CLINICAL INVESTIGATIONS

Anesthesiology
82:331–343, 1995

Phase I Safety Assessment of Intrathecal Neostigmine Methylsulfate in Humans

David D. Hood, M.D., James C. Eisenach, M.D.,* Robin Tuttle, R.N.†*

Background: **In dogs, sheep, and rats, spinal neostigmine produces analgesia alone and enhances analgesia from α_2-adrenergic agonists. This study assesses side effects and analgesia from intrathecal neostigmine in healthy volunteers.**

Methods: **After institutional review board approval and informed consent, 28 healthy volunteers were studied. The first 14 volunteers received neostigmine (50–750 μg) through a #19.5 spinal needle followed by insertion of a spinal catheter. The remaining 14 volunteers received neostigmine through a #25 or #27 spinal needle without a catheter. Safety measurements included blood pressure, heart rate, oxyhemoglobin saturation, end-tidal carbon dioxide, neurologic evaluation, and computer tests of vigilance and memory. Analgesia in response to ice water immersion was measured.**

Results: **Neostigmine (50 μg) through the #19.5 needle did not affect any measured variable. Neostigmine (150 μg) caused mild nausea, and 500–750 μg caused severe nausea and vomiting. Neostigmine (150–750 μg) produced subjective leg weakness, decreased deep tendon reflexes, and sedation. The 750-μg dose was associated with anxiety, increased blood pressure and heart rate, and decreased end-tidal carbon dioxide. Neostigmine (100–200 μg) in saline, injected through a #25 or #27 needle, caused protracted, severe nausea, and vomiting. This did not occur when dextrose was added to neostigmine. Neostigmine by either method of administration reduced visual analog pain scores to immersion of the foot in ice water.**

Conclusions: **The incidence and severity of these adverse events from intrathecal neostigmine appears to be affected by dose, method of administration, and baricity of solution. These effects in humans are consistent with studies in animals. Because no unexpected or dangerous side effects occurred, cautious examination of intrathecal neostigmine alone and in combination with other agents for analgesia is warranted. (Key words: Analgesia. Anesthesia, spinal. Cholinesterase, inhibitors: neostigmine. Nausea. Pain.)**

This article is accompanied by an editorial. Please see: Collins JG: Spinally administered neostigmine: Something to celebrate. ANESTHESIOLOGY 82:327–328, 1995.

INTRATHECAL neostigmine represents a novel approach to providing analgesia. Unlike local anesthetics, neostigmine does not cause nonspecific axonal blockade. Unlike opioids and α_2-adrenergic agonists, neostigmine is not a direct agonist, stimulating all receptors of a certain type, including those responsible for unwanted side effects. Rather, neostigmine inhibits breakdown of an endogenous spinal neurotransmitter, acetylcholine, which has been shown to cause analgesia.[1–4] Because acetylcholine has actions at other spinal sites (inhibition of motoneuron activity, excitation of sympathetic outflow),[1,3,5] the degree to which analgesia and these side effects can be separated after spinal neostigmine administration will depend on the amount of tonic release of acetylcholine at each of these sites.

Before clinical trials of a new agent for spinal use, a series of toxicologic assessments in animals is required.[6,7] In the case of neostigmine, safety assessment primarily is restricted to actions in the spinal cord and central nervous system, because actions, side effects, and treatment of side effects of systemically absorbed neostigmine are well established.[8] The three major toxicologic aspects of intrathecal neostigmine of interest are its pharmacologic effects, effect on spinal cord blood flow, and effect on neural tissue and function. These three aspects have been evaluated extensively after lumbar, thoracic, and cervical intrathecal injection of neostigmine in sheep and lumbar intrathecal injection in rats and dogs, which have included cardiorespiratory and behavioral monitoring, spinal cord blood flow measurement, cerebrospinal fluid (CSF) chemistry, and histopathologic examination.[9–13] Because these animal studies raised no toxicologic concerns and demonstrated

* Associate Professor of Anesthesia.

† Research Nurse.

Received from the Department of Anesthesia, Wake Forest University Medical Center, Winston-Salem, North Carolina. Submitted for publication May 10, 1994. Accepted for publication September 29, 1994. Supported in part by National Institutes of Health grants GM48085 and M01 RR07122.

Address correspondence and reprint requests to Dr. Hood: Department of Anesthesia, Wake Forest University Medical Center, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. Address electronic mail to: dhood@isnet.is.wfu.edu.

efficacy, proceeding to cautious phase I clinical safety studies is appropriate.

Initial clinical trials of any new agent or new route of administration are typically performed using an open-label, dose-escalating design, generally in healthy patients or volunteers.[14] Although efficacy may be examined, the focus is to assess safety and estimate the relationship between dose and incidence of side effects. Safety concerns of particular interest for assessment in a phase I study of spinal neostigmine are possible hypertension, tachycardia, urinary retention, motor weakness, and cephalad movement in CSF, which could lead to a central cholinergic crisis.

The purposes of this study were fourfold: (1) to describe and assess dose-dependent effects of spinal neostigmine on blood pressure, heart rate, a sympathetic-mediated reflex, neurologic function, and vigilance; (2) to sample CSF to define pharmacokinetics and pharmacodynamics of neostigmine in causing these effects and to correlate these changes with changes in CSF acetylcholine and other neurotransmitters; this will be the subject of a subsequent report; (3) to examine whether adverse events from spinal neostigmine differ between the experimental setting, in which a large-gauge spinal needle and catheter are used, and the clinical setting, in which a single injection with a small-gauge needle would be used; and (4) to provide preliminary, descriptive evidence concerning efficacy of spinal neostigmine in producing analgesia.

Methods

The study was divided into two parts: an initial study of 14 volunteers in whom spinal neostigmine was injected through a large-gauge needle and a spinal catheter inserted for CSF sampling, and a second study of 14 other volunteers who received a single injection of spinal neostigmine through a small-gauge needle. Both studies were approved by the Clinical Research Practices Committee, written informed consent was obtained, and volunteers reported to the inpatient General Clinical Research Center at 7 AM, having had nothing to eat or drink since midnight. In each study, a peripheral intravenous catheter was inserted for infusion of lactated Ringer's solution at 50–100 ml/h and a second intravenous catheter inserted and capped for sampling of venous blood. The venous blood analysis data are the subject of another report concerning neostigmine pharmacokinetics. Baseline measures were taken before neostigmine injection and thereafter as indicated.

All patients were closely monitored by a research nurse and/or one of the physician authors until they were stable and without significant ongoing symptoms. If the volunteer was stable after the 6th hour of measurements, the volunteer was cared for by the nursing personnel in the General Clinical Research Center until the 12- and 24-h points, when one of the investigators evaluated the volunteer (part 1, catheter study). Volunteers participating in part 2, spinal needle injection of neostigmine, were discharged home when they were without significant symptoms (6–12 h). After the study, each volunteer was contacted by phone daily for 5 days and at 2 weeks, 1 month, and 6 months. Volunteers were questioned about symptoms of headache or neurologic complaints.

