Mixtures of Local Anesthetics Are No More Toxic than the Parent Drugs

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Mixtures of local anesthetics can combine the best features of both components. The authors assayed the systemic toxicity of local anesthetic mixtures given subcutaneously to mice. Convulsions regularly preceded death. The median convulsant dose (CD$_{50}$) of bupivacaine was one-fourth that of lidocaine, and one-seventh that of chloroprocaine. The median lethal dose (LD$_{50}$) of chloroprocaine was twice the CD$_{50}$, whereas the LD$_{50}$ of bupivacaine was but little greater than the CD$_{50}$. Hence, the more potent the agent, the greater is the chance of death from a convulsant dose of local anesthetic.

Conversion to lidocaine-equivalent doses permitted comparisons between mixtures. None of the mixtures were more convulsant or more lethal than their parent components; lidocaine-containing mixtures were significantly less lethal than the lidocaine norm. Mixing increased the distance between convulsant and lethal doses, with survival from convulsions induced by bupivacaine-containing mixtures enhanced in particular. It is concluded that local anesthetic toxicity is essentially additive. (Key words: Anesthetics, local: bupivacaine; chloroprocaine; lidocaine; mixtures of. Toxicity: convulsions; deaths.)

MIXTURES of local anesthetics are used by many clinicians to combine fast onset contributed by one agent with long duration of nerve block by another agent. For instance, chloroprocaine often is mixed with bupivacaine to take advantage of the short latency of the former, and the long duration of analgesia imparted by the latter. Though large-scale clinical studies do not disclose a higher incidence of toxic reactions in patients,[1] laboratory studies have yielded conflicting conclusions regarding the toxicity of local anesthetic mixtures.[2-4]

Since drug absorption following subcutaneous injection resembles in many respects conditions following infiltration anesthesia and peripheral nerve block,[5] we assayed, in mice, the subcutaneous toxicity of three different local anesthetics, given singly or in combination. We found that mixtures of local anesthetics are no more toxic than when the agents are given alone; that is to say, local anesthetic toxicity is essentially additive.

Methods

A total of 369 healthy white female adult (aged 8–10 weeks) virgin Charles River mice, weighing 30 to 35 g each, were injected between 9 A.M. and 11 A.M. with local anesthetic or a mixture of local anesthetics. The mice were housed five to a cage, away from males, so as to suppress estrus.[6] Solutions were injected with a 25-gauge needle into the subcutaneous areolar tissue of the back of the chest, after which the animal was put in a warmed cage for individual observation. We recorded time of onset of ataxia, aimless running motions, arching of tail, neck and back, convulsions, and time of recovery or death. Deaths occurring within four hours of injection were attributed to the acute effects of local anesthetic administration.[7]

We assayed a short-acting (lidocaine) and long-acting (bupivacaine) amide agent, and a brief-acting representative of the ester-class of local anesthetics (chloroprocaine). Anesthetics were donated by the respective manufacturers as sterile solutions of the hydrochloride salt in saline. In keeping with the logarithmic properties of the dose-response curve, we increased or decreased doses in geometric proportion till we bracketed the median value with four or five points for each drug. Replicate end-values of 0 per

TABLE 1. Composition of Local Anesthetic Mixtures
(proportional to baseline $CD_{50}s$)

	Bupivacaine (mg/ml)	Lidocaine (mg/ml)	Chloro-procaine (mg/ml)
Bupivacaine + lidocaine	2.58	9.66	——
Bupivacaine + chloroprocaine	2.81	——	18.75
Lidocaine + chloroprocaine	——	9.19	16.22

cent or 100 per cent were excluded from final computations.

We first determined the individual median convulsant dose (CD_{50}) and median lethal dose (LD_{50}) (see Results) of bupivacaine, lidocaine, and chloroprocaine in 179 mice. We then made solutions containing two of the local anesthetics in proportion to their respective $CD_{50}s$ (table 1) and determined the CD_{50} and LD_{50} of the mixtures in an additional 190 mice. Animals were used only once.

Results of the population study were analyzed with an iterative curve-fitting technique that fits single yes–no observations individually to a logistic curve.‡ Individual fitting prevents the mathematical dilemmas of grouped percentage assays such as management of 0 per cent and 100 per cent responses.[8,9] We computed the CD_{50} and LD_{50}, plus standard error and log dose-logit response equation, for each anesthetic and anesthetic combination. Differences between median toxic values were evaluated with the two-tailed t test.

