Edrophonium Increases Mivacurium Concentrations during Constant Mivacurium Infusion, and Large Doses Minimally Antagonize Paralysis

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Background: Mivacurium, a nondepolarizing muscle relaxant, is metabolized by plasma cholinesterase. Although edrophonium does not alter plasma cholinesterase activity, we have observed that doses of edrophonium that antagonize paralysis from other nondepolarizing muscle relaxants are less effective with mivacurium. We speculated that edrophonium might alter metabolism of mivacurium, thereby hindering antagonism of paralysis. Accordingly, we determined the effect of edrophonium on neuromuscular function and plasma mivacurium concentrations during constant mivacurium infusion.

Methods: We infused mivacurium to maintain 90% depression of adductor pollicis twitch tension and then gave edrophonium in doses ranging from 125–2,000 μg/kg without altering the mivacurium infusion. Peak twitch tension after edrophonium was determined to estimate the dose of edrophonium antagonizing 50% of twitch depression for antagonism of mivacurium; plasma cholinesterase activity and mivacurium concentrations before and after edrophonium were measured. Additional subjects were given 500 μg/kg edrophonium to antagonize continuous infusions of d-tubocurarine and vecuronium.

Results: With mivacurium, edrophonium increased twitch tension in a dose-dependent manner; the dose of edrophonium antagonizing 50% of twitch depression was 810 μg/kg. The largest dose of edrophonium (2,000 μg/kg) produced only 45 ± 7% antagonism. Edrophonium, 500 μg/kg, antagonized mivacurium markedly less than it antagonized d-tubocurarine and vecuronium. Edrophonium increased plasma concentrations of the two potent stereoisomers of mivacurium 48% and 79%, these peaking at 1–2 min; plasma cholinesterase activity was unchanged.

Conclusions: Edrophonium doses that antagonize d-tubocurarine and vecuronium are less effective in antagonizing the neuromuscular effects of mivacurium during constant infusion. Edrophonium increases plasma mivacurium concentrations, partly or completely explaining its limited efficacy; the mechanism by which edrophonium increases mivacurium concentrations remains unexplained. Our results demonstrate that antagonism of mivacurium by edrophonium is impaired, and therefore we question whether edrophonium should be used to antagonize mivacurium. (Key words: Antagonists, neuromuscular; edrophonium; Enzymes, cholinesterase; plasma. Neuromuscular relaxants, nondepolarizing: mivacurium.)

MIVACURIUM has a shorter duration of action than other nondepolarizing muscle relaxants, presumably because of its rapid clearance by plasma cholinesterase.1 However, in certain instances, spontaneous recovery from mivacurium is sufficiently slow that a neuromuscular antagonist (e.g., edrophonium) is required. In the clinical setting, we have occasionally observed incomplete or delayed antagonism by 0.5–1.0 mg/kg edrophonium. In that edrophonium is reported not to alter plasma cholinesterase activity2 or the degradation of mivacurium in vitro,3 this observation was unexpected. However, little is known about the dose-response relation for edrophonium antagonism of mivacurium and whether edrophonium antagonizes mivacurium concentrations in vivo. Accordingly, we determined the dose-response relation for edrophonium-induced antagonism of mivacurium and whether edrophonium

Materials

With approval from the Research and Human Subjects Board and after obtaining informed consent from 25 American Society of Anesthesiologists' status 1 and status 2 patients undergoing elective procedures, we studied each subject's body weight, height, age, sex, cardiovascular, and respiratory status, medications, and renal function with an electrocardiogram.

After 1–2 h of i.v. saline, subjects were induced with a controlled induction regimen of 3 mg/kg i.v. sodium thiopental. Anesthesia was maintained with 100% oxygen and small tidal isoflurane concentrations. Aortic, pulmonary, and femoral arterial catheters were inserted (noninvasive monitoring performed in case a monitored arterial line was unavailable).

