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Quantitative design of optimal analgesic combination of acetaminophen, caffeine, and butalbital[1]

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KEY WORDS combination drug therapy; drug interactions; drug synergism; drug dose-response relationship; analgesia; acetaminophen; butalbital; caffeine

ABSTRACT

AIM: To quantitatively seek an optimal analgesic combination of acetaminophen (Ace), butalbital (Bul), and caffeine (Caf), and to characterize the pharmacodynamics of interaction among the three drugs. **METHODS**: The models of acute inflammatory pain in carrageenin-injected rats were applied to measure the vocalization threshold to paw pressure. Six groups with different ratios and doses were set to seek an optimal combination of Ace, Caf, and Bul, analyzed by the weighted modification method. Based on the ratio and doses in the optimal combination, four continuous doses were set to analyze the interactions of therapeutic effects by the reflection method. The interaction of the acute toxicity was evaluated by the parameter method. **RESULTS**: According to the degree of importance to the combined analgesic effect, Ace > Caf > Bul; Ace showed a significant dose-response relationship, whereas in Caf and Bul, this relationship was not apparent. A new combination was obtained by the theoretical analysis and confirmed further by experimentation. Namely, at a ratio of 8.6:1:1.5 Ace + Caf + Bul (240 + 28 + 42 mg/kg, ig) was an optimal combination. Both Caf and Bul had a synergism to Ace, but Caf was a stronger synergist in contrast to Bul. Such synergism increased the therapeutic effects in the range of Ace 153.6 - 300 mg/kg combined with Caf 17.9 - 35 mg/kg and Bul 26.8 - 52.5 mg/kg (8.6:1:1.5). However, the dose of Ace + Caf + Bul at 300 + 35 + 52.5 mg/kg resulted in sedation in rats. The peak latency was approximately 1 h for all four continuous doses, but the peak amplitude was dose-related, and the duration of the therapeutic effect was less than 2 h. The acute toxicity of the three drugs in combination remained the same. **CONCLUSION**: Ace + Caf + Bul at a dose of (240 + 28 + 42) mg/kg (ig) results in an optimal combination. The therapeutic window of the combination is located in the range of Ace (153.6 - 240 mg/kg) + Caf (17.9 - 28 mg/kg) + Bul (26.8 - 42 mg/kg) (8.6:1:1.5). Caf has a stronger synergism with Ace than Bul.

INTRODUCTION

Combination of acetaminophen (Ace) with other analgesics or adjuvants is a common therapeutic strategy in an attempt to enhance its analgesic action, and to minimize its side effects and toxicity. Of all the analgesic adjuvants, caffeine (Caf) and butalbital (Bul) are the most frequently used as over-the-counter and prescription oral combinations. Although rationality for many such combinations, for example, aspirin and Caf, is readily apparent, other combinations have less apparent validity[1-4]. One of such combination is Ace combined with Caf and Bul. This understanding about rational combination drug therapy will advance only when there is more scientific information available on drug interaction. However, owing to the limitations of the quantitative method, there are few rigorous evaluations of more than two-drug combination like in Ace + Caf + Bul.

In order to obtain a feasible combination, a full quantitative evaluation includes (1) seeking an optimal combination with appropriate constituents, doses, and ratio; (2) the interaction of therapeutic effects for selecting a therapeutic window of the optimal combination; (3) the interaction of toxicity (or adverse effect) for safety. In addition, this evaluation may need an appropriate experi-

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mental method, animal model and species. In this study, some new evaluation methods were performed to fully evaluate the combined analgesic effect of Ace, Caf, and Bul. The weighted modification method[5] was applied to seek an optimal combination. Then based on the combination, the interactions of therapeutic effects were further analyzed by the reflection method[6] and the interactions of acute toxicity by the parameter method[7]. We used carrageenin-injected rats as an animal model of acute inflammatory pain.

MATERIALS AND METHODS

Drugs Ace powder was obtained from Jilin Pharmaceutical Factory (purity, 99.5 %), Bul powder from Tonghua Pharmaceutical Factory (purity, 99.1 %), and Caf powder from Sunan Pharmaceutical Factory.

