Relative Contribution of Adjuvants to Local Anesthetic for Prolonging the Duration of Peripheral Nerve Blocks in Rats

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Background and Objectives: A chemically compatible, safe 4-drug multimodal formulation of bupivacaine combined with 3 adjuvants (clonidine, buprenorphine, and dexamethasone) has been proposed for long-lasting single-injection peripheral nerve blocks in patients. However, the relative importance of each of the adjuvants of the 4-drug formulation in producing long-lasting nerve blocks has not been determined. The aim of this study in rats was to determine which adjuvants (clonidine, buprenorphine, or dexamethasone) are essential for producing a long-lasting nerve block.

Methods: After baseline sensory and motor responses were recorded, 0.1 mL of drug solution was injected into the sciatic notch of rats. Animals were reevaluated at 10-minute intervals after injection for the absence or presence of sensory and motor response in the sciatic nerve. The 4-drug formulation of 0.25% bupivacaine plus all 3 adjuvants (clonidine, buprenorphine, and dexamethasone), 0.25% bupivacaine with 1 or 2 of the adjuvants added separately, and 0.25% bupivacaine alone were compared for duration of nerve block.

Results: The 4-drug multimodal solution produced a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (P < 0.0001). Bupivacaine plus clonidine also produced a longer duration of nerve block than 0.25% bupivacaine alone (P = 0.0157), but bupivacaine plus buprenorphine or bupivacaine plus dexamethasone did not prolong nerve block compared to bupivacaine alone. There was no difference (P = 0.1414) in the duration of nerve block between the 4-drug multimodal solution versus bupivacaine plus clonidine.

Conclusions: This animal study confirmed that the 4-drug multimodal formulation proposed for clinical nerve block produces superior duration of action compared to local anesthetic alone. This rat sciatic nerve model also indicated that one of the 3 adjuvants, clonidine, could by itself account for the extended duration of nerve block of bupivacaine.

DOI: 10.1097/AAP.0000000000000453

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during that week (the 4-drug formulation is stable after 14 days of refrigeration).7

**Measurement of Nerve Block Duration**

Before the sciatic nerve block injection, rats were accommodated to a fine wire mesh grid and the sensory response was evaluated by withdrawal of the leg when a sharp pin touched the plantar midline surface of each hind paw (distribution of tibial nerve sensory fibers).5,8,9 The motor response was evaluated by the presence or absence of the toe-spreading reflex (distribution of peroneal nerve motor fibers), which is a vestibular reflex evoked by lifting the rat and observing the toes spreading in an extended position.5,8,9 The pin withdrawal response was tested twice, and the toe-spreading reflex once. The duration of the sensory nerve block was measured from the end of nerve injection to the return of pin withdrawal response, and the duration of the motor nerve block was measured from the end of injection to the reappearance of a full toe-spreading reflex. The person who evaluated the sensory and motor block was blinded to the drug treatment.

**Drug Injection Protocol**

After baseline sensory and motor responses were recorded, 0.1 mL of drug solution was injected into the sciatic notch. The percutaneous sciatic nerve blockade technique has been previously described in detail,8,9 using methods similar to Thalhammer et al.10 Animals were briefly anesthetized with 1.2% isoflurane in oxygen, and a standard 27-gauge, 12-mm length needle, with prefilled 1 mL Luer-tip syringe attached, was inserted percutaneously into the sciatic notch, between the greater trochanter and the ischial tuberosity, pointing toward the ischium.8,9,10 Stimulating pulses (0.2 mA, 2 milliseconds, 1 Hz, negative polarity) were delivered to the top of the needle and the syringe/needle advanced until a vigorous ipsilateral hind-leg kick was observed.5,8,9 Subsequently, 0.1 mL of drug solution was injected over 5 seconds and the needle removed after another 5 seconds. Animals were then reevaluated at 10-minute intervals for the absence or presence of sensory and motor response. All animals were observed for 2 days after recovery from nerve block to assess any gait abnormalities.

Each experiment (each graph in the Results) consisted of 16 rats tested with 2 different drug combinations. On the first injection day, on the left sciatic nerve, 8 rats received drug combination A and 8 rats another drug combination B (and in all experiments except one, either A or B was 0.25% bupivacaine alone). Two days later, on the contralateral right sciatic nerve, the order of the same drugs A and B was reversed. So if rats #1 to 8 received drug A on the first injection, they received drug B on the second injection; and if rats #9 to 16 received drug B on the first injection, they received drug A on the second injection. The data from the 2 injection days were then combined for each experiment, yielding n = 16 per drug combination group.

Before starting these experiments, we examined the duration of analgesic action of each of the adjuvant drugs after a single injection. From our earlier paper,4 we knew that the duration of analgesic action for adding 3-μg/mL clonidine to a local anesthetic in a rat was about a 50% increase in block duration. So for a mean duration of analgesia of 0.25% bupivacaine alone of approximately 2 hours, we estimated that the maximum duration of analgesia of 0.25% bupivacaine plus 3-μg/mL clonidine (present experiment) would be 3 hours. As for a systemic analgesic effect of clonidine (up to 1 mg/kg) in the hotplate test in mice, this analgesic effect was of short duration.12 For dexamethasone, there are no papers on the duration of analgesia in the rat, so we used a pharmacokinetic study. After intramuscular administration of 1 mg/kg dexamethasone phosphate in rats, the terminal half-life is 2.3 hours.13 For buprenorphine, there are many references on duration of analgesic effect after systemic administration.14,16 The consensus is that the duration of analgesic effect of systemic buprenorphine hydrochloride is 8 hours or less. So by waiting 48 hours between buprenorphine injections, that is 6 times longer than the duration of analgesic effect.