Part 1: Catheter Study

Drug Administration. Based upon data obtained in animals, an initial dose of 50 μg neostigmine was chosen as likely to be approximately <fr1/2> the minimal therapeutic dose. In this dose-escalation design, the first four volunteers received 50 μg neostigmine, the next four received 150 μg, the next four received 500 μg, and the last two received 750 μg. According to the study design, escalation to the next higher dose would occur only in the absence of serious side effects at the previous dose, defined as more than one of four volunteers experiencing >30% change in mean arterial pressure or heart rate or evidence of central cholinergic syndrome (confusion, ataxia) not responsive to therapy. Only two volunteers were studied at the 750 μg dose because of severe side effects. Neostigmine was diluted in a 4-ml volume with preservative-free normal saline and injected through a Sprotte-tipped, 19.5-G spinal needle that had been inserted at a lower lumbar interspace. Two minutes after injection, a 21-G catheter was inserted through the spinal needle and the needle withdrawn. CSF samples (total withdrawn volume per sample = 1.5 ml) were obtained 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360, 720, and 1,440 min after neostigmine injection for neostigmine and neurochemical assays. The results of these assays will be the subject of a subsequent report.

Cardiorespiratory Monitoring. Blood pressure and heart rate were measured by a noninvasive oscillometric device every 5 min after injection for 60 min, then every 15 min for 2 h, every 30 min for 2 h, hourly for 6 h, and at 12, 18, and 24 h after spinal injection. Oxyhemoglobin saturation by pulse oximetry, end-tidal

carbon dioxide by capnography, and respiratory rate were measured before and at 30 and 60 min, hourly until 6 h, and at 12 and 24 h after injection. Both finger and toe skin blood flows were determined by laser Doppler at these same times. Skin temperature was heated to 37°C before Doppler flow measurements with a regulated heating pad and confirmed by temperature measurement with a thermistor taped to the finger or toe. In addition to baseline flow, activity of a sympathetic-mediated reflex was determined at these times by measuring changes in skin blood flow to a deep inspiratory gasp.[15]

Neurologic Monitoring. Cephalad spread of neostigmine in CSF from lumbar to cervical and brainstem sites is possible. Assuming spinal neostigmine has a local spinal cord effect, concurrent neurologic and analgesia screening of lower and upper extremities, along with the computerized screening of cognitive function, might provide evidence for cephalad migration of neostigmine in CSF.

At the same times as Doppler blood flow measurements, a screening neurologic examination was performed, consisting of assessment of extraocular movements and, in both upper and lower extremities, light touch, cold temperature, gross motor strength, and deep tendon reflexes. The results of each of the extremity neurologic tests were scored as increased, decreased, absent, or equal to the baseline findings. In addition, volunteers were questioned regarding any unusual sensations, including subjective weakness. Four tests were used to screen for central cholinergic stimulation at these same times: pupil size was determined using a pupillometer (Essilor, Creteil, France), short-term memory and attention were tested using standardized and validated tests on a computer-based testing station (Psychological Software Services, Indianapolis, IN), and motor coordination was tested using a standardized peg board (Smith & Nephew Roylan, Germantown, WI). Computerized tests consisted of the flasher test, in which short-term memory is tested by recalling the number of small boxes that appear briefly on the screen, and the p-q test, in which attention is tested by pressing a key to time the recognition of one letter in any array that changes from p to q or from q to p. In addition, level of sedation was measured with a 10-cm visual analog scale (VAS) anchored at "not drowsy at all" and "as drowsy as possible," and anxiety was measured with a 10-cm VAS anchored at "not anxious at all" to "as anxious as possible."

Analgesia. After CSF sampling and Doppler blood flow measurements, pain report by 10-cm VAS was obtained after immersion of hand and, 5 min later, foot in stirred ice water. A 60-s cutoff time was used, although volunteers were allowed to remove their hand or foot before this time if they experienced unbearable pain.

Part 2: Small Spinal Needle Study

Drug Administration. Based on part 1 of the current study, we chose a dose of 500 μg as a dose that would produce near-maximum analgesia without severe side effects. Because it is possible that neostigmine could be removed from the intrathecal space by the large dural hole and withdrawal through the catheter in part 1 but might not after single injection with a small-gauge spinal needle, we arbitrarily reduced the initial dose of neostigmine in part 2 to 200 μg through a #25 or #27 Whitacre spinal needle. The first volunteer received 200 μg neostigmine and experienced protracted, severe side effects, as did the second volunteer, who received 100 μg. The third volunteer had only mild side effects after 50 μg neostigmine, as discussed in the results. These three volunteers received neostigmine in a 2-ml volume of normal saline. We speculated that greater cephalad spread of neostigmine by this method of administration (*i.e.,* without catheter insertion and aspiration of CSF) was the cause of the severity of side effects and that injection in hyperbaric solution might diminish the likelihood of these side effects. The next volunteer received 50 μg neostigmine in hyperbaric solution (5% dextrose in saline) and did not experience severe nausea. The remaining ten volunteers received neostigmine, 100 μg (n = 5) or 200 μg (n = 5), in 1 ml containing 5% dextrose. All dextrose-containing injections were administered in the sitting position, and the head of the bed was elevated at least 30° throughout the study. In all volunteers in this second part, a second #25 or #27 Whitacre needle was inserted at the same lumbar interspace 60 min after neostigmine administration, and 3 ml CSF was aspirated for neostigmine and neurochemical assays (to be reported in a subsequent pharmacokinetic analysis). This study ended 6 h after spinal injection.

Cardiorespiratory Monitoring. Blood pressure, heart rate, oxyhemoglobin saturation, and end-tidal carbon dioxide were measured as described in part 1, but no measurements were obtained beyond 6 h from spinal injection. Skin blood flow measurements were not obtained.

Table 1. Volunteer Demographics in Part 1: Catheter Study

Gender	F (n = 9)/M (n = 5)
Age (yr)	38 ± 1.7
Height (cm)	167 ± 2.8
Weight (kg)	75 ± 3.8

Values are mean ± SEM.

Neurologic Monitoring. Screening neurologic examinations and pupil size by pupillometry were obtained as described in part 1, but no measurements were obtained beyond 6 h from spinal injection. Computer and peg board tests were not performed.

Analgesia. Foot and hand analgesia measurements were obtained as described in part 1, but no measurements were obtained beyond 6 h from spinal injection.

Drugs. Atropine was obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ). Glycopyrrolate was obtained from AH Robins Co., Inc. (Richmond, VA). Hydralazine was obtained from Ciba Pharmaceutical Co. (Summit, NJ). Phenergan was obtained from Wyeth-Ayerst Laboratories (Philadelphia, PA). Midazolam was obtained from Roche Laboratories (Nutley, NJ). Ondansetron was obtained from Ceremax (Research Triangle Park, NC). Neostigmine was obtained under IND approval by the Food and Drug Administration in preservative-free saline from International Medication Systems, Ltd. (El Monte, CA). Neostigmine from this same commercial source had been used in preclinical toxicity studies.

Statistics. Unless otherwise indicated, data are presented as mean ± SEM. Statistical comparisons were not performed in this open-labeling, dose-ranging, initial safety assessment. Rather, descriptive statistics are provided to aid in power calculations for future hypothesis-driven protocols, as described in the Discussion.

