Fig 1. Principle of the reflection method.

4 Compared E_o, an observed effect (or fitted value) produced by dose D_{a+b} ($= D_a + D_b$), with E_t, an expected effect reflected by dose $D_{a'+b'}$ ($= D_{a'} + D_{b'}$) on the curve of combination (Fig 1), Equation 6 is obtained according to traditional method to set up model.

$$Q = E_o/E_t \quad (Q = 1 \text{ addition}, Q < -1 \text{ antagonism}, Q > 1 \text{ synergism}) \quad (6)$$

Some shortcomings exist in Equation 6: not considering the equieffective criterion of a special field, the number of data points, and experimental error. In order to overcome these shortcomings, L is proposed, described by the reference[5].

We find from Equation 1:

1 E_t in Equation 1 has the same principle with that in Equation 6, and takes the equieffective criterion of special field and laboratory error into account. When the different doses of the same drug are combined for analysis, $Q = 0$.

2 Points analysis: The analysis was made at some points on dose-effect curve. To small number of data point just like clinical trial, it is necessary to fit a dose-effect curve in combination, but the dose-effect points of each drug used alone can be decreased to even 1 point as shown in Fig 1.

3 Systematic analysis: Suppose all dose-effect curves of drugs used alone and in combination with a fixed proportion are made, the series of doses from the curves of drugs used alone reflect to the curve of combination, and a series of Q can be obtained for a systematic analysis and drawing a graph of Q values.

4 An appropriate mathematical model and its fitting method are selected according to data nature. We suggest Equation 7 for quantitative data and Equation 8 for qualitative data.

$$E = \frac{E_{max} \cdot D^H}{K^H + D^H} \quad (7)$$

$$E = \frac{D^H}{K^H + D^H} \quad (8)$$

5 The above steps can also analyze interaction belong more than 2 drugs in combined drug therapy.

EXPERIMENTS

Materials Chlorpromazine (Chl), product of Hefong Pharmaceutical Co, Shanghai, № 941202; scopolamine (Sco), product of Qiaoguang Pharmaceutical Factory, Guangzhou, № 941019-6; epinephrine (Epi), product of Tianfong Pharmaceutical Factory, Shanghai, № 93092; isoprenaline (Iso), product of Tianfong Pharmaceutical Factory, Shanghai, № 940102; acetaminophen (Ace), product of Jiling Pharmaceutical Factory, purity 99.5 %; butabital (But), product of Tonghua Pharmaceutical Factory, purity 99.1 %, № 970512; caffeine (Caf), product of Shunan Pharmaceutical Factory, purity 99.4 %. Kunming mice (19－22 g) were purchased from Experimental Animal Center, Shanghai Research Institute of Biological product (Certificate № 21－1, Grade Ⅱ), and a mongrel dog (15 kg) from Experimental Animal Center, Wannan Medical College.

Measurement of the latency of convulsion Chl was diluted with normal saline to a series of doses (3.2, 6.4, 12.8, 25.6 mg·kg^{-1}), and Sco was diluted as 0.1, 0.2, 0.4, 0.6, 0.8, 1.6 mg·kg^{-1}. Chl and Sco in combination were prepared as 3.2＋0.1, 6.4＋0.2, 12.8＋0.4, 25.6＋0.8 mg·kg^{-1}. All solutions were prepared by the time used. Mice were randomly assigned into 15 groups of 10 mice. First, 1 group was injected iv metrazol (0.2 mL·min^{-1}) through tail vein with a constant-speed pump. The latencies of convulsion were observed and their mean value was taken as a baseline. Second, 4 groups were injected iv a series of doses of Chl, 6 groups were injected iv a series of doses of Sco, and other 4 groups were injected iv Chl and Sco (Chl∶Sco＝32∶1). 5 min after above the administration (10 mL·kg^{-1}), metrazol (0.2 mL·min^{-1}) was injected iv through tail vein with the pump. The increased value of each animal latency (latency observed-baseline) was recorded.

