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Cerebrospinal Fluid Pharmacokinetics and Pharmacodynamics of Intrathecal Neostigmine Methylsulfate in Humans

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Background: **This study defines the cerebrospinal fluid (CSF) pharmacokinetics of neostigmine after intrathecal injection in humans and its effect on CSF acetylcholine, and it correlates physiologic effects with neostigmine dose and CSF acetylcholine concentrations.**

Methods: **The CSF was sampled *via* an indwelling spinal catheter in 12 volunteers receiving intrathecal neostigmine (50–750 μg) and analyzed for neostigmine and acetylcholine. Pharmacokinetic and pharmacodynamic analyses were performed with NONMEM. Effect-site models linked the time course of the neostigmine concentration with the time course of analgesia.**

Results: **Acetylcholine concentrations increased from <20 pmol/ml at baseline to >100 pmol/ml within 15 min of neostigmine injection. The pharmacokinetics of intrathecal neostigmine were best described by a triexponential function with an absorption phase. Individual predicted concentrations varied 100-fold. *Post hoc* Bayesian estimates described the observed neostigmine concentrations with a median error of 22% and did not show systematic model misspecification. Individual estimates of effect site concentration producing a 50% maximal effect for foot visual analog scale analgesia correlated with the magnitude of individual CSF neostigmine concentrations.**

Conclusions: **Intrathecal neostigmine concentrations can be well described by a triexponential disposition function, but the intersubject variability is large. The correlation between intersubject variability in concentration and intersubject variability in 50% maximal effect for foot analgesia suggests that both are offset by a common scalar, possibly the distance from the site of injection to the sampling and effect sites. These data provide the basis for the hypothesis of "observation at a distance" to describe the pharmacodynamics of intrathecally administered drugs. (Key words: Acetylcholine; analgesia; cholinergic; nausea.)**

LABORATORY studies suggest that spinal cholinergic activation produces analgesia. For example, there is dense binding of cholinergic ligands in the superficial dorsal horn,[1] and microinjection of cholinergic agonists in this area inhibits excitation of dorsal horn neurons by electrical stimulation.[2] Intrathecal injection of cholinergic agonists yields behavioral analgesia in animals, an effect blocked by muscarinic but not nicotinic antagonists.[3,4] The clinical utility of intrathecally administered cholinergic agonists may, however, be limited by motor weakness caused by direct stimulation in the spinal cord ventral horn.[3]

Intrathecal injection of cholinesterase inhibitors represents another method to exploit cholinergic mechanisms of spinal analgesia. Intrathecally administered neostigmine produces behavioral analgesia in rats[5] and potentiates analgesia from intrathecally administered α_2-adrenergic agonists in rats and sheep,[6,7] which themselves stimulated acetylcholine release. In a phase 1 safety assessment in humans, intrathecal neostigmine was shown to cause dose-dependent analgesia but also side effects (nausea and vomiting, weakness, sedation).[8]

Description of CSF pharmacokinetics of intraspinally administered drugs may be useful in predicting clinical actions and in understanding mechanisms of pharmacologic action. Absolute concentrations of drugs at steady state and residence time in CSF have been correlated with analgesic and other effects with prolonged infusions and duration of action after single bolus adminis-

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The data from this study, and the NONMEM control files, can be found on the World Wide Web at: http://pkpd.icon.palo-alto.med.va.gov in directory/data.dir/intrathecal neostigmine.

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tration, respectively, for opioids[9] and for the α_2-adrenergic agonist clonidine.[10–12] However, detailed pharmacodynamic studies have not been performed with intrathecally administered analgesics, and the CSF space clearly invalidates most of the basic assumptions of traditional pharmacokinetic-dynamic modeling. This is the first such systematic study of CSF pharmacokinetics and dynamics and leads to several testable hypotheses regarding the fundamental relation, if any, between CSF drug concentration after bolus intrathecal injection and drug effect.

As part of a phase 1 safety assessment of intrathecally administered neostigmine in humans, we collected CSF to study neostigmine pharmacokinetics and pharmacodynamics, including the effect of neostigmine on intrathecal CSF acetylcholine concentrations and on measures of analgesia. A description of the pharmacologic effects of intrathecal neostigmine in these volunteers was the subject of a previous report.[8]

Methods

The study was divided into two parts: an initial study of 14 volunteers in whom spinal catheters were inserted 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 in-patient General Clinical Research Center at 7:00 A.M. having had nothing to eat or drink since midnight. In each study, a peripheral intravenous catheter was inserted to infuse lactated Ringer's solution at 50–100 ml/h, and a second intravenous catheter was inserted and capped to sample venous blood. Baseline measures were taken before neostigmine injection and at times thereafter as indicated. Neostigmine was obtained under IND approval by the Food and Drug Administration in preservative-free saline from International Medication Systems (El Monte, CA). Neostigmine from this same commercial source was used in preclinical toxicity studies.

Part 1: Catheter Study

Based on data obtained in animals, an initial dose of 50 g neostigmine was chosen as likely to be approximately one half 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. Neostigmine was diluted in a 4-ml volume with preservative-free normal saline and injected over 30 s through a Sprotte-tipped, 19.5-gauge spinal needle that had been inserted at the L3–L4 or L4–L5 interspace. Volunteers were positioned in a lateral position. Two minutes after neostigmine injection, a 21-gauge catheter was inserted through the Sprotte needle and advanced 3–5 cm beyond the needle tip. The spinal needle was then withdrawn. The CSF samples (total withdrawn volume per sample was 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 acetylcholine assays. For each sample the initial 0.5 ml, representing 2× catheter dead space, was discarded, 0.5 ml was collected for the neostigmine assay, and 0.5 ml was collected in a tube containing 10^{-5} M neostigmine for the acetylcholine assay.

Part 2: Small Spinal Needle Study

Drug Administration. Based on the pharmacologic effects observed in part 1, we chose an initial dose of neostigmine in part 2 of 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. 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 decrease 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 10 volunteers received 100 μg (n = 5) or 200 μg (n = 5) neostigmine 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 part 2 of this study, a second #25 or #27 Whitacre needle was inserted at the same lumbar interspace as the original injection 60 min after neostigmine administration, and 1.5 ml CSF was aspirated for neostigmine and acetylcholine assays. This study ended 6 h after spinal injection. All CSF samples were immediately frozen on dry ice and stored at −70°C until analysis.

Analgesia and Side Effect Monitoring. The following measurements were obtained before and at 30 and 60 min, then hourly until 6 h after injection: blood pressure and heart rate (by a noninvasive oscillometric device), oxyhemoglobin saturation by pulse oximetry, end tidal carbon dioxide by capnography, respiratory rate, finger and toe skin blood flows by laser Doppler flowmetry, a screening neurologic examination, computer tests for attention and for short-term memory, motor coordination tests to screen for central cholinergic stimulation, assessments for level of sedation using a 10-cm visual analog scale (VAS), anchored at 0 = "not drowsy at all" to "as drowsy as possible," and assessments for level of anxiety using a 10-cm VAS, anchored at 0 = "not anxious at all" to "as anxious as possible." Analgesia was assessed at these same times by pain report using a 10-cm VAS after immersion of the hand, and 5 min later immersion of the 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. In part 1 of the study, these measurements were also obtained at 12 and 24 h after injection. Details of these measurements may be found in the report of pharmacologic effects in these volunteers.[8]

Sample Analysis. Neostigmine was extracted and analyzed by high-pressure liquid chromatography with ultraviolet detection, as previously described.[13] The limit of detection was 0.5 ng/ml neostigmine, with an interassay coefficient of variation of 12%. Acetylcholine concentrations were determined by a different high-pressure liquid chromatography–electrochemical detection method. This method has an interassay coefficient of variation of 8% and a detection limit of 50 fmol.[7]

Pharmacokinetic Analysis. The linearity of the pharmacokinetics with increasing dose was assessed by visual analysis of the dose-normalized concentration *versus* time curves. Parametric pharmacokinetic analysis was performed with NONMEM,‖ using first-order conditional estimates. Both population (typical) pharmacokinetic and *post hoc* Bayesian (individual) pharmacokinetic parameters were estimated.