After induction, atracurium 0.5 mg/kg was stimulated with a tendon stimulator at the wrist. Supramaximal stimuli were delivered in the presence of atracurium 0.5 mg/kg and 0.1 mg/kg and followed by vecuronium 0.1 mg/kg and 0.05 mg/kg. Changes in the evoked response were recorded on an oscilloscope. Before muscle relaxation, isoflurane concentration was lowered to 1% and the first dose of mivacurium administered for more than 2 h.

For 17 subjects, the isoflurane concentration was lowered to 0.25% before mivacurium administration for more than 2 h.

Mivacurium is a competitive neuromuscular blocking agent that blocking action is terminated by cholinesterase enzymes.
altered mivacurium concentrations during constant mivacurium infusion.

Materials and Methods

With approval of our local Institutional Review Board and after obtaining informed consent, we studied 25 American Society of Anesthesiologists physical status 1 and 2 patients, 18–52 yr old, scheduled for elective surgery. Patients exceeding 130% of ideal body weight, those with renal, hepatic, neuromuscular, and/or electrolyte disorders, or those taking medication known to interfere with neuromuscular function were excluded.

After 1–2 mg intravenous midazolam, anesthesia was induced with 3–5 µg/kg intravenous fentanyl and 2–3 mg/kg intravenous propofol. Tracheal intubation was performed without neuromuscular blockade and ventilation was controlled to maintain normocapnia (end-tidal carbon dioxide tension 30–35 mmHg). Anesthesia was maintained with 70% nitrous oxide and 30% end-tidal isoflurane (Datex Ultima, Helsinki, Finland). Cardiac rhythm (by electrocardiography), hemoglobin oxygen saturation (by pulse oximetry), blood pressure (noninvasively), and esophageal temperature were monitored continuously. Esophageal temperature was maintained at 35.5–37.0°C.

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 (Digitist II, Neuro Technology, Houston, TX). Preload was maintained at 200–400 g. The evoked twitch tension of the adductor pollicis muscle was measured using a calibrated force transducer (Myotrace, Houston, TX) and amplified (DC Bridge Signal Conditioner, Gould Electronics, Valley View, OH). Twitch tension was then digitized (NB-M10-16, National Instruments, Austin, TX), displayed (LabView, National Instruments), and recorded on line. In addition, a strip chart recorded the evoked twitch tension (TA240, Gould Electronics). Before muscle relaxant administration, end-tidal isoflurane concentration was stable for more than 20 min and the first twitch response of each train was stable for more than 10 min (the control twitch tension).

For 17 subjects, mivacurium was infused at 1–3 µg·kg⁻¹·min⁻¹. When twitch tension stabilized, the mivacurium infusion rate was adjusted based on the Hill equation, targeting 90% twitch depression. When twitch tension had been stable at approximately 10% of the control value for more than 10 min and the mivacurium infusion rate had remained unchanged for more than 15 min, patients received atropine (10 µg/kg, not to exceed 1 mg) and edrophonium. The mivacurium infusion was continued unchanged throughout the remainder of the study. Patients were randomly allocated to receive one of four edrophonium doses (125, 250, 500, or 1,000 µg/kg). When an initial study demonstrated minimal effect of 125 µg/kg edrophonium, that dose was replaced with 2,000 µg/kg for subsequent studies. Thus, 1 patient received 125 µg/kg edrophonium, and 16 received 250–2,000 µg/kg (4 for each dose).

Venous blood samples (two aliquots of 5 ml each) were obtained before edrophonium (two samples separated by 10 min) and 1, 2, 4, and 8 min after edrophonium. One aliquot was used to determine plasma cholinesterase activity and dibucaine inhibition (SmithKline Beecham Clinical Laboratories, Van Nuys, CA), the other to determine mivacurium concentrations. To prevent mivacurium from degrading in vitro, 1.25 mg Phospholine Iodide (Wyeth-Ayerst, Philadelphia, PA) in 100 µl water was added to these samples immediately; samples were iced within 1 min and separated and frozen within 1 h. Plasma cholinesterase activity was determined photometrically using acetylthiocholine as a substrate. Mivacurium concentrations were determined by high-pressure liquid chromatography using a modification of the technique described by Brown et al. and a spectrofluorometric detector (RF-511PC, Shimadzu, Tokyo, Japan). The assay is sensitive to 5 ng/ml for each of the three stereoisomers and has a coefficient of variation less than or equal to 16% at that concentration; the assay is not affected by the presence of edrophonium.

