Analysis of drug interactions in combined drug therapy
by reflection method

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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 eqieffective test a new mathematical model was set as $Q = E_o - E_i / L < 1$ or $Q \leq 1$ synergism $Q \geq 1$ synergism where $E_o$ is an observed value or its fitted value of combined effect $E_i$ is an expected value of combined effect $L$ is an eqieffective cutoff between $E_o$ and $E_i$ decided by the eqieffective 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 method. 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

INTRODUCTION

Addition is a basic concept in analysis of multidrug effects based on two types of interaction similar action 1 independent action in which drugs were supposed to have the same mechanisms of action to act at the same sites and to behave like different doses of the same drug in combination and 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 exclusive and non-exclusive case 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 may 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 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
SETTING UP THE METHOD

Formula \( Q = \frac{E_o - E_i}{\sqrt{L}} - 1 < Q < 1 \) addition\( Q \leq -1 \) antagonism\( Q \geq 1 \) synergism\( Q \) when \( E_o \) is a combined effect or its fitted value\( Q \) and \( E_i \) is an expected value of the combined effect. \( L \) is an equi-effective cutoff\( Q \) between \( E_o \) and \( E_i \) calculated by
\[
L = | E_i \cdot W - \bar{s}_{x-o} \cdot T |
\]
where \( W \) is an equi-effective criterion decided by a special field\( n \) generally equals \( 0.05 - 0.1 \) in an experiment in vivo according to new drug biological statistics. \( T \) is a value of one-sided \( t_{n,0.05} \) and its degree of freedom\( f = N - n - 1 \) where \( n \) is the number of combined drug\( i = 1 \) to \( n \) and \( N \) is the sum of dose points in single drug and in combined drugs. \( s_{x-o} \) is a standard error of \( E_o \) and \( E_i \) calculated by
\[
s_{x-o} = \sqrt{s_{x-o}^2 + \bar{s}_{x-o}^2 + \bar{s}_{x-o}^2}
\]
where \( s_{x-o} \) is the standard error of \( E_o \) and \( E_i \), respectively. To repeated and discontinued dose-effect data which can not be fitted as a curve\( s_{x-o} \) the standard error of observed effects is calculated by Equation 4. To continuous dose-effect data\( s_{x-o} \) the standard error of a fitted effect is calculated by Equation 5 and to repeated and unrepeated observed effect or effect rate single point\( s_{x-o} \) its standard error\( s_{x-o} \) equals 0.
\[
s_{x-o} = \sqrt{\sum_{i=1}^{N} E_i^2 \cdot \frac{1}{R} \cdot \sum_{j=1}^{N} E_i^2 \cdot \frac{1}{R}}
\]
where \( R \) is number of repeated effects\( E_i \) at a dose.
\[
s_{x-o} = \sqrt{\sum_{j=1}^{M} E_j \cdot \frac{1}{M-2} \cdot \sum_{i=1}^{N} D_i \cdot \frac{1}{M} \cdot \sum_{j=1}^{N} D_j \cdot \frac{1}{M-2}}
\]
where \( E_j \) is a fitted value of observed effect\( E_i \) and \( D \) is the mean dose and \( M \) is the number of dose points for fitting a dose-effect curve of single 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\( D_A \) and \( D_B \) equi-effective dose of A and B in combination\( D_R \) is obtained\( D_A \) and \( D_B \) is the obtained is the same as \( D_A \) Fig 1.

**Fig 1. Principle of the reflection method.**

4. Compared \( E_A \) an observed effect\( E_i \) or fitted value\( E_i \) produced by dose \( D_A \) with \( E_i \) an expected effect reflected by dose \( D_A \) on the curve of combination\( Q \) is obtained according to traditional method to set up model.

\[ Q = E_o \cdot E_i \]

Some shortcomings exist in Equation 6 not considering the equi-effective criterion of a special field\( Q \) the number of data points and experimental error. In order to overcome these shortcomings\( L \) is proposed described by the reference\( Q \).

We find from Equation 1

1. \( E_i \) in Equation 1 has the same principle with that in Equation 6 and takes the equi-effective 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.
\[ E = \frac{E_{\text{aff}} \cdot D^H}{K^H + D^H} \]
\[ E = \frac{D^H}{K^H + D^H} \]

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

EXPERIMENTS

Materials Chlorpromazine \( \text{Chl} \) product of Hefong Pharmaceutical Co Ltd Shanghai No 941202 \( \text{Scopolamine} \) \( \text{Sco} \) product of Qiaoguang Pharmaceutical Factory Guangzhou No 941019-6 \( \text{Epinephrine} \) \( \text{Epi} \) product of Tianfong Pharmaceutical Factory Shanghai No 93902 \( \text{Isoprenaline} \) \( \text{Iso} \) product of Tianfong Pharmaceutical Factory Shanghai No 941012 \( \text{Acetaminophen} \) \( \text{Ace} \) product of Jiling Pharmaceutical Factory purity 99.5 % \( \text{Butabital} \) \( \text{But} \) product of Tonghua Pharmaceutical Factory purity 99.1 % No 970512 \( \text{Caffeine} \) \( \text{Caf} \) 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 No 21 – 1 Grade 3 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 the above 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 on SBP of dog

