

Anesthesiology
1996; 85:1403-12
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Interaction of Morphine and Clonidine on Gastrointestinal Transit in Mice

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Background: Combinations of drugs are frequently used to achieve effective analgesia while minimizing side effects. Although the analgesic effects of morphine and clonidine seem to be synergistic, few studies have investigated other effects. Their role in inhibiting gastrointestinal transit was evaluated using different methods of analysis.

Methods: Percentage inhibition of transit induced by morphine, clonidine, or their combination was measured in mice that had been given an intragastric charcoal meal. Dose-response curves were obtained for each drug individually; for morphine:clonidine at 1:3, 1:1, and 1:0.33 ratios; and for morphine in the presence of 0.0138 mg/kg clonidine. The interaction was evaluated by isobolograms, combination indexes, and fixed-dose analysis.

Results: Each drug and their combinations inhibited transit in a dose-related manner. Combinations of morphine and clonidine produced interaction that depended on the ratio and level of response. The interaction analyzed by isobolograms and combination indexes showed that combinations in 1:1 and 1:3 proportions were synergistic at the median effective doses or less and were antagonistic at larger doses. Fixed-dose analysis using different ratios showed similar results. The effects of the combination (median effective dose at 1:1 ratio) were antagonized by efaroan but not by naloxone, suggesting a predominant role of alpha-2-mediated effects.

Conclusions: Investigations into drug interactions should include several levels of response and combinations at different ratios. Isobolographic analysis permits the statistical evaluation of results without making assumptions about mechanisms of action of the drugs or their interactions. In this study, the combination of morphine and clonidine should produce

synergy, antagonism, or no interaction depending on the relative doses and the level of effect. (Key words: Alpha-2 adrenergic agonists. Clonidine. Constipation. Drug combinations. Drug interactions. Gastrointestinal transit. Morphine. Opioids.)

COMBINATIONS of drugs are frequently used in clinical practice to achieve effective analgesia while minimizing side effects. Opioids have long been used as analgesics, but clonidine (CL), an alpha-2 partial agonist, has only recently been introduced for this purpose in humans¹⁻³; however, its combination with morphine (MS) seems to have clinical advantages because of their interaction in relieving pain.⁴⁻⁶ Each type of drug produces various but similar side effects, including sedation, cardiovascular and respiratory depression, and constipation. Although recent studies show that the analgesic effects of these drugs appear to be synergistic in different animal models,⁷⁻¹⁰ few studies have investigated other effects of their interaction. One aspect that has been carefully evaluated is their possible interaction in producing respiratory depression. The administration of clonidine failed to alter the respiratory depression induced by morphine in humans,¹¹ although this aspect is controversial. In different animal species (rat, mice), each drug inhibits gastrointestinal transit (GIT) and results in constipation.¹²⁻¹⁴ From a clinical point of view, this effect could assume great importance both during acute and chronic administration; thus, opioid-induced ileus in the postoperative period, and constipation after chronic use, present medical and financial implications.

Since the interaction of MS and CL on antinociception is synergistic in rodents, but no interaction has been demonstrated for other actions, we designed a series of experiments to document their combined effects on constipation. Our working hypotheses was that the effects of the combination were simply additive (zero interaction); an interaction would be present if the observed effects (obtained experimentally) were significantly different from expected, that is if they were more or less than additive. In order to quantify the type of

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Received from the Department of Anesthesiology, Hospital Universitario del Mar, IMIM, Barcelona, Spain. Submitted for publication February 21, 1996. Accepted for publication July 29, 1996. Supported by grants from Fondo de Investigaciones Sanitarias, 92/0017-01 and 94/1380, Madrid, Spain. Presented at the ESA Annual Congress, London, England, June 1-5, 1996.

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interaction, several methods of analysis could be used. However, due to the different mechanisms of action of MS and CL, the most reliable would be those based on the evaluation the dose-response effects of each drug individually and their combination. Since the type of interaction can vary according to the drug ratios and the level of effect,¹⁵ we utilized various MS:CL combinations (1:1, 1:3, 1:0.3, and so on) and estimated the type of interaction at several levels of effect (20, 50, 80%). In addition, it has been speculated that different methods of analysis applied to the same data could produce different results, and thus we attempted to quantify the interaction of MS and CL on constipation by using more than one approach.¹⁵

This study had two purposes: to evaluate the nature of the interaction of morphine and clonidine on GIT, and to explore the utility and complexity of different methods of analysis. Thus we determined the interaction of morphine and clonidine on GIT using isobolograms, combination indexes, and fixed-dose analysis. We assessed the results from each of these methods and their utility.

Materials and Methods

Estimating Gastrointestinal Transit

The study protocol was approved by the local Committee of Animal Use and Care. Experiments were performed on adult male Swiss CD-1 mice, weighing 20 to 25 g. Animals were housed under 12-h light and 12-h dark conditions in a room with controlled temperature (22°C) and humidity (66%). Animals had free access to food and water and were allowed to become acclimated to their housing conditions for at least 1 week before the study. All experiments were conducted between 9:00 A.M. and 2:00 P.M.

Before the experiments, animals were fasted for 18 h, although they had free access to water for the entire study. Gastrointestinal transit was measured according to procedures used in our previous studies.^{14,16} Briefly, mice were given an intragastric meal consisting of 0.25 ml of a suspension of charcoal comprised of 10% vegetable charcoal in 5% gum acacia (Sigma Chemical Co., St. Louis, MO). Animals were killed 20 min after being given the charcoal meal. The stomach and small intestine were separated from the omentum to avoid stretching. The length of the intestine from the pyloric sphincter to the ileocecal junction and the distance traveled by the charcoal meal were measured and recorded. In

saline-treated mice (controls), mean GIT was $48.8 \pm 0.9\%$ ($n = 20$); the variability of the test was of 8.2% when the coefficient of variability was estimated according to the equation coefficient of variability = $SD/\text{mean} \times 100$.

The inhibitory effects of drugs on GIT are expressed as a percentage of inhibition of the transit in a drug-treated animal when compared with the mean transit obtained in a group of saline-treated mice. Thus

% inhibition

$$= [(\text{saline GIT} - \text{test GIT})/(\text{saline GIT})] \times 100$$

Experiments Performed

Several experiments were done. First, dose-response curves for morphine and clonidine administered subcutaneously were obtained and their median effective doses (ED_{50} s) were calculated. This was defined as the dose of a drug, or a combination of drugs, that produces a 50% inhibition of GIT. Similarly, the ED_{20} and ED_{80} correspond to the doses that produce 20% and 80% inhibition, respectively.