Results

Table 2 shows the computed $CD_{50}s$ and $LD_{50}s$ (expressed in mg/kg with standard error) of individual local anesthetics and their mixtures. Median toxic doses for the components of the mixtures were calculated from median volumes and composition of the solutions as shown in table 1. Notable is that sub-

‡ de Jong RH: DOSRES: Quantal Dose-Response Analysis. DECUS Program Library #11-411, Maynard, Massachusetts, 1979.

cutaneously administered bupivacaine is a nearly four times more powerful convulsant than lidocaine, and nearly seven times more convulsant than chloroprocaine. The linearized dose-death curves for the three solitary agents are shown in figure 1; the slopes of the lines did not deviate significantly from parallelism by t test.

The lethal dose of lidocaine and chloroprocaine is about twice the convulsant dose, whereas with bupivacaine the lethal dose is just 7 per cent greater than the convulsant dose. Thus, when the lethal doses of local anesthetics given subcutaneously are compared, bupivacaine turns out to be about five times more toxic than lidocaine, and over 12 times more toxic than chloroprocaine. Said differently, if sufficient bupivacaine is given to induce convulsions, chances of recovery are slim if the animals are left untreated, whereas chances of recovery from lidocaine- or chloroprocaine-induced convulsions are good.

To facilitate interpretation of the results with mixtures, we converted mass units of drug combinations to "lidocaine equivalents" by multiplying the drug dose with the ratio of the lidocaine CD_{50} (or LD_{50}) to the drug's CD_{50} (or LD_{50})[4] (table 3). Because the log dose-convulsions and the log dose-death lines within each group did not deviate significantly from parallelism, we merely shifted with this maneuver the dose-response curves to new locations without altering their slopes.

Normalizing the median toxic values will, of course, yield the same numbers for bupivacaine and chloroprocaine as for lidocaine; therefore, only the lidocaine values are shown in table 3. Since the local anesthetics were mixed in equal proportion to their $CD_{50}s$, the equivalent convulsant doses of the components of a mixture are equal (within the constraints of rounding) in table 3. This is not so for the lethal doses, however.

In the rightmost column of table 3, the component $CD_{50}s$ or $LD_{50}s$ are summed to permit comparisons between mixtures. Of the three mixtures assayed here,

TABLE 2. Median Toxic Subcutaneous Doses of Local Anesthetics (mg/kg ± SE)

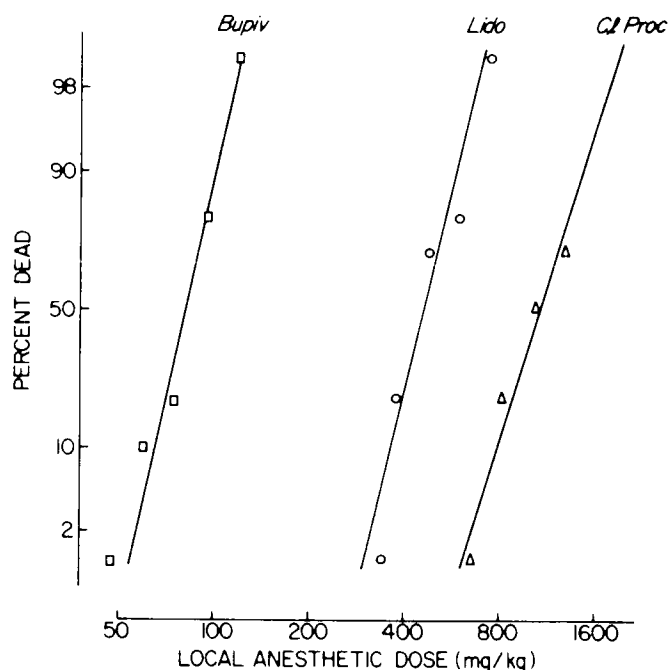|	Bupivacaine		Lidocaine		Chloroprocaine	
	CD_{50}	LD_{50}	CD_{50}	LD_{50}	CD_{50}	LD_{50}
Bupivacaine (n = 50)	77.4 ± 4.3	83.0 ± 4.0	——	——	——	——
Lidocaine (n = 50)	——	——	289.4 ± 13.4	462.7 ± 23.8	——	——
Chloroprocaine (n = 40)	——	——	——	——	510.8 ± 36.3	1069.0 ± 75.1
Bupivacaine + lidocaine (n = 50)	41.2 ± 2.6	68.5 ± 2.7	153.2 ± 9.7	256.4 ± 10.1	——	——
Bupivacaine + chloroprocaine (n = 50)	39.1 ± 2.8	60.2 ± 3.8	——	——	261.2 ± 18.8	401.6 ± 25.7
Lidocaine + chloroprocaine (n = 40)	——	——	161.9 ± 11.8	400.4 ± 25.4	285.8 ± 20.9	706.7 ± 44.8

