Dose–Response Effects of Spinal Neostigmine Added to Bupivacaine Spinal Anesthesia in Volunteers

Spencer S. Liu, M.D., Peter S. Hodgson, M.D., James M. Moore, M.D., Walter J. Trautman, M.D., Daniel L. Burkhead, M.D.

Background: Intrathecal adjuncts often are used to enhance small-dose spinal bupivacaine for ambulatory anesthesia. Neostigmine is a novel spinal analgesic that could be a useful adjunct, but no data exist to assess the effects of neostigmine on small-dose bupivacaine spinal anesthesia.

Methods: Eighteen volunteers received two bupivacaine spinal anesthetics (7.5 mg) in a randomized, double-blinded, crossover design. Dextrose, 5% (1 ml), was added to one spinal infusion and 6.25, 12.5, or 50 μg neostigmine in dextrose, 5%, was added to the other spinal. Sensory block was assessed with pinprick; by the duration of tolerance to electric stimulation equivalent to surgical incision at the pabuls, knee, and ankle; and by the duration of tolerance to thigh tourniquet. Motor block at the quadriceps was assessed with surface electromyography. Side effects (nausea, vomiting, pruritus, and sedation) were noted. Hemodynamic and respiratory parameters were recorded every 5 min. Dose–response relations were assessed with analysis of variance, paired t tests, or Spearman rank correlation.

Results: The addition of 50 μg neostigmine significantly increased the duration of sensory and motor block and the time until discharge criteria were achieved. The addition of neostigmine produced dose-dependent nausea (33–67%) and vomiting (17–50%). Neostigmine at these doses had no effect on hemodynamic or respiratory parameters.

Conclusions: The addition of 50 μg neostigmine prolonged the duration of sensory and motor block. However, high incidences of side effects and delayed recovery from anesthesia with the addition of 6.25 to 50 μg neostigmine may limit the clinical use of these doses for outpatient spinal anesthesia. (Key words: Ambulatory surgery; electric stimulation; tourniquet pain.)

SPINAL anesthesia with small doses of bupivacaine is a common technique for outpatient anesthesia. Small doses of bupivacaine are appropriate for rapid anesthetic recovery but may result in high anesthetic failure rates (as high as 2%). Intrathecal adjuncts, such as opioids, vasoconstrictors, and α₂-adrenergic agonists, are often added to improve the reliability of outpatient spinal anesthesia. These adjuncts are effective in increasing the success of small-dose bupivacaine spinal anesthesia, but they have side effects that may be limiting. The addition of opioids may result in nausea, pruritus, and respiratory depression. The addition of vasoconstrictors may prolong recovery from anesthesia and may increase the incidence of transient neurologic problems. The addition of α₂-adrenergic agonists may result in bradycardia, hypotension, and sedation. Thus, an ideal adjunct for outpatient spinal anesthesia has not been identified.

Neostigmine is a novel spinal analgesic with potential side effects of nausea and motor weakness. In preliminary dose–response studies in volunteers and patients undergoing surgery, intrathecal neostigmine provided analgesia in doses ≥ 10 μg (in surgical patients) to ≥ 50 μg (in volunteers). Larger doses of neostigmine caused nausea (≥ 100 μg) and lower extremity weakness (≥ 150 μg), but even very large doses (750 μg) did not cause sedation, pruritus, respiratory depression, or hemodynamic depression. These preliminary studies suggest that small doses of neostigmine (≤ 50 μg) could enhance sensory anesthesia with few side effects when added to small-dose bupivacaine spinal anesthesia. This is the first study to assess quantitatively the effects of small doses of neostigmine on sensory block, motor block, and side effects from small-dose bupivacaine spinal anesthesia.
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Materials and Methods

After we received institutional review board approval and informed participant consent, 18 healthy volunteers were enrolled in this study (9 men and 9 women). In a randomized, double-blinded, balanced, crossover design, each participant received two spinal anesthetics with 7.5 mg hyperbaric bupivacaine (0.75% bupivacaine in 8.25% dextrose; Astra USA, Westborough, MA). For one spinal anesthetic, 1 ml dextrose, 5%, was added to the bupivacaine (2 ml total volume). For the other spinal anesthetic, 1 ml dextrose, 5%, containing 6.25, 12.5, or 50 μg neostigmine (Genesia Pharmaceuticals, Irvine, CA) was added to the bupivacaine. Administration of the two spinal anesthetics was separated by at least 48 h in each volunteer.