Results

Part 1: Catheter Study

General Observations. Demographic variables are shown in table 1. Spinal neostigmine caused nausea and vomiting, which increased in intensity and duration with increasing dose (table 2). Some volunteers complained of abdominal cramping before the onset of nausea, suggesting the possibility of increased peripheral cholinergic effects. Treatment of severe nausea and vomiting included 0.2–0.75 mg intravenous glycopyrrolate in one volunteer receiving 500 μg and both volunteers receiving 750 μg neostigmine and 0.8 mg intravenous atropine in one volunteer receiving 750 μg. These treatments may have caused minor reductions in nausea but did not abolish it. In some cases, severe nausea and vomiting interfered with the ability to obtain other experimental measures.

Several genitourinary symptoms were observed after higher doses of spinal neostigmine. Urinary retention occurred in two female volunteers, receiving 150 and 500 μg neostigmine, although both subsequently voided within 4 h of neostigmine injection. Both volunteers receiving 750 μg neostigmine, one female and one male, had fecal incontinence, and the female volunteer also had urinary incontinence. It was unclear whether this was due to protracted retching and vomiting or a direct action of the drug. Two volunteers, one receiving 150 μg and one receiving 750 μg, had a transient sensation of urinary urgency but did not void. One male volunteer receiving 500 μg neostigmine experienced a sense of urinary urgency followed by ejaculation. One woman receiving 500 μg neostigmine experienced painless, rhythmic vaginal muscular contractions.

Cardiorespiratory Effects. The observational nature of this small initial phase I safety study precludes

Table 2. Incidence of Nausea and Vomiting in Part 1: Catheter Study

Dose	Time (h)									Cumulative Incidence
	0	0.5	1	2	3	4	6	12	24	
50 μg (n = 4)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0%/0%
150 μg (n = 4)	0/0	0/0	1/0	2/0	0/0	0/0	0/0	0/0	0/0	75%/0%
500 μg (n = 4)	0/1	1/0	1/0	1/1	2/1	2/1	2/0	1/0	0/0	75%/50%
750 μg (n = 2)	0/0	2/0	2/2	2/1	2/0	1/1	1/1	0/0	1/0	100%/100%

Values are number of volunteers with nausea/vomiting at each time period, and cumulative incidence (%) of presence of these symptoms at any time during the study.

statistical analysis to small effects due to drug injection. The observed cardiorespiratory effects of spinal neostigmine were within an absolute range of 5–15% of the baseline for both short-term effects (<6 h) and long-term effects (24 h). Spinal neostigmine (10–500 μg) did not consistently alter mean arterial blood pressure, heart rate, oxyhemoglobin saturation, or end-tidal carbon dioxide by more than 5–15% from baseline values, except end-tidal carbon dioxide, which decreased from 44 mmHg before to 37–38 mmHg 1–4 h after spinal injection of 500 μg neostigmine (table 3). The two volunteers receiving 750 μg neostigmine exhibited numerical increases in blood pressure, heart rate, and respiratory rate and decreases in end-tidal carbon dioxide without change in oxyhemoglobin saturation (fig. 1). Neostigmine produced no consistent pattern of change in either resting finger or toe skin blood flow at baseline or after a deep inspiratory gasp (table 4).

Neurologic Effects. Neostigmine decreased motor strength and deep tendon reflexes (fig. 2). The incidence of noting a decrease in deep tendon reflexes in the lower extremities at any time after injection increased from 50% after 50 μg to 75% after 150 or 500 μg to 100% after 750 μg. Subjective weakness and reflex changes occurred earlier and were more pronounced in lower than in upper extremities (fig. 2). Neostigmine reduced both light touch and cold temperature sensation in lower more than upper extremities but did not consistently affect pupil size or computer or peg board tests (table 5). VAS sedation increased numerically in a dose-related manner, but only the 750-μg dose increased VAS anxiety (table 5). The most common terms used by the volunteers to describe sedation from neostigmine were "relaxed" and "mellow."

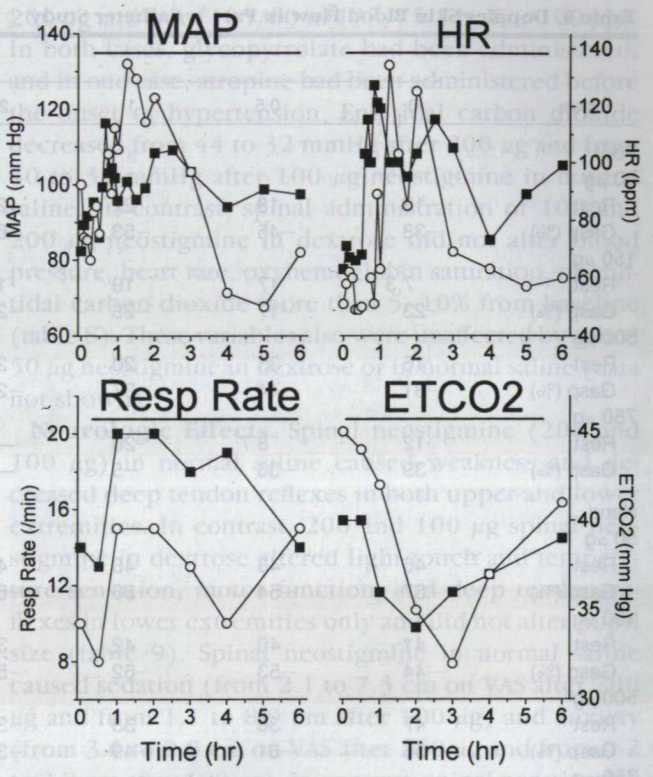

Fig. 1. Mean arterial blood pressure (MAP), heart rate (HR), respiratory rate (Resp Rate), and end-tidal carbon dioxide (ET_{CO_2}) in two volunteers (○, ■) receiving 750 μg neostigmine at time 0 in part 1.

Table 3. Cardiorespiratory Effects in Part 1: Catheter Study

Variable	Dose (μg)	Time (h) 0	0.5	1	2	3	4	5	6	12	18	24
MAP (mmHg)	50	95	89	89	92	95	92	94	97	90	94	98
	150	94	90	92	92	91	93	89	93	88	84	87
	500	90	77	88	93	95	103	89	86	98	100	94
Heart rate (beats/min)	50	80	69	69	70	74	78	86	81	81	73	81
	150	61	62	65	64	64	69	73	69	67	63	70
	500	65	61	59	60	69	66	74	69	75	79	72
Oxyhemoglobin saturation (%)	50	97	98	97	96	96	97	—	95	96	—	97
	150	98	98	99	98	98	98	—	98	97	—	98
	500	97	97	98	97	98	99	—	99	98	—	98
ET_{CO_2} (mmHg)	50	43	41	41	42	42	40	—	43	42	—	42
	150	41	40	40	39	39	39	—	40	41	—	40
	500	44	42	38	38	37	37	—	39	41	—	42

Values are means of four volunteers in each dose group.