Second, similar curves were obtained for combinations of morphine and clonidine. The actual doses used to obtain each curve were combinations of morphine and clonidine in which the quantities of each drug were kept at the same ratio as their individual ED_{50} s. In these experiments, three combinations were used: 1:1, 1:0.33, and 1:3 morphine-to-clonidine mixtures. Thus the proportions used in the study are multiples of the ED_{50} s of each drug and do not represent actual weights (in milligrams). In both series of experiments, the potencies were calculated according to the method of Tallarida and Murray.¹⁷ The reversibility of the effects (ED_{50} s) produced by each drug individually and their combination in a 1:1 ratio was evaluated after administering naloxone (a mixed opioid antagonist) and efaranxan (α -2-imidazolin-1 antagonist).¹⁸

Third, experiments were done in which a complete dose-response curve to morphine was obtained in the presence of a fixed, single dose of clonidine.¹⁹⁻²¹

Data Analysis

The results are expressed as percentages of GIT inhibition. Statistical calculations were performed as described by Tallarida and Murray.¹⁷ Effective doses (ED) at 20%, 50%, and 80% \pm SEM were determined by linear regression analysis of dose-response relations based on at least five different doses and ten or more mice per

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dose. The reversibility of the effects of morphine, clonidine, and their combination (1:1 ratio) was assessed by one-way analysis of variance. All data points shown are mean values \pm SEM, and in the figures vertical bars represent the SEM.

To evaluate the interaction of morphine and clonidine, we used three methods of analysis: isobolographic analysis, combination indexes, and fixed-dose analysis; in addition, we analyzed the results by a two-way analysis of variance, which is a controversial method to establish drug interactions.

Construction of Isoboles. Isoboles are graphic representations of equally effective doses of two (or more) agents. The dose of each drug that produces a given level of response (20%, 50%, 60%, and 80%) are plotted on the axes of the graph. A diagonal line is drawn to join the isoeffective doses on the axis. Doses of drug combinations producing the same effect are then plotted. Points falling on the diagonal line represent zero interaction (additivity), while those located above and below are antagonistic and synergistic, respectively. Mean and SEM were calculated for all the doses plotted, and points were considered to differ significantly from additivity if their SEMs did not overlap. The diagonal noninteraction line is described by the equation $da/Da + db/Db = 1$. The solution to the equation is called the interaction index; if it differs from 1, an interaction is present: either synergy (index < 1) or antagonism (index > 1).^{22,23}

Multiple-drug Effect Analysis. For this procedure the same dose-response curves were used. With the curves obtained from the individual agents, a calculation was made (using Hill coefficients and ED_{50} s) of the doses that, if combined, would produce a particular degree of inhibition under the assumption that the drugs were merely additive. Actual ED_{50} s were obtained from the dose-response curves of the combinations of drugs, and these doses were compared with those previously calculated. The ratio of the actual overexpected doses is the combination index. A combination index of 1 indicates no interaction (additivity), and combination indexes less than or greater than 1 indicate synergy or antagonism, respectively. Combination indexes were calculated for each of several degrees of inhibition. The resulting graph shows the nature of the interaction at different levels of inhibitory response. This procedure was repeated for each of the proportions (morphine:clonidine) previously noted. These calculations were performed using the computerized method of Chou and Chou.²⁴

Fixed-dose Analysis. Fixed-dose experiments compare a dose response to morphine alone with a dose response to morphine in the presence of a fixed (small) dose of clonidine (0.013 mg/kg). When the dose-response curve of the combination is shifted to the left, a more-than-additive interaction is presumed, whereas if it is shifted to the right a subadditive interaction could be present. To demonstrate interaction, the sum of the effects produced by each agent alone (expected effect) must be significantly different from the observed effect of the combination. If the observed effects are greater or smaller than expected, synergy and antagonism are present.¹⁹

Analysis of Variance. Analysis of variance determines the presence of statistical differences between two treatment groups; in this investigation, the groups compared were the dose-response curve of morphine alone and of morphine in the presence of 0.013 mg/kg clonidine. Using two-factor analysis of variance, the effect of the treatment and dose and their interaction were determined.²¹ In this calculation, a significant effect of the treatment indicates a statistical difference between the two groups of study; a significant effect of the dose indicates that the observed responses vary significantly in a dose-related manner, and a significant interaction shows that the differences between the treatment groups depend of the dose administered. If all terms (treatment, dose, and interaction) are significantly different, the analysis indicates that the effects of the combination are different from additivity, whereas if the interaction is not statistically significant the effects are considered additive. To identify the type of interaction, the dose-response curves obtained with each treatment are graphically represented. Synergy and antagonism are suggested by divergent curves shifted to the left and right, respectively. However, this method is not applicable when dose-response curves are parallel, because it cannot differentiate between parallel-additive and parallel-synergistic or antagonistic reactions.^{20,25}

Drugs

Drugs used in the study were obtained from several sources: morphine hydrochloride from Alcaiber S.A., Madrid, Spain; clonidine hydrochloride from Research Biochemicals, Wayland, MA; naloxone hydrochloride from Sigma Chemical Co.; and efaroan hydrochloride from Research Biochemicals. All drugs and their combinations were prepared in pyrogen-free water just before use and administered in a final volume of 10 ml/kg. Agonists were injected subcutaneously at the nape of

Table 1. Potency of Morphine (MS) and Clonidine (CL) at Different Levels of Effect

	ED ₂₀ (mg · kg ⁻¹)	ED ₅₀ (mg · kg ⁻¹)	ED ₈₀ (mg · kg ⁻¹)
Morphine	0.317 ± 0.134	1.61 ± 0.48	8.16 ± 2.31
Clonidine	0.021 ± 0.015	0.101 ± 0.006	0.50 ± 0.32
Ratio of MS/CL	15.1	16.1	16.3

ED values were obtained from the log dose-response curves of MS and CL, and represent the mean ± SEM.

the neck 30 min before the administration of charcoal, and antagonists were given intraperitoneally 15 min before the marker.