The participants fasted for 6 h and voided immediately before the studies. Lactated Ringer’s solution was administered via an 18-gauge intravenous catheter as a bolus of 6 ml/kg given progressively in 15 min before subarachnoid block, followed by 8 ml · kg⁻¹ · h⁻¹ for the first hour, and then by a maintenance infusion at a rate of 2 ml · kg⁻¹ · h⁻¹, which was regulated manually by investigators. Lumbar puncture was performed in the left lateral decubitus position with a 25-gauge Whitacre spinal needle (Becton Dickinson, Franklin Lakes, NJ) through a 20-gauge introducer at the L2–L3 interspace using a midline technique. With the spinal needle’s orifice turned cephalad, a volume of 0.2 ml cerebrospinal fluid was aspirated, and the study solution was injected at approximately 0.25 ml/s. Immediately after injection, the volunteers were placed supine and remained in that position for the rest of the study. Heart rate and oxymepohgbilin saturation were recorded using a pulse oximeter (Ohmeda Biox 3700; BOC Group, Liberty Corner, NJ) every 5 min. Blood pressure was recorded from an automated blood pressure cuff (model 90603A; SpaceLabs, Redmond, WA) every 5 min. Respiratory rate and end-tidal carbon dioxide levels were recorded using a nasal cannula (nasal CO2 sample line; Salter Labs, Arvin, CA) from an infrared gas monitor (Poet IQ; Criticare Systems, Milwaukee, WI).

Tolerance of transcutaneous electric stimulation (TES) was tested, as previously described. Transcutaneous electric stimulation leads were placed at three common surgical sites: the midline at the pubis and bilaterally at the knee (8 cm cephalad to the mediolateral prominence of tibial plateau) and the ankle (immediately cephalad to the lateral malleolus of the ankle). Tolerance to TES (nerve stimulator model NS252; Fisher & Paykel, Auck-

land, New Zealand) was assessed 4 min after the spinal solution was injected and tested again every 10 min thereafter by initially testing with 10 mA and then increasing in 10-mA increments to a maximum of 60 mA for 5 s. Sixty milliamperes was used as a cutoff, because previous studies indicated that this intensity of stimulation is comparable to surgical incision. Each TES location was tested in a systematic order, moving from distal to proximal sites, and the duration of tolerance of 60 mA TES after spinal injection was recorded. In addition, dermatomal levels to pinprick (using an 18-gauge needle) were measured every 5 min after the spinal solution was injected until normal pinprick sensation was recovered at S2. Transcutaneous electric stimulation and pinprick data for the left and right sides were averaged at each location.

Tourniquet pain was assessed using previously reported methods. Thirty minutes after injection of the spinal solution, the left lower extremity was exsanguinated by gravity, and a 7-cm orthopedic pneumatic tourniquet was inflated around the left mid thigh to 300 mmHg. During the first session, each volunteer was shown a visual analog scale marked from 0–100 mm, with 0 representing no discomfort and 100 representing the worst discomfort imaginable. The volunteers were told that the tourniquet could be deflated to relieve discomfort at any time. They were then asked to rate their discomfort on the visual analog scale and to memorize the degree of discomfort. During the subsequent session, the volunteers were shown their level of discomfort on the visual analog scale and instructed to request tourniquet deflation at the same level of discomfort. Tourniquets were left inflated until the volunteers requested deflation or for a maximum of 2 h after inflation.

