Effect of Edrophonium and Neostigmine on the Pharmacokinetics and Neuromuscular Effects of Mivacurium

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Background: Previous studies demonstrated that both edrophonium and neostigmine affect mivacurium’s pharmacokinetics, thereby potentially affecting its recovery profile. However, those studies were not clinically relevant because mivacurium was still infused after the antagonists were given. In the present study, the authors gave antagonists (or placebo) after discontinuing a mivacurium infusion, thereby obtaining data that are more clinically relevant.

Methods: In 18 patients, mivacurium was infused at 10 μg kg⁻¹ min⁻¹ for 40 min, the infusion was discontinued for 15 min and then restarted at the same rate for another 40 min. Patients were randomized to receive 500 μg/kg edrophonium, 50 μg/kg neostigmine, or saline at discontinuation of the second infusion; all subjects received 1 mg atropine. Plasma was sampled during the final 10 min of each infusion to determine steady state mivacurium concentrations and for 15 min after each infusion. Twitch tension was recorded. Mivacurium concentrations after each of the two infusions were compared.

Results: After discontinuation of the second infusion, mivacurium concentrations were larger than those after the first infusion at 2 min with edrophonium and at 2, 4, and 7 min with neostigmine. With both neostigmine and edrophonium, twitch tension recovered after infusion #2 more rapidly than after infusion #1; however, the magnitude of this effect was small.

Conclusion: Edrophonium transiently slows the rate at which mivacurium concentrations decrease; this is consistent with our previous findings. Neostigmine has a similar, although longer, effect. Despite altering mivacurium’s elimination characteristics, both drugs facilitate neuromuscular recovery, although their benefit is small. (Key words: Antagonists; drug interaction; muscle relaxants.)

ANTAGONISM of neuromuscular blockade by edrophonium and neostigmine depends on an increase in the ratio of acetylcholine to that of muscle relaxant at the neuromuscular junction, followed by continued elimination of the relaxant from plasma. Presumably, plasma concentrations of most relaxants are not affected by these antagonists.¹ ² However, we recently demonstrated that during continuous infusion, edrophonium¹ and neostigmine² both increase mivacurium concentration. This may explain the inconsistent findings regarding the ability of these antagonists to facilitate recovery from mivacurium-induced paralysis, in which some studies report that the antagonists facilitated recovery, whereas others report no facilitation.

To address whether antagonist-induced changes in mivacurium’s elimination affects recovery, we considered that the optimal design would incorporate two features. First, each subject should act as his or her own control so that interindividual variability does not obscure the antagonist’s effect. Second, mivacurium concentrations should be measured to assess whether the antagonists slowed mivacurium’s decay. Although our previous studies¹ ² demonstrated that these antagonists affected mivacurium’s elimination, those studies were performed during a continuous infusion of mivacurium, so that the effect of the antagonist was to increase mivacurium concentrations. In contrast, when antagonists are given after discontinuing mivacurium infusion, the antagonists would likely slow the rate at which mivacurium concentrations decreased rather than produce an increase.
In the present study, we gave the antagonists (or placebo) after discontinuing infusion of mivacurium. In addition, each subject received sequential infusions of mivacurium, the first of which was not followed by an antagonist. Because mivacurium’s potent stereoisomers are eliminated rapidly (half-life < 2 min),3 residual mivacurium concentrations from the first infusion were unlikely to affect twitch tension during the second. This permitted each subject to act as his or her own control for both the expected rate of decrease of mivacurium concentrations and twitch recovery.

Methods

After obtaining approval from our institutional review board and informed consent from each subject, we studied 20 American Society of Anesthesiologists physical status 1 patients undergoing peripheral surgery. Anesthesia was induced with 5 µg/kg fentanyl and 2–3 mg/kg propofol and maintained with 60% nitrous oxide and 0.8% end-tidal isoflurane. Patients were kept normothermic and normocarbic. No other drugs known to influence neuromuscular response were given. Before mivacurium was given, blood was sampled to measure plasma cholinesterase activity photometrically using acetylthiocholine as a substrate. The first 18 patients were randomly assigned to receive edrophonium (n = 6), neostigmine (n = 6), or placebo (n = 6). Because data from two subjects given edrophonium could not be used in the analysis (see Results), the final two patients received edrophonium.

