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# *Pentobarbital and Thiopental Anesthetic Interactions with Midazolam*

*Igor Kissin, M.D., Ph.D.,* John O. Mason III, B.S.,† Edwin L. Bradley, Jr. Ph.D.‡*

**The effects of midazolam-pentobarbital and midazolam-thiopental combinations on the righting reflex were studied in rats. Doses that block the righting reflex for the agents given alone and for their combinations were determined with a probit procedure and compared with an isobolographic analysis. Interactions between pentobarbital or thiopental and midazolam were found to be synergistic (supra-additive). The degree of synergism was almost identical with both combinations. The ability of barbiturates to increase the affinity of benzodiazepine receptors to midazolam may be the underlying mechanism for the observed synergism. (Key words: Anesthetics, intravenous: midazolam; pentobarbital; thiopental. Interactions (drugs): barbiturates; benzodiazepines. Pharmacology: barbiturates; benzodiazepines.)**

IT HAS BEEN SHOWN that barbiturates in pharmacologically relevant concentrations increase binding of benzodiazepines to the benzodiazepine receptor (enhanced receptor affinity).[1–4] If barbiturates, along with their own anesthetic action, also enhance benzodiazepine receptor affinity, then the combined administration of a barbiturate and a benzodiazepine should result in a synergism in relation to anesthesia (in other words, the conjoint effect of a barbiturate and a benzodiazepine should be more than the sum of the effects of two agents acting separately).

The aim of the present study was to define the type of interaction between pentobarbital or thiopental and midazolam.

## Methods and Materials

Experiments were performed on 190 male Sprague-Dawley rats weighing 275–325 g. The righting reflex test was regarded as positive (by a blinded observer) if the rat failed to right itself (with all four feet on the table) within 15 s after being placed on its side. Each animal was given one predetermined dose of an agent or a combination of agents. The following agents were used: pentobarbital sodium (Elkins-Sinn), thiopental sodium (Abbott), and midazolam hydrochloride (Roche). The agents or their combinations were injected into the saphenous vein over 30 s in a volume of 0.3 ml/100 g. Times between injections of agents and the righting reflex test were based on the times to peak effect for these agents: 15 min for pentobarbital, 3 min for midazolam, and 2 min for thiopental. With combined drug administration, both drugs were injected so that synchronization of the peak effect would occur.[5] For example, with the pentobarbital-midazolam combination, pentobarbital was injected 15 min and midazolam 3 min before the end point determination. The peak times were chosen after preliminary experiments in which peak actions were determined by measuring the latency of the righting reflex to the pressure on the tail (1 kg/0.25 $cm^2$). All experiments were carried out between 8:00 AM and 12 Noon.

Three series of experiments were performed. Two of them (table 1), series I (midazolam-pentobarbital) and series II (midazolam-thiopental), were performed with the use of isobolographic analysis.[5,6] Isobolographic analysis is a technique that allows one to readily visualize the nature of the interaction. An isobol is a line on a dose-dose surface denoting all dose combinations which elicit the same magnitude of response.[7,8] In each of the isobolographic series, the interaction between the agents was determined in two steps:[9] First, dose-effect curves were obtained and $ED_{50}$ values were calculated; second, isobolographic analysis was performed to define the type of drug interaction. With the first step, three dose-effect curves (three subseries of experiments) were determined in each series of experiments (table 1): two with the components given alone (A and C subseries), and the third subseries (B) with a combination of the components. Five groups of five animals were used to determine the dose-effect curve for a drug or a drug combination in each subseries of experiments with doses equally spread to give a range of doses that block the righting reflex in none or all of the animals in a group. On the basis of the results obtained in the experiments where agents were given alone, the ratios of their $ED_{50}$ values were calculated to determine doses for the combined subseries of experiments (table 1).