Because of the appearance of an initial "absorption" phase, which was likely to be the diffusion of drug from the injection site to the sampling sate, biexponential models of the form: $C_{csf} = A_c^{-at} - A_c^{-\beta t}$ and triexponential models of the form $C_{csf} = A_c^{-\alpha t} + B_c^{-\beta t} - (A - B)e^{-107>t}$ were explored. Interindividual and intraindividual errors were modeled as log normally distributed. To model the intraindividual error as log normal, the log of the predicted concentrations were fitted to the log of the observed concentrations using an additive intraindividual error model.

The NONMEM objective function was −2 times the log likelihood. Goodness of fit was visually assessed by comparing the observed concentrations with the predicted concentration over time, and by visually examining the measured–predicted concentrations over time. Bias was calculated as the median weighted residual, and accuracy was calculated as the median absolute weighted residual, as previously described.[14]

Pharmacodynamic Analysis. The analgesic response in the hand and foot, measured on the VAS scale, was related to the neostigmine concentration using a sigmoidal-E_{max} relation:

$$VAS = E_{max} - E_{max} \frac{C^{\gamma}}{C^{\gamma}_{50} + C^{\gamma}}$$

where E_{max} is the baseline pain response, $-E_{max}$ is the maximum analgesic response (*i.e.*, VAS = 0 [no pain]), C is the CSF neostigmine concentration, C_{50} is the neostigmine concentration associated with 50% of the peak response, and γ is the steepness of the sigmoidal concentration–response relation. An effect-site model was explored, where the effect-site neostigmine concentration was calculated as:

$$Ce(t) = Dose\ k_e0 \left(\frac{A}{k_{e0} - \alpha} (e^{-t\alpha} - e^{-tk_{e0}}) + \frac{B}{k_{e0} - \beta} (e^{-tk_{e0}}) - \frac{A + B}{k_{e0} - \gamma} (e^{-t\gamma} - e^{-tk_{e0}}) \right)$$

where Ce(t) is the effect-site neostigmine concentration at time t and k_{eO} is the equilibration rate constant between the CSF and the site of drug effect, as defined by Sheiner *et al.*[15] Two different sets of the five pharmacokinetic parameters (A, B, α, β, γ) were explored: population estimates and individual *post hoc* Bayesian estimates. The effect-site neostigmine concentration was related to the analgesic response in the hand and foot using a sigmoidal-E_{max} relation:

$$VAS = E_{max} - E_{max} \frac{Ce^{\gamma}}{Ce^{\gamma}_{50} + Ce^{\gamma}}$$

where Ce is the effect site concentration, and the other terms are as previously described.

‖Beal SL, Sheiner LB: NONMEM Users Guide. University of California San Francisco, NONMEM Project Group, San Francisco, 1992

Goodness of fit for the pharmacodynamic measures was measured by the median residual and the median absolute residual, where the residual was the measured VAS response − the predicted VAS response.

The resulting pharmacokinetic–pharmacodynamic model was solved for the time of peak effect using the Solver function of Microsoft Excel (Redmond, WA). The pharmacodynamic model was then expressed as the dose associated with a given drug effect at the time of peak effect:

$$VAS_{peak\ effect} = E_{max} - E_{max} \frac{D^{\gamma}}{D^{\gamma}_{50} + D^{\gamma}}$$

where D is the dose of neostigmine in micrograms, and D_{50} is the dose associated with 50% of the peak analgesic response. This conversion was made by dividing the C_{50} for analgesia by the effect-site concentration at the time of peak effect after a unit bolus.

Statistics

Unless otherwise indicated, data are presented as mean ± SEM. Pearson product moment correlation was used to determine the relation between log CSF neostigmine and log CSF acetylcholine and between these variables and analgesia or side effects. This analysis was also used to determine the relation between the administered dose and log CSF neostigmine and log acetylcholine. For graphic depiction, linear regression with 95% confidence limits was used. Because of multiple correlation testing, $P < 0.01$ was considered significant.

Results

Of the 28 volunteers, 3 were excluded from data analysis in this report. Catheters from two volunteers (one volunteer who received 50 μg and the other who received 150 μg neostigmine) stopped functioning, and it was not possible to obtain CSF within 60 min of neostigmine injection. The first volunteer in part 2 of this study, who received 200 μg neostigmine in saline, had severe nausea and vomiting that precluded our obtaining CSF 60 min after injection.

CSF Pharmacokinetics

Figure 1 shows the CSF neostigmine *versus* time data (upper panel), and the dose-normalized concentrations over time and the individual unit disposition functions (lower panel) for the 13 volunteers. Linearity with respect to dose was established by the lack of a relation between dose and the magnitude of the dose-normalized concentrations over time. The triexponential disposition function was preferred over the biexponential disposition function, based on an improvement in the NONMEM objective function of 282. Table 1 summarizes the pharmacokinetics of neostigmine. The CSF neostigmine concentrations were best described by a triexponential disposition function of the form

$$C_{CSF} = Dose \times (-9.95e^{-0.21t} + 9.89e^{-0.0275t} + 0.0607e^{-0.000416t})$$

in which the most rapid half-life was 3.3 min and the terminal half-life was 1,666 min. The terminal half-life accounted for <1% of the decrease in neostigmine concentration after bolus intrathecal injection.

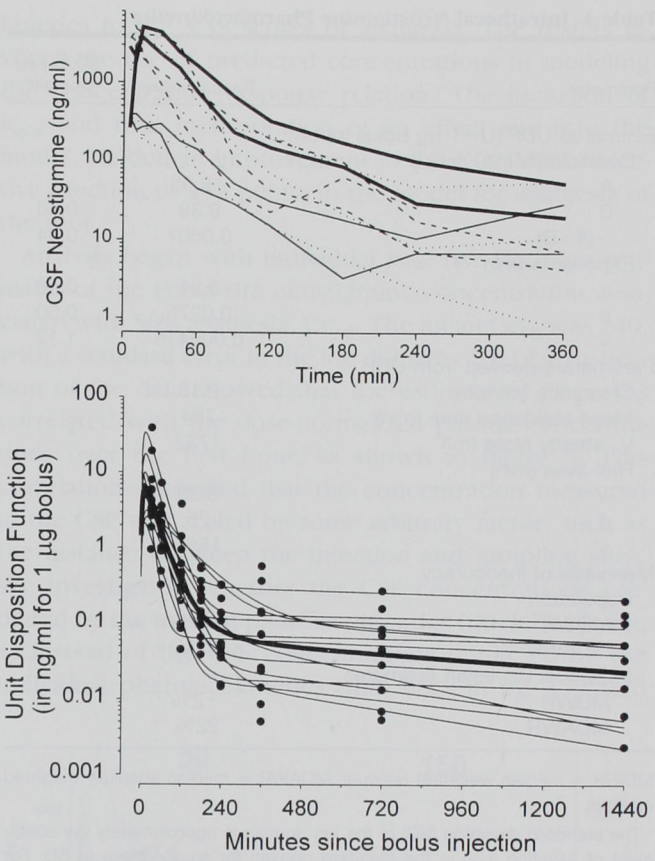

Fig. 1. (*Upper*) Cerebrospinal fluid (CSF) neostigmine concentrations over time in volunteers for 6 h after intrathecal injection at time 0 of neostigmine, 50 μg (*solid thin line*), 150 μg (*dotted line*), 500 μg (*dashed line*), or 750 μg (*solid thick line*). (*Lower*) Unit disposition curves and dose-normalized concentrations of neostigmine in all patients. The thin solid lines are the *post hoc* Bayesian estimates of the pharmacokinetics, and the thick line represents the population estimate of the pharmacokinetics (table 1).

Table 1. Intrathecal Neostigmine Pharmacokinetics

Parameter	Typical Value	SD in Log Domain*
Estimated UDF (U=1 μg bolus input)		
Coefficients (ng · ml^{-1})		
A	−9.95	
B	9.89	0.86
−(A+B)	0.0607	0.94
Exponents (min^{-1})		
α	0.21	0.48
β	0.0275	0.50
γ	0.000416	1.12
Parameters derived from UDF		
Clearance (ml/min)	2.18	
Mean residence time (min)	794	
V_d steady state (ml)	1732	
Half-lives (min)		
α	3.30	
β	25	
γ	1666	
Measures of inaccuracy		
Population		
MDWR	−2%	
MDAWR	52%	
Individual Bayesian estimates		
MDWR	−2%	
MDAWR	22%	

MDWR = median weighted residual; MDAWR = median absolute weighted residual.