After induction of anesthesia, the ulnar nerve was stimulated via subcutaneous needle electrodes at the wrist. Supramaximal stimuli of 0.2 ms duration were delivered in a train-of-four at 2 Hz every 12 s (Digistim II, Neuro Technology, Houston, TX), preceded initially by a 5-s 50-Hz tetanus.4 Preload of 200–400 g was maintained constant. Adductor pollicis twitch tension was measured using a calibrated force transducer (Myotrace, Houston, TX), amplified (DC Bridge Signal Conditioner, Gould Electronics, Valley View, OH), digitized (NB-M10-16, National Instruments, Austin, TX), and recorded on-line (Quadra 800, Apple Computer, Hayward, CA). End-tidal isoflurane concentration was stable at 0.8% for > 20 min, and the first twitch response of each train (T1) was stable for > 10 min before mivacurium administration.

Mivacurium, 500 µg/ml, was infused (Model 908 Infusion Pump, Harvard Apparatus, South Natick, MA) at 10 µg · kg⁻¹ · min⁻¹ for 40 min (infusion #1), then the infusion was discontinued for 15 min and no antagonists were administered. Mivacurium was then infused at the same rate for 40 min (infusion #2, fig. 1). One minute before infusion #2 ended, 1 mg atropine was given through a dedicated peripheral venous catheter. Immediately after the mivacurium infusion, 500 µg/kg edrophonium, 50 µg/kg neostigmine, or an equivalent volume of saline was given through the dedicated catheter; after each injection, the catheter was flushed with 5 ml saline.

Radial arterial blood was sampled before mivacurium administration (blank sample), during each infusion (10, 5, and 1 min before each infusion was discontinued), and 1, 2, 4, 7, 10, and 15 min after discontinuation of each infusion. Samples were obtained over 4–6 s. To prevent mivacurium from degrading in vitro, phospholine iodide (1.25 mg in 100 µl H₂O) was added to samples within 10 s; samples were iced within 1 min and the plasma phase separated and frozen within 1 h. Mivacurium concentrations were determined by high-performance liquid chromatography.5 The assay is sensitive to 5 ng/ml for each of the three isomers and has a coefficient of variation = 16% at that concentration; the assay is not affected by edrophonium or neostigmine.

The “active” concentration of mivacurium was determined as the sum of the concentrations of the cis-trans and trans-trans isomers, (i.e., the cis-cis isomer was assumed to have no neuromuscular effect and the cis-trans and trans-trans isomers were assumed to be equipotent). Steady state concentration for each infusion was determined as the average of the three values during that infusion. The ratio of the steady state concentrations during the two infusions (infusion #2/infusion #1) was determined; the average of these ratios was compared

Fig. 1. “Active” mivacurium concentrations (the sum of the concentrations of the cis-trans and trans-trans isomers) during and after each of two infusions (open triangles for infusion #1, closed triangles for infusion #2) in a patient given neostigmine after infusion #2. The time scale is relative to the end of each infusion.

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with the expected average ratio (1.0) using a one-sample test (statistics detailed later).

For each subject, each “active” mivacurium concentration after each infusion was divided by the steady state value obtained during that infusion. To determine whether groups differed in their pharmacokinetics during infusion #1, these normalized values at 1, 2, 4, 7, 10, and 15 min postinfusion #1 were compared among groups using an unpaired test. To determine whether antagonists or placebo influenced mivacurium’s pharmacokinetics, the normalized values at each time interval after infusion #2 were divided by the corresponding values after infusion #1. For example, if 2 min after infusion #2, a subject’s value was 15% of the steady state concentration during that infusion, and 2 min after infusion #1, the value was 9% of the steady state concentration during that infusion, the resulting ratio was 1.67 (15%/9%). For each group, average values of these ratios were determined at each time point and compared with the expected value (1.0) using a one-sample test. To determine whether mivacurium’s cis-cis isomer accumulated, the three values for each subject during each infusion were averaged. The ratio of the values for infusion #2 and infusion #1 was determined; the average of these ratios for all subjects was compared with the expected average ratio (1.0) using a one-sample test.

Twitch tension during the final 10 min of each infusion was averaged for each individual. To determine whether the steady state response to each of the two infusions of mivacurium was similar among the three groups, mean values for twitch tension during each infusion were compared among groups using an unpaired test. Values for twitch tension were determined at 1-min intervals for 15 min after termination of each infusion. To determine whether groups differed in the rate of recovery after the first infusion, mean values for twitch tension at each time interval were compared among groups using an unpaired test. To determine whether placebo or antagonists influenced the rate of recovery, the difference between twitch tension values after infusions #1 and #2 was determined. For each group, mean values for this difference in twitch tension at each time interval were compared with the expected value (0.0) using a one-sample test. Train-of-four ratio 15 min after discontinuation of each infusion was measured. For each group, mean values for these train-of-four ratios during the two infusions were compared using a paired test.