Table 4. Doppler Skin Blood Flow in Part 1: Catheter Study

	Time (h)								
Dose	0	0.5	1	2	3	4	6	12	24
Foot									
50 μg									
Rest	13	18	22	28	24	26	31	25	25
Gasp (%)	−38	−45	−53	−65	−63	−67	−63	−57	−63
150 μg									
Rest	7.3	17	19	17	17	11	13	18	8.1
Gasp (%)	−23	−17	−28	−26	−31	−32	−35	−48	−34
500 μg									
Rest	16	35	20	22	23	20	19	17	8.2
Gasp (%)	−31	−44	−37	−21	−34	−31	−23	−49	−40
750 μg									
Rest	12	8.7	20	—	11	13	10	14	16
Gasp (%)	−39	−36	−5	—	−28	−20	−49	−59	−40
Hand									
50 μg									
Rest	46	43	48	48	50	49	52	53	48
Gasp (%)	−57	−54	−56	−69	−59	−58	−66	−58	−63
150 μg									
Rest	41	40	42	38	38	34	32	47	39
Gasp (%)	−44	−53	−62	−56	−51	−60	−58	−55	−75
500 μg									
Rest	47	36	33	35	35	31	37	42	35
Gasp (%)	−56	−61	−49	−30	−47	−51	−54	−59	−66
750 μg									
Rest	38	42	43	—	46	42	48	34	39
Gasp (%)	−39	−31	−3	—	−20	−28	−46	−39	−26

Each value represents the mean of resting blood flow, in arbitrary units, and the percent reduction in blood flow following inspiratory gasp in four volunteers (two volunteers at 750 μg).

Other than postdural puncture headache, there were no short- or long-term neurologic symptoms or complaints. Seven of 14 volunteers had clinical symptoms of postdural puncture headache within 48 h of the study, 1 experiencing spontaneous headache resolution and 6 receiving successful epidural blood patch treatment.

Analgesia. Neostigmine reduced VAS pain report in the foot at the same time that it increased the duration of tolerance to ice immersion (fig. 3). This effect was more pronounced after 150 μg than after 50 μg, and 500- and 750-μg doses exhibited similar numeric reductions in pain report to 150 μg (fig. 3). Analgesia occurred within 30–60 min and lasted 4–6 h. In contrast, only the 750-μg dose reduced pain report to ice water immersion in the hand (fig. 3).

Part 2: Small Spinal Needle Study

General Observations. Volunteer demographics are shown in table 6. Spinal neostigmine (200 μg) in normal saline caused severe nausea and vomiting in the first volunteer, beginning 30 min after injection and lasting 6 h, accompanied by loss of deep tendon reflexes and marked weakness in both lower and upper extremities. After intravenous administration of 0.5 mg glycopyrrolate and 0.2 mg atropine, 120 min after spinal neostigmine, she exhibited hypertension, paranoid ideation and anxiety, which were treated effectively with 15 mg intravenous hydralazine and 3 mg intravenous midazolam. Because of these unexpectedly severe adverse events, the next volunteer received 100 μg spinal neostigmine in normal saline. Unlike volunteers who had received 150 μg neostigmine in part 1 (two of four having mild nausea for 1–2 h), this volunteer experienced severe nausea and vomiting lasting 3 h, despite 0.7 mg glycopyrrolate and 50 mg intravenous Phenergan. The next volunteer received 50 μg neostigmine in normal saline, which yielded 2 h of nausea and vomiting, despite treatment with 0.3 mg glycopyrrolate and 8 mg intravenous ondansetron.

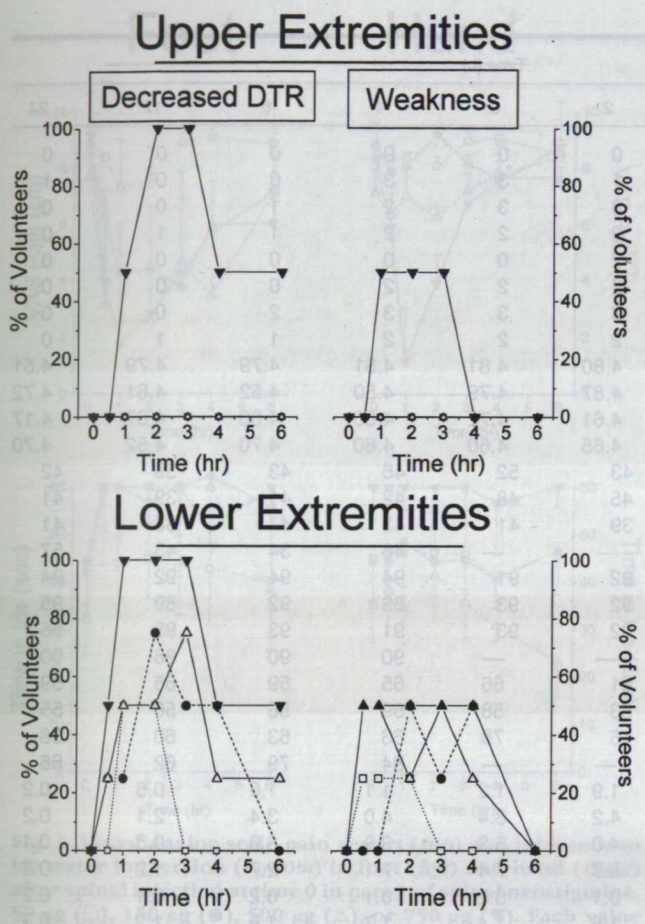

Fig. 2. Percentage of volunteers with subjective motor weakness (*right*) and decreased or absent deep tendon reflexes (*left*) in upper extremities (*top*) or lower extremities (*bottom*) after spinal injection of neostigmine at time 0, 50 μg (□), 150 μg (●), 500 μg (△), or 750 μg (▼) in part 1.

To diminish the likelihood of what were thought to be adverse effects from cephalad spread in CSF, subsequent volunteers received neostigmine in a 1.0-ml volume of 5% dextrose, with volunteers maintained head-up in bed. The first volunteer received 50 μg neostigmine by this method and had 1 h of mild nausea. The remaining ten volunteers received 100 or 200 μg neostigmine, as described in Methods. These volunteers had minor nausea (table 7) without genitourinary side effects. One volunteer receiving 200 μg neostigmine by this method received 0.4 mg glycopyrrolate and 8 mg intravenous ondansetron for nausea, with minimal apparent effect.

Cardiorespiratory Effects. Arterial blood pressure increased after spinal injection of neostigmine in normal saline (from 135/85 before to 200/120 1 h after 200 μg and from 105/60 to 133/75 1 h after 100 μg). In both cases, glycopyrrolate had been administered, and in one case, atropine had been administered before the onset of hypertension. End-tidal carbon dioxide decreased from 44 to 32 mmHg after 200 μg and from 40 to 31 mmHg after 100 μg neostigmine in normal saline. In contrast, spinal administration of 100 and 200 μg neostigmine in dextrose did not alter blood pressure, heart rate, oxyhemoglobin saturation, or end-tidal carbon dioxide more than 5–10% from baseline (table 8). These variables also were unaffected by spinal 50 μg neostigmine in dextrose or in normal saline (data not shown).