**Statistics**

**Sample Size**

In a previous study on the clonidine prolongation of lidocaine analgesia,5 we used 9 to 10 rats per group (comparing sensory nerve block duration of 1% lidocaine vs 1% lidocaine with 2.5-μg/mL clonidine). However, the effect of adding the other adjuvants, dexamethasone and buprenorphine, to the local anesthetic may be more subtle than with clonidine added, and so we increased our sample size to n = 16 for each drug combination.

The 7 experiments comparing 0.25% bupivacaine alone, versus the 4-, 3-, or 2-drug multimodal solutions, were analyzed with a general linear model, with least squares means and Dunnett adjustment for multiple comparisons (SAS version 9.2; SAS Institute, Cary, North Carolina) with P < 0.05 considered significant. The one experiment comparing the 4-drug multimodal solution versus 0.25% bupivacaine with clonidine was analyzed with a general linear model, with least squares means (equivalent to independent samples t test).

**RESULTS**

The 4-drug multimodal solution produced a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 1, A and B). In all of the experiments, there was no difference between the duration of sensory versus motor block, so only the sensory data are shown in the succeeding graphs. This lack of difference between the duration of sensory versus motor block matches previous local anesthetic studies in rats.5,8,9 Examining the 2-drug multimodal solutions, bupivacaine plus clonidine also produced a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 1C). However, bupivacaine plus buprenorphine did not produce a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 1D). Also, bupivacaine plus dexamethasone did not produce a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 1E).

In the experiment with a direct comparison of the 2 best formulations, the 4-drug multimodal solution versus bupivacaine plus clonidine, there was no difference (P = 0.1414) in the duration of sensory and motor nerve block (Fig. 2).

Examining the 3-drug multimodal (bupivacaine plus 2 adjuvants) solutions, bupivacaine plus clonidine plus buprenorphine produced a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 3A). Bupivacaine plus clonidine plus dexamethasone also produced a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 3B). However, bupivacaine plus buprenorphine plus dexamethasone did not produce a longer duration of sensory and motor nerve block than 0.25% bupivacaine alone (Fig. 3C).

In all experiments, no animal showed gait abnormalities by 2 days after sciatic nerve drug injection, and all had normal baseline pinprick and toe-spreading responses at that time; however, we did not verify the safety of the drugs with histopathology as is in a previous study with the same drugs.5

**DISCUSSION**

The main finding of this study was that although the 4-drug multimodal formulation proposed for clinical nerve block is
indeed superior to local anesthetic alone, one of the 3 adjuvants, clonidine, could by itself account for the extended duration of nerve block of bupivacaine in a rat sciatic nerve model. So although the original animal study showed no harm in administering all 4 drugs, our findings beg the question of whether it is worth the time and cost of making a more complicated formulation for general use in the hospital population. There is a significant cost of formulations prepared by hospital pharmacies, so even low-cost generic drugs can seem expensive if a more involved formulation is required.

Although the rat sciatic nerve is widely used to test local anesthetic drug potencies, we are cognizant that we cannot decisively extrapolate findings in rats to humans. One limitation of the animal study is that the rat sciatic nerve at the injection site is a single fascicle, although most nerve blocks in patients affect

**FIGURE 1.** Duration of sciatic nerve block in rats. A, Increased duration of sensory block with 4-drug multimodal formulation versus 0.25% bupivacaine alone. B, Increased duration of motor block with 4-drug multimodal formulation versus 0.25% bupivacaine alone. C, Increased duration of sensory block with bupivacaine plus clonidine versus 0.25% bupivacaine alone. D, No difference in sensory block duration of bupivacaine plus buprenorphine versus 0.25% bupivacaine alone. E, No difference in sensory block duration of bupivacaine plus dexamethasone versus 0.25% bupivacaine alone. All graphs show mean ± SE. n = 16 per group. P < 0.05 is significant.

**FIGURE 2.** Duration of sciatic nerve sensory block in rats showing no difference in duration of 4-drug multimodal formulation versus bupivacaine plus clonidine. Graphs shows mean ± SE. n = 16 per group.
In conclusion, our findings indicate a potential need for clinical studies to assess whether comparable clinical benefit may accrue from use of clonidine as a single adjuvant in contrast to multimodal approaches. Finally, block durations with local anesthetics like bupivacaine are much shorter in rats than humans. Nevertheless, the relative efficacy of local anesthetics in animal studies is consistent with their clinical efficacy (e.g., bupivacaine is always longer lasting than lidocaine in both rats and humans).

In conclusion, our findings indicate a potential need for clinical studies to assess whether comparable clinical benefit may accrue from use of clonidine as a single adjuvant in contrast to multimodal adjuvants to bupivacaine for peripheral nerve blocks.

REFERENCES