* The standard deviation (SD) in the log domain is approximately the coefficient of variation (CV) in the standard domain for small values of SD. For values above 0.5 (50%), the standard deviation in the log domain diverges sufficiently from the CV that we elected to not refer to it as the "approximate CV."

The triexponential disposition function described the general shape of the observations over time (fig. 1). The prediction was also unbiased over time, and the magnitude of the prediction errors was consistent over time (fig. 2), both for the population estimates (fig. 2, top graph) and the *post hoc* individual Bayesian estimates (fig. 2, bottom graph). Although the fit was unbiased (median weighted residual = 2% for both the population and individual estimates (table 1), there was considerable variability in the population fit (median absolute weighted residual = 52%) because of interindividual variability.

Examination of the residual errors in the time domain (fig. 2) and concentration domain (fig. 3) does not show evidence of model misspecification. Indeed, the individual triexponential disposition functions predicted the observed CSF concentrations across four orders of magnitude with a median error of 25% (fig. 3, bottom graph). Thus the inaccuracy of the population estimate is due to interindividual variability and not to a fundamental limitation in the shape of a triexponential disposition curve to describe the observations.

The plasma neostigmine concentrations were at or near the limit of detection at all times (92% of values were <10 ng/ml with no dose dependency).

CSF Acetylcholine

In volunteers in part 1 of this study, CSF acetylcholine increased from 13 ± 2.6 pmol/ml before injection to >100 pmol/ml after injection. In contrast to CSF neostigmine, CSF acetylcholine concentrations remained at a plateau of 100–300 pmol/ml for 4–6 h after neostigmine injection in most volunteers, and with no dependency on neostigmine dose administered (fig. 4). Similarly, there was no significant difference between CSF acetylcholine concentration 60 min after neostigmine injection in hyperbaric solution (median, 149 pmol/ml) and that after isobaric injection in the catheter study (median, 193 pmol/ml). There was no significant correlation between CSF neostigmine and CSF acetylcholine concentrations over time, consistent with their differing time courses and dose dependencies (data not shown).

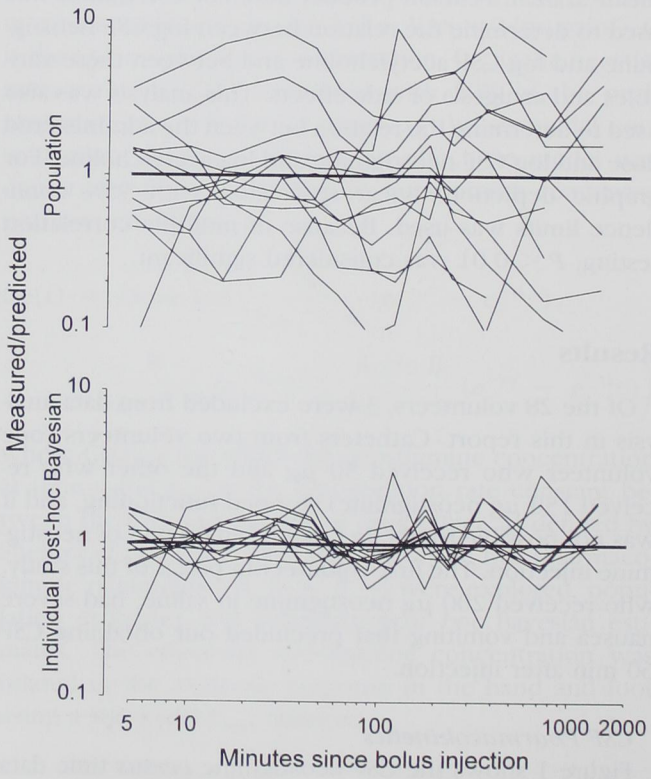

Fig. 2. The residual errors, calculated as measured concentration (ng/ml) divided by predicted, over time for each volunteer, both based on the population pharmacokinetics (*top graph*) and the *post hoc* Bayesian estimates of individual pharmacokinetics (*bottom graph*).

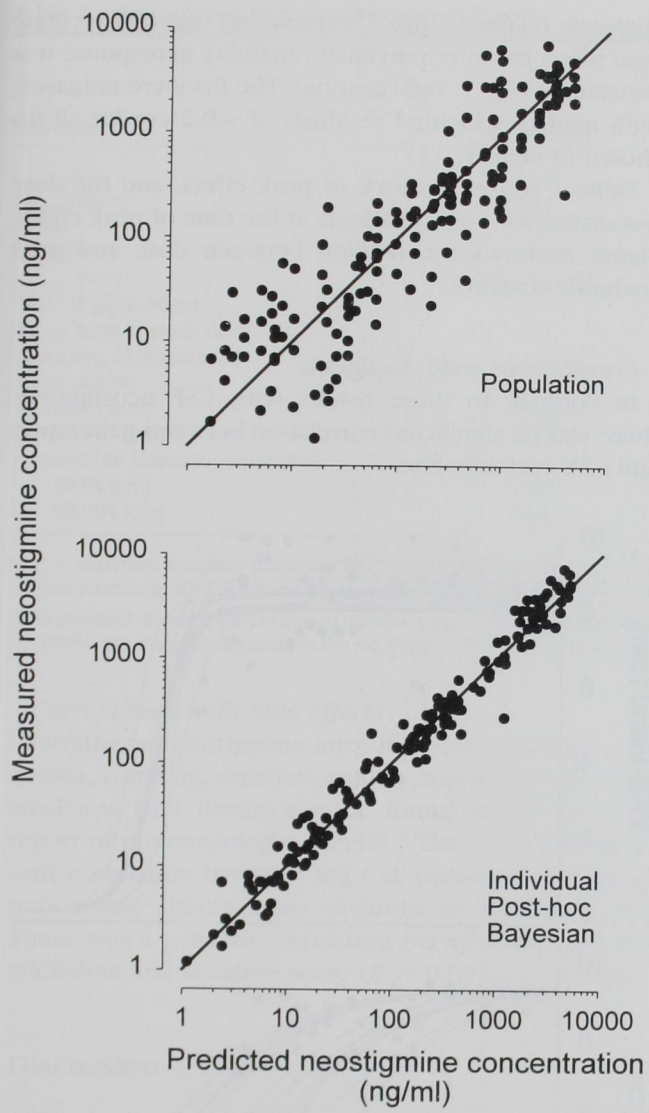

Fig. 3. Predicted *versus* measured neostigmine concentrations, based on the population pharmacokinetics (*top graph*) and the *post hoc* Bayesian estimates of individual pharmacokinetics (*bottom graph*). Neither graph suggests systematic model misspecification over the four orders of magnitude encompassed in the study data.

Pharmacodynamic Analysis

Graphs relating CSF neostigmine concentrations to VAS scores of pain in the hands and feet demonstrated poor correlations. The parameters of a sigmoidal-E_{max} model were estimated to relate both measured neostigmine concentrations and neostigmine concentrations predicted by the individual *post hoc* Bayesian pharmacokinetics to the VAS scores of analgesia. The results favored the use of predicted concentrations in modeling the concentration–response relation. The inclusion of k_{eO}, and thus incorporation of an effect site into the model, yielded an improvement in the NONMEM objective function of 138 points in the model for analgesia of the foot.