Neurologic Effects. Spinal neostigmine (200 and 100 μg) in normal saline caused weakness and decreased deep tendon reflexes in both upper and lower extremities. In contrast, 200 and 100 μg spinal neostigmine in dextrose altered light touch and temperature sensation, motor function, and deep tendon reflexes in lower extremities only and did not alter pupil size (table 9). Spinal neostigmine in normal saline caused sedation (from 2.1 to 7.3 cm on VAS after 200 μg and from 1.1 to 8.9 cm after 100 μg) and anxiety (from 3.6 to 9.0 cm on VAS after 200 μg and from 0.2 to 2.8 cm after 100 μg). In contrast, spinal neostigmine in dextrose did not alter VAS sedation or anxiety more than 1–2 cm from baseline (data not shown).

Other than postdural puncture headache, there were no short- or long-term neurologic symptoms or complaints. Two of 14 volunteers had clinical symptoms of postdural puncture headache within 48 h of the study, 1 experiencing spontaneous headache resolution and 1 receiving successful epidural blood patch treatment.

Analgesia. Spinal neostigmine (200 μg) in dextrose caused analgesia in foot and in hand (fig. 4), which was numerically greater than that after 100 μg. Spinal neostigmine (50 μg) in dextrose did not alter VAS pain more than 1 cm in either foot or hand. When injected in normal saline solution, 200 μg neostigmine reduced VAS pain report in foot (from 5.8 to 0.3 cm) and in hand (from 7.2 to 0.3 cm), whereas 100 μg and 50 μg did not reduce VAS pain score in either upper or lower extremity below 7 cm.

Discussion

This is the first clinical trial of spinal neostigmine in healthy humans and demonstrates dose-related analgesia and side effects. Side effects observed (nausea,

Table 5. Neurolotic Effects in Part 1: Catheter Study

Test	Dose (μg)	Time (h) 0	0.5	1	2	3	4	6	12	24
Light touch	50	0	0	0	0	0	0	0	0	0
(number with	150	0	1	1	1	3	3	0	0	1
diminished	500	0	1	2	3	3	2	1	0	0
sensation)	750	0	2	2	2	2	2	1	1	0
Cold temperature	50	0	0	2	2	0	0	0	0	0
(number with	150	0	0	1	2	2	2	0	0	0
diminished	500	0	3	3	3	3	3	2	0	0
sensation)	750	0	2	2	2	2	2	1	1	0
Pupil size (mm)	50	4.77	4.81	4.54	4.80	4.81	4.81	4.79	4.79	4.51
	150	4.92	4.76	4.74	4.87	4.79	4.50	4.52	4.61	4.72
	500	4.57	4.57	4.67	4.61	4.72	4.60	4.60	4.67	4.17
	750	4.62	4.67	4.65	4.65	4.60	4.60	4.70	4.52	4.70
Attention (p&q	50	44	43	44	43	52	46	43	39	42
test) reaction	150	46	40	41	45	48	42	41	39	41
time to change	500	39	43	43	39	41	43	47	38	41
(s/100)	750	39	—	—	—	—	46	34	43	57
Memory (flasher)	50	91	92	91	92	91	94	94	92	94
(% correct	150	91	91	93	92	93	89	92	89	95
responses)	500	91	92	87	92	93	91	93	95	95
	750	92	—	—	—	—	90	90	96	90
Coordination (time	50	62	61	66	61	66	65	59	65	59
[s] for the	150	57	60	59	63	58	59	56	56	55
pegboard test)	500	61	68	62	76	70	63	63	60	58
	750	56	72	—	—	—	84	79	62	66
VAS sedation (cm)	50	0.2	0.6	0.8	1.9	1.2	1.1	1.6	0.5	0.2
	150	2.7	0.8	4.2	4.2	3.4	4.0	3.4	2.1	0.2
	500	0.3	1.5	1.7	4.0	5.3	3.9	3.0	0.5	0.1
	750	0.1	3.8	9.2	8.8	7.4	7.1	2.7	1.2	0.2
VAS anxiety (cm)	50	0.3	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
	150	1.4	0.5	1.5	1.5	1.4	1.7	1.9	1.2	0.3
	500	2.9	3.1	1.4	1.4	3.4	2.0	1.3	0.2	0.1
	750	0.1	0.9	7.6	7.6	3.0	3.6	1.8	0.4	0.1

Values are number of volunteers or mean of four volunteers for all dose groups except 750 μg, which had two volunteers.

vomiting, urinary retention, motor weakness and decreased deep tendon reflexes) are similar to those observed in a study of larger doses of spinal neostigmine in hemiplegic patients performed in 1943.[16] None of the side effects in either study were unexpected. Spinal and supraspinal anatomic mechanisms of these adverse effects are well described and are discussed below.

Cardiorespiratory Effects

In contrast to systemic administration, relatively large doses of spinally administered cholinergic agonists or cholinesterase inhibitors increase blood pressure and heart rate.[9,17–19] Cardiovascular stimulation from neostigmine, due to excitatory actions on preganglionic sympathetic neurons, are more pronounced after injection directly into the intermediolateral cell column[1] than after intrathecal injection in animals with a small spinal cord (rats)[19] and are even less pronounced after intrathecal injection in sheep[9] with a spinal cord similar in size to that of humans. This and the smaller drug dose may explain the lack of cardiovascular stimulation observed with ≤500 μg neostigmine. Whether increased blood pressure and heart rate after 750 μg spinal neostigmine was due to a direct spinal effect is uncertain, given concomitant administration of anticholinergic agents for severe nausea. There was no evidence of bradycardia from systemic absorption of neostigmine in the doses used.

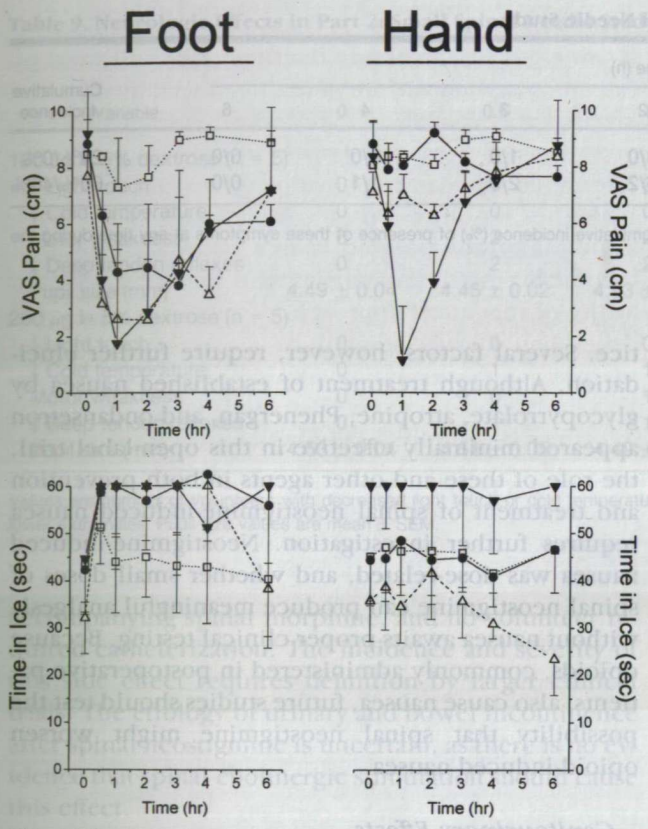

Fig. 3. Visual analog scale pain report (*top*) and tolerance to ice water immersion (*bottom*) in foot (*left*) and hand (*right*) after spinal injection at time 0 in part 1 of spinal neostigmine, 50 μg (□), 150 μg (●), 500 μg (△), or 750 μg (▼). Each value represents mean ± SEM of two to four volunteers.