In the midazolam-pentobarbital series of experiments, the midazolam dose range was from 3 $mg \cdot kg^{-1}$ to 13 $mg \cdot kg^{-1}$ (A subseries), and the pentobarbital dose

* Professor of Anesthesiology, Department of Anesthesiology.
† Senior Research Technician, Department of Anesthesiology.
‡ Associate Professor of Biostatistics, Department of Biostatistics and Biomathematics.
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Address reprint requests to Dr. Kissin.

TABLE 1. Series of Experiments for Isobolographic Analysis

| Series | Subseries | Groups | Number of Animals | Agents | Doses ($mg \cdot kg^{-1}$) | Weight Ratio of Doses |
|---|---|---|---|---|---|---|
| I | A | 1 | 5 | Midazolam | 3 | — |
| | | 2 | 5 | Midazolam | 5 | — |
| | | 3 | 5 | Midazolam | 7 | — |
| | | 4 | 5 | Midazolam | 10 | — |
| | | 5 | 5 | Midazolam | 13 | — |
| | B | 1 | 5 | Pentobarbital, midazolam | 2.0<br>1.0 | 1:0.5 |
| | | 2 | 5 | Pentobarbital, midazolam | 2.5<br>1.2 | 1:0.5 |
| | | 3 | 5 | Pentobarbital, midazolam | 3.0<br>1.5 | 1:0.5 |
| | | 4 | 5 | Pentobarbital, midazolam | 4.0<br>2.0 | 1:0.5 |
| | | 5 | 5 | Pentobarbital, midazolam | 5.0<br>2.5 | 1:0.5 |
| | C | 1 | 5 | Pentobarbital | 15.0 | — |
| | | 2 | 5 | Pentobarbital | 15.5 | — |
| | | 3 | 5 | Pentobarbital | 16.0 | — |
| | | 4 | 5 | Pentobarbital | 17.0 | — |
| | | 5 | 5 | Pentobarbital | 18.0 | — |
| II | A | 1 | 5 | Midazolam | 3 | — |
| | | 2 | 5 | Midazolam | 5 | — |
| | | 3 | 5 | Midazolam | 7 | — |
| | | 4 | 5 | Midazolam | 10 | — |
| | | 5 | 5 | Midazolam | 13 | — |
| | B | 1 | 5 | Thiopental, midazolam | 1.4<br>1.0 | 1:0.7 |
| | | 2 | 5 | Thiopental, midazolam | 1.8<br>1.3 | 1:0.7 |
| | | 3 | 5 | Thiopental, midazolam | 2.2<br>1.6 | 1:0.7 |
| | | 4 | 5 | Thiopental, midazolam | 2.8<br>2.0 | 1:0.7 |
| | | 5 | 5 | Thiopental, midazolam | 3.4<br>2.5 | 1:0.7 |
| | C | 1 | 5 | Thiopental | 9 | — |
| | | 2 | 5 | Thiopental | 10 | — |
| | | 3 | 5 | Thiopental | 11 | — |
| | | 4 | 5 | Thiopental | 12 | — |
| | | 5 | 5 | Thiopental | 14 | — |

range was from 15 $mg \cdot kg^{-1}$ to 18 $mg \cdot kg^{-1}$ (C subseries). In the B subseries of experiments, pentobarbital and midazolam were administered as a combination with the dose ratio of the two drugs always 1:0.5. Doses were given over a range that blocked the righting reflex in none or all of the animals in a group: the dose range was from 1 $mg \cdot kg^{-1}$ to 2.5 $mg \cdot kg^{-1}$ for midazolam, and 2 $mg \cdot kg^{-1}$ to 5 $mg \cdot kg^{-1}$ for pentobarbital.