Results

Dose-Response Curves to Morphine and Clonidine

Morphine and clonidine produced dose-related inhibition of GIT, with correlation coefficients of 0.98 and 0.99, respectively. Statistical analysis of the curves revealed that their slopes were not significantly different, demonstrating parallelism (42.5 ± 7 and 44.5 ± 4.8 for morphine and clonidine, respectively). To compare the potencies of each drug at different levels of effect, effective doses at 20%, 50%, and 80% response were calculated (table 1). The results show that at all levels of effect, clonidine was approximately 15 times more potent than morphine in inhibiting GIT.

Dose-Response Curves to Morphine and Clonidine at Fixed Ratios

Dose-response curves were obtained for combinations of morphine and clonidine in three proportions. These were chosen to evaluate the type of interaction when (1) the proportion of the drugs was the same (*i.e.*, ED₅₀s of each drug at a 1:1 mixture), (2) there was a surplus of morphine (ED₅₀s in a ratio of 1:0.33), and (3) there was a similar surplus of clonidine (ED₅₀s in a ratio of 1:3). Thus the proportions used in the study are multiples of the ED₅₀s of each drug and do not represent actual weights (in milligrams). In constructing the curves, each point was a mixture of morphine and clonidine fixed at the previously noted proportions. Figure 1 shows linear log dose-response curves for the three combinations of morphine and clonidine. The curves obtained for 1:3 and 1:0.33 mixtures were linear between 20% and 80% of the maximal

response. When the drugs were used in a 1:1 proportion, the dose-response curve showed a plateau at approximately 60% effect. Thus with this particular mixture a higher level of response was unattainable. From these curves we calculated the actual amounts of morphine and clonidine (in each of the combinations) that produced a 20%, 50%, or 80% response (values displayed in table 2). In determining the ED₂₀ and ED₅₀ of the 1:1 proportion, data points greater than 1.28 mg/kg (plateau; fig. 1) were not included because they skewed the calculations. The columns in table 2 represent particular levels of response, and each pair of doses of morphine to clonidine within a column produced the same effect. These values were used to evaluate the type of interaction by the construction of isobolograms

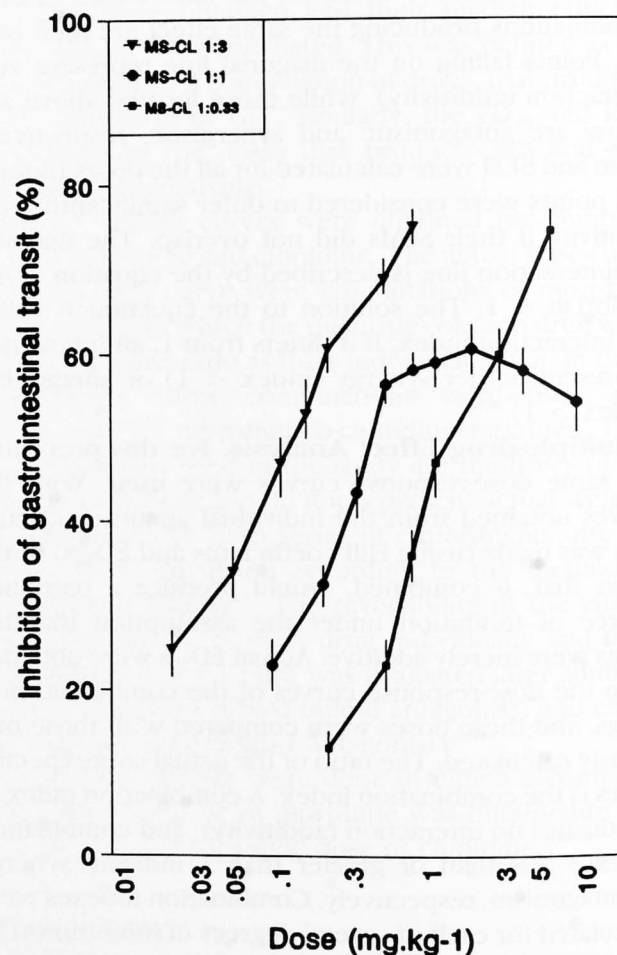


Fig. 1. Log dose-response curves of morphine (MS) and clonidine (CL) combined at proportions of 1:3 (triangles), 1:1 (circles), and 1:0.33 (squares). The abscissa represents the sum of the doses of morphine and clonidine (measured in milligrams per kilogram), and the ordinate the percentage inhibition of gastrointestinal transit. Each point is the mean ± SEM of ten or more mice.

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Table 2. Potency of Morphine (MS) and Clonidine (CL) in Different Proportions and at Three Levels of Response

Proportion	MS/CL	ED ₂₀ (mg · kg ⁻¹)	ED ₅₀ (mg · kg ⁻¹)	ED ₈₀ (mg · kg ⁻¹)
1:3	MS	0.0093 ± 0.002	0.063 ± 0.006	0.77 ± 0.35
	CL	0.0025 ± 0.0001	0.059 ± 0.004	0.68 ± 0.30
1:1	MS	0.1028 ± 0.02	0.44 ± 0.02	—
	CL	0.0056 ± 0.001	0.028 ± 0.003	—
1:0.33	MS	0.391 ± 0.06	1.72 ± 0.26	7.61 ± 2.90
	CL	0.0016 ± 0.0002	0.007 ± 0.001	0.0321 ± 0.005

Values were obtained from the log dose-response curves depicted in fig. 1. Each pair of values shows the individual doses of MS and CL that must be combined to produce the indicated level of response. The ED₈₀ of the 1:1 proportion was unobtainable.

and by multiple-drug-effect analysis (combination indexes).

Isobolographic Analysis of the Interaction of Morphine and Clonidine

Isoboles were constructed for 20%, 50%, 60%, and 80% levels of response. Figures 2 and 3 show a series of isoboles with the ordinate and abscissae representing the doses of clonidine and morphine, respectively. In all four graphs, the zero interaction line connects the doses of each agent that produced a given level of response; points located at the right and left of the zero interaction line show antagonism and synergy, respectively. In figure 2, the concave-up lines show the isoboles at 20% and 50% responses. Figure 3 displays isoboles for 60% and 80% levels of response; we plotted the ED₈₀s obtained from the dose-response curves of the 1:3 and 1:0.33 mixtures (fig. 1). However, with the 1:1 mixture, an 80% effect was unattainable and thus, for this specific combination, we represented the maximal effect obtained with the combination (ED₆₀; that is, the response to the highest total dose on the plateau). The results show that at high levels of response (60% and 80% of maximum effect), the interaction is antagonistic when drugs are combined in 1:1 and 1:3 ratios.