We used Doppler measurements of skin blood flow in the hand and foot and reflex reduction in flow in response to a deep inspiratory gasp to estimate sympathetic neural activity to the skin.[15] We have used this method,[20] as well as a photoplethysmographic one,[21] to demonstrate segmental *decreases* in sympathetic activity after epidurally administered clonidine. There was no evidence using this indirect methodology, however, of segmental or generalized increases in basal sympathetic activity or in a sympathetically mediated reflex after spinally administered neostigmine.

Systemic administration of cholinesterase inhibitors that cross the blood-brain barrier can increase respiratory rate and functionally reverse respiratory depression from a variety of drug classes.[22–24] In support of this observation are the respiratory stimulant effects of cholinergic agonists or cholinesterase inhibitors near pontine centers of respiratory control.[25,26] As such, one would expect either no effect of spinal neostigmine on respiration or mild stimulation from cephalad distribution in CSF. This is in marked contrast to other clinically used spinal analgesics, α_2-adrenergic agonists, and opioids, which can cause mild or severe respiratory depression, respectively.[21] We observed no effect of spinal neostigmine on respiration, except numerically decreased end-tidal carbon dioxide after the largest dose, 750 μg. As with hemodynamic effects, it is uncertain whether this was a direct effect on respiratory centers of secondary to anxiety and protracted nausea.

Neurologic Effects

Volunteers receiving spinal neostigmine in the current study exhibited motor weakness and reduction in deep tendon reflexes in the lower extremities and, after larger doses, later onset of the same effects in the upper extremity. Ascending motor effects over time is consistent with cephalad spread of neostigmine in CSF and has been observed in humans receiving much larger doses of spinal neostigmine.[16] Similar motor effects are observed in animals receiving spinal neostigmine or cholinergic agonists[3,27] and are thought to be due to direct actions on motoneuron outflow rather than to ischemia or neurotoxicity, because neostigmine in larger doses does not reduce spinal cord blood flow or cause histopathologic changes.[12,13] Although all volunteers could lift their knees, and some walked to the bathroom during the period of subjective weakness, this side effect may limit the utility of large doses of spinal neostigmine for postoperative or chronic pain management.

Both sedation and anxiety were associated with spinal injection of 750 μg neostigmine, and these symptoms could be due to central cholinergic stimulation, as could paranoid ideation in the volunteer who received 200 μg neostigmine in normal saline in the second part of the current study. However, protracted nausea and use of atropine in these patients makes this diagnosis far from certain. In addition, mydriasis, which occurs in patients after organophosphate poisoning and central

Table 6. Volunteer Demographics in Part 2: Small Spinal Needle Study

Gender	F (n = 8)/M (n = 6)
Age (yr)	31 ± 2.4
Height (cm)	171 ± 3.0
Weight (kg)	83 ± 5.1

Values are mean ± SEM.

Table 7. Incidence of Nausea and Vomiting in Part 2: Small Spinal Needle Study

Dose (in 5 % dextrose)	Time (h)							Cumulative Incidence
	0	0.5	1	2	3	4	6	
100 μg (n = 5)	0/0	0/0	0/0	0/0	1/0	0/0	0/0	20%/0%
200 μg (n = 5)	0/0	0/0	1/1	3/2	2/2	1/1	0/0	60%/40%

Values are number of volunteers with nausea/vomiting at each time period, and cumulative incidence (%) of presence of these symptoms at any time during the study.

cholinergic stimulation,[28] was not present after any dose of spinal neostigmine. Similarly, spinal neostigmine did not cause even subtle changes in attention, memory, or motor coordination in this study, all of which can occur from central cholinergic stimulation. These results do not exclude the possibility of delayed central cholinergic crisis from spinal neostigmine but suggest that it may be unlikely to occur.

Nausea and vomiting occurred in a dose-related manner after spinal neostigmine in both this and a previous study.[16] The most likely site of this effect is in the brainstem, as its appearance was delayed 30–90 min after spinal injection. Although systemically administered neostigmine can cause abdominal cramping and nausea, a peripheral site of action after spinal administration is unlikely, because plasma concentrations after these small spinal doses would be expected to be low and because systemic administration of the peripheral anticholinergic agent glycopyrrolate was minimally effective in treating the nausea.

The current study suggests that nausea and vomiting is the most likely bothersome side effect that could limit the utility of spinal neostigmine in clinical practice. Several factors, however, require further elucidation. Although treatment of established nausea by glycopyrrolate, atropine, Phenergan, and ondansetron appeared minimally effective in this open-label trial, the role of these and other agents in both prevention and treatment of spinal neostigmine-induced nausea requires further investigation. Neostigmine-induced nausea was dose-related, and whether small doses of spinal neostigmine can produce meaningful analgesia without nausea awaits proper clinical testing. Because opioids, commonly administered in postoperative patients, also cause nausea, future studies should test the possibility that spinal neostigmine might worsen opioid-induced nausea.

Genitourinary Effects

Systemic administration of cholinergic agonists causes increased intravesical pressure in the bladder, although the role of spinal muscarinic receptors in bladder reflexes is not described.[29] Although urinary retention was observed both in the current study and in a previous one with larger spinal neostigmine doses,[16] the duration of urinary retention was brief in comparison to that

Table 8. Cardiorespiratory Effects in Part 2: Small Spinal Needle Study

Dose/Variable	Time (h)						
	0	0.5	1	2	3	4	6
100 μg in 5% dextrose (n = 5)							
MAP (mmHg)	84 ± 7.6	87 ± 3.6	87 ± 3.4	91 ± 2.6	92 ± 4.8	95 ± 6.0	89 ± 4.7
HR (beats/min)	73 ± 4.1	68 ± 4.5	73 ± 4.0	62 ± 3.4	70 ± 4.7	72 ± 3.4	76 ± 5.0
Oxyhemoglobin saturation (%)	98 ± 0.6	98 ± 0.6	98 ± 0.6	98 ± 0.7	98 ± 0.0	98 ± 0.7	98 ± 0.8
ET_{CO_2} (mmHg)	42 ± 0.9	39 ± 0.4	39 ± 1.2	40 ± 1.4	40 ± 0.9	39 ± 0.9	40 ± 0.4
200 μg in 5% dextrose (n = 5)							
MAP (mmHg)	93 ± 6.2	86 ± 6.9	85 ± 8.5	86 ± 4.4	83 ± 2.5	82 ± 3.8	88 ± 2.6
HR (beats/min)	69 ± 4.5	65 ± 6.3	69 ± 6.8	71 ± 11	75 ± 10	72 ± 9.0	74 ± 7.1
Oxyhemoglobin saturation (%)	99 ± 0.6	99 ± 0.5	99 ± 0.6	98 ± 0.9	98 ± 0.7	99 ± 0.6	99 ± 0.7
ET_{CO_2} (mmHg)	40 ± 2.7	38 ± 2.0	38 ± 2.8	42 ± 2.0	40 ± 2.7	38 ± 2.8	38 ± 2.8

Values are mean ± SEM.

