STUDIES ON COMBINATION DOSING (III)
ASPIRIN AND ETHENZAMIDE

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Abstract—In our studies with drug combinations, we searched for mixtures which
would enhance the effectiveness of the related active substances. Ethenzamide was
found to possess a specific suppressive effect on the gastric damage induced by aspirin.
Such effect could not be demonstrated in analgesic agents such as salicylamide, bucutin,
acetaminophen and phenacetin. The combination of aspirin with ethenzamide had
a potentiating effect on analgesic activity and reduced motor incoordination and loss
of righting reflex. We calculated the safety margins of various ratios of combinations
and concluded that the best was aspirin and ethenzamide at a ratio of 2:3.

The general principle in considering drug combinations containing two or more in-
gredients is to evaluate not only the enhancement of the main effect and/or the magnification
of the pharmacological spectrum of each ingredient but also the usefulness of the combi-
nation from the standpoint of total effect. We further propose that this principle should
include increasing the safety margin so that drug combinations have greater safety margins
than do the ingredients as single entities (1, 2, 3).

Aspirin although in wide use does induce gastrointestinal damage. As such an untoward
effect often limits extensive clinical application, we searched for a compound which would
enhance the usefulness of aspirin when used in combination with other agents.

MATERIALS AND METHODS

Aspirin, ethenzamide, salicylamide, bucutin, acetaminophen, phenacetin and five
mixtures of aspirin and ethenzamide were used. Such were prepared in accordance with
the ratios shown in Table 1. All orally administered test agents were suspended in 1%
gum arabic solution. SLC-ddY male mice weighing 24±2 g were used. For each dose
studied, a group of either 5 or 10 mice was employed in each experiment.

1. Gastric damages induced by aspirin combined with various mild analgesics

The method of Fukawa et al (4) was followed. The mice were deprived of solid food
but allowed free access to drinking water for 18 hr prior to experiments. The animals were
sacrificed by cervical dislocation 4 hr after administration of drugs. The stomachs were
rapidly exposed and fixed with 5% formalin for 3 min. Gastric damages were observed
under a stereoscopic microscope, and the length of erosive lesions (L.E.) was measured.
The cumulative frequency was obtained from frequency distribution of 10 mice by L.E.
and converted to the cumulative percentage. The cumulative percentage (cumulative frequency distribution) against L.E. was plotted on normal probability paper and the relative frequency of 50% on L.E. (L.E. [50]) was obtained on the paper. The L.E. [50] values were used for evaluating the drug-induced gastric damage.

2. Analgesic effect and gastric damage

Following the method of Fukawa et al. (5), the analgesic effect and gastric damage were observed in the same animals. The same preparative procedures were followed here. Analgesic effect was measured by the stretching method. The writhing and stretching responses were induced by administration of 0.1 ml/10 g of 0.6% acetic acid given i.p.. The test drugs were administered p.o. 30 min before injecting the acetic acid, after which the frequency of response was counted in each animal for 20 min. The analgesic activity was expressed as the ED50 value calculated by the Litchfield & Wilcoxon method (6). Evaluation of gastric damage was carried out as described in experiment 1.

3. Effect on motor coordination (inclined screen test)

Mice administered the test drug were placed on an inclined glass screen with a 25° angle at set intervals of time. Motor incoordination in mice was evaluated according to whether or not mice slid down the screen. Motor incoordination was expressed as a 50% side effective dose (SD50) calculated by the Litchfield & Wilcoxon method from the percentage of mice which slid down the screen.

4. Effect on righting reflex

Mice administered the test drug were observed for righting reflex at set intervals of time. Loss of righting reflex was expressed as a 50% side effective dose on righting reflex (RD50) calculated by the Litchfield & Wilcoxon method from the percentage of mice losing the reflex.

5. Acute toxicity

The LD50's of the mixtures (I~VII) were calculated by the Litchfield & Wilcoxon method from the mortality on the eighth day after administration of the mixtures.

### Table 1. Combination ratios

| Mixture | Combination ratio |
|---------|------------------|
|         | Aspirin : Ethenzamide |
| I (aspirin) | 1 : 0 |
| II      | 4 : 1 |
| III     | 3 : 2 |
| IV*     | 1 : 0.92 |
| V       | 2 : 3 |
| VI      | 1 : 4 |
| VII (ethenzamide) | 0 : 1 |

* Equimolecular mixture of aspirin and ethenzamide
RESULTS

1. Gastric damage induced by aspirin combined with various mild analgesics

Gastric damage following single doses of 100 and 200 mg/kg of aspirin were 13.0 and 27.0-33.0 at the L.E.[50], respectively (Table 2). Gastric damaging effect of aspirin was not significantly altered with combinations of salicylamide, bucetin, acetaminophen or phenacetin (Table 2). On the other hand, the administration of aspirin-ethenzamide combinations reduced markedly aspirin-induced gastric damage. Gastric damage induced by doses of 100 and 200 mg/kg of aspirin was drastically reduced with addition of more than 25 and 100 mg/kg of ethenzamide, respectively. Thus, the suppressive effect of ethenzamide on aspirin-induced gastric damage was obtained by combining ethenzamide with aspirin at the ratio of 1:2-1:4.