In table 3 we present a summary of the results of the isobolographic analysis of the combination of morphine and clonidine. The table shows that when morphine is combined with a small dose of clonidine (as in the 1:0.33 proportion), the drugs do not interact and their effects are additive. In contrast, combining morphine with larger doses of clonidine (1:3 and 1:1 proportions) produces an interaction that depends on the level of effect. Thus, at responses that are equal or less than half maximal (ED₂₀ to ED₅₀), the interaction is synergistic, whereas at high levels of response (more than ED₅₀) the interaction is antagonistic (fig. 3). In table 3, interaction

indexes were calculated according to the noninteraction inequality (see Materials and Methods).

Multiple-drug-effect Analysis (Combination Indexes) of Morphine and Clonidine

Figure 4 shows combination indexes (an indication of the nature of the interaction) at different degrees of inhibition. The combination indexes for levels of inhibition that were less than 20% or more than 80% are extrapolations from actual experimental data. When morphine and clonidine were combined in a 1:0.33 proportion, the interaction was antagonistic up to 60% inhibition, at which point it became synergistic. Combinations of drugs in a 1:3 proportion showed synergism at 75% or less effect and antagonism thereafter. Finally, in the 1:1 mixture of the interaction between 20% and 60%, inhibition is synergistic. Points greater than 60% effect are not shown because these levels of response were unattainable. Table 4 summarizes the results showing the type of interaction at 20%, 50%, and 80% inhibition. Combination indexes were generated according to the method of Chou and Chou.²⁴

Dose-Response Curve of Morphine in the Presence of a Fixed Dose of Clonidine

Figure 5 shows log dose-response curves of morphine alone and in the presence of 0.013 mg/kg clonidine. Clonidine induced a shift to the left of the dose-response curve to morphine at doses of morphine ranging from 0.16 to 0.81 mg/kg. However, at large doses of morphine (1.8 to 9.1 mg/kg), the presence of clonidine induced a plateau in the curve.

According to the fixed-dose analysis (see Materials and Methods), synergy is present if the dose-response curve of the combination is displaced to the left, and if the sum of the observed effects of each agent individually (at each point tested) is smaller than the effect of the

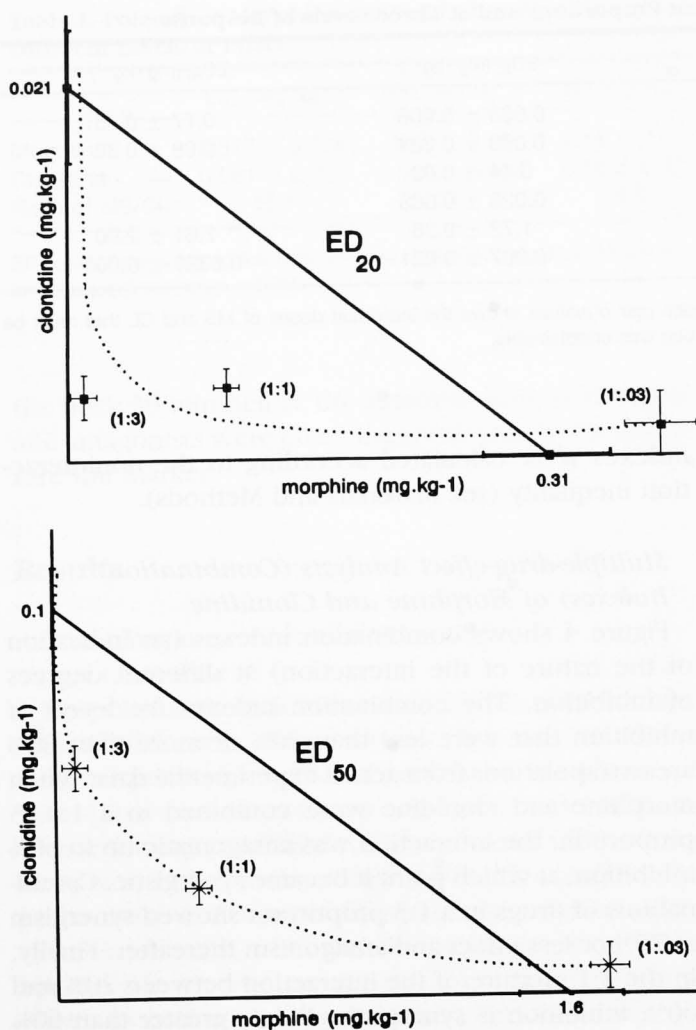


Fig. 2. Isobolographic representation of the interaction of morphine and clonidine at 20% and 50% levels of response. In both graphs, the axis indicate the mean doses (\pm SEM) of morphine (abscissa) and clonidine (ordinates) that produced 20% or 50% of maximal response when administered separately. Bold diagonal lines connect equieffective doses of each drug alone and designate additivity (no interaction). All points in the graphs were obtained by plotting each of the paired values \pm SEM found in table 2 (morphine:clonidine in different proportions), and concave-up lines are the isoboles of morphine and clonidine at the indicated levels of response.

combination. Conversely antagonism is manifested in the graph by a curve negatively displaced, where increasing doses of morphine in the presence of clonidine produce less effect than morphine alone. In our experiments, a dose of 0.013 mg/kg clonidine produced a $6.1 \pm 1.5\%$ inhibition of GIT. When the effect produced by clonidine alone (6%) was added to the effect produced by each dose of morphine alone up to 0.81 mg/kg, the observed effects of the combinations were always

greater than expected. However, at larger doses of morphine (between 3 and 10 mg/kg), the observed effects were smaller than expected, demonstrating antagonism.