Animals Kunming strain mice of either sex (weighing 18 – 20 g) were purchased from the Animal Center of Nanjing Medical University (Grade Ⅱ, Certificate № 97001), and male Wister rats (200 – 250 g) from Shanghai Research Institute of Biological Product (Grade Ⅱ, Certificate № 21 – 1).

Nociceptive behavioral measurements Before the experiments, animals were fasted for 12 h, with free access to water for the entire study. Carrageenin (1 % solution of lambda carrageenin in saline) was prepared 24 h before each experiment and injected in a volume of 0.2 mL sc into the right plantar hindpaw.

Using a pressure meter, the vocalization thresholds to paw pressure (TPP) was determined by applying increasing pressure to the right hindpaw until an audible squeak was elicited[8]. The cutoff value of 300 mmHg was used to avoid injury to the paw. This criterion was chosen according to pre-experimental observation. Thirty minutes after the carrageenin injection, the drug combinations were administered (ig). The TPP of each animal was measured before (TPP_0) and at different time (TPP_t) after the administration, and the increased analgesic effect rate at the time was expressed as $RTPP_t = (TPP_t - TPP_0)/TPP_0$.

Analgesic effects in the six combination groups The range of each drug from the minimal to the maximal safe dose (D_{max}) in combination was given in Tab 1 and determined by pre-experimental observation. Each range was divided into 6 doses, which were distributed into 6 groups evenly according to the weighted modification method[5]. The administration of the six groups ($n = 10$ in each group) was performed as Tab 1. For each animal, TPP was determined before and 1 h after the administration, and $RTPP_{1\,h}$ was recorded.

Tab 1. Doses in the six combination groups.

Groups	Ratios Ace:Caf:Bul	Ace/ mg·kg^{-1}	Caf/ mg·kg^{-1}	Bul/ mg·kg^{-1}
G1	6.87:1:2.87	79 (1)	11.5 (2)	33 (3)
G2	5.47:1:3.63	98 (2)	17.9 (4)	65 (6)
G3	4.39:1:0.96	123 (3)	28.0 (6)	27 (2)
G4	16.74:1:5.65	154 (4)	9.2 (1)	52 (5)
G5	13.43:1:1.47	192 (5)	14.3 (3)	21 (1)
G6	10.71:1:1.88	240 (6)	22.4 (5)	42 (4)
$\bar{x}$		148	17.2	40
D_{max}		240	28.0	65

Ordinal number of dose level is expressed in ( ).

Analgesic effects of Ace alone and Ace + Caf + Bul on the optimal ratio Based on the optimal combination with the ratio and the doses from the above experimentation, four continuous combination doses were selected to further observe the timed analgesic effects in rats. For each animal, the TPP was determined before and 0.5, 1, 2, and 4 h after the administration, and $RTPP_t$ of each time point was recorded. When Ace was used alone, the $RTPP_{1\,h}$ was only observed.

Acute toxicity of Ace + Caf + Bul on the optimal ratio Twenty groups of 10 mice were administered (ig) Ace, Bul, and Caf alone and in combinations, respectively. All doses were listed in Tab 4 (see Results) and the volume of administration (ig) was kept as 0.02 mL/g body weight. The mortality within 3 d was recorded in each group.

Data analysis and evaluation For optimizing combination, the weighted modification method[5] was used, and the data of dose (d)-effect (E) were fitted to the following equation:

$$E = E'_{max} \cdot \frac{b_1 d_1 + b_2 d_2 + b_3 d_3}{1 + b_1 d_1 + b_2 d_2 + b_3 d_3} \qquad (1)$$

where E'_{max} is the sum of the maximal mean of effect (E_{max}) and its deviation (s) in the six groups, d is a quotient of a dose divided by the mean dose, and b is a weighted coefficient which reflects the dose-effect relationship of a drug and also is a mark of its degree importance in the combinations.

The group with E_{max} was called $G_{E_{max}}$, in which the dose of each drug was labeled as its $D_{E_{max}}$. According to the magnitude of $|b|$ and its F-test, the doses in combination were optimized according to Tab 2.

Tab 2. Drug types and their doses optimized.