FIG. 1. Linearized dose-response curves for bupivacaine (Bupiv), lidocaine (Lido) and chloroprocaine (ClProc) given subcutaneously to mice. Drug dose, expressed in mg/kg, is on the logarithmic horizontal axis. The vertical axis represents percentage of deaths on a probit-transformed response scale.

the lidocaine–chloroprocaine combination was the least lethal, while the bupivacaine–chloroprocaine mixture was the most toxic in that it alone did not differ significantly from the lidocaine standard. Both lidocaine-containing mixtures were significantly less lethal than lidocaine alone, as illustrated in figure 2.

Bearing on clinical practice is the much wider spread between convulsant and lethal doses of the bupivacaine-containing mixtures as opposed to bupivacaine alone. Whereas the difference between bupivacaine's LD_{50} and CD_{50} was only 7 per cent, it was

73 per cent for the bupivacaine–chloroprocaine mixture, and 108 per cent for the bupivacaine–lidocaine mixture. Thus, convulsions from bupivacaine-containing mixtures in mice are less likely to terminate fatally than if bupivacaine were given alone.

With all doses of lidocaine and of chloroprocaine, and with doses of bupivacaine less than the CD_{50}, convulsions always preceded death. (A few mice given supraconvulsant amounts of bupivacaine died quickly without ever convulsing.) Quite consistently, the larger the convulsant dose of any of the drugs, the briefer was the latency to convulsions, and the longer convulsions in survivors lasted. This is illustrated in table 4 for a low- and a high-dose range of each drug and drug combination. Seizures from lidocaine seemed to last much longer than with the other local anesthetics. That trend was evident also with the lidocaine-containing mixtures. The bupivacaine–chloroprocaine mixture produced briefer seizures before recovery than the other mixtures, but it also caused the quickest deaths.

Discussion

We found that the subcutaneous LD_{50} of bupivacaine was approximately five times greater than that of lidocaine, a ratio similar to that obtained by Adams and associates who also used female mice.[10] However, our LD_{50} values for both bupivacaine and lidocaine were approximately twice those obtained by these investigators (45 and 211 mg/kg, respectively). We have no ready explanation for the discrepancy, other than that the drugs must have been absorbed faster from their (unspecified) injection site than from the areolar tissues of the back of the chest. That assumption seems reasonable in that other investigators reported subcutaneous toxicities in mice close to our values for lidocaine (425 mg/kg)[5] and bupivacaine

TABLE 3. Median Toxicities Expressed as "Lidocaine Equivalents" (mg/kg ± SE)*

	Bupivacaine		Lidocaine		Chloroprocaine		Total Drug Mass†	
	CD_{50}	LD_{50}	CD_{50}	LD_{50}	CD_{50}	LD_{50}	CD_{50}	LD_{50}
Lidocaine	—	—	289.4 ± 13.4	462.7 ± 23.8	—	—	289.4 ± 13.4	462.7 ± 23.8
Bupivacaine + lidocaine	154.0 ± 9.6	381.9 ± 15.0	153.2 ± 9.7	256.4 ± 10.1	—	—	307.2 ± 19.3 (0)	638.3 ± 25.1 (‡)
Bupivacaine + chloroprocaine	146.2 ± 10.5	335.6 ± 21.2	—	—	148.0 ± 10.6	173.8 ± 11.1	294.2 ± 21.1 (0)	509.4 ± 32.3 (0)
Lidocaine + chloroprocaine	—	—	161.9 ± 11.8	400.4 ± 25.4	161.9 ± 11.8	305.9 ± 19.4	323.8 ± 23.6 (0)	706.3 ± 44.8 (‡)

* Only lidocaine values are listed, since equivalent convulsant and lethal doses of bupivacaine and chloroprocaine are the same.

