REGIONAL ANESTHESIA AND ACUTE PAIN

ORIGINAL ARTICLE

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.

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There are recent large studies that have demonstrated the benefit of regional anesthesia for orthopedic surgery patients.[1–3] With the increased use of regional anesthesia and analgesia for major orthopedic surgery, extending the duration of single-injection nerve blocks becomes a high priority. Although there are clinically long-acting local anesthetics, such as bupivacaine or ropivacaine, their duration is insufficient after single injection for postoperative analgesia after many surgeries. Continuous perineural infusion can maintain analgesia for days but is met with challenges such as technical expertise, maintaining the catheter, and greater cost than a single injection.[4] One of the proposed solutions to extending the duration of long-acting local anesthetic is the addition of different classes of analgesics with the local anesthetic. Clonidine is one example that has been shown to prolong the duration of analgesia. Our research team has delineated the mechanism by which clonidine prolongs the duration of analgesia in peripheral nerve blocks in a rat model.[5]

Another solution is the 4-drug formulation of bupivacaine combined with 3 adjuvants (clonidine, buprenorphine, and dexamethasone) for long-lasting single-injection peripheral nerve blocks in patients.[6] The formulation is based on safety data in rats that shows no damage to the sciatic nerve after a single perineural injection of the 4-drug formulation.[7] In addition, the 4-drug formulation is water-soluble, chemically compatible, and stable with refrigerated storage.[7] However, the relative importance of each of the adjuvants of the 4-drug formulation in producing long-lasting nerve blocks has not been determined in patients or rats. In addition, for some of these adjuvants used clinically for nerve block, buprenorphine and dexamethasone, there is no clear mechanism by which they would extend the duration of analgesia at the peripheral level. This study in rats assesses which adjuvants (clonidine, buprenorphine, or dexamethasone) are essential for producing a long-lasting nerve block, using the exact same adjuvant doses and 0.25% bupivacaine, as in the safety study.[7] Our hypothesis is that a subset of these 3 adjuvants, or maybe even just one, would be sufficient to produce prolonged analgesia, and this would be more convenient to formulate in the hospital setting.

METHODS

After Institutional Animal Care and Use Committee approval, experiments were performed on 80 male Sprague–Dawley rats (185 g; Sasco, Charles River). Percutaneous sciatic nerve blocks were performed in briefly anesthetized rats (1.5% isoflurane) using the methods of Kroin et al.[8] A single 0.1-mL injection was made and the duration of sensory and motor nerve block of the sciatic nerve was measured.

Drugs Studied

The 4-drug formulation of 0.25% bupivacaine plus all 3 adjuvants, 0.25% bupivacaine with each of the adjuvants added separately, and 0.25% bupivacaine alone were compared for duration of nerve block. The 4-drug multimodal solution (denoted $BVP_{0.25}$-C_3D-B_{18} by Williams et al[7]) consists of the following formulation: bupivacaine hydrochloride, 0.25% (2.5 mg/mL); clonidine hydrochloride, 3 μg/mL; dexamethasone sodium phosphate, 67 μg/mL; and buprenorphine hydrochloride, 18 μg/mL. To produce the formulations used in this study, the following commercial generic drugs (available in our hospital pharmacy) were used as stock solutions: bupivacaine hydrochloride injection, 0.5% (Hospira, Lake Forest, Illinois); clonidine hydrochloride, 100 μg/mL (Mylan, Rockford, Illinois);, dexamethasone sodium phosphate injection, 10 mg/mL (APP Pharmaceuticals, Schaumberg, Illinois); buprenorphine hydrochloride injection, 300 μg/mL (Reckitt Benckiser Pharmaceuticals, Richmond, Virginia); and 0.9% sodium chloride injection (Hospira) as diluent. All formulations were prepared fresh the week of each experiment, and refrigerated for use

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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.[5,10,11] 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,[5] 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–Hsu 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.[7]

DISCUSSION

The main finding of this study was that although the 4-drug multimodal formulation proposed for clinical nerve block[6] is