Type	$b(d_i)$	$b(d_id_j)$	P	Dose optimized
1	>0	–	<0.05	D_{max}
2	–	>0	<0.05	D_{max}
3	Any value	–	>0.05	$D_{E_{max}}$
4	<0	–	<0.05	Low dose or no
5	–	<0	<0.05	Low dose or no

d_id_j = mutual element between the drug of type 1 and other drug ($d_id_j = d_i \times d_j$). If a drug belongs to two types, its dose is optimized to a range like ($D_{E_{max}} - D_{max}$).

For quantitative data, the interactions of analgesic effects were evaluated by the reflection method[6]. The result was expressed as Q value. Briefly, $Q = (E_o - E_t)/L$, where $-1 < Q < 1$ indicated additivity, $Q \leqslant -1$ implied an antagonism, $Q \geqslant 1$ indicated synergism. E_o = an observed 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 value of a special field, the number of data points, and the experimental error. For qualitative data, the interactions of the acute toxicity were analyzed by the parameter method[7]. Q values were the same as above.

All analysis was completed on the computer and the curve was fitted with the CoDrug software.

RESULTS

Optimal combination Among the six groups, G6 was $G_{E_{max}}$ with a maximal effect of 1.88 (Tab 3). $E'_{max} = 1.88 + 0.43 = 2.31$. $D_{E_{max}}$ (mg/kg): Ace = 240, Caf = 22.4, and Bul = 42. All standardized doses (d) and their corresponding effects were fitted to Equation 1 to calculate the weighted coefficients (b) given as follows.

$$\hat{E} = 2.31 \cdot \frac{1.98d_1 - 0.11d_2 - 0.30d_3}{1 + 1.98d_1 - 0.11d_2 - 0.30d_3}$$

$$\hat{E} = 2.31 \cdot \frac{1.11d_1d_2 + 0.45d_1d_3}{1 + 1.11d_1d_2 - 2 + 0.45d_1d_3}$$

Applying the F-test, $b_1 = 1.98$, $P < 0.05$; $b_2 = -0.11$, $P > 0.05$; $b_3 = -0.30$, $P > 0.05$; $b_1(d_1d_2) = 1.11$, $P < 0.05$; $b_2(d_1d_3) = 0.45$, $P > 0.05$. According to the analysis, Ace (b_1) > Caf (b_2) > Bul (b_3) on the degree of importance to the combined analgesic effects. Ace showed a significant dose-response relationship, whereas in Caf and Bul, this relationship was not apparent; the synergism between Ace and Caf was more significant than that between Ace and Bul. So the dose of Ace was adjusted to D_{max} (240 mg/kg), the dose of Caf to D_{max} (28 mg/kg), and the dose of Bul to $D_{E_{max}}$ (42 mg/kg). Thus, an optimized combination was obtained as Ace 240 mg/kg + Caf 28 mg/kg + Bul 42 mg/kg, which did not exist in Tab 1.

All the drug combinations (Tab 4) yielded analgesic effects greatly than that of Ace alone (G3) ($P < 0.05$ or $P < 0.01$) in further experimentation. The difference between G2 and G1 was not statistically significant ($P > 0.05$ by t-test), but the effect of G2 was increased by 12.5 % – 15.8 % in comparison with the two-drug combinations (G4 and G5). The increase had a therapeutic significance. It indicated that Caf and Bul were synergistic to Ace; Ace + Caf + Bul (240 + 28 + 42 mg/kg, ig) was an optimal combination, and the optimal ratio of Ace:Caf:Bul was 8.6:1:1.5.

Interaction of the analgesic effects of Ace + Caf + Bul in the optimal ratio The $RTPP_{1h}$ of Ace (153.6, 192, 240, and 300 mg/kg) was compared with that of combinations in Fig 1. The difference in $RTPP_{1h}$ was not statistically significant ($P > 0.05$ by t-test) between Ace (153.6 mg/kg) and Ace + Caf +

Tab 3. Standardized doses of Ace, Caf, and Bul, and their mutual elements corresponding to the analgesic effects ($RTPP_{1h}$) in the six combination groups. $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* G6 by t-test.