† Total drug mass is the sum of the component lidocaine equiv-

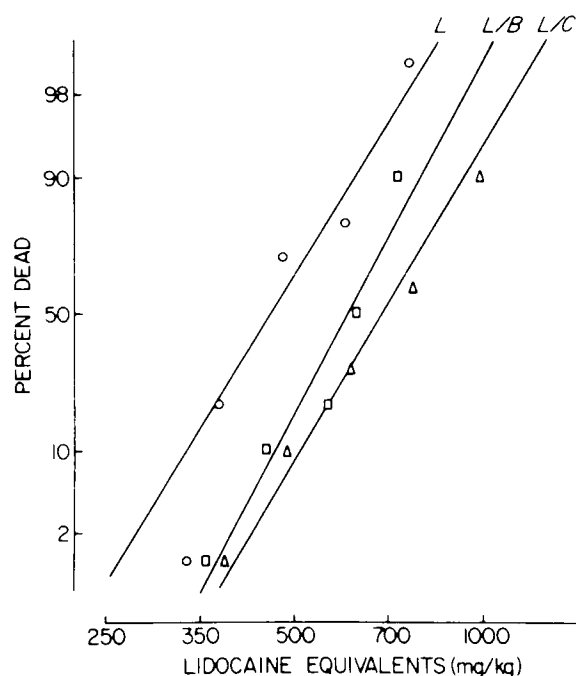alents; ‡ = significant, 0 = no significant difference from lidocaine value.

Fig. 2. The mixture of lidocaine with bupivacaine (L/B) or with chloroprocaine (L/C) is less toxic than when lidocaine (L) alone is given subcutaneously. Local anesthetic doses, converted to "lidocaine equivalents" are on the logarithmic horizontal axis. The vertical axis represents percentage of deaths on a probit-transformed response scale.

that the estrus cycle of the female mouse affects her response to local anesthetics. Accordingly, we housed our animals five to a cage, and away from males, to suppress estrus.[6]

We observed in our mice a phenomenon described recently in dogs where a phase of aimless running motions precedes the onset of convulsions. In the dog, running motions started after an average intravenous lidocaine dose of 22.7 mg/kg, whereas convulsions required half as much again (33.7 mg/kg) of local anesthetic.[14] Since these running motions superficially resemble grand mal convulsions, spuriously low CD_{50}s may have been obtained by earlier investigators.

Hydrolysis of chloroprocaine by human serum esterases is inhibited 38 per cent by bupivacaine _in vitro_, suggesting that mixing bupivacaine and chloroprocaine might intensify chloroprocaine intoxication.[15] Since the mouse is one of the few non-primate species to hydrolyze procaine about as well as does man,[16] and since we found bupivacaine–chloroprocaine to be no more toxic than either drug alone, that hypothesis proved untenable. Nonetheless, the bupivacaine–chloroprocaine mixture was slightly more toxic than either drug mixed with lidocaine.

Our observations are based on mixtures of local anesthetics compounded in proportion to the CD_{50}, a yardstick that represents advanced, but not necessarily irreversible, toxicity. We selected the CD_{50} over the LD_{50} since convulsions from local anesthetic overdosage are a familiar endpoint of severe systemic intoxication in patients given local anesthetics.[17] Though we did not test it, we believe that local anes-

(63 mg/kg).§ The subcutaneous LD_{50} of chloroprocaine we obtained in mice, likewise, was quite close to the value (950 mg/kg) reported by others.[11]

Our work clearly shows that mixtures of local anesthetics tend to be somewhat less convulsant, and somewhat less lethal on an equivalent basis, than are the individual local anesthetics. Though differences were small and mostly insignificant statistically, they were all in the same direction. Thus we are confident that the toxicity of local anesthetics is roughly additive; it certainly is not synergistic as reported in previous studies.[2]

Worth considering here are some of the variables that may affect the seizure threshold to local anesthetics in mice. Time of day was shown to be a major factor by Moore, with a large rise in seizure response in the evening, and a plateau in the morning.[7] For that reason we injected the mice at mid-morning. Sex and age also affect the CD_{50} and LD_{50}. For instance, younger mice survive mepivacaine-induced convulsions better than adults.[12] And a given dose of cocaine kills more female than male mice.[13] Finally, it may be

Table 4. Mean Time to Convulsions, Recovery, and Death

	Dose* (mg/kg)	Time to Convulsions (min)	Duration of Convulsions† (min)	Time to Death‡ (min)
Bupivacaine	224	5.0	9.0	2.0
	450	——	——	1.6
Lidocaine	240	12.5	11.5	——
	480	6.9	91.3	48.0
Chloroprocaine	225	4.0	2.5	——
	460	2.4	8.9	12.0
Bupivacaine + chloroprocaine	200	5.0	5.0	——
	400	1.7	17.5	4.3
Bupivacaine + lidocaine	290	8.6	26.6	——
	579	4.2	49.0	25.9
Lidocaine + chloroprocaine	275	3.0	8.3	——
	550	5.1	36.1	24.0