In the midazolam-thiopental series, the midazolam dose ranged from 3 $mg \cdot kg^{-1}$ to 13 $mg \cdot kg^{-1}$ (A subseries), and the thiopental dose ranged from 9 $mg \cdot kg^{-1}$ to 14 $mg \cdot kg^{-1}$ (C subseries). In the B subseries of experiments, thiopental and midazolam were administered as a combination with the dose ratio of the two drugs approximately 1:0.7; the resulting dose ranges were from 1 $mg \cdot kg^{-1}$ to 2.5 $mg \cdot kg^{-1}$ for midazolam and from 1.4 $mg \cdot kg^{-1}$ to 3.4 $mg \cdot kg^{-1}$ for thiopental. Determination of $ED_{50}$ values from the corresponding dose-effect curves was based on the probit procedure.[10]

Isobolographic analysis was performed at the $ED_{50}$ level. $ED_{50}$ values from all three subseries of midazolam-pentobarbital (or midazolam-thiopental) experiments were plotted in a form of an isobologram (fig. 1). Single-drug $ED_{50}$ points were placed on the dose coordinates of the isobologram (points A and C) and a combined $ED_{50}$ point (B) in the dose-field. The deviation of a combined $ED_{50}$ point from an additive line (straight line joining single-drug $ED_{50}$ points) was measured as

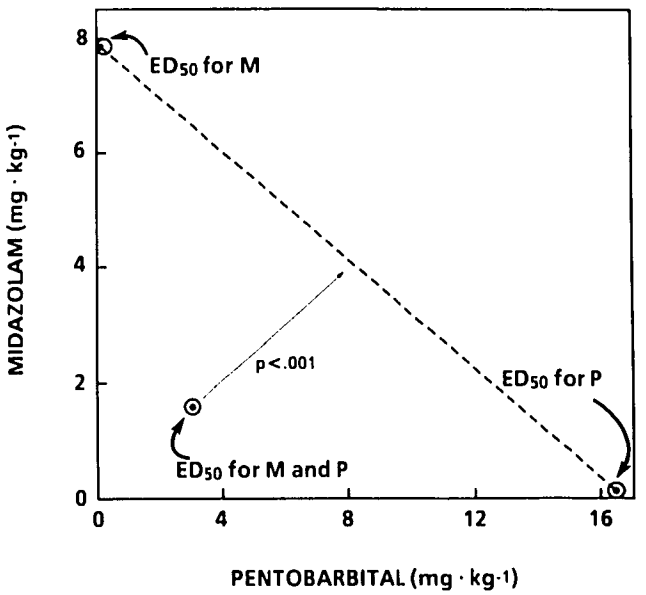


FIG. 1. $ED_{50}$ isobologram for the interaction of midazolam and pentobarbital as characterized by blockade of the righting reflex. $ED_{50}$ values generated by probit analysis indicate the dose level that provides the effect in 50% of the animals. Points shown are $ED_{50}$ values for midazolam (M) and pentobarbital (P) given alone, and the $ED_{50}$ value for the midazolam-pentobarbital (M-P) combination. The dashed straight line connecting the single-drug $ED_{50}$ points is an additive line. *P* value indicates the level of statistical significance for deviation of the combined $ED_{50}$ point from the additive line.

the length along a line running from the point in question to the additive line perpendicularly to it. This distance was used to determine if a statistically significant difference was present. The standard error of this distance was computed by the method of propagation of error,§ and error estimates from a combined $ED_{50}$ point, as well as single-drug $ED_{50}$ points, were used. An approximate *t* test used to test the assumption of additivity was then obtained as the ratio of the measured distance to its standard error.[11]

An additional third series of experiments was also performed to verify the results obtained with the isobolographic technique. In the third series of experiments, the thiopental-midazolam interaction was assessed with the Poch-Holzmann technique modified for analysis of quantal responses.[12] With this technique, a midazolam dose-response curve for loss of the righting reflex was determined with and without the addition of a fixed dose of thiopental. As a result, two subseries of experiments were performed. One subseries consisted of four groups of five animals with the following doses of midazolam ($mg \cdot kg^{-1}$): 2, 5, 10, and 25. In another subseries, four groups of five animals which received midazolam followed by thiopental. The dose of thiopental was kept constant at 10 $mg \cdot kg^{-1}$ in all five groups of animals; the doses of midazolam were as follows ($mg \cdot kg^{-1}$): 0.1, 0.05, 0.03, and 0.02. The dose of thiopental (10 $mg \cdot kg^{-1}$) represented the $ED_{20}$ level on the thiopental dose-response curve obtained in the second series of experiments. The actual dose-response curve for the midazolam-thiopental combination was determined and compared with a theoretical additive dose-response curve for this combination constructed on the basis of the midazolam dose-response curve (see Results). The comparison was made at the $ED_{50}$ level using a *t* test.

