

## The Effects of Interaction Between Lidocaine and Pentobarbital on Toxicity in Mice and Guinea Pig Atria

Richard K. Richards, M.D.,\* N. Ty Smith, M.D.,†  
and Jordan Katz, M.D.‡

The LD<sub>50</sub> of intravenous lidocaine in albino mice was determined to be 30.3 mg./kg. Small amounts of pentobarbital prevented the convulsive action of lidocaine. The LD<sub>50</sub> of lidocaine was significantly increased to 44.7 mg./kg. by the addition of 20 mg./kg. pentobarbital, remained essentially unchanged with 30 mg./kg. pentobarbital, and fell gradually with higher barbiturate doses to a value below that for lidocaine alone. Death occurred within 1-2 minutes. At this time, the atria usually contracted fairly normally but the ventricles contracted slowly and ineffectually. For this reason, and because of the known cardiotoxicity of high concentrations of both drugs, their interaction on the isolated heart was studied. Isolated guinea pig atria were exposed to various concentrations of lidocaine (2-54 µg./ml.) and pentobarbital (1-18 mg./100 ml.) and recordings were made of depression of isometric contraction height. Concentration-effect curves were determined. To determine the concentrations of each drug alone and in a combination that produced an equal effect, isobolograms were constructed. These showed a synergistic effect between the two agents under all conditions tested.

Although it is experimentally possible to reduce the toxicity of lidocaine by pentobarbital within narrow limits, the use of such mixtures for intravenous administration clinically appears inadvisable.

Toxic, sometimes fatal, reactions to local anesthetics have been reported since the advent of their clinical use. Gordh<sup>1</sup> recently reported

17 deaths after nerve blocks and infiltration with local anesthetics, and restated the axiom that most toxic reactions are due to too-rapid absorption of the drug into the blood stream. In addition, there has been a revival of interest in the intravenous injection of local anesthetics for a variety of anesthetic and medical procedures, among which are: supplementation of general anesthesia<sup>2</sup>; isolated-extremity anesthesia<sup>3,4</sup>; treatment of cardiac arrhythmias<sup>5,6</sup>; and control of epileptic seizures.<sup>7</sup>

Prevention and control of toxic actions of local anesthetics encompass, among other measures, the use of drugs. The most dramatic toxic manifestations in experimental and clinical overdosages with local anesthetics are those arising in the central nervous system, particularly convulsions. Their pharmacologic management has been the focal point of the classic work by Tatum *et al.*<sup>8</sup> on the protective and therapeutic use of barbiturates. Although this work was confirmed in principle by other investigators, it was soon realized that this antagonism did not extend to the cardiotoxic action of local anesthetics. Sollman<sup>9</sup> has summarized some of the important findings on this subject.

When physicians began to administer local anesthetics intravenously, this problem gained new importance, particularly since the doses used approached and even surpassed those capable of producing toxic reactions. It appeared worthwhile, therefore, to reinvestigate experimentally, the interaction of local anesthetics and the barbiturates over a significant dose range, by simultaneous intravenous administration. To our knowledge, little information is available in the literature. Lidocaine hydrochloride (Xylocaine) and pento-

\* Lecturer in Anesthesia and Pharmacology.

† Assistant Professor of Anesthesia; recipient of NIH Career Development Award 1-K3-GM-31757.

‡ Assistant Professor of Anesthesia.

Received from the Department of Anesthesia, Stanford Medical Center, Palo Alto, California. Accepted for publication December 15, 1967. Supported by Program Project USPHS GM-12537.

Presented at the fall meeting of the American Society of Anesthesiology, October 2, 1967, in Las Vegas, Nevada.