Measurement of systolic blood pressure (SBP)[5] Epi was diluted with normal saline to 0.46, 1.37, 4.55, 13.66, 45.52, and 136.6 nmol·L^{-1} and Iso to 6, 18, 54, 162, 486 and 1458 nmol·L^{-1}. The series of doses of Epi and Iso were prepared in combination (proportion fixed as 1∶1.44, 1∶22.2, and 1∶44.4 respectively). The value of SBP increased (SBP↑) was recorded.

Measurement of analgesic effect Mice, fasting for 6 h, were randomly assigned into 21 groups of 10 mice. The latency to withdraw tail from a focused light stimulus was measured by a radiant apparatus in the mouse heat radiant tail-flick test[6]. Analgesia was defined as prolongation of latency to twice as baseline in the normal control group or even longer. The normal control group did not use any drug, and each of other 20 groups was given (ig) a dose as follow∶Ace 60.0, 42.0, 29.4, 20.6, 14.4 mg·kg^{-1}; But 15.0, 10.5, 7.4, 5.1, 3.6 mg·kg^{-1}; Caf 7.0, 4.9, 3.4, 2.4, 1.7 mg·kg^{-1}; doses in combination showed in Tab 2. The volume of administration (ig) was kept at 0.2 mL for per 10 g body weight. Analgesia rate of each group was recorded.

Data analysis Equation 7 was selected to fit the dose-effect curves for quantitative date with the simplex method, and Equation 8 for qualitative data. All curve fittings were of statistical significance ($P < 0.05$). Computer completed all calculations according to this reflection method.

RESULTS

Interactions of Chl and Sco in combination (32∶1) on the latency of convulsion induced by metrazol in mice There were different dose-effect curves on Fig 2 as Chl and Sco used alone and in combination (32∶1). Chl combined with Sco at the same proportion could lengthen the latency of convulsion induced by metrazol in mice, which changed along with the dose in combination at the same proportion. According to the systematic analysis by this method at same proportion, Chl (19.2－25.6 mg·kg^{-1}) combined Sco (0.6－0.8 mg·kg^{-1}) exhibited addition, and below the range exhibited synergism (Fig 3).

Interactions of Epi and Iso in combinations with different proportions on SBP↑ of dog The dose-effect equations∶ Epi∶$\hat{E} = 7.45 \times D^{0.75}/(6.39^{0.75} + D^{0.75})$; Iso∶$\hat{E} = 8.32 \times D^{0.39}/(189.5^{0.39} + D^{0.39})$; Epi and Iso in combination (1∶1.44)∶$\hat{E} = 50.8 \times D^{0.99}/(464.3^{0.99} + D^{0.99})$; Epi and Iso in combination (1∶22.2)∶$\hat{E} = 1637 \times D^{0.73}/(470916^{0.73} + D^{0.73})$; Epi and Iso in combination (1∶44.4)∶$\hat{E} = 16.40 \times D^{0.71}/(289.4^{0.71} + D^{0.71})$; As the proportions (Epi∶Iso) changed

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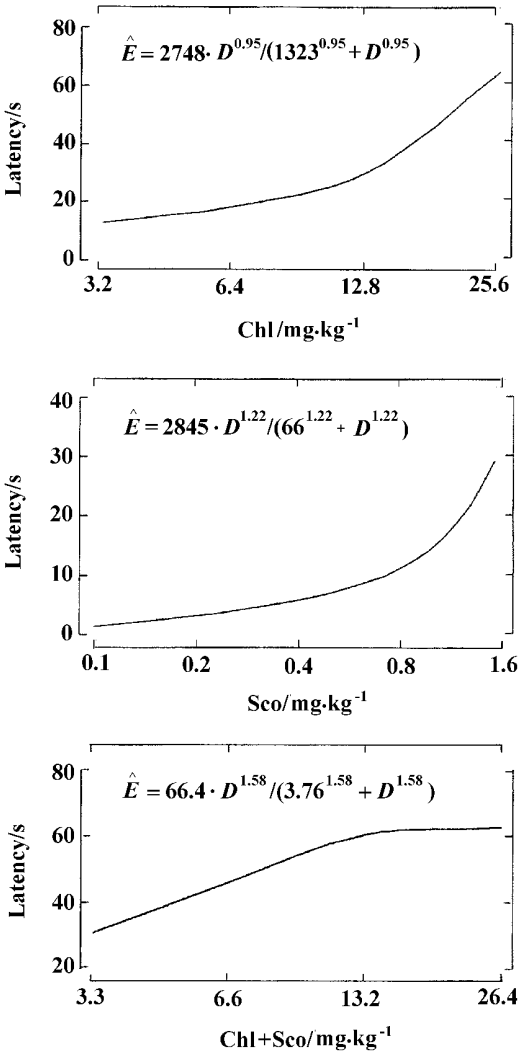