The remaining eight subjects received the identical anesthetic and neuromuscular monitoring as described earlier except that d-tubocurarine (dTC) (n = 4) or vecuronium (n = 4) was infused to maintain 90% twitch depression. Atropine (10 µg/kg, not to exceed 1 mg), and 500 µg/kg edrophonium were then given, and the infusion was continued unchanged. Plasma samples (5 ml each) were obtained to determine vecuronium (but not dTC) concentrations before 1, 2, and 4 min after edrophonium. Vecuronium concentrations were determined by gas-liquid chromatography, sensitive to 10 ng/ml with a coefficient of variation less than 15% at that concentration; the assay is not affected by the presence of edrophonium.
Peak twitch tension after antagonism and time to peak antagonism were determined. Antagonism was then calculated as:

\[
\text{Antagonism} = \frac{(\text{peak twitch tension after antagonism} - \text{baseline})}{(100\% - \text{baseline})} 
\]

where baseline is the twitch tension immediately before edrophonium administration. For example, if twitch tension recovered from 11% of control to 53% of control, antagonism was \((53\% - 11\%)/(100\% - 11\%) = 47\%\) (Fig. 1). The dose of edrophonium antagonizing 50% of twitch depression for antagonism of mivacurium was determined by linear regression of antagonism versus log dose. In addition, the effect of 500 µg/kg edrophonium in antagonizing mivacurium, dTc, and vecuronium was compared using analysis of variance and Dunnett’s ttest.

To document that concentrations of each of the mivacurium stereoisomers were at steady state before edrophonium administration, the two plasma concentrations obtained before edrophonium were compared using Student’s ttest for paired data. Mivacurium concentrations after edrophonium were compared with the average of the two control values using repeated measures analysis of variance. Peak mivacurium concentrations after edrophonium were determined; the percentage increase in peak mivacurium isomer concentrations after edrophonium was compared with edrophonium dose by analysis of linear regression.

Plasma cholinesterase values after edrophonium were compared with control values using repeated measures analysis of variance. Plasma vecuronium concentrations were analyzed in a manner similar to that for mivacurium. Values are reported as mean ± SD. Statistical significance was accepted when \(P < 0.05\).

**Results**

With mivacurium, twitch tension immediately before edrophonium administration was 9.6 ± 1.4%. The mivacurium infusion rates to maintain steady state 90% twitch depression ranged from 1.3–5.0 µg·kg\(^{-1}\)·min\(^{-1}\) (2.8 ± 1.1 µg·kg\(^{-1}\)·min\(^{-1}\)). Concentrations of each of the mivacurium stereoisomers did not vary before edrophonium administration. Baseline plasma cholinesterase activity and values for dibucaine inhibition were normal for all subjects. Values for plasma cholinesterase activity after edrophonium were obtained in only 14 of 17 subjects because of technical difficulties with blood sampling. In these 14 subjects, edrophonium did not alter plasma cholinesterase activity (Fig. 2).

With mivacurium, time to peak antagonism was less than 2 min for all patients. Antagonism increased with increasing dose of edrophonium (Fig. 3, \(P < 0.001\)). Edrophonium, 2,000 µg/kg, produced 45 ± 7% antagonism. The dose of edrophonium antagonizing 50% of twitch depression for antagonism of mivacurium was 2,810 µg/kg (a value exceeding our largest dose of edrophonium). With dTc and vecuronium, twitch tension immediately before edrophonium was 9.5 ± 1.4% and 9.5 ± 1.0%, respectively. After 500 µg/kg edrophonium, antagonism was less with mivacurium (37 ± 7%) than with dTc (45 ± 7%) and vecuronium (45 ± 7%).