Because the curves obtained in these experiments (fig. 5) were not parallel, an analysis of variance could be used to document an interaction. Because of the plateau obtained with the combination, each segment of the curve was analyzed separately by two-way analysis of variance. At lower doses, the analysis showed

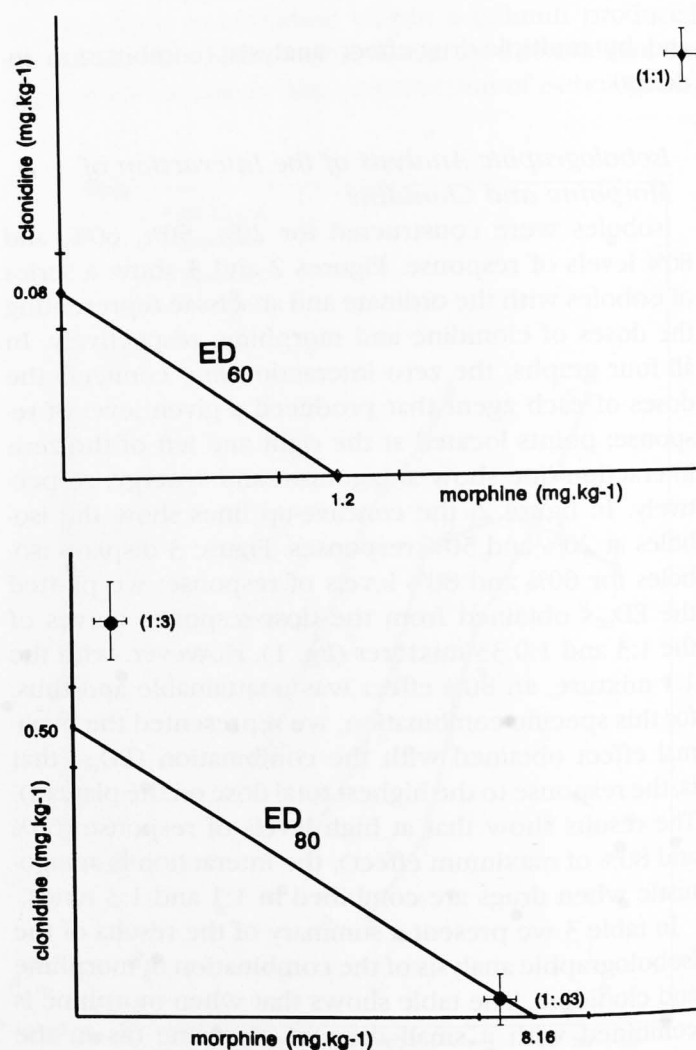


Fig. 3. Isobolographic representation of the interaction of morphine and clonidine at 60% and 80% levels of response. In both graphs, the axis indicates the mean doses (\pm SEM) of morphine (abscissa) and clonidine (ordinates) that individually produced 60% or 80% of maximal response. Diagonal lines connect equieffective doses of each drug alone and designate additivity. All points were obtained by plotting the values \pm SEM from figure 1 (ED_{60}) and table 2 (ED_{80}). At both levels of effect, morphine and clonidine combined in 1:1 and 1:3 ratios showed antagonism.

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Table 3. Summary of the Results of the Isobolographic Analysis of the Combination of Morphine (MS) and Clonidine (CL), Showing the Nature of Their Interaction

Proportion MS/CL	ED ₂₀	ED ₅₀	ED ₈₀
1:3	Synergy (0.15)	Synergy (0.63)	Antagonism (1.45)
1:1	Synergy (0.57)	Synergy (0.55)	NA
1:0.33	Additive (1.31)	Additive (1.10)	Additive (0.99)

NA = not available (see fig. 3). Synergy and antagonism represent statistically significant deviations from additivity with a $P < 0.05$. Values in parentheses show interaction indexes.

Table 4. Summary of the Results of the Multiple Drug Effect Analysis Using Combination Indexes According to the Method of Chou and Chou²⁴

Proportion MS/CL	ED ₂₀	ED ₅₀	ED ₈₀
1:3	Synergy (0.30)	Synergy (0.59)	Antagonism (1.13)
1:1	Synergy (0.30)	Synergy (0.66)	NA
1:0.33	Antagonism (1.45)	Antagonism (1.13)	Synergy (0.89)

MS = morphine; CL = clonidine; NA = not available. The method of calculation does not permit statistical analysis. Numbers in parentheses are the combination indexes generated by the method of Chou and Chou.²⁴ Values above and below 1 indicate antagonism and synergy, respectively.

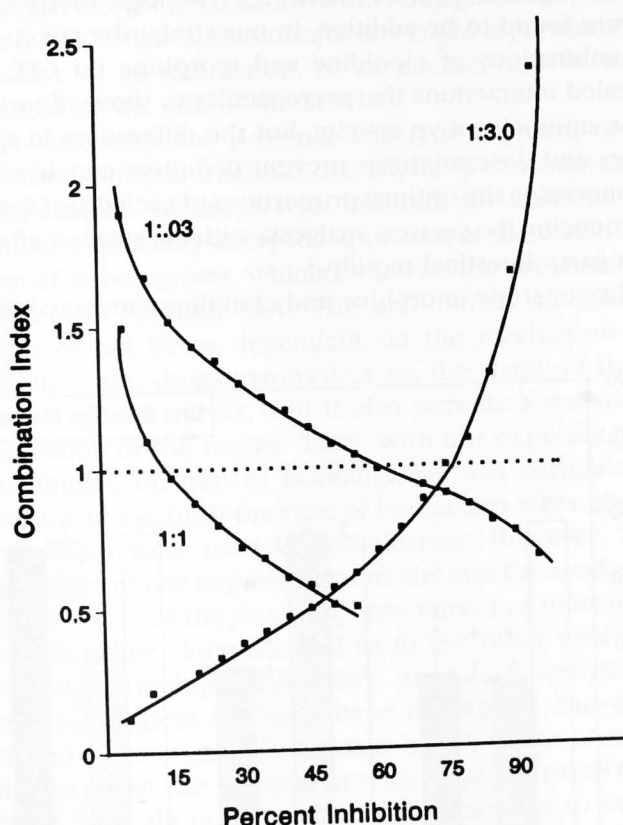


Fig. 4. Multiple-drug-effect analysis of combinations of morphine and clonidine. Combination indexes (CI) are plotted (ordinate) at various degrees of inhibition (abscissa) for the three mixtures of drugs. A combination index of 1 indicates no interaction (additive), and values above and below the horizontal line indicate antagonism and synergy, respectively. Combination indexes for levels of inhibition less than 20% or more than 80% are extrapolated from actual experimental data obtained using the computerized method of Chou and Chou.²⁴

significant effects of treatment, dose, and their interaction ($P < 0.0001$). At higher doses, no significant effect of the treatment was observed ($P < 0.77$), whereas the dose ($P < 0.001$) and the interaction ($P < 0.0001$) remained very significant. Thus the analysis suggests that at levels of effect less than 60% the interaction is synergistic, whereas it becomes antagonistic thereafter.