Surface electromyography (MyoTrac 2; Thought Technology, Montreal, Canada) was used to assess 5-isometric maximal force contraction of the right quadriceps, as previously reported. Electromyography pads (Triode, Thought Technology) were placed on the right quadriceps after the skin was shaved and prepared with an abrasive alcohol pad. The electromyography pad was placed 60% of the distance from the greater trochanter to the knee crease at the lateral joint line. The volunteers were trained to contract isometrically the quadriceps muscle with the knee fully extended. Measurements were performed at baseline and every 10 min after injection of the drug solution until return to 90% of baseline. Measurements were performed in triplicate with a
Table 1. Subject Demographics, Side Effects, and Sensory Block to Pinprick from Spinal Bupivacaine with and without Neostigmine

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Dose of Neostigmine Added to Spinal Bupivacaine (7.5 mg)</th>
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<tbody>
<tr>
<td></td>
<td>0 μg (n = 18)</td>
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<tr>
<td>Age (yr)</td>
<td>—</td>
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<td>Height (cm)</td>
<td>—</td>
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<td>Weight (kg)</td>
<td>—</td>
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<tr>
<td>Gender (female/male)</td>
<td>—</td>
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<tr>
<td>Side effects (% incidence)</td>
<td></td>
</tr>
<tr>
<td>Nausea (range of verbal scores in subjects with nausea)*</td>
<td>0 33 (6–9)</td>
</tr>
<tr>
<td>Vomiting*</td>
<td>0 17</td>
</tr>
<tr>
<td>Pruritus*</td>
<td>0 0</td>
</tr>
<tr>
<td>Sedation</td>
<td>17</td>
</tr>
<tr>
<td>Pinprick</td>
<td></td>
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<tr>
<td>Peak level (median and range)</td>
<td>T4 (T7–T1)</td>
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<tr>
<td>Regression to S2 (min)</td>
<td>T5 (T8–T2)</td>
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<td></td>
<td>T4 (T6–T2)</td>
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</table>

Values are mean (standard error) unless otherwise noted. All 18 subjects received one spinal with plain bupivacaine and one spinal with bupivacaine + neostigmine. Verbal scores for severity of nausea ranged from 0 = none to 10 = worst. No subject had a sedation score >2.

* Dose-response relationship between dose of neostigmine and side effect determined with Spearman Rank Correlation (P < 0.004).

1-min resting period between efforts and then averaged at each measurement interval.

Sedation was assessed by a blinded observer every 5 min using a four-point scale (1 = awake, 2 = drowsy but responsive to verbal stimulus, 3 = drowsy but responsive to physical stimulus, 4 = unresponsive). At the end of each study session, the volunteers were asked to give verbal scores (0.0–10.0, with decimals allowed) for the degree of pruritus and nausea. Vomiting initially was treated with ondansetron (4 mg given intravenously in two doses) followed by propofol in 10-mg increments.

The volunteers underwent a simulated clinical discharge pathway. When bilateral sensation to pinprick at the S2 dermatome was recovered, the volunteers tried to walk without assistance. If they could walk, then they tried to void. If ambulation or voiding was unsuccessful, then repeated attempts were made every 15 min. The volunteers were contacted 1 and 7 days after each spinal anesthetic and questioned about any general or neurologic symptoms.

Statistical Analysis

Paired t tests with Bonferroni correction for multiple testing were used to assess differences between the addition of dextrose and neostigmine for the duration of tolerance to TES, motor block, tolerance of thigh tourniquet, and time until discharge criteria were achieved. Differences in heart rate, systolic blood pressure, respiratory rate, end-tidal carbon dioxide level, and oxyhemoglobin saturation were analyzed with repeated-measures analysis of variance followed by post hoc testing with Scheftec’s F test. The incidence of side effects was analyzed using Spearman rank correlation. Significance was defined as P < 0.05.

Results

The demographics of the volunteers in the three neostigmine groups were comparable (table 1). The addition of 50 μg neostigmine to spinal bupivacaine significantly increased the duration of sensory block to TES equivalent to surgical stimulation and the duration of tolerance to pneumatic thigh tourniquet (figs. 1–4). The addition of 50 μg neostigmine to spinal bupivacaine significantly increased the duration of motor block at the quadiceps (fig. 5). The addition of 12.5 and 50 μg neostigmine to spinal bupivacaine significantly increased the time until discharge criteria were achieved (fig. 6).