**Statistics**

Values are reported as mean ± SD. One-sample tests were performed using Student one-sample t test or its nonparametric equivalent. Unpaired tests were analysis of variance and the Student–Newman–Keuls test or their nonparametric equivalents. Paired tests were repeated-measures analysis of variance and the Student–Newman–Keuls test or their nonparametric equivalents. For all comparisons, P < 0.05 was considered statistically significant.

**Results**

The three groups were comparable in age, weight, and gender distribution. Average weight was 79 ± 17 kg, average age was 32 ± 9 yr, and there was one woman in each group. All patients had normal plasma cholinesterase activity; mean activity values did not differ among groups. One patient given edrophonium had steady state mivacurium concentrations that differed 10-fold between the two infusions, a result of partial disconnection of the intravenous tubing. In another subject given edrophonium, mivacurium concentrations fluctuated markedly during both infusions (during which twitch was ablated), then increased transiently after infusion #1 was discontinued. Data from these subjects were excluded from all analyses. This resulted in a sample size of six in each group.

All mivacurium isomers could be identified in all patients during each infusion and for at least 2 min after each infusion. One (or both) potent isomers could not be identified in one patient (in the edrophonium group) at 4 min after the infusions, in seven patients at 7 min (one in the neostigmine group, three in each of the other groups), and in 11 or more patients at subsequent times. Because of the small number of samples in which the potent isomers could be identified after 7 min, plasma concentration values > 7 min after the infusion were not included in analyses.

“Active” concentrations of mivacurium for all patients varied minimally during each infusion (coefficient of variation averaged 6% during the first infusion and 4% during the second infusion). For all patients, steady state concentrations during infusion #2 (219 ± 59 ng/ml) were 4% more (P = 0.05) than those during infusion #1 (211 ± 59 ng/ml). After the first infusion (at which time no antagonists were given), the rate at which mivacurium concentrations decreased differed among groups only at 7 min (P < 0.05), at which time the concentra-
tion in the placebo group (6.6 ± 0.3% of the value during steady state) was less than that in the neostigmine (10.2 ± 1.0%) and edrophonium (10.8 ± 4.1%) groups. Concentrations of the cis-cis isomer were 31 ± 12% larger during infusion #2 (89 ± 8 ng/ml) than during infusion #1 (68 ± 15 ng/ml; P < 0.05).

The rate at which "active" mivacurium concentrations decreased postinfusion did not differ between infusions in the placebo group (figs. 2 and 3). With neostigmine, concentrations after the second infusion were larger than those after first infusion 2, 4, and 7 min after antagonist administration. With edrophonium, concentrations after infusion #2 were larger than those after infusion #1 only 2 min after antagonist administration.

During the final 10 min of both infusions, twitch tension was ablated in three patients in each group; in the remainder, it ranged from 2 to 11% of control. Twitch tension during mivacurium administration did not differ among groups during infusion #1 or infusion #2. After infusion #1, recovery of twitch tension did not differ among groups. After infusion #2, recovery at 11–15 min with placebo was less than that in infusion #1 (P < 0.05 at each time point, fig. 4); with both neostigmine (at 4–14 min) and edrophonium (at 4–15 min), the magnitude of recovery was larger (P < 0.05 at each time point). For both neostigmine and edrophonium, train-of-four ratio was larger 15 min after infusion #2 compared with infusion #1 (table 1). In the placebo group, train-of-four ratio 15 min after the two infusions did not differ.

Discussion

Both edrophonium and neostigmine slowed the expected decrease in concentrations of mivacurium's potent isomers after a mivacurium infusion was discontinued. This was evident 2 min after edrophonium was given and did not persist. With neostigmine, the effect occurred later and persisted longer. Both findings are consistent with previous studies from our group, despite differences in design. For example, in one study, mivacurium was infused to maintain 90% twitch depression, then 125–2,000 µg/kg edrophonium was given and mivacurium was infused at the same rate. The intent was to determine the dose-response relation for antagonism of mivacurium, because our clinical impression was that antagonists were less effective for mivacurium than for other muscle relaxants. We selected a continuous dosing regimen because we assumed that edrophonium did not affect mivacurium’s elimination in vitro, leading us to...
assume that edrophonium would not affect mivacurium’s elimination in vivo. In contrast, edrophonium increased mivacurium concentration 48–79%, peaking at 1–2 min. This transient increase in mivacurium concentrations impeded antagonism, compared with antagonism of vecuronium-induced paralysis using a similar study design. A similar study with neostigmine demonstrated that mivacurium concentrations also increased, although to a lesser magnitude and later than with edrophonium.