Pharmacologic Antagonism of the Effects by Naloxone and Efaroxan

The effects on GIT produced by the ED₅₀s of morphine, clonidine, and their combination (at 1:1 ratio) were evaluated after administering naloxone (0.1 mg/kg) and efaroxan (2 mg/kg). The doses of antagonists were selected based on previous studies with these agents and their ability to antagonize the effects mediated at mu, delta, and kappa opiate receptors (naloxone),^{14,16} and alpha-2-imidazoline-1 sites (efaroxan).¹⁸ Figure 6 shows that the administration of naloxone and efaroxan (solid bars) did not alter percentage GIT in our experimental model. Naloxone completely reversed the effects of morphine, but did not alter those of clonidine or the morphine-clonidine combination. On the other hand, efaroxan antagonized the effects of clonidine and the morphine and clonidine mixture.

Discussion

In the treatment of pain, a widely used therapeutic strategy is to combine drugs with similar actions in an attempt to preserve or enhance their analgesic action and minimize side effects.^{26,27} Opioids and alpha-2 agonists are both used as analgesics, and recent reports suggest that when used in combination, they produce

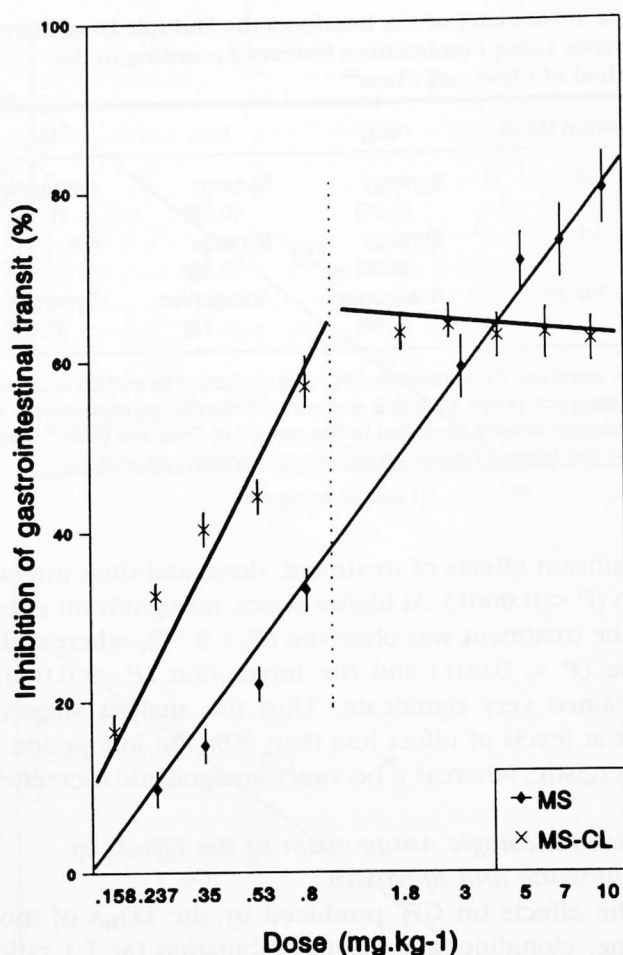


Fig. 5. Log dose-response curves to morphine alone (open circles) and morphine in the presence of a fixed dose (0.0138 mg/kg) of clonidine (filled circles). In the ordinates we have represented percentage inhibition of GIT, and in the abscissa log doses in milligrams per kilogram are shown. Each point is the mean \pm SEM of ten or more mice.

a more potent and longer lasting analgesia.^{3,6,28} In addition to analgesia, both drugs can cause ileus or constipation by inhibiting gastrointestinal motility. Our study documented the effect of clonidine and morphine on GIT in mice and investigated their interaction when both drugs are used together.

Systemic administration of morphine and clonidine produce a dose-related antinociceptive effect in various species and tests. For example, studies conducted in our laboratory showed that morphine was effective in mice when using three different nociceptive stimuli (chemical, thermal, and pressure). In these experiments, the ED₅₀ of morphine varied with the stimuli used: 0.78, 1.77, and 1.02 mg/kg in the writhing, tail-flick, and tail-pinch tests, respectively.²⁹ The data show

that the ED₅₀ of morphine in the mouse tail-flick test was similar to the ED₅₀ for inhibiting mouse GIT reported in this study (1.61 ± 0.48 mg/kg). Similarly, the ED₅₀ for the antinociceptive effect of clonidine has been reported to be 0.03 and 0.108 mg/kg in the writhing and tail-flick tests, respectively^{30,31}; in our study, the ED₅₀ of clonidine in mouse GIT was 0.101 ± 0.006 mg/kg. Thus the analgesic potencies of morphine and clonidine in the tail-flick test (a thermal spinal reflex model) closely resemble their potencies for inhibiting GIT.

Studies of the effects of combinations of systemically administered opiates and alpha-2 agonists have demonstrated synergy in the rat tail-flick³² and hot-plate tests.⁹ In the latter report, clonidine and morphine were administered in four dose combinations. In two of these mixtures (1:10 and 1:30 clonidine:morphine), isobolograms of ED₅₀s show synergistic interactions, whereas other mixtures (1:100 and 1:3 clonidine:morphine) were found to be additive. In our study, the effects of combinations of clonidine and morphine on GIT revealed interactions that were similar to those reported for antinociceptive activity, but the differences in species and dose mixtures prevent definitive conclusions concerning the optimal proportions of each drug (doses producing the greatest analgesia with the smallest effect on gastrointestinal motility).