The addition of neostigmine to spinal bupivacaine produced dose-dependent increases in the incidence of nausea and vomiting (table 1). The addition of neostigmine to spinal bupivacaine did not affect hemodynamic (heart rate and systolic blood pressure) or respiratory (respira-
Fig. 1. The duration of tolerance of transcutaneous electrical stimulation (TES) at the ankle with and without the addition of neostigmine for all 18 volunteers. N = neostigmine. The addition of 50 μg significantly prolonged the duration of the volunteers' tolerance of TES.

Fig. 2. The duration of tolerance of transcutaneous electrical stimulation (TES) at the knee with and without the addition of neostigmine for all 18 volunteers. N = neostigmine. The addition of 50 μg significantly prolonged the duration of the volunteers' tolerance of TES.

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tory rate, end-tidal carbon dioxide, and pulse oximeter saturation) parameters over time. Sedation and pruritus were uncommon in all groups. No volunteer had a sedation score more than 2. At 1 and 7 days after spinal injection, no volunteer reported any problems except mild backache (39%), which resolved by 7 days. The incidence of backache was comparable to the addition of saline or neostigmine. Backaches were mild, required little treatment, and were revealed only after direct questioning.

Discussion

Our data show that the addition of 50 μg neostigmine to bupivacaine spinal anesthesia prolongs the duration...
Fig. 3. The duration of tolerance of transcutaneous electrical stimulation (TES) at the pubis with and without the addition of neostigmine for all 18 volunteers. N = neostigmine. The addition of 50 µg significantly prolonged the volunteers' duration of tolerance of TES.

of sensory and motor block and significantly increases the incidence of nausea and vomiting and the time until discharge criteria are achieved. Smaller doses of neostigmine (6.25 and 12.5 µg) did not significantly prolong sensory and motor block but did increase the incidence of nausea and vomiting and prolong the time until discharge criteria were achieved.

Enhancement of sensory block by neostigmine can be explained by its intrinsic analgesic efficacy. Spinal administration of neostigmine produces analgesia in a novel manner. Neostigmine does not cause nonspecific neural blockade (such as local anesthetics), and it is not a direct agonist for receptors mediating analgesic pathways (such as opioids and α₂-adrenergic agonists). Instead, neostigmine inhibits the breakdown of an endogenous spinal neurotransmitter, acetylcholine, that induces analgesia. Pain, systemic opioids, and spinal α₂-agonists stimulate the release of acetylcholine in the

Fig. 4. The duration of tolerance of pneumatic thigh tourniquet with and without the addition of neostigmine for all 18 volunteers. N = neostigmine. The addition of 50 µg significantly prolonged the duration of tolerance of thigh tourniquet.
spinal cord. Further analgesic effects of acetylcholine may involve the stimulation of nitric oxide production because increased levels of spinal cord nitrite are observed after the spinal administration of acetylcholine.

Our observations have clinical relevance because they suggest that the addition of 50 μg neostigmine will enhance the efficacy of small-dose bupivacaine spinal injections for surgical anesthesia. Our data correspond with those of previous volunteer studies that evaluated the analgesic effects of intrathecal neostigmine alone. These studies also observed a threshold dose for analgesia of approximately 50 μg. In contrast, dose-response studies in patients undergoing surgery showed effective postoperative analgesia in much smaller doses (≥ 10 μg). The enhanced analgesic efficacy of neostigmine in the postoperative setting was proposed...
to result from greater release of spinal acetylcholine from the more intense and more prolonged discomfort of postoperative pain compared with intermittent ice water immersion in volunteers. Therefore, further clinical studies evaluating augmentation of spinal bupivacaine with small doses of neostigmine may be warranted in the surgical setting because intrathecal neostigmine may have greater analgesic effects in that situation.

The addition of 50 μg neostigmine prolonged motor block at the quadriceps from bupivacaine spinal anesthesia. Large doses of intrathecal neostigmine alone can cause lower-extremity motor weakness in animals and volunteers (≥ 150 μg) because of an acetylcholine-mediated reduction in motor neuron outflow. In addition to the potential direct inhibition of motor activity by administration of neostigmine, we speculate that increased spinal levels of acetylcholine may augment motor block as a result of axonal conduction block from spinal bupivacaine. Neostigmine-enhanced motor block from spinal bupivacaine may be useful in the clinical setting. Many lower-extremity surgical procedures require muscle relaxation, and spinal bupivacaine alone provides only modest motor block.