Animal care standards in this study were in accordance with federal and institutional policy and standards of the American Association for Accreditation of Laboratory Animal Care as specified in the *Guide for the Care and Use of Laboratory Animals.*¶

## Results

The midazolam-pentobarbital isobologram is presented in figure 1. The combined $ED_{50}$ point for blockade of the righting reflex deviates to the left of the additive line (joining single-drug $ED_{50}$ points) indicating synergism (supra-additive interaction). Comparison of the combined and single-drug $ED_{50}$ doses for the midazolam-pentobarbital series is presented in table 2. In combination the sum of the fractional doses was significantly lower than a single drug fractional dose (0.39 *vs.* 1.00, $P < 0.001$). The ratio of a single drug dose to a combined dose was 2.65.

The midazolam-thiopental isobologram for blockade of the righting reflex is shown in figure 2, and the combined and single-drug $ED_{50}$ doses are shown in table 3. The midazolam-thiopental interaction was also supra-additive with almost the same degree of synergism as for the midazolam-pentobarbital interaction.

The results of the drug interaction analysis with the use of the Poch-Holzmann method is presented in figure 3. Curve A represents a dose-response curve for midazolam administered alone. Curve B represents an actual dose-response curve resulting from the combined administration of different doses of midazolam with the fixed dose of thiopental 10 $mg \cdot kg^{-1}$. For construction of the curve B, the fixed dose of thiopental was expressed as a midazolam equivalent. Since 10 $mg \cdot kg^{-1}$

§ Ku HH. Notes on the use of propagation of error formulas. J Res Natl Bureau Stand 70:263–273, 1966

¶ Guide for the Care and Use of Laboratory Animals. Washington, U. S. Government Printing Office, 1978: DHEW Publication No. (NIH) 78–23

TABLE 2. Midazolam-pentobarbital Anesthetic Interaction

| Subseries | Equi-effective Doses ($ED_{50}$) of Midazolam-pentobarbital Combination | | | | | Ratio* |
|---|---|---|---|---|---|---|
| | Midazolam Component | | Pentobarbital Component | | Sum of Fractional Doses | |
| | Fraction of $ED_{50}$ | Dose in $mg \cdot kg^{-1}$ | Fraction of $ED_{50}$ | Dose in $mg \cdot kg^{-1}$ | | |
| A | 1.00 | 7.9 (5.2, 21.9) | 0.00 | 0.0 | 1.00 | |
| B | 0.20 | 1.6 (1.2, 1.9) | 0.19 | 3.17 (2.4, 3.7) | 0.39 $P < 0.001$ | 2.56 |
| C | 0.00 | 0.0 | 1.00 | 16.4 (15.6, 17.0) | 1.00 | |

Confidence limits in parenthesis.
* Ratio of single-drug fractional dose to combined fractional dose.

The $P$ value denotes the significance of the difference between combined fractional dose and single-drug fractional doses.

of thiopental gave the same effect as 6 $mg \cdot kg^{-1}$ of midazolam (see a dashed line "e" in figure 3), the midazolam equivalent for 10 $mg \cdot kg^{-1}$ of thiopental was set as 6 $mg \cdot kg^{-1}$. The 6 $mg \cdot kg^{-1}$ equivalent was then added to every dose of midazolam administered in the combined experiments, and the curve B was plotted using actual responses obtained in the experiments. Curve C represents a theoretical additive dose-response curve for the midazolam-thiopental combination, which was constructed in the following way. The 6 $mg \cdot kg^{-1}$ midazolam equivalent (for 10 $mg \cdot kg^{-1}$ of thiopental) was added to the following doses of midazolam: 2, 5, 10, and 25 $mg \cdot kg^{-1}$ with the resulting combined doses of 8, 11, 16, and 31 $mg \cdot kg^{-1}$. Then, the additive response for every combined dose was derived from the midazolam dose-response curve A (see a, b, c, and d in figure 3). These theoretical responses and combined doses were used to construct the additive curve C. The comparison of the actual dose-response curve for the midazolam-thiopental combination (curve B) with the theoretical additive dose-response curve for this combination (curve C) shows that the curve B is shifted to the left from the curve C, indicating a significant ($P < 0.02$) synergism between midazolam and thiopental.