2. Analgesic effect and gastric damage

All test mixtures (I-VII) in dose ranges from 100 to 400 mg/kg progressively depressed the frequency of acetic acid-induced writhing syndrome with the increase in dosage. The ED50 values of these mixtures are shown in Table 3. The ED50 value of I (aspirin) was 310 mg/kg and the values of other mixtures decreased proportionally with increase in the

| TABLE 2. Gastric damage induced by the combination of aspirin and various mild analgesics in mice |
|---------------------------------------------|-------------------------------|------------------|
| Compound                                    | Exp. No. | Dose (mg/kg p.o.) | L.E.[50] |
| Aspirin                                     | 1        | 100              | 13.0     |
|                                             | 1        | 200              | 33.0     |
|                                             | 2        | 200              | 27.0     |
| Aspirin + Ethenzamide                       | 1        | 100+12.5         | 11.0     |
|                                             | 1        | 100+25           | 3.5      |
|                                             | 1        | 100+50           | 2.5      |
|                                             | 1        | 100+100          | 5.0      |
|                                             | 1        | 100+200          | 3.0      |
|                                             | 1        | 100+400          | 1.0      |
|                                             | 1        | 100+450          | 0        |
|                                             | 1        | 200+25           | 32.5     |
|                                             | 1        | 200+50           | 24.0     |
|                                             | 1        | 200+100          | 9.5      |
|                                             | 1        | 200+200          | 4.5      |
| Aspirin + Salicylamide                      | 2        | 200+50           | 32.5     |
|                                             | 2        | 200              | 25.0     |
|                                             | 2        | 200+200          | 23.0     |
| Aspirin + Bucetin                           | 2        | 200+100          | 30.0     |
|                                             | 2        | 200+400          | 24.0     |
| Aspirin + Acetaminophen                     | 2        | 200+200          | 28.0     |
| Aspirin - Phenacetin                        | 2        | 200+200          | 32.5     |

Each group included 10 mice.
dose of ethenzamide in the mixtures, mixture V showing the minimal value (140 mg/kg). The analgesic activity of mixture V was more than twice as potent as aspirin (I) and almost equal that of ethenzamide (VII).

Aspirin (I) in the dose range from 50 to 400 mg/kg induced gastric damage dose-dependently. The aspirin-induced gastric damage at 50 mg/kg and 400 mg/kg was 5.0 and 39.0 at the L.E. [50], respectively. The gastric damage was depressed significantly by ethenzamide mixed with aspirin (Fig. 1). Degree of gastric damage seen with mixtures II, III and IV was reduced markedly as compared with I (aspirin). The relative potencies of II, III and IV to I on the gastric damage calculated by the parallel line assay were 0.34

![Fig. 1 Dose response curves for aspirin, ethenzamide and five mixtures of aspirin and ethenzamide on gastric damage in mice](image)

**Table 3. Pharmacological effects of mixtures composed of various ratios of aspirin and ethenzamide**

| Mixture | Analgesic effect | Gastric damage | Inclined screen test | Loss of righting reflex | Acute toxicity |
|---------|------------------|----------------|----------------------|------------------------|---------------|
|         | ED50 (mg/kg)     | NED (mg/kg)   | SD50 (mg/kg)         | RD50 (mg/kg)           | LD50 (mg/kg)  |
| I       | 310 (207~465)    | 48             | 850 (785~930)        | 1,170 (1,140~1,220)    | 1,170 (1,140~1,220) |
| II      | 320 (221~464)    | 115            | 1175 (1,085~1,290)   | 1,440 (1,370~1,520)    | 1,440 (1,370~1,520) |
| III     | 215 (157~295)    | 110            | 980 (880~1,090)      | 1,350 (1,280~1,420)    | 1,410 (1,320~1,510) |
| IV      | 150 (116~234)    | 150            | 850 (810~890)        | 1,290 (1,290~1,390)    | 1,410          |
| V       | 140 (112~175)    | >400           | 930 (855~1,010)      | 1,350 (1,260~1,445)    | 2,240 (2,060~2,440) |
| VI      | 160 (107~240)    | 200            | 675 (615~744)        | 1,230 (1,150~1,350)    | 2,040          |
| VII     | 165 (107~254)    | >400           | 700 (570~860)        | 1,260 (1,150~1,370)    | 2,690          |

Figures in parentheses are the 95th confidence limits.
NED (non-effective dose) values obtained from the dose response curves in Fig. 1
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(0.26~0.55), 0.27 (0.22~0.32) and 0.18 (0.14~0.23), respectively. Mixtures V, VI and VII in the dose range from 100 to 400 mg/kg induced little gastric damage.

The L.E.'s of one hundred mice given 0.1 ml/10 g of 1% gum arabic solution p.o. were examined and cumulative frequency distribution of the mice by L.E. was plotted on normal probability paper. The normal range of gastric damage has been defined to be the point which shows a relative frequency of 95% obtained by testing 100 normal mice. The value of 3.0 corresponding to the point was obtained at the L.E. (4).