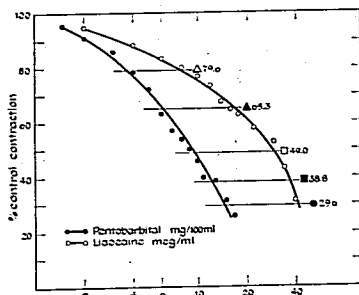


FIG. 1. Two concentration-effect curves drawn on the same graph; one for pentobarbital and one for lidocaine vs. the percentage of control contraction amplitude in an electrically-driven atrium. Five equal-effect planes were drawn through the concentration-effect curves. The intersections of each line with the concentration-effect curves are plotted on the axes of new linear coordinates (fig. 2). The symbol and percentage effect to the right of each plane correspond to the symbols for the additive isobole and the combined concentration of pentobarbital and lidocaine in figure 2.

barbital (Nembutal) sodium were chosen as representative drugs. Albino mice were used as experimental animals to determine dose-lethality curves. In addition, the isolated guinea pig atrium was used to study the interaction of these drugs in an *in vitro* preparation.

### Methods

#### TOXICITY STUDIES IN MICE

Six hundred and thirty-seven albino mice of either sex, weighing between 18 and 26 Gm., were used. They were maintained on a commercial laboratory diet. Each animal was used only once. Lidocaine hydrochloride in 0.1–0.2 per cent concentration and pentobarbital sodium 0.4–0.8 per cent were prepared in physiologic saline solutions made fresh daily. The volume injected into the tail vein of each animal was adjusted to 0.5 mL, and the speed of injection was standardized at 10 seconds. Mixtures of these drugs proved stable for the period of the experiment. Since the addition of pentobarbital sodium (pH 8.3) shifts the pH of lidocaine hydrochloride solution (pH 4.8) to the alkaline side (pH 7.55), a series of toxicity experiments with lidocaine hydro-

chloride alone, buffered to this pH value, were undertaken; no significant changes in symptoms, course of action or lethality were found.

Sleep, in pentobarbital-treated mice, was defined as the onset of a lateral recumbent position and absence of spontaneous righting for at least one minute. All animals were observed until the acute drug effects had subsided, for a minimum of two hours.

In order to study the interaction of these drugs without interference, no attempt was made to resuscitate the animals.

#### ISOLATED GUINEA PIG ATRIA

Six guinea pigs, 300 to 500 Gm. in weight, were killed by cervical dislocation. The heart was quickly removed and transferred to a shallow dish containing modified Krebs' solution at 4° C. Part of the ventricles and all extra-

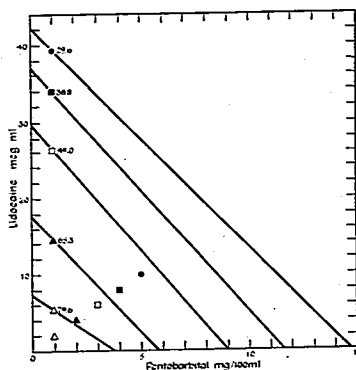


FIG. 2. Isobolograms constructed from figure 1. The additive isobole is simply a straight line drawn between the two points on the x and y axes representing the intersection of an equal-effect line with the concentration-effect curves. Each symbol relates a line to a combined concentration of lidocaine and pentobarbital. For example, the closed squares, corresponding to the 38.8 per cent effect plane in figure 1, denote the additive isobole and the combined concentrations of pentobarbital and lidocaine which produced the 38.8 per cent effect. As the closed square in the lower part of this figure indicates, a combination of about 8  $\mu$ g./ml. lidocaine with 4 mg./100 ml. pentobarbital produced a 38.8 per cent depression equal to either 37  $\mu$ g./ml. lidocaine or 11.5 mg./100 ml. pentobarbital alone.

neous tissues were removed. The remaining part of the ventricles was tied to a plastic muscle holder and the preparation transferred to a 50-ml. constant-temperature bath containing Krebs' solution at 28° C., pH 7.40-7.43, saturated with 95 per cent oxygen and 5 per cent carbon dioxide. One liter of modified Krebs' solution contains 144.8 millimoles sodium, 6.09 millimoles potassium, 2.54 millimoles calcium, 128.38 millimoles chloride, 1.35 millimoles biphosphate, 26.3 millimoles bicarbonate, and 2.0 Gm. glucose.