In our study, morphine and clonidine interacted in a

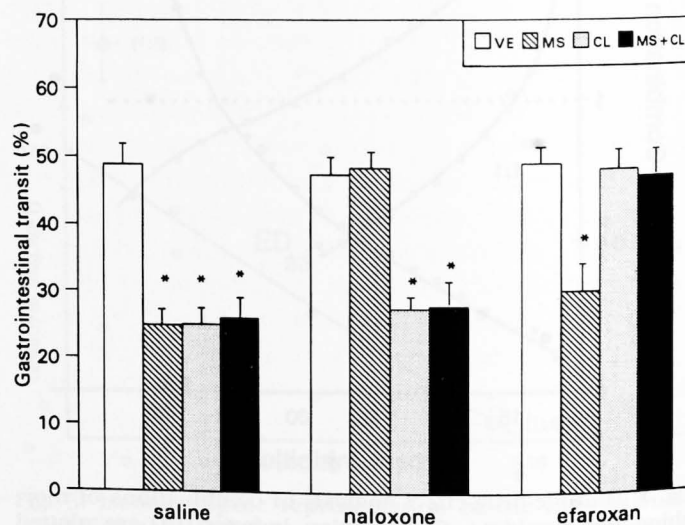


Fig. 6. Pharmacologic antagonism for the median effective doses of morphine, clonidine, and their 1:1 combination by naloxone and efaroxan. In the ordinates we have represented percentage gastrointestinal transit, and in abscissa the different groups of study are shown. Each bar represents the mean \pm SEM of ten or more mice. * $P < 0.05$ when compared with vehicle (Student's Newman-Keuls test). Naloxone was given at 0.1 mg/kg and efaroxan at 2 mg/kg.

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manner that depended on the proportions of each drug on the level of response. When drugs were mixed in ratios of 1:3 and 1:1, isobolograms and multiple-drug-effect analysis demonstrated similar results; that is, synergy at or below ED_{50} s and antagonism at high levels of response. In evaluating the interaction(s), our use of the term *antagonism* makes no assumption about the mechanism of the interaction but merely describes a response that is less than additive. When morphine was in excess (1:0.33 ratio), the interaction was difficult to analyze. This could be attributed to the definition of synergy and antagonism used in isobolographic analysis, each being a statistically significant deviation from the line that designates zero interaction (additivity). Thus in table 3, synergy and antagonism were established when the values in the combinations were significantly different from the zero interaction line. Combinations that were considered additive did not significantly deviate from this line and consequently could represent the absence of an interaction, or an interaction that was masked by the size of the SEM.

To determine the presence and type of interaction, we used different methods of analysis. When analyzing combinations of drugs with different mechanisms of action, a generally accepted approach is the construction of isobolograms, which can show the presence or absence of an interaction. This method has the advantages of not being dependent on the mechanism of action of the drugs involved or on the shape of their dose-response curves, and it also permits a statistical evaluation of the results. Thus, with our experimental conditions, the use of isobolograms was particularly suitable to establish the type of interaction when these two drugs were used in combination. However, the simplicity of our experimental model and the similarity (parallelism) of the dose-response curves of morphine and clonidine alone allowed us to use other methods of analysis (multiple-drug effect, fixed dose, analysis of variance). These methods have advantages and deficiencies: although isobolograms enable us to identify the nature of the interaction with some statistical certainty, they do not permit us to extrapolate to other levels of response or drug ratios. In contrast, multiple-drug-effect analysis does not allow a statistical comparison, but it does differ from isobolograms in its ability to demonstrate trends that can later be tested.

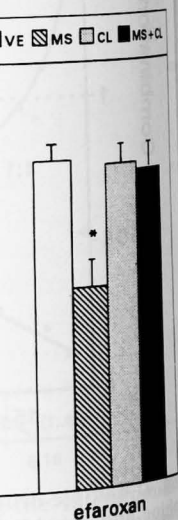
Fixed-dose analysis (using different dose ratios) uses a simple experimental design that is valuable in clinical settings. By comparing the expected and observed effects statistically, it also permits us to demonstrate the

type of interaction. In our experiments, the results we obtained using this method corroborated the results of the isobolographic analysis: mainly that at low levels of response the interaction between morphine and clonidine was synergistic and it became antagonistic when the response was 60% or more of maximal response.

The analysis of variance has been criticized because it can only be used with drugs with dose-response curves of a similar general type.¹⁵ Furthermore, when the dose-response curves of the individual agents and the combinations of agents are parallel, the method cannot be used to establish the presence of an interaction (see Materials and Methods). However, in our study, the dose-response curves to morphine and the morphine:clonidine combination were not parallel (fig. 5), and thus a two-way analysis of variance could be applied. The results were consistent with those obtained with the other methods of analysis: synergy at low levels of response and antagonism at high levels of response. However, the ratios of morphine and clonidine used in the fixed-dose and in the analysis of variance experiments were not analogous to those used in previous experiments. Because of this disparity, we could not make a detailed comparison with the results obtained from isobolograms or multiple-drug-effect analysis.

When the results are evaluated globally, all methods of analysis showed a synergistic interaction between MS and CL when the level of response was 50% or less of maximum effect, and it became antagonistic at levels of response that were more than 50% of maximum. Thus, even if the different analytical methods produced similar results, only isobolograms permitted us to reach unequivocal conclusions (with a certain probability) about the type of interaction at a particular drug ratio and level of response.

The pharmacologic antagonism of the effects of morphine and clonidine alone by naloxone and efaroxan shows that, in our experimental conditions, no significant cross-antagonism occurred between the α -2-imidazoline and opioid systems. The results correlate with those obtained by other investigators in different models.¹⁰ When the morphine:clonidine combination (1:1 ratio) was evaluated, efaroxan completely reversed the effects on GIT, whereas naloxone failed to do so. This finding suggests a primary or predominant role of the α -2 system for this particular combination. The ability of one specific antagonist to reduce the effect of the combination further supports a synergistic interaction at this level of response^{19,33} and suggests a poten-



the median effective dose (ED₅₀) of the 1:1 combination by efaroxan. We have represented the ED₅₀ on the abscissa. The different bars represent the mean ED₅₀ values when compared with the control (naloxone was given at 1 mg/kg).

tial use of alpha-2 antagonists to treat constipation induced by this combination of agents. Despite of the results of our study, we cannot speculate about the mechanism of action of the morphine:clonidine combination.