Table 9. Neurologic Effects in Part 2: Small Spinal Needle Study

Variable	Time (h) 0	0.5	1	2	3	4	5
100 μg in 5% dextrose (n = 5)							
↓ Light touch	0	0	0	0	0	0	0
↓ Cold temperature	0	0	0	1	0	0	0
Motor weakness	0	2	2	4	2	2	0
↓ Deep tendon reflexes	0	2	2	2	2	2	1
Pupil size (mm)	4.49 ± 0.04	4.45 ± 0.02	4.53 ± 0.02	4.53 ± 0.03	4.50 ± 0.03	4.46 ± 0.03	4.44 ± 0.03
200 μg in 5% dextrose (n = 5)							
↓ Light touch	0	0	0	2	2	0	0
↓ Cold temperature	0	1	1	3	2	0	0
Motor weakness	0	0	1	1	1	2	1
↓ Deep tendon reflexes	0	1	1	2	2	2	0
Pupil size (mm)	4.53 ± 0.04	4.51 ± 0.05	4.49 ± 0.08	4.56 ± 0.04	4.46 ± 0.06	4.79 ± 0.27	4.50 ± 0.07

Values are number of volunteers with decreased light touch or cold temperature sensation or subjective motor weakness or decreased deep tendon reflexes in lower extremities. Pupil size values are mean ± SEM.

accompanying spinal morphine, and no volunteer required catheterization. The incidence and severity of this side effect requires definition by larger clinical trials. The etiology of urinary and bowel incontinence after spinal neostigmine is uncertain, as there is no evidence that spinal cholinergic stimulation should cause this effect.

Sexual responses involve both sympathetic and parasympathetic influences, some of which are reflexes mediated by the spinal cord. Vaginal contractions and ejaculation after large doses of spinal neostigmine in the current study may reflect spinal sympathetic stimulation.[30] The incidence of these side effects should be carefully monitored in initial clinical trials of spinal neostigmine, and should they occur with clinically useful doses, patients should be warned of the possibilities of such effects.

Analgesia

The purposes of this phase I study were to describe side effects and assess safety before large-scale safety and efficacy studies are undertaken. Some information regarding therapeutic dose range and efficacy were obtained, however. Although spinally administered cholinergic agonists produce analgesia in all species tested, analgesia from cholinesterase inhibitors depends on the degree of tonic spinal cholinergic tone and occurs in some but not all species.[4,10] The current study suggests tonic spinal cholinergic activity in normal humans is adequate for neostigmine to produce meaningful analgesia alone.

The therapeutic dose of spinal neostigmine, based on responses to experimental pain, probably lies between 50 and 500 μg. We chose ice water immersion as a pain test, as we and others have demonstrated a close correlation and near equivalency for the opioid dose and plasma concentration response relationship for this experimental pain stimulus and for acute postoperative pain.[21,31] Based on these data, 150–500 μg spinal neostigmine should cause near-total relief of postoperative pain, lasting 4–6 h. The duration of enhancement, if any, by neostigmine, of intraspinally administered α_2-adrenergic agonists and the neostigmine dose required for such enhancement are not addressed in the current study.

Method of Administration

The method of spinal neostigmine administration in the initial dose-ranging part of this study (injection through a large-gauge needle, subsequent insertion of a catheter, and multiple aspirations of CSF) is unlikely to be used clinically. For this reason, we examined, in a few additional volunteers, side effects from single lumbar spinal injections of neostigmine through a small-gauge needle (25- or 27-gauge). Severe side effects after 100 and 200 μg neostigmine in normal saline by this method but not by 150 μg neostigmine in the catheter study suggest but do not prove a difference exists between these methods in distribution of neostigmine in CSF. It is possible that, in the catheter study, leak of CSF around the catheter into the epidural space and withdrawal of CSF from the catheter limited ceph-

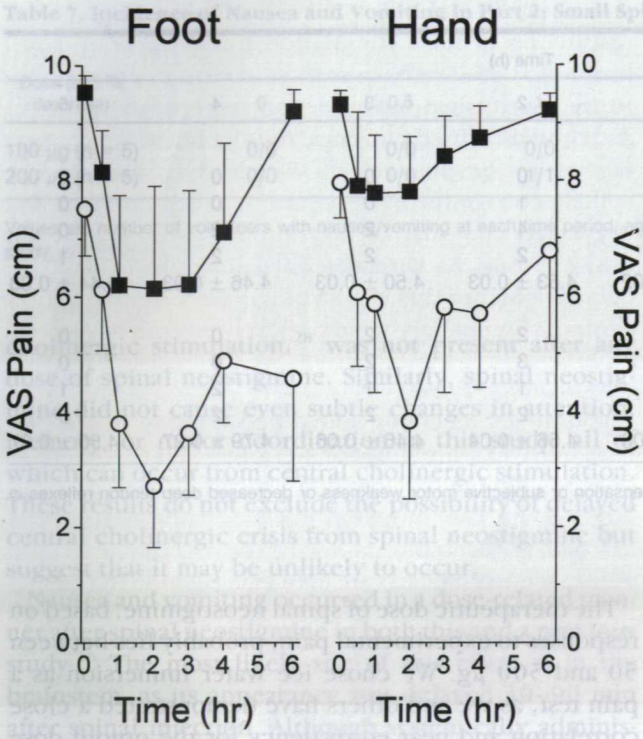

Fig. 4. Visual analog scale pain report and time of tolerance to ice water immersion in hand (*right*) and foot (*left*) after spinal injection at time 0 of spinal neostigmine, 100 μg (■) or 200 μg (○) in 5% dextrose in part 2. Each value represents mean ± SEM of five volunteers.

alad spread compared to single injection through a small-gauge needle. Injection of 200 μg neostigmine in hyperbaric solution resulted in analgesia without severe nausea, further substantiating this theory.

These preliminary results raise the possibility that neostigmine's side effects, especially nausea, may be reduced by injection in hyperbaric solution and maintaining a head-up position. Again, several factors remain unanswered and should be addressed in hypothesis-testing clinical trials. Such factors include injected volume and baricity, patient positioning and timing of change in positioning, and the incidence of spinally mediated side effects (motor and genitourinary) with injection of solutions of differing baricity.

