

Transdermal Nitroglycerine Enhances Spinal Neostigmine Postoperative Analgesia following Gynecological Surgery

Gabriela R. Lauretti, M.D., M.Sc., Ph.D., Ana-Paula M. Oliveira, M.D.,† Maria-do-Carmo C. Julião, M.D.,† Marlene P. Reis, M.D., M.Sc., Ph.D.,‡ Newton L. Pereira, B.Pharm., M.Sc., Ph.D.§*

Background: **Intrathecal neostigmine causes analgesia by inhibiting the breakdown of acetylcholine. Experimental data suggest that the production of endogenous nitric oxide is necessary for tonic cholinergic inhibition of spinal pain transmission. The purpose of this study was to determine whether association of transdermal nitroglycerine would enhance analgesia from a low dose of intrathecal neostigmine in patients undergoing gynecologic surgery during spinal anesthesia.**

Methods: **Forty-eight patients were randomized to one of four groups. Patients were premedicated with use of 0.05–0.1 mg/kg intravenous midazolam and received 15 mg bupivacaine plus 1 ml test drug intrathecally (saline or neostigmine, 5 μg). Twenty to 30 min after the spinal puncture, a transdermal patch of either 5 mg nitroglycerin or placebo was applied. The control (Con) group received spinal saline and transdermal placebo. The neostigmine group received spinal neostigmine and transdermal placebo. The nitroglycerin group received spinal saline and a transdermal nitroglycerine patch. Finally, the neostigmine–nitroglycerin group received spinal neostigmine and transdermal nitroglycerine. Pain and adverse effects were evaluated using a 10-cm visual analog scale.**

Results: **Patients in the groups were similar regarding age, weight, height, and American Society of Anesthesiologists status. Sensory level to pin prick at 10 min, surgical duration, anesthetic duration, and visual analog scale score for pain at the time of administration of first rescue medication were statistically the same for all groups. The time to administration of first rescue analgesic (min) was longer in the neostigmine–nitroglycerin group (550 min; range, 458–1,440 min; median, 25–75th percentile) compared with the other groups ($P < 0.001$). The neostigmine–nitroglycerin group required fewer rescue analgesics in 24 h than did the control group ($P < 0.0005$), whereas the neostigmine group required less analgesics compared with the control group ($P < 0.02$). The incidence of perioperative adverse effects (nausea, vomiting, headache, back pain) was similar among groups ($P > 0.05$).**

Conclusion: **Although neither intrathecal 5 μg neostigmine alone nor transdermal nitroglycerine alone (5 mg/day) delayed the time to administration of first rescue analgesics, the combination of both provided an average of 14 h of effective postoperative analgesia after vaginoplasty, suggesting that transdermal nitroglycerin and the central cholinergic agent neostigmine may enhance each other's antinociceptive effects at the dose studied. (Key words: Intrathecal anticholinesterase; nitric oxide donator.)**

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* Assistant Professor of Anesthesiology, † Postgraduate Student, ‡ Associate Professor of Anesthesiology, Department of Surgery, Orthopedics, and Traumatology, Faculty of Medicine; § Assistant Professor of Pharmacy, Faculty of Pharmacy, Department of Pharmacy and Sciences, Ribeirão Preto, University of São Paulo.

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Address reprint requests to Dr. Lauretti: Rua-Campos Sales, 330, apto. 44, Ribeirão Preto- São Paulo, Brazil 14015 110. Address electronic mail to: grlauret@fmrp.usp.br. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

INTRATHECAL neostigmine causes dose-dependent postoperative analgesia[1–3] by inhibiting the breakdown of acetylcholine in the dorsal horn[4,5] and spinal meninges.[6] Acetylcholine may cause analgesia through direct action on spinal cholinergic muscarinic receptors M_1 and M_3,[7] and nicotinic[8] receptors subtypes and indirectly through stimulation of release of the second-messenger nitric oxide in the spinal cord.[9]

The purpose of this study was to determine whether the application of transdermal nitroglycerine would enhance analgesia from single intrathecal low-dose neostigmine administration in patients undergoing gynecologic surgery during intrathecal anesthesia.

Methods

The Ethical Committee of the Teaching Hospital of the University of São Paulo, Ribeirão Preto, approved the study protocol. After giving informed consent, 48 patients, American Society of Anesthesiologists physical status I and II, scheduled to undergo vaginoplasty were randomized with use of a computer to one of four groups and prospectively studied using a placebo-controlled double-blind design to evaluate analgesia and adverse effects. The concept of visual analog scale (VAS), which consisted of a 10-cm line, with 0 equaling "no nausea" or "no pain at all" and 10 equaling "worst possible nausea" or "the worst possible pain" was introduced before surgery.