Analysis began with individual *post hoc* Bayesian estimates of the effect-site neostigmine concentration associated with 50% analgesia, Ce_{50}. The mean Ce_{50} was 240, with a standard error in the log domain of 1.14.# Inspection of the data showed that the estimate of Ce_{50} was correlated with the dose-normalized plasma concentrations over the first hour, as shown in figure 5. This correlation suggested that the concentration measured in the CSF was scaled by some arbitrary factor, such as the distance between the injection and sampling sites. We investigated whether the CSF concentrations predicted by the median pharmacokinetics (thick line in fig. 1) instead of the CSF concentrations predicted by the individual pharmacokinetics (thin lines in fig. 1) could

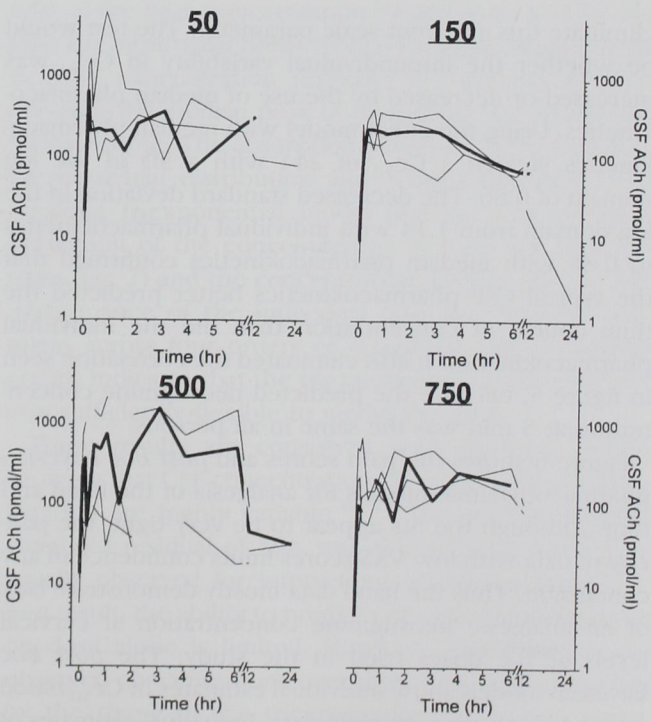

Fig. 4. Cerebrospinal fluid acetylcholine concentrations over time in 12 volunteers in part 1 of the study who received 50–750 g intrathecal neostigmine at time 0. Each thin line represents the value for each volunteer, and each thick line represents the mean of those persons who received that dose. Some lines are disconnected because of missing values. Panels are arranged according to the administered dose of neostigmine.

#An error of 1.14 in the log domain means that the 95% confidence limits range $10^{\wedge}(4 \cdot 1.14) = 36{,}000$.

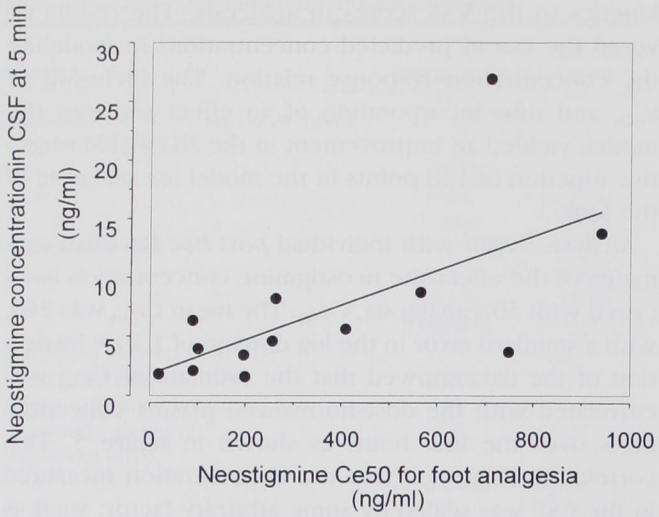

Fig. 5. The correlation between neostigmine Ce_{50} for foot analgesia and the concentration predicted by the *post hoc* Bayesian individual pharmacokinetics 5 min after a bolus of 1 μg. Similar correlations between the magnitude of the predicted concentration after a unit bolus and the Ce_{50} for foot analgesia were seen at every time point from 1 min to 60 min after neostigmine.

eliminate this apparent scale parameter. The test would be whether the intraindividual variability in Ce_{50} was increased or decreased by the use of median pharmacokinetics. Using the effect model with median pharmacokinetics yielded a Ce_{50} of 244 with a SD in the log domain of 0.86. The decreased standard deviation in the log domain from 1.14 with individual pharmacokinetics to 0.86 with median pharmacokinetics confirmed that the typical CSF pharmacokinetics better predicted the time course of concentration than did the individual pharmacokinetics. It also eliminated the correlation seen in figure 5, because the predicted neostigmine concentration at 5 min was the same in all persons.

Figure 6 shows the pain scores and *post hoc* Bayesian pharmacodynamic models for analgesia of the hand and foot. Although the fits appear to be very tight, the paucity of data with low VAS scores limits confidence in any conclusion. Thus the hand data mostly demonstrate lack of an analgesic neostigmine concentration at cervical levels at the doses used in the study. The *post hoc* Bayesian models show individual estimates of Ce_{50} based on median pharmacokinetic data. Individual estimates of Ce_{50} for the foot ranged from 49 to 1,110 ng/ml.

Table 2 shows the estimates of the pharmacodynamic parameters for the analgesic response of the foot (left columns) and hand (right columns). If the interindividual variability in Ce_{50} is not considered, the prediction of the analgesic response (fig. 7, left graphs) was less accurate than when the interindividual variability in response was considered (fig. 7, right graphs). The fits were unbiased, with median weighted residuals of <0.2 cm for all fits shown in table 2.

Table 2 shows the time of peak effect, and the dose associated with 50% analgesia at the time of peak effect. Figure 8 shows the relation between dose and peak analgesic response.

Correlations with Analgesia

In contrast to these results with CSF neostigmine, there was no significant correlation between pain report and CSF acetylcholine.

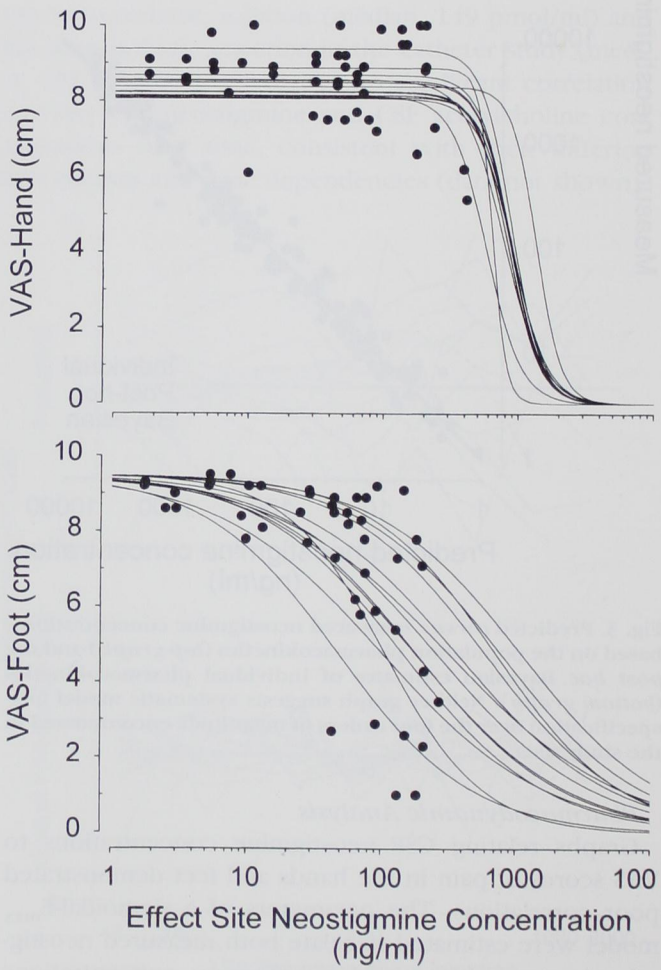

Fig. 6. All observations of hand analgesia (*top graph*) and foot analgesia (*bottom graph*) and individual *post hoc* Bayesian estimates of the relation between analgesia and effect-site neostigmine concentration. The concentrations are referenced to a CSF sampling site at an unknownable distance from the site of injection. Thus the units of concentration are arbitrary.