Despite enhancement of sensory and motor block, we suggest that the clinical usefulness of neostigmine, in the doses studied, may be limited as an adjunct for small-dose bupivacaine spinal anesthesia because of side effects and prolongation of the time until discharge criteria are achieved. The addition of even the smallest dose of neostigmine (6.25 μg) produced a high incidence of nausea and vomiting. Nausea and vomiting were delayed in onset (60–90 min after spinal injection), severe (see verbal nausea scores in table 1), repetitive, prolonged (2–6 h), and resistant to pharmacologic therapy. Ondansetron was ineffective and propofol was effective only briefly in treating nausea and vomiting. Previous studies reported similar difficulty in preventing or treating nausea and vomiting with spinal neostigmine. In contrast to our findings, previous volunteer studies observed nausea and vomiting with doses greater than 50 μg neostigmine alone. However, clinical studies in patients undergoing surgery observed nausea (50%) and vomiting (16.5%) with intrathecal doses as small as 10 μg when administered with epidural anesthesia. Because central neuraxial anesthesia (epidural and spinal) alone is associated with nausea and vomiting, the interaction of spinal anesthesia and intrathecal neostigmine may explain severe nausea and vomiting with such small doses (6.25–50 μg) of neostigmine.

The addition of 12.5 and 50 μg neostigmine prolonged the time until discharge criteria were achieved. Discharge criteria used for this study were return of pinprick sensation to a dermatomal level of S2 bilaterally and the ability to walk and to urinate. The magnitude of delay in achievement of discharge criteria with the addition of neostigmine was either comparable to or greater than the prolongation of sensory and motor block. Assuming that these results are consistent in patients undergoing surgery, an increased duration of sensory and motor block from the addition of neostigmine would be offset by a comparable or greater delay until patient discharge.

Mild decreases in heart rate and blood pressure developed in our volunteers with spinal bupivacaine, but they were not affected by the addition of neostigmine. Spinal neostigmine alone increases the activity of sympathetic neurons counteracts the sympatholytic effects of spinal anesthesia, and prevents hypotension during spinal anesthesia in animals. However, cardiovascular stimulatory effects of spinal neostigmine are seen only with large doses (750 μg) in humans. Our use of much smaller doses of neostigmine probably explains the lack of effect of neostigmine on hemodynamics during spinal anesthesia.

We used a commercially available neostigmine preparation containing methyl and propylparabens as antioxidants. A preservative-free preparation of neostigmine would be preferable but is no longer available (since 1996). Previous animal and human testing of paraben-containing neostigmine solutions have not yielded behavioral or histopathologic evidence of neurotoxicity after spinal administration. Our small study adds further evidence of the safety of spinal administration of paraben-containing neostigmine because no volunteer had signs or symptoms of neurotoxicity 1 or 7 days after spinal administration.

Certain aspects of our study design merit discussion. We evaluated only one dose of spinal bupivacaine. However, this dose is clinically relevant and is suitable for ambulatory anesthesia. We used a crossover design to maximize our ability to detect the effects of the addition of neostigmine. Previous studies noted large intersubject variability with small doses of bupivacaine, whereas repeated spinal anesthesia with bupivacaine is consistent within the same subject.

In conclusion, the addition of 50 μg neostigmine to bupivacaine spinal anesthesia prolonged the duration of sensory and motor block and increased the incidence of nausea and vomiting and the time until discharge criteria were achieved. Smaller doses of neostigmine (6.25 and
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12.5 µg) did not significantly enhance sensory or motor block but did increase the incidences of nausea and vomiting and the time until discharge criteria were achieved. The high incidence of nausea and vomiting and the prolonged time until discharge criteria were achieved suggests that the clinical usefulness of neostigmine is limited, in these doses, for outpatient spinal anesthesia.

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