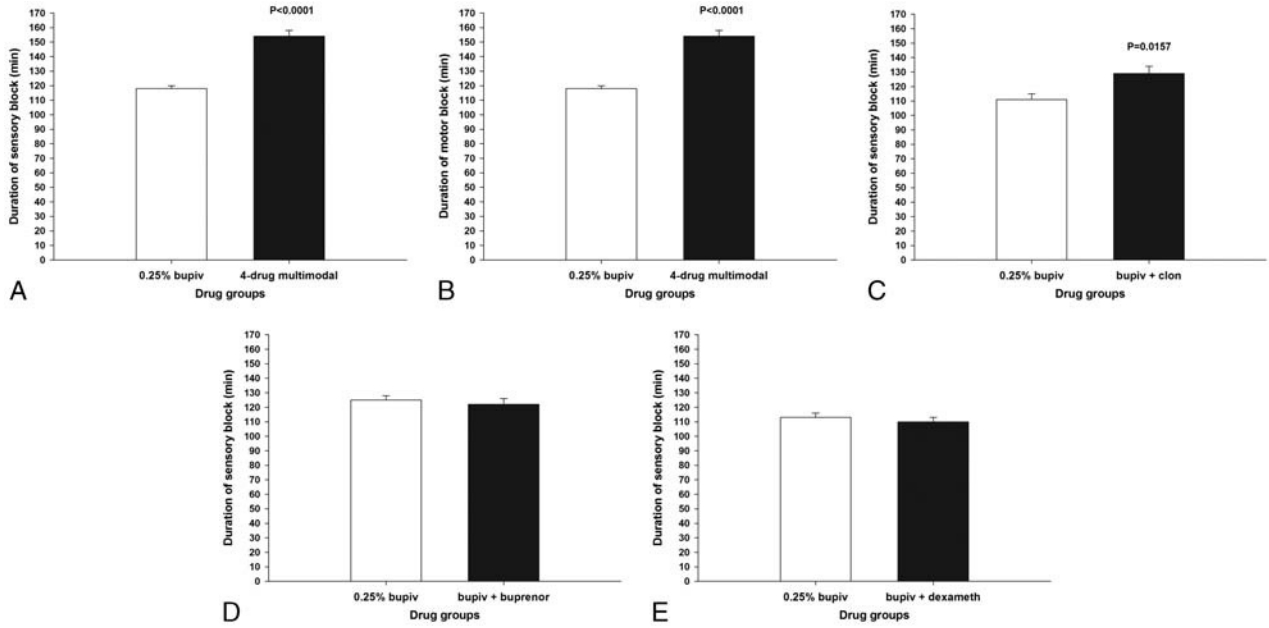

FIGURE 1. Duration of sciatic nerve block in rats. A, Increased duration of sensory block with 4-drug multimodal formulation versus 0.25% bupivacaine alone. B, Increased duration of motor block with 4-drug multimodal formulation versus 0.25% bupivacaine alone. C, Increased duration of sensory block with 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.

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,[7] 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

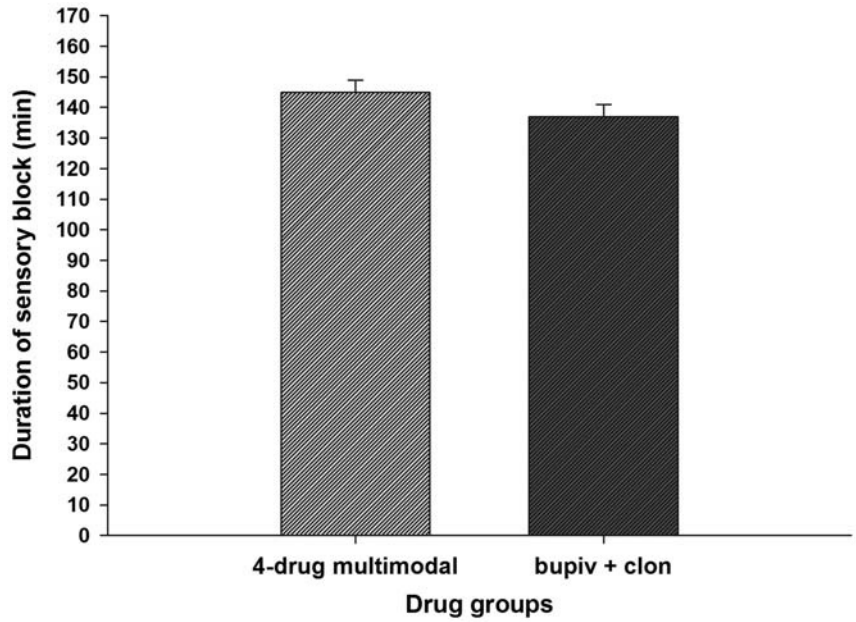

FIGURE 2. Duration of sciatic nerve sensory block in rats showing no difference in duration of 4-drug multimodal formulation versus bupivacaine plus clonidine. Graphs shows mean ± SE. n = 16 per group.