Table 2. Intratecal Neostigmine Pharmacodynamics

Parameter	VAS–Foot		VAS–Hand	
	Typical Value	SD in Log Domain*	Typical Value	SD in Log Domain*
E_{max} (cm)	8.94		8.42	0.06
Ce_{50} (ng/ml)	244	0.86	860	0.31
Slope	1.5		3.68	0.33
k_{e0} (min^{-1})	0.00604	0.56	0.00706	0.67
$t_{1/2\ k_{e0}}$ (min)	114.759467		98.17948733	
Time of peak effect	77		73	
D_{50} at time of peak effect (μg)	195		615	
Measures of inaccuracy				
Population				
MDR (cm)	0.13		0.09	
MDAR (cm)	1.38		0.80	
Individual Bayesian estimates				
MDR (cm)	0.02		0.08	
MDAR (cm)	0.69		0.58	

E_{max} = maximum analgesic response; Ce_{50} = neostigmine concentration associated with 50% of the maximum response; $T_{1/2_{keo}}$ = half-life for effect site; D_{50} = dose producing 50% of the maximum response; MDR = median residual; MDAR = median absolute residual.

* The standard deviation (SD) in the log domain is approximately the coefficient of variation (CV) in the standard domain for small values of SD. For values above 0.5 (50%), the standard deviation in the log domain diverges sufficiently from the CV that we elected to not refer to it as the "approximate CV."

Correlations with Side Effects

Intrathecal neostigmine injection was associated with nausea, vomiting, sedation, anxiety, and lower extremity weakness (full details can be found in the previous report of pharmacologic effects).[8] There was no significant correlation between log CSF neostigmine concentration and simultaneous sedation or anxiety scores. There was a positive correlation between log CSF acetylcholine and sedation score ($P = 0.001$; $r = 0.462$).

Discussion

This is the first description of CSF pharmacokinetics and pharmacodynamics of neostigmine after intrathecal administration in humans. These results raise interesting questions regarding disposition of drugs in the intrathecal space and provide guidance in the introduction of intrathecal neostigmine in clinical practice.

Cerebrospinal Fluid Pharmacokinetics

The pharmacokinetics of neostigmine administered by bolus injection are linear with respect to bolus dose. These data do not indicate whether similar pharmacokinetics could be expected with an infusion, so we cannot extrapolate these findings beyond bolus dosing. Intrathecal neostigmine concentrations were best described by a three-compartment model with an absorption phase. The absorption phase was likely the delay between drug injection and diffusion to the sampling catheter. The time of the peak concentration ranged from 5 to 30 min after the intrathecal bolus. This is the same range in time to peak concentration reported by Sjöström *et al.*,[16] although the median peak in their study was at the first 5-min sample. The absorption phase was followed by a biexponential distribution and elimination phase. The resulting triexponential model produced an unbiased prediction of the concentrations in both the time domain (fig. 2) and the concentration domain (fig. 3). The performance of the individual *post hoc* Bayesian estimates across four orders of magnitude (fig. 3, bottom graph) indicates that the triexponential model structure was sufficiently flexible to model the data.

These results are consistent with previous studies showing that CSF concentrations after intrathecal administration are highly variable.[16–19] The median absolute weighted residual of 56% was almost twice as large as usually observed for intravenous anesthetic drugs.[20,21] As a result, the ability to predict CSF concentration based on dose alone is limited. However, the shape of the observed concentrations over time were well described by the shape of the triexponential disposition curve. This suggests that although the absolute magnitude of the concentration cannot be accurately predicted from dose alone, the relative change in concentration over time can be accurately predicted.

Pharmacokinetic models are often expressed as vol-

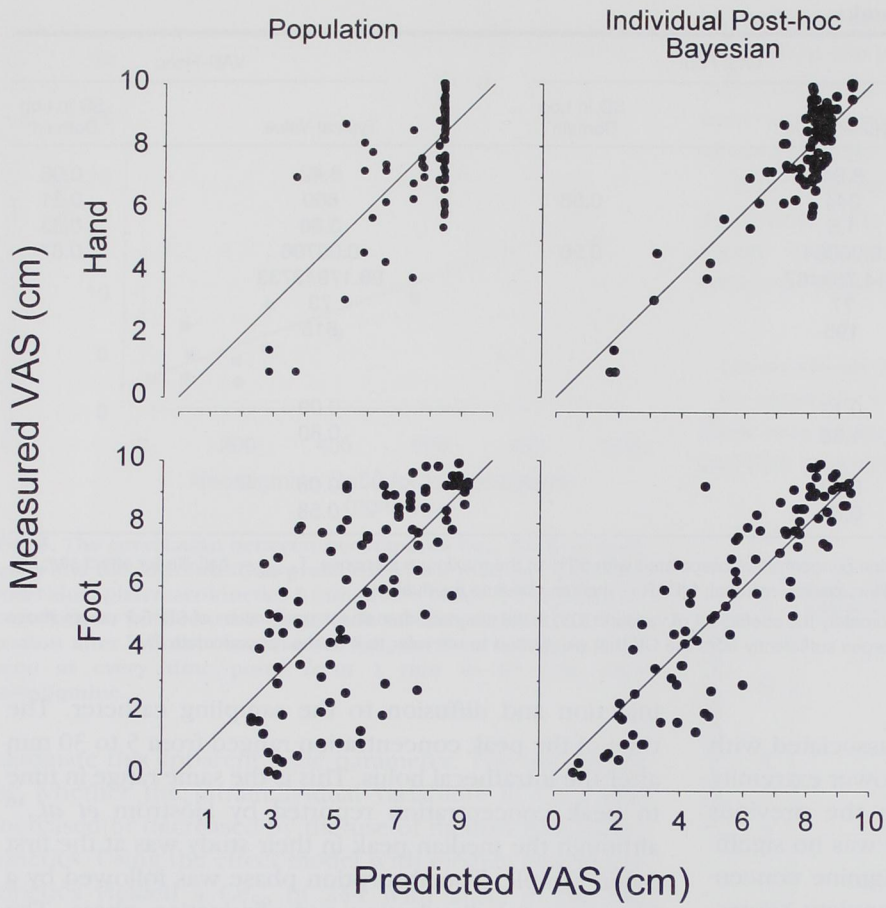

Fig. 7. Predicted *versus* measured analgesia in the hand (*top panels*) and foot (*bottom panels*), according to the population pharmacodynamic model (*left panels*) and the model based on the individual *post hoc* Bayesian estimates of the pharmacodynamics (*right panels*). Failure to incorporate interindividual variability in potency (*left panels*) reduced the accuracy of the prediction of the analgesic response. Analysis of analgesia in the hand was complicated by fairly few analgesic responses.

umes and clearances to better understand physiologic implications of the pharmacokinetic modeling. In this case we believe that the polyexponential shape of the curve reflects, at least in part, the diffusion of neostigmine away from the site of injection. Diffusion is a fundamentally different process than distribution and clearance that govern systemic pharmacokinetics. Therefore we have not calculated volumes and clearances from our polyexponential disposition function.

Pharmacodynamics of Analgesia

The pharmacodynamic modeling demonstrates a concentration *versus* response relation between effect-site neostigmine concentrations and analgesia in the feet and hands. There was considerable intersubject variability in the Ce_{50} values for analgesia, so estimation of the concentration–response relation required use of population modeling approaches and examination of individual concentration-response relations based on *post hoc* Bayesian estimates of Ce_{50} in each individual.

The initial results from our pharmacokinetic and pharmacodynamic analysis showed an unexpected correlation between the magnitude of the observations and the drug potency (fig. 5). This can be explained by viewing the CSF samples as observations taken at a distance from both the sites of injection and drug effect. Consistently high concentrations indicate that the sampling site is close to the site of injection, and consistently low concentrations indicate that the sampling site is distant from the site of injection. These interindividual differences in magnitude thus do not reflect true differences in the time course drug concentration in the CSF and thus must be removed from the analysis. The concept of "observation at a distance" is explained in detail in Appendix 1.