Patients were premedicated with use of 0.05–0.1 mg/kg intravenous midazolam in the holding room. Hydration consisted of 10 ml/kg Ringer's lactate solution preoperatively and 10 $ml \cdot kg^{-1} \cdot h^{-1}$ after spinal anesthesia. Spinal anesthesia was performed in the operating room at the L3–L4 interspace, with the patient in the sitting position. A volume of 4 ml was injected over 30 s through a 25-gauge spinal needle. The intrathecal drugs included 15 mg hyperbaric bupivacaine, 0.5% (3 ml), plus the test drug (1 ml). Patients were placed in the supine position immediately after spinal injection. The

Table 1. Groups

	Control Group	Neostigmine Group	Nitroglycerine Group	Neostigmine–Nitroglycerine Group
Intrathecal supplement (1 ml)	Saline	Neostigmine (5 μg)	Saline	Neostigmine (5 μg)
Transdermal test drug	Placebo	Placebo	Nitroglycerine (5 mg/24 hours)	Nitroglycerine (5 mg/24 hours)

transdermal patch was applied at the thorax (ventral, between T2–T4), in a nonanesthetized area, 20–30 min after the spinal puncture (after hemodynamic stabilization). The drugs (intrathecal and transdermal) were prepared by one anesthesiologist. A second anesthesiologist who was blind to the drugs selected performed the lumbar puncture and drug injection, stayed during the intraoperative period, and checked postoperative analgesia and adverse effects. The groups (control, neostigmine, nitroglycerin, and neostigmine–nitroglycerin) are described in table 1.

Intraoperative sensory loss assessment included the pin prick test 10 min after spinal injection. Blood pressure was monitored noninvasively every 5 min throughout surgery, and heart rate and oxyhemoglobin saturation (Spo_2) were monitored continuously during surgery. A decrease in mean arterial pressure greater than 15% below the preanesthetic baseline value was treated by incremental doses of ephedrine, 4 mg intravenously. Decreases in heart rate below 50 beats/min were treated with incremental doses of atropine, 0.25 mg intravenously. Intraoperative nausea was scored by the patient using the 10-cm VAS N (nausea). The number of patients having nausea (of any degree) or vomiting at any point intraoperatively was noted. Nausea greater than 2 cm on a 10 cm scale at any time or vomiting during the study were treated initially with 10 mg intravenous metoclopramide, followed by 0.5 mg intravenous droperidol, if necessary. For patients who had more than one episode of nausea, the VAS scores were averaged.

Postoperative assessment included pain scores, adverse effects and the duration of motor block, measured from anesthetic injection until the time to reach a Bromage score of 2.[10] Patients were allowed to receive rescue analgesics, and there was always someone from the staff available to administer the analgesic at the time requested. Intramuscular diclofenac (75 mg) was available. Pain was assessed at the time of administration of first rescue analgesic and 24 h after the spinal puncture by the anesthesiologist who was blind to the treatment. Nausea and occurrence of vomiting were continuously assessed intraoperatively and 24 h after the spinal puncture by the same anesthesiologist, blind to the treatment. Duration of effective analgesia was measured as time from the intrathecal drug administration to the patient's first request for analgesic administration, either in the recovery room or the infirmary, and was recorded in minutes. The VAS at the time of administration of first rescue analgesic medication was measured using the 10-cm VAS. The 24-h VAS pain score and VAS for nausea reflected the patient's overall impression of the 24 h after spinal injection.

Statistical Analysis

The number of subjects was based on preliminary experimental data. We hypothesized that intrathecal neostigmine would increase the time to administration of first rescue analgesic by 20% in the population studied and that the association of transdermal nitroglycerin patch would increase the time to administration of first rescue analgesic by 100% when compared with the control group. If we estimated a standard deviation for this prospective power analysis as 40% and an α value of 0.05, these assumptions would necessitate inclusion of five patients in each group to show a 100% increase in the time to administration of the first rescue analgesics. To further increase the power, we elected to observe 12 patients in each group.