Our previous study provided no insight regarding whether the transient increase in mivacurium concentrations induced by edrophonium resulted from a decrease in the rate at which mivacurium was metabolized or from edrophonium displacing mivacurium from tissue into plasma. We expected that if edrophonium affected mivacurium’s metabolism, the rate at which mivacurium concentrations decreased after infusion #2 would parallel those after infusion #1 but would be displaced upward. In contrast, if edrophonium displaced mivacurium from tissue, mivacurium concentrations would increase transiently, then would return to the values observed after infusion #1. Our results—an effect of edrophonium at only a single time—suggest that edrophonium displaces mivacurium. In contrast, neostigmine-induced changes in the rate at which mivacurium concentrations decrease persisted, consistent with neostigmine decreasing plasma cholinesterase activity and thereby decreasing the rate of mivacurium metabolism.

The second finding of the present study is that both neostigmine and edrophonium facilitated recovery of twitch tension; however, the magnitude of this benefit was small. For example, with both antagonists, twitch

<table>
<thead>
<tr>
<th>Infusion No. 1</th>
<th>Infusion No. 2</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>0.51 ± 0.23</td>
</tr>
<tr>
<td>Neostigmine</td>
<td>0.31 ± 0.20</td>
</tr>
<tr>
<td>Edrophonium</td>
<td>0.44 ± 0.25</td>
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Values are mean ± SD.

* Different from infusion no. 1 by Student t test for paired data. Comparisons were between infusions, not between groups.

Fig. 3. Values (mean ± SD) for the relative rate (infusion #2/infusion #1) of decrease of “active” mivacurium concentrations after discontinuation of a mivacurium infusion (see text for explanation). Asterisks indicate that values differ from the expected ratio of 1.0.

Fig. 4. Values (mean ± SD) for twitch tension after an infusion of mivacurium are displayed for each of the two infusions. Some error bars are omitted for clarity. With placebo, recovery from infusion #2 was less (P < 0.05) than that after infusion #1 at 11–15 min. With neostigmine, recovery from infusion #2 was more (P < 0.05) than after infusion #1 at 4–14 min. With edrophonium, recovery from infusion #2 was more (P < 0.05) than after infusion #1 at 4–15 min.
tension recovered only 2–3 min faster after the second infusion than after the first (fig. 4). Numerous studies6–15 have examined the effect of antagonists on mivacurium neuromuscular blockade. Some demonstrate that both neostigmine6,10,11,15 and edrophonium8,9,11–14 decrease the time to reach end points such as twitch of 90% of control or a train-of-four ratio > 0.7; in addition, studies8,9,11 including both neostigmine and edrophonium do not demonstrate differences between these drugs. The findings of those studies are consistent with ours. However, three studies7–9 report that neostigmine or edrophonium do not improve the time to certain end points. Connolly et al.8 report that 0.75 mg/kg edrophonium did not decrease the time to T1 reaching 90% of control or a train-of-four ratio > 0.7. Although trends in their data are consistent with edrophonium speeding recovery, their large number of groups and small number of patients per group probably prevented them from attaining statistical significance. Devcic et al.8 infused mivacurium to maintain 92–99% twitch depression. Although both 1 mg/kg edrophonium and 70 μg/kg neostigmine facilitated recovery of twitch to 50% of control compared with a group given placebo, neostigmine did not affect time for twitch to reach 75% of control or time for the train-of-four ratio to reach 70%. Similarly, Kao and Le9 infused mivacurium to depress twitch 97–98% during isoflurane anesthesia. Although both 1 mg/kg edrophonium and 70 μg/kg neostigmine facilitated recovery of twitch to 25%, 50%, and 75% of control compared with a group given no antagonist, neostigmine delayed time for twitch to recover to 90% of control and for the train-of-four ratio to reach 90%. Neostigmine’s failure to improve time to train-of-four recovery in these latter studies might result from a larger magnitude of twitch depression before reversal compared with other groups, a finding that is difficult to assess when all subjects have profound twitch depression.8,9 In addition, studies in which neostigmine did not speed recovery gave larger neostigmine doses (70 μg/kg) than did those studies in which neostigmine sped recovery (20–40 μg/kg). A larger neostigmine dose may impair mivacurium’s elimination to a larger magnitude and for a longer period than a smaller dose, thereby slowing the rate of neuromuscular recovery.