Fig 2. Dose-effect curves of Chl and Sco used alone and in combination.

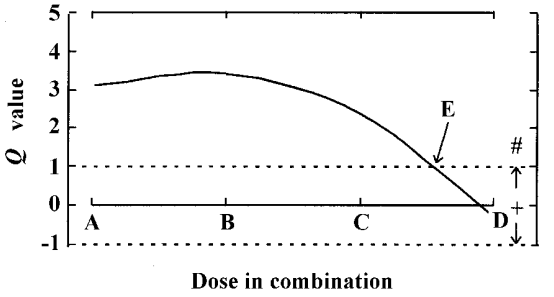

Fig 3. Systematic analysis of interactions of Chl and Sco used in combination (32∶1) on the latency of convulsion induced by metrazol in mice. Equieffective criterion∶ $W = 5$ %. Chl + Sco∶A) 3.2 + 0.1；B) 6.4 + 0.2；C) 12.8 + 0.4；D) 25.6 + 0.8；E) 19.2 + 0.6 (mg·kg^{-1}). #∶synergism, +∶addition.

from 1∶1.44 to 1∶44.4, Q values changed towards 1, namely antagonism and synergism had tendency to addition (Tab 1), which indicated that the contribution values of Epi in combined effect were decreased with increasing dose of Iso in combination. At the proportion (1∶1.44) it exhibited antagonism at the low and medium doses, and synergism at the high dose (Tab 1). The systematic analysis showed that it exhibited addition at Epi (11.7 − 17.5 nmol·L^{-1}) combined with Iso (51.95 − 77.70 nmol·L^{-1}) at the proportion, synergism above the range and antagonism below the range at the same proportion. The similar results were exhibited at the proportion (1∶22.2). At proportion (1∶44.4), Epi (0.68 − 8.13 nmol·L^{-1}) combined with Iso (31.2 − 361.3 nmol·L^{-1}) exhibited addition, and above the range exhibited synergism.

Interactions of Ace, But and Caf in combination on analgesic effect in mice But (3.6 − 15.0 mg·kg^{-1}) and Caf (1.7 − 7.0 mg·kg^{-1}) had not analgesic effect. The dose-effect equations∶ Ace used alone∶ $\hat{E} = D^{2.15}/(41.59^{2.15} + D^{2.15})$；Ace, But and Caf in combination∶ $\hat{E} = D^{3.38}/(39.67^{3.38} + D^{3.38})$. The analysis of the data points in combination indicated that the interactions exhibited addition at low doses and synergism at high doses (Tab 2).

Tab 1. Interactions of Epi and Iso used in combination with different proportions on SBP ↑ of dog, analyzed by the reflection method (points analysis). $W = 5$ %. $^{+}$∶addition, $^{-}$∶antagonism, $^{\#}$∶synergism.