The spontaneously-beating atria were allowed to stabilize for 30 minutes. A metal clip was attached to one of the atrial tips and tied to a Sanborn FTA-3 transducer for recording isometric contractions on an oscillograph. The preparations were stimulated with punctate platinum electrodes by square-wave pulses of 1 to 5 volts, 5 msec. duration, at a frequency of 120 to 180 per minute. The hearts were driven electrically to eliminate any effect of change in heart rate upon myocardial contractility. Voltage was adjusted to slightly above threshold to minimize the release of norepinephrine.

Peak developed tension was measured for ten consecutive beats, and the results were averaged. In order to compare drug effects, per cent of control contraction was plotted against drug concentration on a log-log scale.

Three separate studies of each atrium were performed in random sequence: 1) 0.25 per cent pentobarbital sodium was added to the bath in increments of 0.2 ml.; 2) lidocaine hydrochloride 0.1 per cent solution was added in increments of 0.1 ml.; and 3) pentobarbital sodium was added in increments of 0.1 or 0.2 ml., together with lidocaine hydrochloride in increments of 0.1 or 0.2 ml. These solutions were prepared freshly for each experiment by adding the pure powder to Krebs' solution. The increase in tonicity of the lidocaine solution was 0.04 per cent; that of the pentobarbital solution 1.0 per cent. Thus, the maximum increase in tonicity of the bath perfusing the atria was 0.06 per cent. Since the total volume of the bath was 50 ml., the concentration of pentobarbital was increased in steps of 0.5 or 1.0 mg./100 ml. Lidocaine was increased by 2 or 4 µg./ml. After each incre-

ment, five minutes were allowed for the full response to develop. After each run, the bath was refilled several times with fresh solution and 45 to 60 minutes allowed for the preparation to stabilize before the next run.

To determine the effects of pentobarbital and lidocaine, and their relationship to the effects of combinations of the two agents, isobolograms were constructed by a method described elsewhere.<sup>10, 11</sup> In brief, concentration-effect curves were constructed in each experiment for each drug alone. Lines parallel to the dose (x axis), were drawn through the concentration-effect curves. The heights of these lines reported the effects produced by various combinations of the two drugs, simultaneously administered (fig. 1). The points of intersection of these lines with the concentration-effect curves were transferred to a new graph with linear coordinates (concentration of lidocaine vs. concentration of pentobarbital) (fig. 2). Straight lines drawn between the pairs of points established the additive isobole. Points representing the combined concentration of drug were then plotted. If a point fell on its corresponding additive isobole, an additive effect was considered present; points falling towards the origin indicated synergism; points falling away from the origin indicated antagonism. Complete inhibition can be obtained in such preparations by higher concentrations of either drug alone or of mixtures.

## Results

### TOXICITY IN MICE

The effect of intravenous pentobarbital alone in doses ranging from 10 to 110 mg./kg., was studied in 90 mice. At the lowest level, 25 per cent were judged asleep; at 20 to 35 mg./kg. about 50 per cent were asleep; and at 50 mg./kg. or more, 100 per cent were asleep. With one exception at 35 mg./kg., no fatalities occurred up to and including 70 mg./kg. Sleep lasted from seven to 95 minutes.

Convulsive manifestations were seen in about half of the animals at 25 mg./kg. lidocaine; they occurred regularly in those surviving 30 mg./kg. With larger doses, rapid collapse followed, and either respiratory movements began again within a minute or death regularly fol-

lowed. The chest was quickly opened in ten animals not breathing within one minute after injection. The heart was found to beat slowly and regularly, but ventricular tone and contractility appeared markedly reduced on inspection and palpation. Animals that survived resumed normal posture and activity after a few minutes in the lateral position.