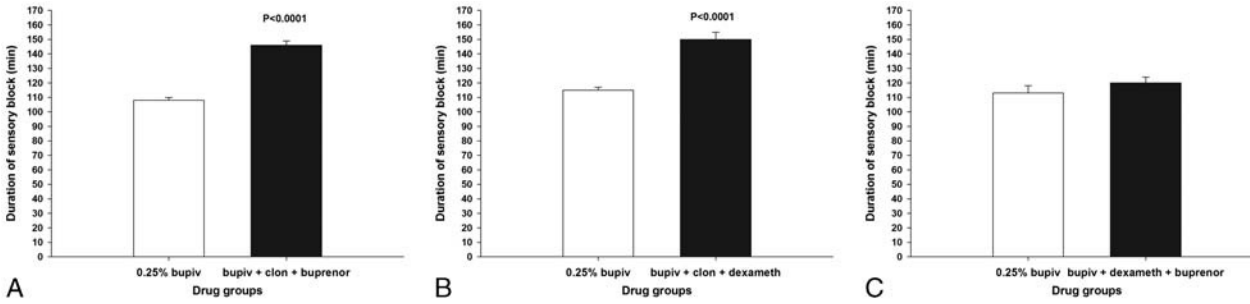

FIGURE 3. Duration of sciatic nerve sensory block in rats. A, Increased duration with bupivacaine plus clonidine plus buprenorphine versus 0.25% bupivacaine alone. B, Increased duration with bupivacaine plus clonidine plus dexamethasone versus 0.25% bupivacaine alone. C, No difference in duration of bupivacaine plus buprenorphine plus dexamethasone versus 0.25% bupivacaine alone. All graphs show mean ± SE. n = 16 per group. $P < 0.05$ is significant.

nerves with multiple fascicles, or combinations of nerves (eg, lumbar or brachial plexus). In addition, human studies involve patient-reported pain scores to determine when the anesthetic is no longer providing meaningful pain relief,[6] whereas the animal study only measures the conduction block of peripheral nerve fibers. Finally, block durations with local anesthetics like bupivacaine are much shorter in rats than humans.[17] Nevertheless, the relative efficacy of local anesthetics in animal studies are consistent with their clinical efficacy (eg, 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

1. Memtsoudis SG, Sun X, Chiu YL, et al. Perioperative comparative effectiveness of anesthetic technique in orthopedic patients. *Anesthesiology*. 2013;118:1046–1058.
2. Pugely AJ, Martin CT, Gao Y, Mendoza-Lattes S, Callaghan JJ. Differences in short-term complications between spinal and general anesthesia for primary total knee arthroplasty. *J Bone Joint Surg Am*. 2013;95:193–199.
3. Helwani MA, Avidan MS, Ben Abdallah A, et al. Effects of regional versus general anesthesia on outcomes after total hip arthroplasty: a retrospective propensity-matched cohort study. *J Bone Joint Surg Am*. 2015;97:186–193.
4. Carvalho B, Yun RD, Mariano ER. Continuous versus single-injection peripheral nerve blocks: a prospective cohort study comparing procedural time and estimated personnel cost. *Open Anesthesiol J*. 2015;9:1–5.
5. Kroin JS, Buvanendran A, Beck DR, Topic JE, Watts DE, Tuman KJ. Clonidine prolongation of lidocaine analgesia after sciatic nerve block in rats is mediated via the hyperpolarization-activated cation current, not by alpha-adrenoreceptors. *Anesthesiology*. 2004;101:488–494.
6. Williams BA, Ibinson JW, Mangione MP, et al. Research priorities regarding multimodal peripheral nerve blocks for postoperative analgesia and anesthesia based on hospital quality data extracted from over 1,300 cases (2011–2014). *Pain Med*. 2015;16:7–12.
7. Williams BA, Butt MT, Zeller JR, Coffee S, Pippi MA. Multimodal perineural analgesia with combined bupivacaine–clonidine–buprenorphine–dexamethasone: safe in vivo and chemically compatible in solution. *Pain Med*. 2015;16:186–198.
8. Kroin JS, Buvanendran A, Tuman KJ, Kerns JM. Effect of acute versus continuous glycemic control on duration of local anesthetic sciatic nerve block in diabetic rats. *Reg Anesth Pain Med*. 2012;37:595–600.
9. Kroin JS, Buvanendran A, Williams DK, et al. Local anesthetic sciatic nerve block and nerve fiber damage in diabetic rats. *Reg Anesth Pain Med*. 2010;35:343–350.
10. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *Anesthesiology*. 1995;82:1013–1025.
11. Kohane DS, Sankar WN, Shubina M, Hu D, Rifai N, Berde CB. Sciatic nerve blockade in infant, adolescent, and adult rats: a comparison of ropivacaine with bupivacaine. *Anesthesiology*. 1998;89:1199–1208.
12. Hirst M, Kavaliers M, Teskey GC. Age and day–night changes in clonidine-induced analgesia in mice. *Can J Physiol Pharmacol*. 1984;62:1102–1105.
13. Samtani MN, Jusko WJ. Comparison of dexamethasone pharmacokinetics in female rats after intravenous and intramuscular administration. *Biopharm Drug Dispos*. 2005;26:85–91.
14. Cowan A, Lewis JW, Macfarlane IR. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. *Br J Pharmacol*. 1977;60:537–545.
15. Gades N, Danneman PJ, Wixson SK, Tolley EA. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. *Contemp Top Lab Anim Sci*. 2000;39:8–13.
16. Yang C, Lan KM, Liu KS, et al. The antinociceptive effect of buprenorphine and its long-acting ester buprenorphine benzoate in rats. *Acta Anaesthesiol Taiwan*. 2004;42:135–139.
17. Berde C. Developing better local anesthetics. *Anesth Analg*. 2015;120:718–720.