Specific predictions can be made about the behavior of pharmacokinetic system in which diffusion plays a major role in drug distribution, and thus observations are local, at an unknown distance from the sites of drug injection and drug effect:

1. The observed concentrations over time should have similar shapes, but with different magnitudes. This

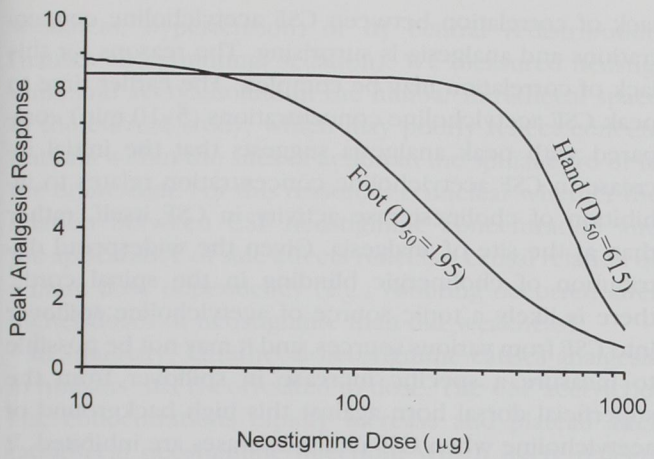

Fig. 8. The relation between the neostigmine bolus dose and peak analgesia of the hand and foot. Unlike the arbitrary units of figure 6, the units of dose are not an arbitrary scale. This removes the postulated effect of the distance between injection and sampling sites.

was observed in this study, as shown in the dose-normalized concentrations and disposition curves seen in figure 1.

2. The magnitude of the concentrations, normalized to dose, will correlate with the apparent potency. This is shown in figure 5. The correlation is a result of both measures sharing a common offset, the distance from the site of injection. Note the distinction between this result and the expectation in "well stirred" models (*e.g.*, intravenous pharmacokinetics) in which persons with higher concentrations after the same dose simply have more profound drug effect, but not a change in potency.
3. The Ce_{50} for analgesia of the hand should be higher than the Ce_{50} for analgesia of the foot. The Ce_{50} for hand analgesia was 860 ng/ml, and the Ce_{50} for foot analgesia was 244 ng/ml. Put another way, compared with the foot, a larger dose is required to produce analgesia in the hand, as shown in figure 8.
4. The variability in magnitude of the concentration *versus* time profile is, in part, an artifact of the distance. This variability contributes to the variability in Ce_{50} for drug effect. If the variability in CSF concentrations is removed, for example by assuming that all persons are described by the population pharmacokinetics, then the variability in Ce_{50} will also be reduced. Calculating the neostigmine effect-site concentrations using population values of the pharmacokinetics (fig. 1, thick line) rather than the individual *post hoc* Bayesian estimates (fig. 1, thin lines) reduced the standard deviation of Ce_{50} in the log domain from 1.14 to 0.86. There was almost no influence on the goodness of fit from ignoring the individual effects incorporated in the *post hoc* Bayesian estimates. Were the "observation at a distance" hypothesis false, then calculating effect-site neostigmine concentrations from the population pharmacokinetics should increase the variability in estimates of Ce_{50}.
5. The t ½ k_{eO} should be more rapid for the more proximal site of drug effect. We did not observe this in our data. T ½ k_{eO} for the hand and foot were 98 and 114 min, respectively. However, this change produced only a trivial change in the time of peak effect in the hand and foot of 73 and 77 min, respectively. Given the wide variability in k_{eO} estimates (table 2), we have given little weight to the discrepancy between our findings a more rapid t ½ k_{eO} in the hand and the prediction of an opposite result by the observation at a distance hypothesis.

Our neostigmine results suggest that CSF samples in this study represent observations at a distance. Because distance affects the magnitude of the observations, we need to find a common scale for CSF concentrations to reduce the variability of the estimates of drug potency. In finding a common scale, the pharmacodynamic modeling used the shape of the typical intrathecal concentrations over time (fig. 1, solid line) as given by the convolution of the input function (dose) and the disposition function:

$$C_{CSF} = \text{Dose} \times (-9.95e^{-0.21t} + 9.89e^{-0.0275t} + 0.0607e^{-0.000416t})$$

that describes the concentrations, in nanograms per milliliter, after a 1-μg bolus injection. This modeling approach reduced the variability in Ce_{50}, implying that individual differences in the pharmacokinetics were mostly an artifact of a scalar, which may be distance from the injection site and did not reflect true differences in drug concentrations in the CSF. Thus an important hypothesis is that CSF samples must be gathered at precisely known distances from the site of injection and the site of drug effect. If this is not possible then typical pharmacokinetic parameters in the population may be more accurate predictors of individual drug exposure than the actual measurements of concentration. This suggests the testable hypothesis that having established the shape of the concentration *versus* time curve for intrathecal neostigmine, under identical experimental circumstances there is little value to drawing intrathecal

neostigmine concentrations in future pharmacodynamic studies. This hypothesis assumes that circumstances are unchanged (same dose, vehicle, subject position, subject population) and that the sampling itself does not alter the CSF pharmacokinetics. If this hypothesis can be demonstrated prospectively, then it may facilitate research in the pharmacodynamics of intrathecally administered drugs by decreasing the necessary instrumentation of the participants.

Cholinesterase Inhibition and Analgesia

Two striking observations in the current study are the sustained plateau of increased acetylcholine concentrations in CSF after intrathecal neostigmine and the lack of correlation between CSF acetylcholine concentration and analgesia. Low basal concentrations of acetylcholine in CSF are similar to those reported by others[22] and in keeping with the presence of cholinesterase activity in human CSF,[23,24] which would limit the accumulation of acetylcholine from synaptic spillover into the interstitial and CSF spaces. Although we did not measure inhibition of cholinesterase activity in the current study, it is likely that CSF neostigmine concentrations even after the lowest dose of neostigmine were adequate to significantly inhibit cholinesterases in CSF and allow a new steady state to be achieved.[25] Assuming a neostigmine concentration of 30 ng/ml is sufficient to cause 50% inhibition of cholinesterase,[20] the time until decay of neostigmine in CSF to this value was sufficiently long, ranging from 100 min for the 50 μg dose to 800 min for the 750 μg dose, to produce sustained increases in acetylcholine over this period. This may explain the sustained and relatively constant increase in CSF acetylcholine after neostigmine injection and the lack of relation to dose within the neostigmine dose range used.

The assumption that neostigmine causes analgesia by a spinal action is supported in the current study by the significant correlation between CSF neostigmine and analgesia in the lower extremities. The marked hysteresis (peak analgesia, 60–90 min after injection) is similar to that produced by morphine[9] and would be anticipated from neostigmine's hydrophilicity. It is assumed, based on the ability of cholinesterase inhibitors of widely varying structure to produce analgesia after intrathecal injection, and on their ability to potentiate analgesia from intrathecal injection of agents that increase spinal cholinergic activity,[6] that neostigmine causes analgesia by inhibiting acetylcholinesterase in the spinal cord dorsal horn.

Given the presumed cholinergic mechanism of neostigmine's analgesic action in the spinal cord, the lack of correlation between CSF acetylcholine concentrations and analgesia is surprising. The reasons for this lack of correlation may be complex. The earlier time to peak CSF acetylcholine concentrations (5–10 min) compared with peak analgesia suggests that the initial increase in CSF acetylcholine concentration relates to inhibition of cholinesterase activity in CSF itself, rather than at the site of analgesia. Given the widespread distribution of cholinergic blinding in the spinal cord,[1] there is likely a tonic source of acetylcholine spillover into CSF from various sources, and it may not be possible to measure a specific increase in spillover from the superficial dorsal horn against this high background of acetylcholine when CSF cholinesterases are inhibited. It would appear that in this circumstance, unlike that when spinal acetylcholine release is directly stimulated (by opioids or $_2$– adrenergic agonists[7]), measurement of acetylcholine in CSF reflects the poorly synaptic acetylcholine concentration in the dorsal horn.

Methods of Administration

The method of neostigmine administration in the initial 14 volunteers of this study (injection through a large-gauge spinal needle followed in 2 min by intrathecal catheter insertion and repeated sampling of CSF) does not mimic the clinical situation. We speculated that ongoing CSF losses because of the intrathecal catheter and dural rent may have limited cephalad spread of neostigmine, explaining the increased incidence of central side effects (nausea and vomiting) when neostigmine is injected in saline through a small needle without a catheter rather than with a catheter.