Plasma mivacurium concentrations in only 14 of 17 subjects were assessed. The mivacurium stereoisomer concentrations in the 14 subjects (Fig. 4) did not correspond to the \(\text{cis}\) and \(\text{trans}\) configuration with an increased slope with respect to the \(\text{cis}\) and \(\text{trans}\) stereoisomers. Plasma mivacurium concentrations ranged between 1 and 5 µg/ml and did not change with 125–2,000 µg/kg edrophonium (164 ± 52 ng/ml before and 159 ± 40 ng/ml after). The mivacurium \(\text{cis}\) stereoisomer was predominant (>95%) in all subjects.

**Discussion**

Antagonism of mivacurium with edrophonium of dTc in our clinic setting with a pretreatment dose of 400 µg/kg produced by mivacurium was studied to examine the stereospecificity of antagonism of dTc and vecuronium. We report the 90% steady-state antagonism of mivacurium in 14 of 17 subjects. The concentration–response curve for the antagonism of mivacurium was continuous. The stereospecificity of antagonism of mivacurium with edrophonium was confirmed and confirmed by our in vivo studies. The stereospecificity of antagonism of mivacurium by edrophonium was confirmed and confirmed by our in vivo studies. The stereospecificity of antagonism of mivacurium by edrophonium was confirmed and confirmed by our in vivo studies.
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Fig. 3. The dose–response relation for edrophonium antagonism of mivacurium (diamonds and line). Values for antagonism of dTc (triangles) and vecuronium (circles) by 500 μg/kg edrophonium are also shown (offset laterally for clarity).

Fig. 4. Concentrations of the three stereoisomers of mivacurium for each individual before and 1, 2, 4, and 8 min after edrophonium antagonism. Values are normalized to the plasma concentration before edrophonium.

Study. In addition, Cronnelly et al.’s7 dose–response relation for edrophonium antagonism of dTc was steeper than ours for antagonism of mivacurium (P < 0.001 by analysis of covariance). Although their study design differs from ours (they gave single switches at 0.1 Hz rather than train-of-four stimuli and administered halothane rather than isoflurane), the effect of

Discussion

Antagonism of a constant infusion of mivacurium by edrophonium appears to be less effective than antagonism of dTc or vecuronium. This finding is consistent with our clinical observations that 0.5–1.0 mg/kg edrophonium may not effectively antagonize paralysis induced by mivacurium. Our protocol was similar to that used to examine the dose–response relation for antagonism of dTc by edrophonium,6 neostigmine,7 and pyridostigmine. In those studies, dTc was infused to 90% steady-state block, after which single bolus doses of the antagonist were administered and the infusion was continued unchanged. Using this approach, Cronnelly et al.6 demonstrated that the dose of edrophonium antagonizing 50% of twitch depression by dTc was 128 μg/kg, a value less than one-tenth that in the current study.
500 μg/kg edrophonium to antagonize dTc is similar in the two studies (61 ± 15% vs. 82 ± 12%, respectively, P > 0.05), suggesting that comparison between our mivacurium and Cronnelly et al.'s dTc data is justified. Combined with the results from the current study, Cronnelly et al.'s findings suggest that antagonism of continuously infused mivacurium by edrophonium is impaired compared with antagonism of continuously infused dTc or vecuronium.

Under the experimental conditions of continuous infusion of dTc, the neuromuscular effect of the antagonist can be evaluated in the presence of a presumed constant plasma concentration of the muscle relaxant, and unchanged anesthetic potentiation and carbon dioxide tension. Although dTc concentrations were not measured in previous experiments, its pharmacokinetic characteristics and its metabolic pathways suggest that its concentrations remained constant from antagonist administration to peak effect. Although edrophonium is reported not to influence plasma cholinesterase or the degradation of mivacurium in vitro, we were concerned that edrophonium might influence mivacurium concentrations during constant infusion in vitro. In fact, our measurements revealed that concentrations of each of the three stereoisomers of mivacurium increased after edrophonium. Thus, our intent to maintain constant mivacurium concentrations (thereby isolating the effect of the antagonist from that of changing muscle relaxant concentrations) failed.