Epi∶Iso = 1∶1.44			Epi∶Iso = 1∶22.2			Epi∶Iso = 1∶44.4		
Epi + Iso /nmol·L^{-1}	SBP /kPa	Q	Epi + Iso /nmol·L^{-1}	SBP /kPa	Q	Epi + Iso /nmol·L^{-1}	SBP /kPa	Q
0.68 + 3.03	0.47	-1.8^{-}	0.68 + 15.1	1.23	-1.5^{-}	0.68 + 30.2	3.47	-0.1^{+}
2.28 + 10.12	1.33	-2.6^{-}	2.28 + 50.6	2.38	-1.9^{-}	2.28 + 101.2	5.33	0.3^{+}
6.83 + 30.32	3.86	$2.4^{\#}$	6.83 + 151.6	4.58	-1.4^{-}	6.83 + 303.2	6.86	1.0^{+}
22.80 + 101.23	10.82	$3.1^{\#}$	22.80 + 506.2	11.78	$3.0^{\#}$	22.80 + 1012.3	12.62	$1.3^{\#}$

Tab 2. Interactions of Ace, But and Caf in combination on analgesia rate in mice, analyzed by the reflection method (points analysis). $W = 5\ \%$. $n = 10$ mice. $^{\#}$: synergism, $^{+}$: addition.

Ace + But + Caf /mg·kg^{-1}	Rate observed	Rate fitted $E_o \pm s_{\bar{x}}$	Rate expected $E_o \pm s_{\bar{x}}$	Q
40.0 + 12.0 + 5.0	0.8	0.78 ± 0.05	0.52 ± 0.02	4.0$^{\#}$
32.0 + 9.6 + 4.0	0.6	0.62 ± 0.04	0.40 ± 0.02	3.1$^{\#}$
25.6 + 7.7 + 3.2	0.4	0.42 ± 0.04	0.29 ± 0.02	1.7$^{\#}$
20.5 + 6.1 + 2.6	0.3	0.25 ± 0.04	0.20 ± 0.02	0.6^{+}
14.4 + 4.9 + 2.0	0.1	0.13 ± 0.05	0.10 ± 0.02	0.0^{+}

DISCUSSION

In combined drug therapy, quantitative data are difficult to be analyzed by most current methods, besides coexistence of 2 types of interactions. This method can solve those problems and deal with data effectively and it is unnecessary to distinguish between independent and similar action, or exclusive and non-exclusive case among drugs. Moreover, the number of combined drugs does not need to be limited. But the experimental data should be enough to fit the dose-effect curve of combined drugs. When all dose-effect curves of drugs for combination were fitted, a series of Q values is obtained from all levels of dose-effect for a systematic analysis. To large animal or human experiment, the points of dose-effect of each drug used alone can be reduced to even 1 point. The analysis results are obtained by taking the criterion of a special field and laboratory error into account in this method. An appropriate mathematical model and its fitting method can be selected according to data nature.

To experimental design in this method, a fixed proportion in combination must be kept among the drugs used alone. The effect parameters, E_{max}, K and H in Equation 7 and 8, are not always of practical significance, so the selected dose fo drug used alone for analysis should be kept in dose range in combination.

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映射法分析药物联合治疗的相互作用[1]

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关键词 药物协同作用；药物拮抗作用；复方合剂；联合药物治疗；药物剂量效应关系；生物统计学；药物相互作用

目的：在联合药物治疗中，建立一种分析药物相互作用的新方法. **方法**：基于联合用药的量效关系曲线和等效性检验的原理，建立一种新的数学模型：$Q = (E_o - E_t)/L$（$-1 < Q < 1$ 相加，$Q \leq -1$ 拮抗，$Q \geq 1$ 协同）其中 E_o 为药物联用实测效应或实测效应拟合值，E_t 为联用药效期望值，L 为 E_o 和 E_t 的等效界值. 同时设计实验，取得不同类型的数据，用此模型对实验结果进行分析. **结果**：本法适用药物联用治疗中的相互作用分析，不考虑相互作用的类型，联用药物数目不受限制. 根据受试对象的不同，其设计可繁可简，药物按某一比例联用的量效方程必不可少，而各单用药实验点可多可少，点多时可求出各单用药的量效曲线方程，作系统分析. 即使各单用药按以上比例只取一点，就可得出此点联用效果的结论. 本法结论能考虑专业要求和实验误差，较为严谨. **结论**：映射法能有效地分析药物联合治疗中的相互作用.

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