The addition of dextrose to local anesthetic solutions can, in conjunction with specific patient positioning, dramatically restrict the distribution of spinal anesthesia. Similarly, the addition of dextrose to neostigmine in the small needle injection part of this study avoided the protracted nausea and vomiting observed in the few volunteers who received neostigmine in saline. Compared with volunteers who received neostigmine in saline, those who received neostigmine in dextrose appeared to have similar or greater lumbar CSF neostigmine concentrations, supporting the concept of restricted cephalad spread with injection of hyperbaric solution. However, the number of volunteers studied was small, and confirmation of this hypothesis requires further testing.

Correlation with Side Effects

Intrathecally administered neostigmine could produce side effects by local, spinal actions (lower extremity

weakness, hypertension) or by central redistribution (nausea and vomiting, sedation). We measured neostigmine and acetylcholine in the lumbar intrathecal space in the current study, which may poorly reflect concentrations within the sites of action in the spinal cord or in the brain stem. For this reason, it is unclear whether the relation between CSF neostigmine concentration and the appearance of side effects reflects a causal relation or a mere dose dependency (*e.g.*, vomiting occurred after higher doses of neostigmine than did weakness).

In summary, intrathecal neostigmine causes analgesia in humans that is correlated to dose. The CSF acetylcholine concentrations rapidly increase and plateau after intrathecal neostigmine injection, which likely reflects inhibition of cholinesterase activity in CSF and probably does not reflect dorsal horn synaptic acetylcholine concentrations. Neostigmine pharmacokinetics in CSF after intrathecal injection are complex and include an absorption phase that likely reflects diffusion from the site of injection to the tip of the sampling catheter. Pharmacodynamic modeling demonstrated a concentration *versus* response relation for both foot and hand analgesia. The pharmacokinetics and pharmacodynamics observed in this study suggested that CSF samples represent "observations at a distance" in which the absolute magnitude of the concentrations are scaled by an arbitrary factor (*e.g.*, distance of the observation site from the catheter and the site of drug effect). The pharmacodynamic model was transformed to a nonarbitrary scale by expressing it as the peak analgesic response after a bolus dose. This pharmacodynamic model would only be useful if the scalar could be known in advance or could be correlated to some physical property (*e.g.*, the distance from the injection site to the effect site, volume of CSF), and the nature of these properties should be sought in hypothesis-driven studies. If the nature of this scalar cannot be determined, these results suggest that knowledge of CSF concentrations of a drug after intrathecal bolus administration tells us nothing about the drug effect.

Appendix 1: Observation at a Distance

Imagine injecting a drop of dye into the center of a clear cylindrical container of still liquid. The dye will gradually spread away from the injection point by the process of diffusion. The concentration of dye observed at every point in the container will be a function of the distance from the point of injection and the time of the observation. The actual relation among dye concentration, time, and distance for diffusion in two dimensions (caudad and cephalad) is given by the equation[26]:

$$C(r,t) = \frac{S}{\sqrt{4\pi Dt}} e^{-\frac{r^2}{4Dt}}$$

where $C(r,t)$ is the concentration at a distance r (representing radius) and at time t. S is an arbitrary scaling factor (set to 10,000 in this example), D is the diffusion coefficient (set to 1 in this example), and e is the base of natural logarithms.

The spread of drug will follow the same relation as the spread of a visible dye. We can gain insight into diffusion of drug throughout the CSF by solving this equation at five different distances: 0, 1, 5, 7, and 11 arbitrary distance units. By definition, drug is injected at r = 0. Let us postulate two sites of drug sampling, r =1 (close to the injection site) and r = 5 (distant from the injection site). Let us also postulate that the spinal site of analgesia for the foot is 7 distance units away from the injection site, and the spinal site of analgesia for the hand is 11 distance units away from the injection site.

Figure 9 shows the result. Dashed lines in figure 9 show unmeasurable values, and solid lines show values that can be measured. The graphs on the left are concentration *versus* time, and the graphs on the right are drug effect *versus* time. Concentrations gathered near the injection site (r = 1) are higher than those gathered distant from the injection site (r = 5). The drug concentrations at the spinal sites of analgesia for the foot and hand, r = 7 and r = 11, respectively, are less than those at the sites of drug sampling.

To simplify this example, we will ignore the requirement that the drug enters the spinal cord to exert drug effect and will simulate the drug effect as though it instantaneously reflects the local CSF drug concentration. We can calculate the time course of drug effect by postulating a relation between CSF drug concentration at the site of drug effect and the VAS score for analgesia:

$$\text{VAS Score} = 8 - 8\frac{C^{\gamma}}{C^{\gamma}_{50} + C^{\gamma}}$$

$$\text{VAS Score} = 8 - 8^{*}\frac{C^{\text{slope}}}{C^{\text{slope}}_{50} + C^{\text{slope}}}$$

where C_{50} is 200, and the slope is 4 (values arbitrarily assigned). From this relation we can calculate the time course of foot (r = 7) and hand (r = 11) response, as shown in the two VAS score *versus* time graphs of figure 9.

Pharmacodynamic modeling allows us to relate the drug concentrations to drug effect. The measures of concentration available in this simulation study are closer to the injection site (one and five units, in this example) than the sites of drug effect. This causes a delay between the time course of drug concentration at the sampling site and the time course of the drug effect. We modeled this delay the same way we modeled the delay with the real data from this study, using the effect-site model proposed by Sheiner *et al.*[15] For the purposes of this example, we implemented the effect site using the "nonparametric" approach described by Fuseau and Sheiner.[27]

Figure 10 shows the results of the pharmacodynamic analysis of the data from the simulation in figure 9. The top panels show the true hand and foot concentration *versus* response relation, which are identical in this simulation. The middle panels show the foot and hand concentration *versus* response relations estimated from the VAS scores and the concentrations sampled from near the injection site (r = 1). The $t_{1/2}$ k_{eO} estimates for foot and hand analgesia were 26 min and 56 min, respectively. The Ce_{50} estimated for the analgesic response was greater than the true Ce_{50} of 200 because the model did not consider the dilution of drug as it