References

- Glynn CJ, Jamous MA, Teddy PJ: Cerebrospinal fluid kinetics of epidural clonidine in man. *Pain* 1992; 49:361-7
- Eisenach JC, Detweiler D, Hood D: Hemodynamic and analgesic actions of epidurally administered clonidine. *ANESTHESIOLOGY* 1993; 78:277-87
- Murga G, Samsó E, Vallés J, Casanovas P, Puig MM: The effect of clonidine on intra-operative requirements of fentanyl during combined epidural/general anaesthesia. *Anaesthesia* 1994; 49:999-1002
- Kitahata LM: Spinal analgesia with morphine and clonidine. *Anesth Analg* 1989; 68:191-3
- Segal IS, Jarvis DA, Duncan SR, White PF, Maze M: Clinical efficacy of oral transdermal clonidine combinations during the peri-operative period. *ANESTHESIOLOGY* 1991; 74:220-5
- Capogna G, Celleno D, Zangrillo A, Costantino P, Foresta S: Addition of clonidine to epidural morphine enhances postoperative analgesia after cesarean delivery. *Reg Anesth* 1995; 20:57-61
- Yaksh TL, Reddy SVR: Studies in the primate on the analgesic effects associated with intrathecal actions of opiates, alpha-adrenergic agonists and baclofen. Their pharmacology in the primate. *ANESTHESIOLOGY* 1981; 54:451-67
- Harada Y, Nishioka K, Kitahata LM, Kishikawa K, Collins JG: Visceral antinociceptive effects of spinal clonidine combined with morphine, [D-Pen2, D-Pen5] enkephalin, or U50,488H. *ANESTHESIOLOGY* 1995; 83:344-52
- Ossipov MH, Harris S, Lloyd P, Messineo E: An isobolographic analysis of the antinociceptive effect of systematically and intrathecally administered combinations of clonidine and opiates. *J Pharmacol Exp Ther* 1990; 255:1107-16
- Omote K, Kitahata LM, Collins JG, Nakatani K, Nakagawa I: Interaction between opiate subtype and alpha-2 adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. *ANESTHESIOLOGY* 1991; 74:737-43
- Benhamou D, Narchi P, Hamza J, Marx M, Peyrol MT, Sembeil F: Addition of oral clonidine to postoperative patient-controlled analgesia with i.v. morphine. *Br J Anaesth* 1994; 72:537-40
- Ruwart MJ, Kelper MS, Rush BD: Clonidine delays small intestinal transit in the rat. *J Pharmacol Exp Ther* 1980; 212:487-90
- Ramabadran K, Bansinath M, Turndorf H, Puig MM: Streptozotocin-diabetes attenuates alpha-2-adrenoceptor agonist-induced delay in small intestinal transit in mice. *J Auton Pharmacol* 1990; 10:163-71
- Pol O, Ferrer I, Puig MM: Diarrhea associated with intestinal inflammation increases the potency of mu and delta opioids on the inhibition of gastrointestinal transit in mice. *J Pharmacol Exp Ther* 1994; 270:386-91
- Berenbaum MC: What is synergy? *Pharmacol Rev* 1989; 41:93-141
- Pol O, Planas E, Puig MM: Peripheral effects of naloxone in mice with acute diarrhea associated with intestinal inflammation. *J Pharmacol Exp Ther* 1995; 272:1271-6
- Tallarida RJ, Murray RB: *Manual of Pharmacologic Calculations with Computer Programs*. New York, Springer Verlag, 1986, pp 26-31
- Carlisle MA, Smyth DD, Glavin GB: Efaroxan acts peripherally to block the antisecretory and gastroprotective effects of moxonidine in rats. *J Pharmacol Exp Ther* 1995; 274:598-601
- Lee Y-W, Yaksh TL: Analysis of drug interaction between intrathecal clonidine and MK-801 in a peripheral neuropathic pain rat model. *ANESTHESIOLOGY* 1995; 82:741-8
- Caudle RM, Williams GM: The misuse of analysis of variance to detect synergy in combination drug studies. *Pain* 1993; 55:313-7
- Miaskowski C, Sutters KA, Taiwo YO, Levine JD: Antinociceptive and motor effects of delta/mu and kappa/mu combinations of intrathecal opioid agonists. *Pain* 1992; 49:137-44
- Tallarida RJ, Porreca F, Cowan A: Statistical analysis of drug-drug and site-site interactions with isobolograms. *Life Sci* 1989; 45:947-61
- Tallarida RJ: Statistical analysis of drug combinations for synergism. *Pain* 1992; 49:93-7
- Chou J, Chou TC: *Dose-effect Analysis with Microcomputers*. Cambridge, Elsevier-BIOSOFT, 1987, pp 19-32
- Tallarida RJ: A further comment on testing for drug synergism. *Pain* 1992; 51:381-2
- Ruffolo RR, Nichols AJ, Stadel JM, Hieble JP: Pharmacologic and therapeutic applications of alpha-2-adrenoceptor subtypes. *Annu Rev Pharmacol Toxicol* 1993; 32:243-79
- Kehlet H, Dahl JB: The value of "multimodal" or "balanced analgesia" in postoperative pain treatment. *Anesth Analg* 1993; 77:1048-56
- Anzai Y, Nishikawa T: Thoracic epidural clonidine and morphine for postoperative pain relief. *Can J Anaesth* 1995; 42:292-7
- Vargas ML, Bansinath M, Turndorf H, Puig MM: Antinociceptive effects of azapexole (BHT 933) in mice. *Pain* 1989; 36:117-23
- Fielding S, Wilker J, Hynes M, Szewczak M, Novick W, Lal H: A comparison of clonidine with morphine for antinociception and antiwithdrawal actions. *J Pharmacol Exp Ther* 1978; 207:899-905
- Spaulding TC, Fielding S, Venafo JJ, Lal H: Antinociceptive activity of clonidine and its potentiation of morphine analgesia. *Eur J Pharmacol* 1979; 58:19-25
- Meert TF, De Kock M: Potentiation of the analgesic properties of fentanyl-like opioids with alpha-2 adrenoceptor agonists in rats. *ANESTHESIOLOGY* 1994; 81:677-88
- Malmberg AB, Yaksh TL: Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and isobolographic analysis of the NSAID interaction. *ANESTHESIOLOGY* 1993; 79:270-81