Previously, investigators questioned whether antagonists should be given when twitch is absent, which was the situation for many of our subjects. For example, Magorian et al.16 gave neostigmine either 15 min after vecuronium (when twitch was absent) or when T1 reached 10% of control. Early administration of neostigmine neither sped nor hindered time to recovery of T1 to 90% of control or the train-of-four ratio to > 0.7. Our study provides no insight regarding whether giving these antagonists when twitch tension reached 5–10% of control (instead of immediately after the infusion) or 2 min after terminating an infusion17 would further facilitate recovery. Both edrophonium and neostigmine sped recovery despite slowing the rate at which mivacurium concentrations decreased, indicating that the antagonist-induced increase in acetylcholine overcomes the slowing of the rate at which mivacurium concentrations decrease.

With placebo, recovery was nearly identical during the first 8 min after the two infusions (fig. 4). This similar rate of initial recovery presumably results from plasma and effect site concentrations of mivacurium’s potent isomers decreasing rapidly (and supports our use of a paired design). However, after 8 min, recovery after infusion #2 was slower than after infusion #1 (i.e., a cumulative effect). Concentrations of the cis-cis isomer were larger during the second infusion than during the first. Previous investigators ignored any neuromuscular effect of the cis-cis isomer, assuming that it is less potent than the other isomers and because it comprises only 6% of the administered drug. However, potency of the cis-cis isomer in humans is unknown. In addition, its clearance (7 ml·kg⁻¹·min⁻¹)³ is markedly less than that of the potent isomers (29–46 ml·kg⁻¹·min⁻¹),¹⁸ so that during continuous infusion its concentration reaches that of the other isomers.¹⁸ Additionally, its elimination half-life (53 ± 20 min)¹⁸ is longer than those of the potent isomers (< 2 min)³; therefore, plasma concentrations decrease slowly after infusion. Thus, slower late recovery after the second infusion could result from accumulation of the cis-cis isomer. In turn, ignoring a neuromuscular effect of the cis-cis isomer may be at least partially flawed. Two other differences between our sequential infusions might contribute to a cumulative effect. First, atropine—which might alter circulatory time, thereby altering the pharmacokinetics of a drug eliminated in plasma—was given near the end of the second infusion but not the first. Second, isoflurane’s potentiation may increase with time; however, this

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**For illustration, if one group received 20 μg·kg⁻¹·min⁻¹ mivacurium and another 10 μg·kg⁻¹·min⁻¹, all patients would have profound twitch depression. Average twitch tension would be similar in the two groups, thereby providing no insight that mivacurium concentrations were higher in the first group. In addition, recovery would be slower in the first group.**

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would have minimized our likelihood of demonstrating that edrophonium and neostigmine facilitated recovery. Several aspects of our study design warrant comment. First, we normalized all mivacurium concentrations to the average value during the infusion. This normalization is necessary to prevent values from patients with larger plasma concentrations (e.g., those with a smaller plasma cholinesterase activity) from overwhelming values from subjects with smaller plasma concentrations, thereby increasing our statistical power. Second, rather than comparing the rate at which mivacurium concentrations decreased among groups, we compared values after infusion #2 to those after infusion #1; using each patient as his or her control also improved our statistical power. Finally, we selected commonly used doses of neostigmine and edrophonium, recognizing that these doses may not be equipotent. Although it would have been ideal to examine the dose-response relation for each antagonist, that would have been impractical and costly with the present study design.

Both edrophonium and neostigmine alter the rate at which mivacurium concentrations decrease after a mivacurium infusion is discontinued. As in our previous studies, edrophonium’s effect on mivacurium’s plasma concentrations is transient, whereas neostigmine’s effect persists. The magnitude of edrophonium’s effect on mivacurium’s plasma concentrations is smaller than we reported previously, presumably because of differences in study design. Because the design of the current study more closely resembles clinical practice, the current results are probably more clinically relevant. Both 500 μg/kg edrophonium and 50 μg/kg neostigmine facilitate neuromuscular recovery, although the magnitude of their benefit is small. Finally, in the placebo group, the slower rate of neuromuscular recovery after the second infusion, coupled with accumulation of the cis-cis isomer, suggests that the cis-cis isomer contributes to mivacurium’s neuromuscular effect, particularly after prolonged administration.

References