In summary, preclinical toxicity testing of intrathecal neostigmine is reasonably complete. In normal human volunteers, 50–750 μg intrathecal neostigmine followed by catheter insertion and aspiration of CSF for analysis produces dose-related motor weakness, decreases in deep tendon reflexes, urinary incontinence, genitourinary stimulation, and nausea and vomiting. Nausea and vomiting are perhaps more pronounced after single spinal injection than injection followed by catheter insertion and are perhaps reduced by injection in a hyperbaric solution. Intrathecal neostigmine alone produces definitive analgesia in humans. However, its efficacy alone and in combination with a variety of other analgesics requires considerable clinical study. Only acute safety was assessed in this study; the effects of chronic dosing are unknown at this time. The ultimate utility of this novel approach to spinal analgesia will depend on efficacy, strength of interaction with other analgesics, and separating analgesia from side effects. This study provides a safety basis and defines side effects to be monitored for subsequent clinical trials.

The authors thank Carswell Jackson, M.D., for assistance in obtaining some measurements; Thomas Smith, Ph.D., for assistance in Doppler skin blood flow studies; and Herbert M. Floyd, M.D., for assistance in the design of the second part of the study.

References

1. Bartolini A, Ghelardini C, Fantetti L, Malcangio M, Malmberg-Aiello P, Giotti A: Role of muscarinic receptor subtypes in central antinociception. Br J Pharmacol 105:77–82, 1992

2. Iwamoto ET, Marion L: Characterization of the antinociception produced by intrathecally administered muscarinic agonists in rats. J Pharmacol Exp Ther 266:329–338, 1993

3. Yaksh TL, Dirksen R, Harty GJ: Antinociceptive effects of intrathecally injected cholinomimetic drugs in the rat and cat. Eur J Pharmacol 117:81–88, 1985

4. Gordh T Jr, Jansson I, Hartvig P, Gillberg PG, Post C: Interactions between noradrenergic and cholinergic mechanisms involved in spinal nociceptive processing. Acta Anaesthesiol Scand 33:39–47, 1989

5. Calaresu FR, McKitrick DJ, Weernink EJ: Microinjection of substance P and ACh into rat intermediolateral nucleus elicits cardiovascular responses. Am J Physiol 259:R357–R361, 1990

6. Coombs DW, Fratkin JD: Neurotoxicology of spinal agents. ANESTHESIOLOGY 66:724–726, 1987

7. Yaksh TL, Collins JG: Studies in animals should precede human use of spinally administered drugs. ANESTHESIOLOGY 70:4–6, 1989

8. Calvey TN, Wareing M, Williams NE: Pharmacokinetics and pharmacological effects of neostigmine in man. Br J Clin Pharmacol 7:149–155, 1979

9. Williams JS, Tong C, Eisenach JC: Neostigmine counteracts spinal clonidine-induced hypotension in sheep. ANESTHESIOLOGY 78:301–307, 1993

10. Detweiler DJ, Eisenach JC, Tong C, Jackson C: A cholinergic interaction in *alpha*$_2$ adrenoceptor-mediated antinociception in sheep. J Pharmacol Exp Ther 265:536–542, 1993

11. Tong C, Li P, Lothe A, Eisenach JC: Spinal clonidine hypotension: Mechanism of reversal by spinal neostigmine (abstract). Soc Neurosci Abstr 19:491, 1993

12. Yaksh TL, Grafe MR, Malkmus S, Rathbun ML, Eisenach JC: Studies on the safety of chronically administered intrathecal neostigmine methylsulfate in rats and dogs. ANESTHESIOLOGY 82:412–427, 1995

13. Hood DD, Eisenach JC, Tong C, Tommasi E, Yaksh TL: Cardiorespiratory and spinal cord blood flow effects of intrathecal neostigmine methylsulfate, clonidine, and their combination in sheep. ANESTHESIOLOGY 82:428–435, 1995

14. Kessler DA: The regulation of investigational drugs. N Engl J Med 320:277–288, 1989

15. Valley MA, Bourke DL, McKenzie AM, Raja SN: Quantitative testing of sympathetic function with laser doppler flowmetry (abstract). ANESTHESIOLOGY 77:A882, 1992

16. Kremer M: Action of intrathecally injected prostigmine, acetylcholine, and eserine on the central nervous system in man. Q J Exp Physiol 31:337–357, 1942

17. Sundaram K, Murugaian J, Krieger A, Sapru H: Microinjections of cholinergic agonists into the intermediolateral cell column of the spinal cord at T_1-T_3 increase heart rate and contractility. Brain Res 503:22–31, 1989

18. Eisenach JC, Tong C: Site of hemodynamic effects of intrathecal α_2-adrenergic agonists. ANESTHESIOLOGY 74:766–771, 1991

19. Takahashi H, Buccafusco JJ: The sympathoexcitatory response following selective activation of a spinal cholinergic system in anesthetized rats. J Auton Nerv Syst 34:59–68, 1991

20. Tuttle R, Hood DD, Eisenach JC, Shafer S: Computer controlled epidural clonidine infusion in humans (abstract). Reg Anesth 18:16, 1993

21. Eisenach J, Detweiler D, Hood D: Hemodynamic and analgesic actions of epidurally administered clonidine. ANESTHESIOLOGY 78:277–287, 1993

22. Snir-Mor I, Weinstock M, Davidson JT, Bahar M: Physostigmine antagonizes morphine-induced respiratory depression in human subjects. ANESTHESIOLOGY 59:6–9, 1983

23. Weinstock M, Roll D, Erez E, Bahar M: Physostigmine antagonizes morphine-induced respiratory depression but not analgesia in dogs and rabbits. Br J Anaesth 52:1171–1176, 1980

24. Elmalem E, Chorev M, Weinstock M: Antagonism of morphine-induced respiratory depression by novel anticholinesterase agents. Neuropharmacology 30:1059–1064, 1991

25. Willette RN, Doorley BM, Sapru HN: Activation of cholinergic mechanisms in the medulla oblongata reverse intravenous opioid-induced respiratory depression. J Pharmacol Exp Ther 240:352–358, 1987

26. Ovid Trouth C, Millis RM, Bernard DG, Pan Y, Whittaker JA, Archer PW: Cholinergic-opioid interactions at brainstem respiratory chemosensitive areas in cats. Neurotoxicology 14:459–468, 1993

27. Gillberg PG, Hartvig P, Gordh T, Sottile A, Jansson I, Archer T, Post C: Behavioral effects after intrathecal administration of cholinergic receptor agonists in the rat. Psychopharmacology (Berl) 100:464–469, 1990

28. Byth W, Logue NA, Bell P, Best SJ, King DJ: The McCollough effect as a measure of central cholinergic activity in man. Psychopharmacology (Berl) 106:75–84, 1992

29. Durant PAC, Yaksh TL: Drug effects on urinary bladder tone during spinal morphine-induced inhibition of the micturition reflex in unanesthetized rats. ANESTHESIOLOGY 68:325–334, 1988

30. Young WK: Ejaculatory disturbances, Andrology. Edited by Pryor JP, Lipshultz LI. Boston, Butterworth, 1987, pp 183–216

31. Van den Nieuwenhuyzen MCO, Engbers FHM, Burm AGL, Lemmens HJM, Vletter AA, van Kleef JW, Bovill JG: Computer-controlled infusion of alfentanil for postoperative analgesia: A pharmacokinetic and pharmacodynamic evaluation. ANESTHESIOLOGY 79:481–492, 1993