* Doses expressed in lidocaine equivalents.
† Duration of convulsions in survivors.
‡ Time to death following injection.

thetics mixed in different proportions (*e.g.*, lethal ratio or potency ratio) also would exhibit the property of simple additive toxicity as demonstrated here for the convulsant ratio. It is interesting to note that the subcutaneous LD_{50} of bupivacaine in this study was about one-fifth that of lidocaine, whereas it was about two-fifth that of lidocaine when the drugs were given intraperitoneally.[18] Even so, mixtures of bupivacaine, chloroprocaine and lidocaine given intraperitoneally still proved to be significantly less lethal (by a factor of 20 per cent) than the sum of the lethalities of the two parent drugs.[19] It appears that, regardless of differences in relative toxicities associated with different rates of absorption, the toxicity of local anesthetic mixtures is additive at most.

We conclude that mixtures of local anesthetics, administered under experimental conditions approaching those of peripheral nerve block in man, exhibit essentially additive toxicity, and thus are no more toxic than if the agents were injected singly.

References

1. Moore DC, Bridenbaugh LD, Bridenbaugh PO, et al: Does compounding of local anesthetic agents increase their toxicity in humans? Anesth Analg (Cleve) 51:579–585, 1972
2. Akamatsu TJ, Siebold KH: The synergistic toxicity of local anesthetics. ANESTHESIOLOGY 28:238, 1967
3. Brecher MJ, Greenberg RA, Greene NM: Drug interactions (toxicity) associated with drug combinations. Acta Anaesthesiol Scand 16:22–26, 1972
4. Munson ES, Paul WL, Embro WJ: Central-nervous-system toxicity of local anesthetic mixtures in monkeys. ANESTHESIOLOGY 46:179–183, 1977
5. Wielding S: Xylocaine. The Pharamacological Basis of its Clinical Use. Second Edition. Stockholm. Almgvist & Wiksell, 1964.
6. Whitten WK: Occurrence of anaestrus in mice caged in groups. J Endocrinol 18:102–107, 1959
7. Moore PA: Circadian variations in the convulsant response and pharmacokinetics of lidocaine. Thesis, University of Pittsburgh, 1977
8. Waud DR: On biological assays involving quantal responses. J Pharmacol Exp Ther 183:577–607, 1972
9. de Jong RH: Generalized analysis of quantal dose-response curves. Comput Biomed Res 6:588–595, 1973
10. Adams HJ, Kronberg GH, Takman BH: Local anesthetic toxicity and acute toxicity of (±)-2-(N-Ethylpropylamino)-2',6'-butyroxylidide, a new long-acting agent. J Pharm Sci 61:1829–1831, 1972
11. de Jong RH: Local Anesthetics. Charles C Thomas, Springfield, 1977, p 280
12. Goldberg NB, Goldberg AF: Mepivacaine toxicity—the effect of ambient P_{O_2} variation. Pharmacol Ther Dent 1:56–58, 1970
13. Evans MA, Harbison RD: Cocaine-induced hepatotoxicity in mice. Toxicol Appl Pharmacol 45:739–754, 1978
14. Hofman WF, Jerram DC, Gangarosa LP: Cardiorespiratory and behavioral reactions to the lidocaine-induced convulsions in the dog. Res Commun Chem Pathol Pharmacol 16:581–591, 1977
15. Lalka D, Vicuna N, Burrow SR, et al: Bupivacaine and other amide local anesthetics inhibit the hydrolysis of chloroprocaine by human serum. Anesth Analg (Cleve) 57:534–539, 1978
16. Reidenberg MM: Metabolism of procaine in serum of man and other species. Clin Res 19:354, 1971
17. Steen PA, Michenfelder JD: Neurotoxicity of anesthetics. ANESTHESIOLOGY 50:437–453, 1979
18. de Jong RH, Bonin JD: Deaths from local anesthetic-induced convulsions in mice. Anesth Analg (Cleve) 59:401–405, 1980
19. de Jong RH, Bonin JD: Toxicity of local anesthetic mixtures. Toxicol Appl Pharmacol 54:501–507, 1980