Pentobarbital, even at the lowest dose level, prevented the signs of central stimulation completely. However, the majority of the animals, especially with the larger doses of pentobarbital, remained in the lateral position for periods of time which often exceeded the periods produced by corresponding doses of pentobarbital alone. The variation was too great to permit a statistical evaluation of this observation. No consistent or significant differences in the appearances of the hearts of mice dying from the combination, as compared with those of mice dying from lidocaine alone, were evident on gross inspection.

The data (table 1) show that the  $LD_{50}$  for lidocaine increased in the presence of 10, 20 or 30 mg./kg. pentobarbital. Disregarding an unexplained inconsistency at 40 and 50 mg./kg. pentobarbital, the  $LD_{50}$  for lidocaine showed a decrease with larger doses of the barbiturate. Although these trends are unmistakable, scrutiny of the  $LD_{50}$  and the confidence limits reveals that only the values associated with mixtures containing 20 and 30 mg./kg. pentobarbital belong to the sets not

sharing any values with the set for lidocaine alone, thus comprising a different population of results. Statistical analysis for differences in slope showed the slopes for lidocaine alone and those for additions of 10, 20, 40 and 60 mg./kg. pentobarbital not to be significantly different. On this basis, relative toxicity potencies of these sets were calculated (table 2), indicating that under the conditions of these experiments the maximal reduction of toxicity of lidocaine resulted from 20 mg./kg. pentobarbital, but reached only a modest 25 per cent.

The trends for changes of the  $LD_{05}$  in the presence of pentobarbital were comparable with those for the  $LD_{50}$ . The maximal increase of the  $LD_{05}$  of lidocaine was produced by 10 mg./kg. pentobarbital, but the confidence limits overlap with those of lidocaine alone. There was an even greater tendency to increased toxicity with larger doses of the barbiturate. Whereas the  $LD_{50}$  of lidocaine was reduced by about 30 per cent by the presence of 70 mg./kg. pentobarbital, the reduction amounted to about 60 per cent for the  $LD_{05}$ .

#### TOXICITY IN GUINEA PIG ATRIA

Figure 1 shows simultaneous concentration-effect curves constructed from results obtained in one atrium. Figure 2 shows a family of isoboles obtained from figure 1, plus known concentrations of combinations of the drugs.

TABLE 1. Effect of Pentobarbital on Toxicity of Lidocaine (Intravenous) in Mice

Mixture No.	No. of Animals	Pentobarbital mg./kg. Added	Lidocaine mg./kg. $LD_{50}$ (95 per cent conf. limits)	Lidocaine mg./kg. $LD_{05}$ (95 per cent conf. limits)	Slope
1.	47	None	30.35 (25.83-35.62)	21.7 ( 5.0-25.7)	4.93
2.	52	10	33.70 (31.61-37.15)	27.1 (20.4-29.7)	7.57
3.	105	20	44.65 (38.02-88.55)	24.4 ( 4.4-30.7)	2.71
4.	64	30	42.46 (36.54-50.18)	22.6 ( 4.9-30.2)	2.60
5.	74	40	30.01 (25.99-33.62)	17.7 ( 9.6-21.9)	3.12
6.	52	50	35.64 (31.53-40.93)	19.7 (12.2-24.0)	2.78
7.	34	60	28.32 (24.68-32.57)	17.4 (10.1-21.0)	3.37
8.	56	70	22.82 (19.15-29.23)	8.8 ( 3.5-12.1)	1.73

TABLE 2.

If the $LD_{50}$ of lidocaine HCl alone is taken as	1.000
the mixture with 10 mg./kg. pentobarbital has a value of	0.914
the mixture with 20 mg./kg. pentobarbital has a value of	0.755
the mixture with 40 mg./kg. pentobarbital has a value of	1.021
the mixture with 60 mg./kg. pentobarbital has a value of	1.102

The slopes of the dose response curves of the other mixtures (30, 50 and 70 mg./kg.) are not parallel with the one for lidocaine alone.