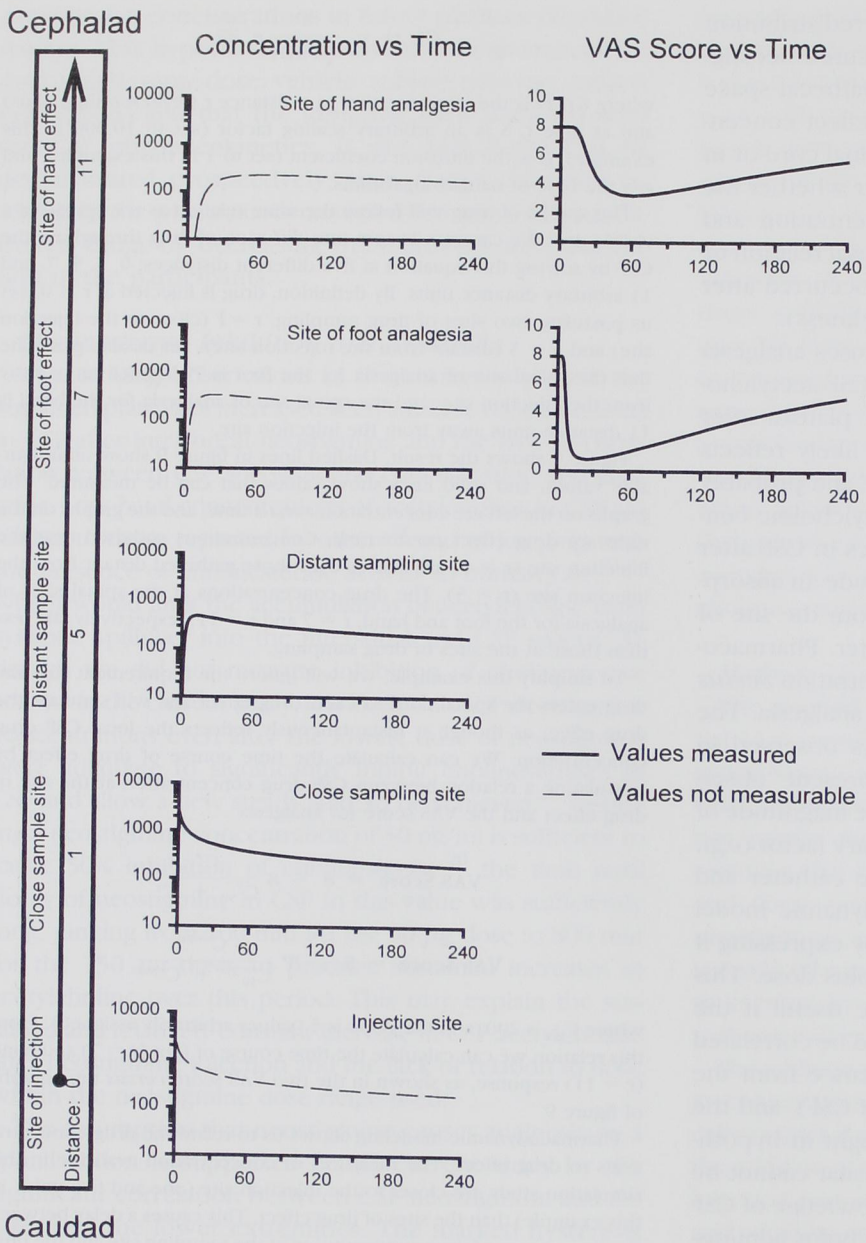

Fig. 9. Simulation showing the time course of drug concentration at 0, 1, 5, 7, and 11 distance units away from a bolus injection at r = 0, based on a model of diffusion in two dimensions. The VAS score in the foot and hand is determined by a pharmacodynamic model, based on the concentration at distances of 7 and 11 units from the site of injection, respectively.

diffused from the sampling site to the site of drug effect. This was more pronounced for the hand than the foot, because there is even greater dilution of drug during diffusion to the hand than to the foot. Thus the Ce_{50} for analgesia in the hand was greater than for the foot. Expressed another way, the model predicts that it will take more drugs to cause analgesia in the hand than in the foot, an expected result.

The lower panels in figure 10 show the concentration *versus* response relations estimated from the VAS scores and the concentrations sampled distant from the site of injection and closer to the site of action (r = 5). The concentrations are lower because the drug has been diluted into additional CSF in the process of diffusing to the sampling site. As a result, the estimates of Ce_{50} for the foot and the hand are less than when the drug was sampled close to the injection site. The $t_{1/2}$ k_{eO} values for hand and foot analgesia based on samples taken at r = 5 are 4 and 25 min, respectively. The $t_{1/2}$ k_{eO} values from concentrations sampled at r = 5 are faster than those based on concentrations sampled at r = 1 because the CSF concentrations at r = 5 already exhibit much of the delay between the time course of concentration and the time course of drug effect. Thus, as the sampling site gets closer to the true site of drug effect, the need to include an effect site in the model decreases.

Figure 11 demonstrates the relation between peak concentration and apparent potency from this simulation of a diffusion model. The numbers in parentheses are the distance of the drug sampling site, in

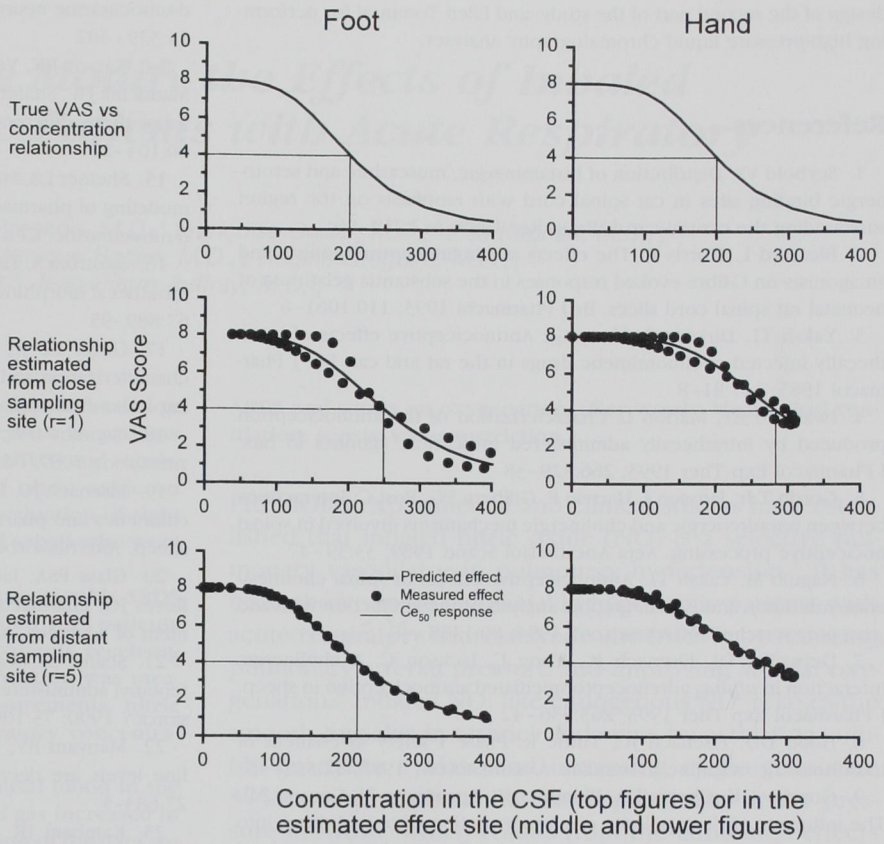

Fig. 10. Simulation of pharmacodynamic modeling of the concentration *versus* effect relation in the foot and hand. The top panels show the true relation, given in the discussion. The middle panels show the relation estimated using concentrations sampled one unit away from the site of injection. The bottom panels show the relation estimated using concentrations sampled five units away from the site of injection. The closer the samples are to the injection site, the higher the apparent concentrations, resulting in decreased apparent potency (higher Ce_{50}) of the drug.

the same arbitrary units, from the site of injection. The position of the numbers in parentheses reflect the concentration at 5 min (x axis) and the Ce_{50} estimated for foot analgesia (y axis). Near the site of injection, the concentrations are very high, and the estimated Ce_{50} is large. Further from the site of injection (r = 0), and hence closer to the site of drug effect (r = 7), the concentrations at 5 min become less and the Ce_{50} approaches the true value of 200.

The ability of the equation for diffusion in two dimensions to produce data resembling those arising from this study might suggest that we abandon polyexponential models entirely and model the pharmacokinetics using diffusion equations. We attempted this using equations for diffusion in two and three dimensions.[26] Our results, not shown, suggested that the appropriate model required inclusion of both diffusion and tissue distribution. In addition, the model required incorporation of diffusion of drug from the CSF into the spinal cord, which was not a part of the simulation. There is no closed-form equation that describes diffusion and distribution. We are developing approximate solutions based on diffusion–distribution that may better specifically incorporate the crucial role of distance in modeling these data. However, the best pharmacokinetic model for these data remains the polyexponential model described in the text.

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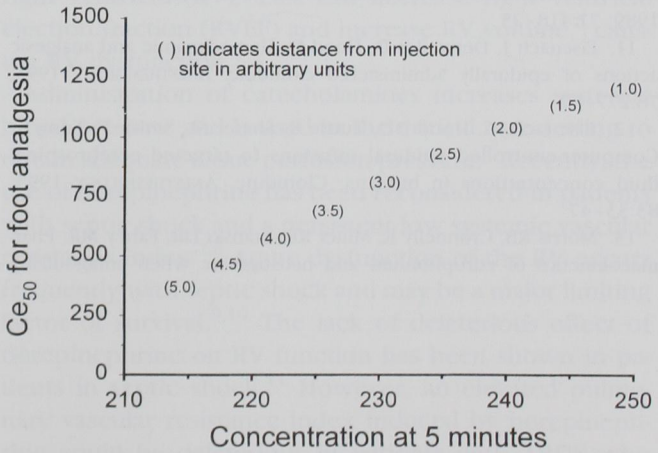

Fig. 11. The relation between concentration at 5 min and the Ce_{50} for foot analgesia, based on the model shown in figures 9 and 10. Each data point is indicated by a number in parentheses. The number itself is the distance units away from the site of injection. As the samples are drawn closer to the site of foot analgesia (r = 7), the Ce_{50} approaches the true value of 200. This figure, based on a model of distance, shows the same pattern as seen in figure 5 with the real data from this study.

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