The increase in plasma mivacurium concentration (in contrast to the stable vecuronium and, presumably, stable dTc concentrations) after edrophonium is likely to contribute to the increased edrophonium dose requirement to antagonize mivacurium. Edrophonium increased plasma concentrations of the two potent stereoisomers of mivacurium 48% and 79% (Fig. 5). Assuming that these two stereoisomers are equipotent and accounting for differences in their plasma concentrations, the increase in the "effective" total plasma concentration is 55%. The peak increase in mivacurium concentrations in the effect compartment is unknown but is probably smaller than this value. Had edrophonium not increased mivacurium concentrations, twitch tension should have increased more. If plasma (and effect) compartment concentrations of mivacurium

**This assumes that effect compartment concentrations increased in proportion to plasma concentrations (55%) and that the 37% antagonism produced by edrophonium, 500 μg/kg, occurred despite this 55% increase in effect compartment mivacurium concentrations.

Figure 5. Concentrations of the three stereoisomers of mivacurium before and 1, 2, 4, and 8 min after edrophonium antagonism. Values are means and SE for each stereoisomer and are normalized to the plasma concentration before edrophonium. Standard deviations for the cis-trans isomer (omitted for clarity) are similar to those for the trans-trans isomer.

were unchanged by edrophonium, we estimate (appendix) that twitch tension would have increased to 74%, corresponding to 71% antagonism. This 71% value is similar to that for antagonism of both vecuronium and dTc (60% and 61%, respectively). If the increase in effect site concentrations was less than 55%, the 71% value is an overestimate. These calculations suggest that the increased response to edrophonium to antagonize mivacurium results, at least partially, from edrophonium's effect on mivacurium concentrations.

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Increase in plasma mivacurium concentration is not unexpected as the drug is not removed from the tissue. Basic science studies did not indicate that edrophonium antagonizes mivacurium because of its effect on the plasma concentration of mivacurium; rather, its effect was attributed to an increase in the duration of muscle relaxation. In another study, in vitro experiments indicated that edrophonium antagonizes mivacurium in vitro and that the concentration of mivacurium at which edrophonium antagonizes is different for the three stereoisomers of mivacurium. However, edrophonium did not significantly alter plasma concentration of mivacurium.

Naguib et al. reported that mivacurium in antagonized by edrophonium in twitch tests in rats and that edrophonium antagonizes mivacurium in patients. In our study, twitch tension increased (Fig. 5). This may be due to the time course of the antagonist. Antagonism of mivacurium by edrophonium occurred after 2 min. However, the most effective concentration of mivacurium was tested at 5 min.

Whether edrophonium can be used in clinical practice as an antagonist is unknown. However, basic science studies have shown that edrophonium antagonizes mivacurium but that the concentration of edrophonium required is greater than that for other antagonists. Whether edrophonium antagonizes mivacurium in patients is unknown. However, basic science studies have shown that edrophonium antagonizes mivacurium but that the concentration of edrophonium required is greater than that for other antagonists.
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Increased plasma mivacurium concentrations should result either from decreased elimination or from increased input such as displacement of mivacurium from tissue. Based on Cook et al.'s report that edrophonium did not influence mivacurium degradation in vitro, we anticipated that edrophonium would not alter mivacurium elimination. However, Cook et al. measured the slope of the mivacurium decay curve, a technique that may be less sensitive to changes in elimination rate than measuring concentrations during constant infusion. In addition, their measurements were performed in vitro whereas ours were in vivo. Our mivacurium concentrations increased after edrophonium despite no change in plasma cholinesterase activity. Perhaps other cholinesterases or esterases contribute to the elimination of mivacurium, and these enzymes may be inhibited by edrophonium. In support of altered metabolism (rather than displacement from sites within the body) explaining the rapid increase in mivacurium concentrations is the difference in the time course of the concentration changes for the three stereoisomers: the two isomers with elimination half-lives less than 2 min peaked early, whereas the cis–cis isomer (which has a 53-min elimination half-life) peaked later.