All points representing the combination of drugs fall well below their corresponding additive isoboles, indicating potentiation of the myocardial depressant effects of pentobarbital and lidocaine at all dose levels. This is in contrast to other drug interactions studied<sup>10</sup> which have shown a synergistic interaction at low-effect levels and an antagonistic interaction at high-effect levels. This synergistic interaction was demonstrated in five of six atria. The sixth atrium showed an antagonistic interaction.

Pentobarbital decreased the amplitude of contraction of the atria at all concentration levels. In two atria, lidocaine alone caused an initial stimulation, followed by depression at levels of 4 to 8  $\mu$ g./ml. or more. Concentrations of lidocaine equivalent to those known to produce convulsions in man (8–10  $\mu$ g./ml.) produced relatively little depression of the atrium.

### Discussion

There is still controversy about the best management of the acute toxic reactions caused by local anesthetics. Moore and Bridenbaugh<sup>12</sup> suggested the use of oxygen and succinylcholine for the control of central nervous symptoms and advised against the use of barbiturates because of their depressant effects. On the other hand, the judicious use of the latter has been found helpful by many experienced anesthesiologists. This matter has been discussed by Adriani,<sup>13</sup> who pointed out that, although the muscular manifestations of convulsive attacks can be controlled by muscle relaxants, the electroencephalographic seizures remain. This was also the experience of Toman.<sup>14</sup> It is highly doubtful that uncontrolled central seizure activity is harmless. As Adriani<sup>13</sup> pointed out, oxygen does not increase the convulsive dose of local anesthetics. How-

ever, the synergistic depressant effect of barbiturates on the heart is, within limits, responsive to vasopressor drugs.

There have been conflicting reports concerning the effects of barbiturates on toxicity of lidocaine in experimental animals. Woods and Haggart<sup>15</sup> found no effect, whereas Hunter<sup>16</sup> reported reduction of toxicity of intravenous lidocaine in mice by pentobarbital, but used only one dose level of each drug.

As evidenced by our results, the  $LD_{50}$  of lidocaine was indeed statistically significantly increased by pentobarbital, at low dosage. It should be noted that this increase was attained with 20 or 30 mg./kg. of pentobarbital, an amount greater than that merely necessary to suppress overt convulsive symptoms (10 mg./kg.). In fact, over the next-higher-dose levels of pentobarbital, there was no marked change in mortality above that with lidocaine alone; finally, additive toxicity became evident. It is interesting that whatever beneficial effect was produced at the  $LD_{50}$  level, this was less pronounced when the  $LD_{05}$  was used as a criterion. It appears reasonable to assume that clinically overdosage will be more likely to occur in amounts comparable to the  $LD_{05}$  than to the  $LD_{50}$ .

Clinical work on a combination of intravenous thiopental and procaine has been reported by Fraser.<sup>17</sup> Though one of the aims in using this mixture was the prevention of procaine toxicity, no detailed case histories were presented.

Lidocaine in low doses has been reported to cause cardiovascular stimulation.<sup>18,19</sup> In our experiments a positive inotropic effect was seen in two preparations. However, as has been demonstrated in our experiments, the usually-negative inotropic actions of lidocaine, as well as those of pentobarbital, proved to be synergistic in the sense of potentiation, espe-

cially with higher concentrations of the local anesthetic.

Relating these observations in the heart to the modest, but definite, protective effect of pentobarbital at certain doses, using mortality as a criterion, we conclude that the barbiturate is protective at a time when the limiting factor for survival probably rests upon prevention of convulsive effects rather than on cardiovascular toxicity. That the latter aspect becomes more evident with higher doses of the barbiturate is demonstrated by the decrease of the fatal dose of lidocaine in mixtures with larger amounts of pentobarbital.