## Discussion

The isobolographic analysis used in the present study demonstrated synergistic (supra-additive) midazolam-pentobarbital and midazolam-thiopental interactions in relation to the loss of the righting reflex. In the combination studies, less than one-fourth of the single drug dose (for each of two agents) was needed to produce the required effect. The degree of synergism obtained was almost identical for both combinations with the ratios of single drug fractional dose to combined fractional dose of 2.56 for midazolam-pentobarbital, and 2.63 for midazolam-thiopental. Because loss of the righting reflex is the classical index of hypnotic action,[13] it is possible that midazolam-pentobarbital (or midazolam-thiopental) interaction has a synergism for the hypnotic component of anesthesia. The additional analysis of the midazolam-thiopental interaction with the use of the Poch-Holzmann method[12] confirmed the isobolographic evidence for the synergistic interaction between these agents. Nonadditive interaction between intravenous

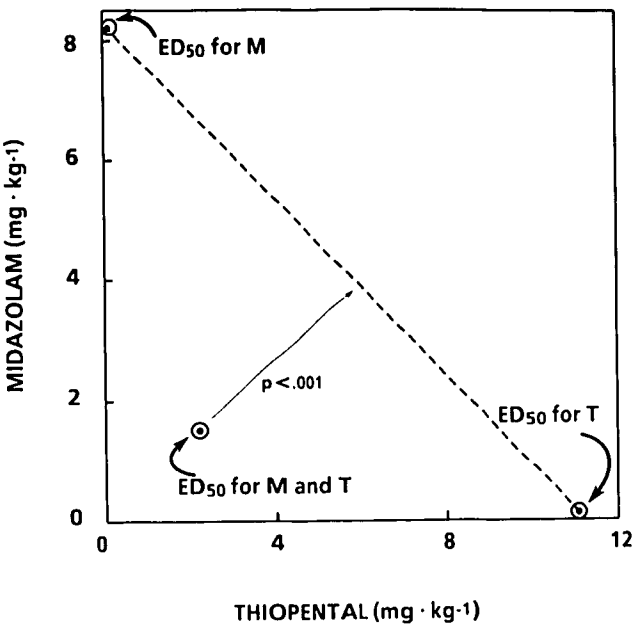


FIG. 2. $ED_{50}$ isobologram for the interaction of midazolam (M) and thiopental (T) as characterized by blockade of the righting reflex. Points shown are $ED_{50}$ values for midazolam and thiopental alone, and the $ED_{50}$ value for their combination.

TABLE 3. Midazolam-thiopental Anesthetic Interaction

| Subseries | Equi-effective Doses ($ED_{50}$) of Midazolam-thiopental Combination | | | | | Ratio* |
|---|---|---|---|---|---|---|
| | Midazolam Component | | Thiopental Component | | | |
| | Fraction of $ED_{50}$ | Dose in $mg \cdot kg^{-1}$ | Fraction of $ED_{50}$ | Dose in $mg \cdot kg^{-1}$ | Sum of Fractional Doses | |
| A | 1.00 | 8.0 (4.9, 20.1) | 0.00 | 0.00 | 1.00 | |
| B | 0.19 | 1.5 (0.9, 2.2) | 0.19 | 2.1 (1.3, 3.0) | 0.38 $P < 0.001$ | 2.63 |
| C | 0.00 | 0.0 | 1.00 | 11.0 (9.8, 12.5) | 1.00 | |

Confidence limits in parenthesis.