Groups	Ace + Caf + Bul			Mutual elements		$RTPP_{1h}$
	d_1	d_2	d_3	d_1d_2	d_1d_3	
G1	0.534	0.667	0.825	0.347	0.454	0.62 ± 0.21^c
G2	0.663	1.039	1.625	0.673	1.110	1.10 ± 0.28^c
G3	0.832	1.626	0.675	1.320	0.578	1.32 ± 0.26^c
G4	1.042	0.534	1.300	0.539	1.396	1.4 ± 0.4^b
G5	1.300	0.830	0.525	1.057	0.702	1.6 ± 0.4
G6	1.625	1.301	1.050	2.060	1.757	1.9 ± 0.4

d_i = quotient of a dose divided by the $\bar{x}$ (see Tab 1). $d_1d_2 = d_1 \times d_2$. $d_1d_3 = d_1 \times d_3$.

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Tab 4. Comparison of the analgesic effects of the different combinations. n = 10 rats. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* G3 by t-test.

Groups	Ace + Caf + Bul mg·kg^{-1}			$RTPP_{1h}$
G1	240	22.4	42	1.9 ± 0.4^c
G2	240	28.0	42	2.0 ± 0.4^c
G3	240	–	–	1.4 ± 0.3
G4	240	–	42	1.7 ± 0.3^b
G5	240	28.0	–	1.8 ± 0.4^b

G1 = $G_{E_{max}}$; G2 = optimized combination

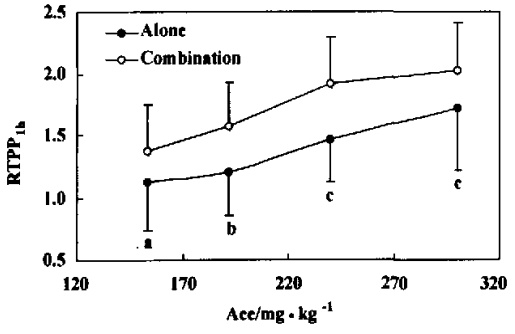

Fig 1. Dose-response ($RTPP_{1h}$) curves of Ace (153.6, 192, 240, 300 mg/kg, ig) used alone and in combinations of Ace + Caf + Bul (153.6 + 17.9 + 26.9, 192 + 22.4 + 33.6, 240 + 28 + 42, 300 + 35 + 52.5 mg/kg) at a ratio of 8.6:1:1.5. n = 10. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* combination.

Bul (153.6 + 17.9 + 26.9 mg/kg). However, the analgesic effect of the combination was increased by 25 %, indicating a therapeutic significance. With increasing doses at the same ratio, the analgesic effects in rest of the combinations were obviously enhanced in comparison with Ace administered alone ($P<0.05$ or $P<0.01$, Fig 1). However, the dose of Ace + Caf + Bul up to 300 + 35 + 52.5 mg/kg yielded obvious sedative side effects in rats.

The dose-related curves of $RTPP_{1h}$ presented a similar slope ($P>0.05$) in Ace 153.6 – 300 mg/kg used alone and in combination with Caf 17.9 – 35 mg/kg and Bul 26.9 – 52.5 mg/kg at a ratio of 8.6:1:1.5, demonstrating parallelism. However, the curve of the combination was observed to shift to the upper (Fig 1). It further suggested that Caf and Bul were synergistic to Ace.

On quantitative evaluation by the reflection method[6], the interaction exhibited a synergism in the above range (Fig 2). In the graph, the larger Q values

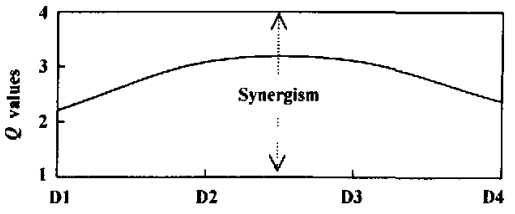

Fig 2. Interaction of the analgesic effects of Ace (A), Caf (C), and Bul (B) combinations in rats. D1 (mg/kg) = A 153.6 + C 17.9 + B 26.9; D2 (mg/kg) = A 192 + C 22.4 + B 33.6; D3 (mg/kg) = A 240 + C 28 + B 42; D4 (mg/kg) = A 300 + C 35 + B 52.5. *Equieffective value*: $w=0.05$. Q is the index for judging the nature of interaction by the reflection method[6]. $Q>1$ indicates synergism.

were located on the plateau of D2 – D3 (Fig 2). It revealed that the dose range between D2 and D3 had a stronger synergism than the lowest dose (D1) and the highest dose (D4). The range was Ace 192 – 240 mg/kg + Caf 22.4 – 28 mg/kg + Bul 3.6 – 42 mg/kg (8.6:1:1.5). The tendency of the synergism was towards additivity (Fig 2).