Naguib et al. have examined edrophonium's effects in antagonizing bolus doses of mivacurium. When twitch tension had recovered to 10% of control, some subjects were allowed to recover spontaneously and others were given 100–1,000 μg/kg edrophonium, and the time course of twitch tension recovery was compared. Although edrophonium facilitated recovery in a dose-dependent manner, Naguib et al.'s study design did not permit them to assess the relative contribution of edrophonium and spontaneous degradation of mivacurium to the eventual recovery.

Whether edrophonium (or another antagonist such as neostigmine) should be given to antagonize mivacurium remains controversial. During initial clinical evaluation of mivacurium, there were no clinical trials to examine its antagonism from profound degrees of neuromuscular blockade, a situation common to many muscle relaxants. Despite minimal data regarding appropriate antagonist doses or the time course of antagonism, Savarese and Diefenbach suggested that spontaneous recovery was preferable. Our study does not identify the preferred antagonist for mivacurium or the optimal dose. In addition, our study design—continuing the infusion of the muscle relaxant after administration of edrophonium—differs from usual clinical practice, thereby limiting the clinical utility of our results. However, we demonstrate that edrophonium antagonism of mivacurium (administered by continuous infusion) is impaired compared with antagonism of dTc or vecuronium.

In summary, we antagonized constant infusions of muscle relaxants with edrophonium, finding less effective antagonism of mivacurium compared with that of dTc or vecuronium. After edrophonium, plasma mivacurium (but not vecuronium) concentrations increased, at least partially explaining this difference. Mivacurium concentrations increased despite stable plasma cholinesterase activity, suggesting the role of other cholinesterases in the elimination of mivacurium. Our findings suggest that edrophonium, in doses as large as 2,000 μg/kg, fails to antagonize adequately the paralysis from mivacurium during constant infusion.

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Appendix: Determination of the Effect of Increasing Muscle Relaxant Concentrations On Antagonism by Edrophonium

Using the Hill equation, twitch depression (effect) can be described as:

\[ \text{effect} = \frac{C_e \gamma \gamma}{C_e \gamma + C_{50} \gamma} \]  \hspace{1cm} (1)

where \( C_e \) is the muscle relaxant concentration in the effect compartment; \( \gamma \) is the Hill coefficient; and \( C_{50} \) is the effect site concentration depressing twitch tension 50%. Equation A1 can be rewritten as:

\[ Ce_{50} = Ce^\gamma \times \frac{1 - \text{effect}}{\text{effect}} \]  \hspace{1cm} (2)

If \( Ce_{\text{unchanged}} \) is the effect compartment concentration if edrophonium did not alter mivacurium concentrations and \( Ce_{\text{changed}} \) is the peak effect compartment concentration after edrophonium, then:

\[ Ce_{50} = Ce_{\text{unchanged}}^\gamma \times \frac{1 - \text{effect}_{\text{unchanged}}}{\text{effect}_{\text{unchanged}}} \]  \hspace{1cm} (3)

\[ Ce_{50} = Ce_{\text{changed}}^\gamma \times \frac{1 - \text{effect}_{\text{changed}}}{\text{effect}_{\text{changed}}} \]  \hspace{1cm} (4)

where \( \text{effect}_{\text{unchanged}} \) and \( \text{effect}_{\text{changed}} \) are the effects associated with the \( Ce_{\text{unchanged}} \) and \( Ce_{\text{changed}} \), respectively. If the right sides of equations A3 and A4 are equated, \( \text{effect}_{\text{unchanged}} \) can be determined as:

\[ \text{effect}_{\text{unchanged}} = \frac{1}{\left(1 + \frac{Ce_{\text{changed}}}{Ce_{\text{unchanged}}} \times \frac{1 - \text{effect}_{\text{changed}}}{\text{effect}_{\text{changed}}} \right)} \]  \hspace{1cm} (5)

Although \( \gamma \) is unknown, we assume a value of 3.\#\# Fisher DM: Unpublished data. 1994.

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