The normality of the distributions was assessed using the Shapiro-Wilk test. Groups were compared with regard to demographic data (age, weight, height) and duration of surgery with use of one-way analysis of variance. Incidence of adverse events, gender, American Society of Anesthesiologists physical status, and adjuvant drug use were compared among groups with use of the chi-square test corrected for multiple comparisons. Probability was considered to be significant if less than 0.0125. Blood pressure, heart rate, level of anesthesia (pin prick), and VAS scores were compared among groups by two-way analysis of variance for repeated measures.[11] Tukey analysis was applied to decrease the probability of type I error. The time to administration of first rescue analgesics was compared using the Kruskal-Wallis test, applied with a nonparametric multiple comparison procedure, and the Wilcoxon rank sum test. Time to administration of first rescue analgesics is ex

Table 2. Demographic Data

Group	ASA Class (I/II)	Age (yrs)	Weight (kg)	Height (cm)
Con group	5/5	51 ± 12	60 ± 13	154 ± 6
Neo group	4/6	52 ± 13	65 ± 13	155 ± 9
Ntg group	4/7	47 ± 12	67 ± 18	156 ± 5
Neo–Ntg group	3/7	53 ± 12	66 ± 12	156 ± 6
P	0.9999	0.7609	0.6789	0.3338

Data are mean ± SD.

ASA = American Society of Anesthesiologists; Con = control; Neo = neostigmine; Neo–Ntg = neostigmine–nitroglycerine; Ntg = nitroglycerine.

Table 3. Intraoperative Data

Group	Pinprick (10 min)*	Surgical Time (min)	Anesthetic Time (min)	Ephedrine (mg)
Con group	8 (6–8)	119 ± 37	149 ± 22	4 ± 8
Neo group	8 (7–8)	105 ± 50	146 ± 30	5 ± 9
Ntg group	8 (6–8)	98 ± 30	138 ± 34	2 ± 2
Neo–Ntg group	8 (6–10)	97 ± 34	138 ± 35	7 ± 5
P	0.2958	0.2716	0.1118	0.7899

Data are mean ± SD. No statistically significant differences were observed. Median (25–75% percentile confidence).

* Pinprick refers to thoracic dermatome anesthesia to a pinprick on the skin.

Con = control; Neo = neostigmine; Neo–Ntg = neostigmine–nitroglycerine; Ntg = nitroglycerine.

pressed as the median (25–75th percentile). $P < 0.05$ was considered to be significant. Data are expressed as mean ± SD, unless otherwise stated.

Results

The four groups showed no differences regarding American Society of Anesthesiologists physical status, age, weight, and height ($P > 0.05$; table 2). The sensory level to pin prick at 10 min, surgical and anesthetic time, and intraoperative ephedrine consumption were similar among groups (table 3).

The postoperative data are represented in table 4. The pain VAS score at the time of administration of first rescue analgesic medication was similar among the four groups ($P > 0.05$). The time to administration of first rescue analgesic medication (min) was longer in the neostigmine–nitroglycerin group compared with the other groups ($P < 0.001$). The analgesic consumption during the first 24 h postoperatively was less for the neostigmine–nitroglycerin compared with the control group ($P < 0.0005$) and also less for the neostigmine group compared with the control group ($P < 0.02$). The analgesic consumption of the other groups were similar.

There were no differences regarding the incidence of perioperative adverse effects ($P > 0.05$). Intraoperatively, none of the patients reported nausea or vomiting. Postoperatively, one patient from the control group reported back pain (VAS 5 cm) and another experienced one episode of vomiting. One patient from the nitroglycerine group reported a headache (VAS 3 cm); another reported back pain (VAS 4 cm); and one other experienced one episode of vomiting. One patient from the neostigmine group reported back pain (VAS 5 cm); another patient reported pain in the knee (VAS 4 cm); and another reported flatulence. Two patients from the neostigmine–nitroglycerin group vomited. The mean overall 24-h nausea VAS score was similar among groups ($P > 0.05$).

Discussion

The results of this study showed that, although neither 5 μg spinal neostigmine nor transdermal nitroglycerine alone delayed the time to administration of first rescue analgesic, the association of 5 mg/day transdermal nitroglycerin patch and intrathecal low-dose neostigmine (5 μg) resulted in an average of 14 h of postoperative analgesia after vaginoplasty during bupivacaine spinal block, compared with 3.5 h in the control group. Nevertheless, the lack of a direct action on the time to administration of first rescue analgesic, patients who received 5 μg intrathecal neostigmine only as analgesic had a lower analgesic consumption during the first 24 h of observation, compared with the control group. This lower consumption may reflect some analgesic effect of the dose used in the population studied.