The timed dose-response curves of all four doses displayed that there was approximately 1 h latency for the maximal effect (the peak of $RTPP_t$), but total peak amplitude was dose-related. The duration of therapeutic response was less than 2 h, and the effect nearly disappeared 4 h after the administration (Fig 3).

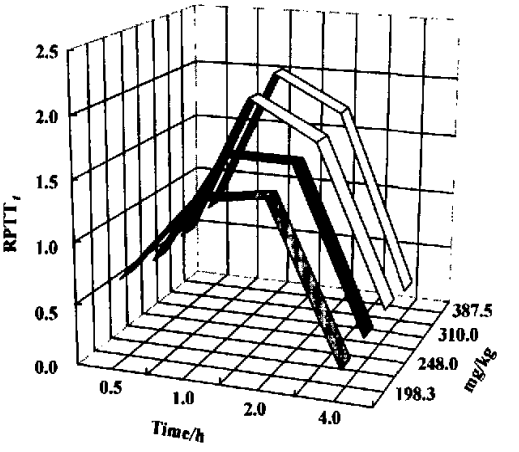

Fig 3. Timed dose-response curves of the different doses of Ace + Caf + Bul in the optimal ratio.

Acute toxicity interaction of Ace + Caf + Bul at the optimal ratio The mice mortalities within 3 d were compared in Ace, Caf, and Bul used alone, and in combination (Tab 5). On quantitative analysis by the parameter method[7], the acute toxicity interaction exhibited additivity in Ace (528.4 – 1290 mg/kg) + Caf (61.4 – 150 mg/kg) + Bul (92.2 – 225 mg/kg) (8.6∶1∶1.5). This indicated that the toxicity did not increase in the combinations. However, the trend was towards synergism (increasing toxicity) as observed on the graph (Fig 4).

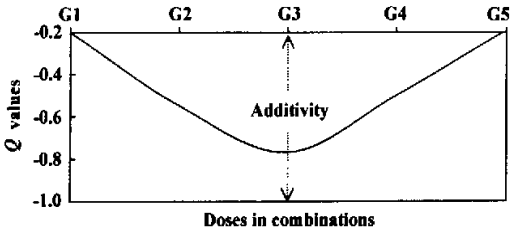

Fig 4. Acute toxicity interaction of the combination of Ace (A), Caf (C), and Bul (B) in mice. G1 (mg/kg) = A 528.4 + C 61.4 + B 92.2; G2 (mg/kg) = A 660.5 + C 76.8 + B 115.2; G3 (mg/kg) = A 825.6 + C 96 + B 144; G4 (mg/kg) = A 1032 + C 120 + B 180; G5 (mg/kg) = A 1290 + C 150 + B 225. Equieffective value: $w = 0.05$. Q is the index for judging the nature of interaction using the parameter method[7]. $-1 < Q < 1$ indicates additivity.

DISCUSSION

Combined drugs therapy is a common approach in the treatment of pain such as headache, toothache, and postoperative pain[7,8]. The design of the therapy should depend on the analytic method of drug interaction. The type of interaction such as synergism, antagonism or additivity can vary according to the drug ratios used and the level of therapeutic effect. Combination drug therapy is required to provide appropriate constituents, doses, and ratios. However, some traditional methods fail to find an optimal combination as was studied in this work, because a total of 18 dose levels of three drugs may form numerous combinations. However, the weighted modification method, utilizing just 6 groups, can provide many important cues to seek an optimal combination. It is noted that the optimal combination must be conformed further by experimentation. Based on an optimal combination, the interaction should be analyzed quantitatively as with the reflection method[6] and qualitatively as with the parameter method[7]. In carrageenin injection rats, the acute inflammatory pain, together with stimulus-evoked pain can be compared with some postoperative pains, toothache, and myalgia.