* Ratio of single-drug fractional dose to combined fractional dose.

anesthetics probably should not be viewed as an unusual phenomenon because, even with inhaled anesthetics (which generally are additive), there are some synergistic or even antagonistic combinations.[14]

As far as the mechanism for the midazolam-barbiturate synergism is concerned, several possibilities should be considered. Because the benzodiazepine receptors and barbiturate binding sites are different entities, the benzodiazepine-barbiturate interaction may have a functional nature (two different agents, acting at different sites, affect the same physiologic function). On the other hand, one hypothesis states that the benzodiazepine receptor, the GABA receptor, and the barbiturate binding sites are part of a supramolecular complex and that binding of a drug to one of the sites of this complex can allosterically modify the benzodiazepine receptor, resulting in altered affinity for a benzodiazepine.[15] The observation that benzodiazepine binding is enhanced by barbiturates supports such a hypothesis.[1-4] The supra-additive benzodiazepine-barbiturate interaction regarding the hypnotic effect may well be explained by an increase in benzodiazepine binding by barbiturates. We could not find any data on the effect of benzodiazepines on the affinity of the barbiturate binding sites for barbiturates. It is conceivable that not only barbiturates potentiate the hypnotic effect of benzodiazepine, but that the reverse is also true: benzodiazepines potentiate the hypnotic effect of barbiturates. Although pharmacodynamic mechanisms seem to be the most likely cause for the supra-additive benzodiazepine-barbiturate interaction, pharmacokinetic factors cannot be excluded from consideration. Analysis of the mechanisms responsible for potentiation of the hypnotic effect of midazolam by barbiturates requires further study.

In conclusion, the interaction between pentobarbital or thiopental and midazolam in relation to blockade of the righting reflex may be defined as supra-additive (synergistic).

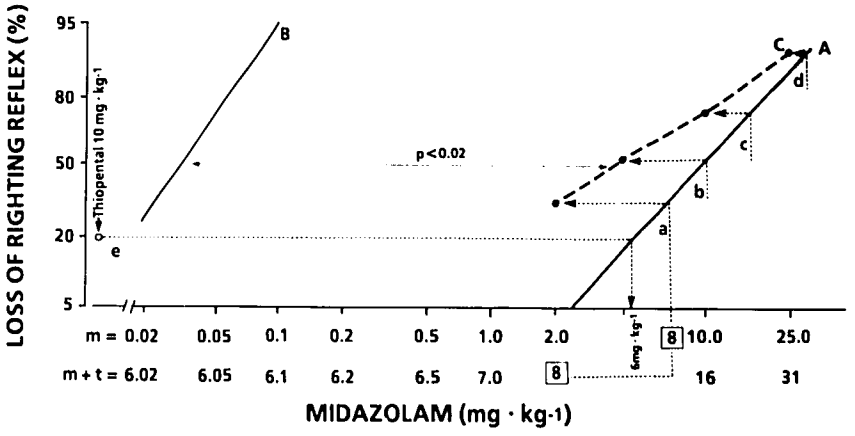


FIG. 3. Assessment of midazolam-thiopental hypnotic interaction with the use of the Poch-Holzmann method. The vertical axis represents the percentage of animals that demonstrated the endpoint (probit scale). The horizontal axis represents the dose of midazolam (log scale). m = midazolam alone; m + t = midazolam dose plus midazolam equivalent of 10 $mg \cdot kg^{-1}$ of thiopental (6 $mg \cdot kg^{-1}$). Thiopental 10 $mg \cdot kg^{-1}$, the $ED_{20}$ levels, depicted as a dashed line e. A = midazolam dose-response curve; B = actual dose-response curve for the midazolam-thiopental combination; C = theoretical additive dose-response curve for the midazolam-thiopental combination. The horizontal line between the curves B and C indicates a deviation from additive interaction toward synergism. Explanation in the text.

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