Previously, we demonstrated that spinal neostigmine causes dose-dependent analgesia in patients undergoing vaginoplasty.[1] Intrathecal neostigmine doses varying from 10 μg to 200 μg were limited by side-effects (namely, nausea and vomiting) when the doses varied between 25 and 200 μg.[2,3,12] In addition, intrathecal neostigmine was more efficient for somatic rather than visceral pain,[13] and the female population may have an advantage with neostigmine analgesia because of the sex difference in spinal cholinergic analgesic nicotinic mecha-

Table 4. Postoperative Data

	Con Group	Neo Group	Ntg Group	Neo–Ntg Group	*P*
Time to first rescue Analgesic (min)	210 (189–245)	420 (178–470)	370 (242–430)	550 (458–1440)	*
VAS at first rescue analgesic	8 ± 2	7 ± 2	7 ± 1	7 ± 2	0.7418
No. IM diclofenac dose injections in 24 h	3 (3–4)	2 (1–2)	2 (1–2)	1 (0–2)	†
Overall 24-h VAS pain	2.2 ± 1.8	1.3 ± 1.2	0.8 ± 0.9	0.7 ± 1	0.3635
Overall 24-h VAS N/V	0.5 ± 1	0.4 ± 1.5	0.5 ± 1	0.7 ± 1.6	0.9999

Time to first rescue analgesic and number of IM diclofenac are expressed as median (25–75% percentile confidence). Other data are mean ± SD.

* Neo–Ntg group > Con group = Neo group = Ntg group. Neo–Ntg group > Con group ($P = 0.000529$); Neo–Ntg group > Neo group ($P = 0.007228$); Neo–Ntg group > Ntg group ($P = 0.0008678$).

† Neo–Ntg group < Con group ($P = 0.000308$); Neo group < Con group ($P = 0.010019$).

Con = control; IM = intramuscular; N/V = nausea and vomiting; Neo = neostigmine; Neo–Ntg = Neostigmine–Nitroglycerine; VAS = visual analogue scale.

nisms.[7] All data directed us to further study female patients undergoing a purely somatic painful stimuli, *i.e.*, vaginoplasty surgery.

Intrathecal neostigmine antinociception is secondary to acetylcholine release and action in the spinal cord tissue.[4,5] During surgical stimuli, a preexistent spinal cholinergic tonus is activated.[14] The presence of acetylcholine in the cerebrospinal fluid has been shown in humans.[15] Acetylcholine from this physiologic cholinergic mechanism and acetylcholine preserved from cholinesterase activity after intrathecal neostigmine will bind to muscarinic[7] and nicotinic[8] nerve terminals in the spinal cord. The remaining acetylcholine that reaches the cerebrospinal fluid is also preserved from cholinesterase activity located within the spinal meninges.[6] This would increase acetylcholine cerebrospinal fluid concentration and improve acetylcholine bioavailability at cholinergic nerves within the spinal cord.

The transdermal nitroglycerin patch has been related to nitric oxide formation during degradation of organic nitrates.[16] In accordance to animal[17] and clinical research,[18] nitric oxide generators did not result in analgesia. Nevertheless, a current study provides evidence that acetylcholine stimulate nitric oxide synthesis in the spinal cord,[9] and this synthesis is necessary for the expression of analgesia secondary to the cholinomimetic agents,[19] such as spinal neostigmine, as much as behavioral analgesia from intrathecal injection of muscarinic agonists in rats is inhibited by nitric oxide synthase blockers.[20]

In addition, the activation of descending pain pathways involves the participation of nitric oxide, which mechanisms of action are likely to include activation of second messengers such as cyclic guanosine monophosphate (cGMP).[21] Wide-dynamic-range neurons in the superficial dorsal horn and high-threshold cells in the superficial or deep layers show reduced response after exposure to cyclic guanosine monophosphate.[22] Therefore, analgesia would be a result of predominant analgesic action on superficial spinal layers.

Anatomic evidence also supports the connection between nitric oxide and acetylcholine. Nitric oxide synthase colocalizes in dorsal horn neurons that contain choline acetyl transferase.[23] Nitric oxide synthase is localized to the superficial dorsal horn and the intermediolateral cell column regions of the spinal cord.[24] Muscarinic receptors have been identified on spinal cord dorsal horn and intermediolateral cell columns.[25]

In conclusion, although intrathecal neostigmine alone (5 μg) or transdermal nitroglycerine alone (5 mg/day) (a nitric oxide generator) did not delay the time to administration of first rescue analgesic, the association of both provided 14 h of postoperative analgesia after vaginoplasty surgery, suggesting that nitric oxide and cholinergic receptors may enhance each other's antinociceptive effects at the dose studied.

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