In our study, a new combination was obtained by theoretical analysis and further confirmed by experimentation. Namely, at 8.6∶1∶1.5 ratio Ace + Caf + Bul (240 + 28 + 42 mg/kg, ig) was an optimal combination for the animal models. With respect to the combined analgesic effect, Ace > Caf > Bul in the six combination groups, Ace showed a significant dose-response relationship, whereas in Caf and Bul, this relationship was not apparent. Both Caf and Bul were synergistic to Ace, but Caf had a stronger synergism as compared to Bul. Such synergism increased the therapeutic effects in the range of Ace (153.6 – 300 mg/kg) combined Caf (17.9 – 35 mg/kg) + Bul (26.8 – 52.5 mg/kg) (8.6∶1∶1.5 ratio). The peak effect latency was approximately 1 h for all four continuous doses, but the peak amplitude was dose-related, and the duration of therapeutic effect was less than 2 h. The acute toxicity was not increased in combination. Because the sedative side effect was found in the highest dose, the therapeutic window was located in the range of Ace (153.6 – 240 mg/kg) + Caf (17.9 – 28 mg/kg) + Bul (26.9 – 42 mg/kg). All these findings have also confirmed the availability of above methods of analysis.

Tab 5. Mortality within 3 d of Ace, Caf, and Bul used (ig) alone and in combination in mice. $n = 10$.

Ace		Caf		Bul		Combinations (8.6∶1∶1.5)					
/mg·kg^{-1}	Mortality	/mg·kg^{-1}	Mortality	/mg·kg^{-1}	Mortality	Ace	+	Caf	+	Bul	Mortality
1638	0.1	415	0.1	92	0.1	528.4		61.4		92.2	0.1
2048	0.2	460	0.2	102	0.3	660.5		76.8		115.2	0.3
2560	0.5	576	0.6	128	0.5	825.6		96		144	0.5
3200	0.8	720	0.8	160	0.8	1032		120		180	0.8
4000	1.0	900	1.0	200	1.0	1290		150		225	1.0

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All analyses were based on an analgesic effect and acute toxicity in our study, and it did not investigate the mechanism related to a pharmacokinetic or pharmacodynamic interaction. However, a pharmacodynamic interaction is more likely[9]. In addition, because Ace has limited effect on inflammation, anti-inflammatory effect did not seem to be related to analgesia in this study.

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醋氨酚、咖啡因和布他比妥联合镇痛组方的定量设计与优化[1]

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关键词 联合药物疗法; 药物相互作用; 药物协同作用; 药物剂量效应关系; 镇痛; 醋氨酚; 布他比妥; 咖啡因

目的: 寻找醋氨酚(Ace), 咖啡因(Caf)和布他比妥(Bul)联合镇痛作用的优化组方, 并定量分析三药相互作用特点. **方法**: 角叉菜致大鼠足爪急性炎症, 以痛阈下降率作为药效指标; 用权重配方法进行组方优选, 映射法分析药效相互作用, 参数法分析急性毒性的相互作用. **结果**: 在联合镇痛组方中的重要程度为 Ace > Caf > Bul. 联用中 Ace 呈现明显的量效关系, 而 Caf 和 Bul 则不甚明显. 理论分析获得一个新的优化组方, 并经确定性实验证实, 其 Ace + Caf + Bul 联用比例为 8.6:1:1.5, 剂量为(240 + 28 + 42) mg/kg (ig). Caf 和 Bul 对 Ace 均有协同作用, 而 Caf 更强. 同样比例, Ace (153.6 - 300 mg/kg)与 Caf (17.9 - 35 mg/kg)和 Bul (26.8 - 52.5 mg/kg)联用, 其镇痛效应明显增加. 剂量至(300 + 35 + 52.5) mg/kg, 对大鼠产生镇静作用. 上述剂量的药效达峰时间约为 1 h, 峰值具有剂量依赖性, 作用持续时间小于 2 h. 联用时急性毒性表现为相加性(即未增加). **结论**: Ace + Caf + Bul (240 + 28 + 42) mg/kg (ig)是一个优化组方; 该方的治疗窗为 Ace (153.6 - 240 mg/kg) + Caf (17.9 - 28 mg/kg) + Bul (26.8 - 42 mg/kg), 其联用比例为 8.6:1:1.5; Caf 对 Ace 的协同作用强于 Bul.

(责任编辑 朱倩蓉)