Epi was diluted with normal saline to 0.46 \( 1.37 \) \( 4.55 \) \( 13.66 \) \( 45.52 \) and 136.6 mmol L\(^{-1} \) and Iso to 6 \( 18 \) \( 54 \) \( 162 \) \( 486 \) and 1458 mmol L\(^{-1} \). The series of doses of Epi and Iso were prepared in combination proportion fixed as 1 : 1.44 \( 1 : 22.2 \) \( 1 : 44.4 \) respectively. The value of SBP increased SBP \( \uparrow \) 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. 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 data 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 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 \( \text{Chl} 19.2 – 25.6 \) mg kg\(^{-1} \) combined \( \text{Sco} 0.6 – 0.8 \) 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 G 7.45 \( \times D^{0.77} \) 6.39 \( \times D^{0.75} \) \( \text{Iso} = 7.32 \times D^{0.39} \) \( 189.5 \times D^{0.39} \) \( D^{0.39} \) Epi and Iso in combination \( 1 : 1.44 \) \( 1 \) \( \times D^{0.99} \) \( 464.3 \times D^{0.99} \) \( D^{0.99} \) Epi and Iso in combination \( 1 : 22.2 \) \( 1 \) \( \times D^{0.72} \) \( 4709 \) \( 76 \) \( D^{0.73} \) Epi and Iso in combination \( 1 : 44.4 \) \( 1 \) \( \times D^{0.97} \) \( 289.4 \times D^{0.71} \) \( D^{0.71} \) As the proportions Epi : Iso changed...
Fig 2. Dose-effect curves of Chl and Sco used alone and in combination.

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 equation Ace used alone, $\hat{E} = D^{2.15} / (41.59^{1.15} + D^{2.15})$, Ace, But and Caf in combination, $E = D^{3.38} / (39.67^{3.38} + D^{3.38})$. The analysis of the data points in combination indicated that the interactions exhibited addition at low doses and synergism at high doses, Tab 2.

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%.

### Table 1. Interactions of Epi and Iso used in combination with different proportions on SBP↑ of dog analyzed by the reflection method

<table>
<thead>
<tr>
<th>Epi: Iso = 1:1.44</th>
<th>Epi: Iso = 1:22.2</th>
<th>Epi: Iso = 1:44.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{0.68 + 3.03}$</td>
<td>$E_{0.68 + 15.1}$</td>
<td>$E_{0.68 + 30.2}$</td>
</tr>
<tr>
<td>$E_{2.28 + 10.12}$</td>
<td>$E_{2.28 + 50.6}$</td>
<td>$E_{2.28 + 101.2}$</td>
</tr>
<tr>
<td>$E_{6.83 + 30.32}$</td>
<td>$E_{6.83 + 151.6}$</td>
<td>$E_{6.83 + 303.2}$</td>
</tr>
<tr>
<td>$E_{22.80 + 101.23}$</td>
<td>$E_{22.80 + 506.2}$</td>
<td>$E_{22.80 + 1012.3}$</td>
</tr>
<tr>
<td>SBP /nmol L⁻¹</td>
<td>SBP /kPa</td>
<td>Q</td>
</tr>
<tr>
<td>0.47</td>
<td>-1.8</td>
<td>1.23</td>
</tr>
<tr>
<td>1.33</td>
<td>-2.6</td>
<td>2.38</td>
</tr>
<tr>
<td>3.86</td>
<td>2.4</td>
<td>4.58</td>
</tr>
<tr>
<td>10.82</td>
<td>3.1</td>
<td>11.78</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Acetaminophen (mg kg$^{-1}$) + Butorphanol (mg kg$^{-1}$)</th>
<th>Rate observed</th>
<th>Rate fitted $E_o \pm s_o$</th>
<th>Rate expected $E_o \pm s_o$</th>
<th>$Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.0 + 12.0 + 5.0</td>
<td>0.8</td>
<td>0.78 ± 0.05</td>
<td>0.52 ± 0.02</td>
<td>4.0*</td>
</tr>
<tr>
<td>32.0 + 9.6 + 4.0</td>
<td>0.6</td>
<td>0.62 ± 0.04</td>
<td>0.40 ± 0.02</td>
<td>3.1*</td>
</tr>
<tr>
<td>25.6 + 7.7 + 3.2</td>
<td>0.4</td>
<td>0.42 ± 0.04</td>
<td>0.29 ± 0.02</td>
<td>1.7*</td>
</tr>
<tr>
<td>20.5 + 6.1 + 2.6</td>
<td>0.3</td>
<td>0.25 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>0.6*</td>
</tr>
<tr>
<td>14.4 + 4.9 + 2.0</td>
<td>0.1</td>
<td>0.13 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>0.0*</td>
</tr>
</tbody>
</table>

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 for drug used alone for analysis should be kept in dose range in combination.

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