From the above results, we propose that the dose corresponding to L.E. [50]=3.0 should be used as the NED (non-effective dose) in evaluating the significance of drug-induced gastric damage (4). The NED's of all tested mixtures (I~VII) were obtained from the dose response curves in Fig. 1. The NED of aspirin (I) was the lowest at 48 mg/kg. The NED's of mixtures V and VII could not be obtained, as the L.E. [50]'s were less than 3.0 (Table 3).

3. Effect on motor coordination (inclined screen test)

Aspirin (I) induced an excitation and clonic convulsion, while ethenzamide dosing resulted in a sedation and muscle relaxation. Slippage down the inclined screen in mice was often due to clonic convulsion or muscle relaxation. The administration of 630 mg/kg of I induced clonic convulsion in one out of five animals. The SD50 value of I was 850 mg/kg (Table 3). Mixtures II, III and IV also produced clonic convulsion at 1,000, 795 and 1,000 mg/kg, respectively.

On the other hand, mixtures V, VI and VII induced only muscle relaxation. The SD50 value of VII was 700 mg/kg. The combination of I with VII showed a tendency towards antagonism in the effect on motor incoordination. The SD50 values of II, III and V were larger than I and VII (Table 3).

4. Effect on righting reflex

In the animals given aspirin (I) the loss of righting reflex was not seen until death as the result of administration of high doses. Thus, the RD50 values of I, II and III containing high percentages of aspirin were similar to the LD50 values, respectively (Table 3). By contrast, increases in the percentage of ethenzamide in aspirin-ethenzamide mixtures resulted in a loss of righting reflex in mice, and the difference between the RD50 and the LD50 values increased proportionally. The RD50 values of mixtures IV, V, VI and VII were much the same (Table 3).

5. Acute toxicity

The LD50 of aspirin (I) was 1,170 mg/kg, while that of ethenzamide (VII) was 2,690 mg/kg. The LD50 values of other mixtures (II~VI) occupied values between those of I and VII and showed an additive effect (Table 3). Mixtures containing a high percentage of ethenzamide were less toxic than those with a high percentage of aspirin.

DISCUSSION

Ethenzamide had a specific suppressive effect on the gastric damage induced by aspirin.
Aspirin-induced gastric damage was reduced significantly by the addition of ethenazamide at the ratio of 1:2~1:4 of ethenazamide to aspirin. This effect was not found in asilicylamide, bucutin, acetaminophen and phenacetin.

We have already reported that when mice were used as experimental animals, a reliable evaluation of drug-induced gastric damage could be made. Davenport (7) suggested that gastric mucosal damage after aspirin dosing in dogs was dependent on the pH of the solution and that aspirin in neutral solution (citrate or Tris buffer, above pH 6) did not break the mucosa.

In our experiments on mice, the pH of aspirin (I) and ethenzamide (VII) in gum arabic solution was 2.9 and 5.4, respectively. Since the mixtures (II~VI) of I and VII were pH 2.9, it is unlikely that the prevention of gastric damage was due to neutralization of the solution of aspirin with the addition of ethenazamide. Gastric damage induced by mixture IV, an equimolecular mixture of aspirin and ethenzamide, was slight. Furthermore, on the assumption that an equimolar complex was formed in the stomach, the amount of free aspirin was calculated by subtracting the molar amount of ethenzamide from that of aspirin in Table 2. A dose response curve, which was obtained from plotting L.E. [50] against the amount of free aspirin, was in accord with that of aspirin in Fig. 1 (Fig. 2). In light of our observations, it appears that the interaction between aspirin and ethenazamide on gastric damage is related to the equimolecular nature of the mixture and that the amount of free aspirin in an aspirin-ethenazamide mixture has a direct relationship to the gastric damage induced.

The combination of aspirin and ethenazamide potentiated the inhibitory effect on the
acetic acid-induced writhing syndrome. This was particularly marked in mixture V. Furthermore, mixture V induced little gastric damage.

Anti-convulsive and muscle relaxing effects of ethenzamide were described by Matsumura (8) and Bornmann and Opitz (9). We also found that ethenzamide antagonized clonic convulsion induced by aspirin, while the exciting effect at high dosages of aspirin antagonized the sedative and muscle relaxing effects of ethenzamide. Thus, the combination of aspirin and ethenzamide reduced motor incoordination and loss of righting reflex. The antagonistic effect by combining the two drugs was obtained in mixtures II, III and V. In acute toxicity, the combinations resulted in an additive effect. The maximum LD50 value in mixtures (II~VI) was seen with mixture V, the toxicity being half that of aspirin and almost equal to that of ethenzamide. These results suggested that the best combination of aspirin and ethenzamide was mixture V in which the ratio of aspirin to ethenzamide was 2:3.

We also examined safety margins of aspirin and ethenzamide combinations, and the most effective for clinical use was determined. The safety margins were calculated by dividing the values of side effect doses (NED, SD50 and RD50) and LD50 by the ED50. Among the mixtures (I~VII), the maximum safety margin was obtained in mixture V (Fig. 3). These results also suggested that mixture V reduced to a minimum the adverse reaction of its ingredients.

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