These experiments may be especially relevant to clinical conditions where these drugs are given intravenously; perhaps less relevant for subcutaneous or other routes of administration. The experimental data indicate that one can devise mixtures of lidocaine and pentobarbital that result in a reduction of the toxicity of the local anesthetic. It appears unlikely, however, that it would be possible to prepare such mixtures with just the right proportions where clinical administration of large doses of lidocaine was intended. Furthermore, signs of central stimulation usually, though not always, precede the cardiac depressing effect of local anesthetics. Thus, the suppression of central symptoms may deprive the anesthesiologist of a valuable warning symptom.

The authors are indebted to Miss Jacquelyn Smith and Miss Nancy Hood for their very effective assistance in performing these experiments.

### References

- Gordh, T.: Complications in connection with local anaesthesia, *Acta Anaesth. Scand. Suppl. XVI*: 137, 1965.
- Steinhaus, J. E., and Gaskin, L.: A study of intravenous lidocaine as a suppressant of cough reflex, *ANESTHESIOLOGY* 24: 285, 1963.
- Holmes, C. M.: Intravenous regional analgesia: A useful method of producing analgesia of the limbs, *Lancet* 1: 245, Feb. 2, 1963.
- Adams, J. P., Dealy, E. J., and Kenmore, P. I.: Intravenous regional anesthesia in hand surgery, *J. Bone Joint Surg.*, 46A: 81, June 1964.
- Harrison, D. C., Sprouse, J. H., and Morrow, A. G.: The antiarrhythmic properties of lidocaine and procaine amide, *Circulation* 28: 486, 1963.
- Frieden, J.: Antiarrhythmic drugs, Part VII, Lidocaine as an antiarrhythmic agent, *Amer. Heart J.* 70: 713, Nov. 1965.
- Bohm, E., Flodmark, S., and Petersen, I.: Effect of lidocaine (Xylocaine) on seizure and interseizure electroencephalograms in epileptics, *AMA Arch. Neurol. Psychiat.* 81: 550, 1959.
- Tatum, A. C., Atkinson, A. J., and Collins, K. H.: Acute cocaine poisoning, its prophylaxis and treatment in laboratory animals, *J. Pharmacol. Exp. Ther.* 26: 325, 1925.
- Sollman, T.: *A Manual of Pharmacology*, 8th edition. Philadelphia, W. B. Saunders Co., 1957.
- Smith, N. T., and Corbascio, A. N.: Interaction between pentobarbital and decreased calcium on guinea pig atria, *Anesth. Analg.* 45: 576, 1966.
- Smith, N. T.: The use of isobolograms in anesthesia, *Anesth. Analg.* 45: 467, 1966.
- Moore, D. C., and Bridenbaugh, L. D.: Oxygen: The antidote for systemic toxic reaction from local anesthetic drugs, *J.A.M.A.* 174: 840, 1965.
- Adriani, J.: Reactions to local anesthetics, *J.A.M.A.* 145: 405, 1960.
- Toman, J. E. P.: Personal communication.
- Woods, L. A., and Haggart, J.: Apneic and hypotensive effects of local anesthetic drugs in dogs and mice under general anesthesia, *ANESTHESIOLOGY* 18: 831, 1957.
- Hunter, H. R.: The toxicity of Xylocaine, *Brit. J. Anaesth.* 23: 153, 1951.
- Frazer, R. J.: Intravenous pentothal-procaine anesthesia, *Anesth. Analg.* 28: 203, 1949.
- Kimmey, J. R., and Steinhaus, J.: Cardiovascular effects of procaine and lidocaine during general anesthesia, *Acta Anaesth. Scand.* 3: 9, 1959.
- Kao, F. F., and Jalow, U.: The central action of lidocaine and its effect on cardiac output, *Brit. J. Pharmacol.* 14: 522, 1959.
- Toman, J. E. P.: Neuroparmacology of the peripheral nerve, *Pharmacol. Rev.* 4: 168, 1952.