Priming with anticholinesterases - the effect of different combinations of anticholinesterases and different priming intervals

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This study was designed to investigate the effect of different combinations of neostigmine and edrophonium when administered in divided doses and the effect of different intervals (priming intervals) between the doses. Seventy-two patients divided into 12 groups ($n = 6$ in each) were included in the study. An initial dose of neostigmine 0.012 mg·kg^{-1} or edrophonium 0.2 mg·kg^{-1} was administered, followed at different priming intervals (1, 2 or 3 min) by either edrophonium 0.8 mg·kg^{-1} or neostigmine 0.048 mg·kg^{-1} for antagonism of atracurium-induced neuromuscular blockade. Reversal was attempted at 10 per cent spontaneous recovery of twitch height. Adequate reversal of neuromuscular block (train-of-four ratio of 0.75) was achieved in all patients. Significant ($p < 0.05$) acceleration of the recovery index (time taken for the twitch height to recover from 25 to 75 per cent of control) and reversal time (time taken from the end of injection of the priming dose until TOF ratio value had reached 0.75) was obtained in groups which received edrophonium-edrophonium combination. Recovery indices and reversal times were found to be significantly shorter ($p < 0.05$) with a 1 min priming interval.

In two additional groups of patients premedicated and anaesthetized as the others equipotent mixtures of the antagonists were administered as a single bolus dose. Reversal times were significantly longer ($p < 0.05$) when compared to those given the same amounts of the combination but in divided doses with a 1 min priming interval.

It is concluded that the administration of edrophonium in divided doses (0.2 mg·kg^{-1} followed one minute later by 0.8 mg·kg^{-1}) is the best combination of those studied for the antagonism of profound (90 per cent depression of T1) neuromuscular blockade induced by atracurium. With this technique of administration, a TOF ratio of 0.75 can be obtained in about 4 min.

Key words

ANTAGONISTS, NEUROMUSCULAR RELAXANTS: edrophonium, neostigmine; PHARMACODYNAMICS: priming principle; NEUROMUSCULAR RELAXANTS: atracurium; NEUROMUSCULAR TRANSMISSION: train-of-four.

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We have demonstrated that the antagonism of atracurium-induced neuromuscular blockade can be accelerated by divided administration of neostigmine[1] or edrophonium[2] when compared to a single bolus administration of the antagonist.

It has been suggested that different anticholinesterases have different affinities for the presynaptic and postsynaptic sites, in addition to their inhibition of acetylcholinesterase. Edrophonium has a predominant presynaptic mechanism, compared to neostigmine or pyridostigmine.[3] Therefore, administration in divided doses of different anticholinesterases which have different sites of action may influence the reversal of neuromuscular blockade.

The present study is an extension of previous studies[1,2] and was undertaken to identify the effect of different combinations of neostigmine and edrophonium when administered in divided doses and the effect of different intervals (priming intervals) between the doses on the recovery rate following antagonism of atracurium induced neuromuscular blockade.

Methods

After institutional approval, seventy-two ASA physical status I or II adult patients of either sex whose ages ranged from 19 to 55 years and who weighed 50 to 82 kg were studied. All patients were undergoing minor elective procedures, were free of neuromuscular, renal or hepatic disease and were not taking any drugs known to interfere with neuromuscular function. Informed consent was obtained. All patients received 0.15 $mg \cdot kg^{-1}$ diazepam PO 90 minutes preoperatively.

An intravenous infusion of lactated Ringer's solution in 5 per cent dextrose was established prior to induction. The ECG and nasopharyngeal temperature were monitored continuously by a Medishield® M1 monitor. Body temperatures varied less than 0.5°C and were in the 36–37°C range throughout the studies. Blood pressure was measured every 5 minutes by an electronic oscillotonometer (Dinamap®).

In all patients, anaesthesia was induced with fentanyl 2.0 $\mu g \cdot kg^{-1}$, thiopentone 5.0 $mg \cdot kg^{-1}$, and was maintained with 70 per cent nitrous oxide in oxygen and halothane (0.5–1.0 per cent). Inspired halothane concentration was monitored by an Engstrom Emma® monitor. End-tidal CO_2 was monitored by a Datex infra-red CO_2 analyzer and ventilation was adjusted to maintain normocapnia (end-tidal CO_2 35–40 mmHg).

The ulnar nerve was stimulated at the wrist with square wave supramaximal stimuli of 0.2 ms duration, delivered in a train-of-four (TOF) sequence at 2 Hz every ten seconds, using a Myotest® peripheral nerve stimulator (Biometer). The resultant contraction of the adductor pollicis was recorded using a force displacement transducer and neuromuscular function analyzer (Myograph 2000, Biometer).[4] Preload tension on the thumb was maintained at 300 g throughout the investigation.

After stabilization of twitch recording, atracurium 0.5 $mg \cdot kg^{-1}$ was administered and tracheal intubation was performed at maximum block. Patients were excluded from the study if additional doses of atracurium were given.

At the end of surgery, when spontaneous recovery had begun and the first twitch (T1) of the TOF returned to ten per cent of the control value, 72 patients were assigned randomly to one of twelve groups (n = 6 in each). Stratified sampling was used to obtain an even sex distribution. An initial dose of neostigmine 0.012 $mg \cdot kg^{-1}$ or edrophonium 0.2 $mg \cdot kg^{-1}$ was administered followed at different intervals (1, 2, or 3 minutes) by either neostigmine 0.048 $mg \cdot kg^{-1}$ or edrophonium 0.8 $mg \cdot kg^{-1}$ (Table I). Atropine 0.5 mg was administered before the first dose of antagonist and the remainder of the 0.02 $mg \cdot kg^{-1}$ atropine dose was administered before the second dose of the antagonist. Patients continued to receive 70 per cent nitrous oxide in oxygen and 0.5–1 per cent halothane until all measurements were complete. Antagonism of block was considered adequate when a TOF ratio (the amplitude of the fourth to the first evoked response; T4/T1) of 0.75 was attained.[5] Further assessment of the patients was carried out in the Recovery Room for 60 minutes, using clinical criteria such as ability to open eyes, cough and sustain a head lift.

The following parameters were calculated: (a) T1 and TOF ratio just before the administration of the second dose of the antagonist; (b) time for the twitch height (T1) to recover from 25 to 75 per cent of control (recovery index); and (c) reversal time, was the time taken from the end of injection of the priming dose of the antagonist until TOF ratio value had reached 0.75.

For the sake of comparison, reversal time was also measured following single dose administration of a combination of edrophonium and neostigmine. Twelve patients divided randomly into 2 groups (n = 6 in each) premedicated and anaesthetized as the others, received neostigmine 0.012 $mg \cdot kg^{-1}$ mixed with edrophonium 0.8 $mg \cdot kg^{-1}$ (Group XIII) or edrophonium 0.2 $mg \cdot kg^{-1}$ mixed with neostigmine 0.048 $mg \cdot kg^{-1}$ (Group XIV), when T1 returned to ten per cent of the control value.

Data analysis

Differences between groups were evaluated with analysis of variance using the classical methods and nonparamateric methods (Kruskal-Wallis test) when needed. Differences were considered statistically significant when $p < 0.05$.

To estimate, simultaneously, the effects of different combinations of the antagonists and the priming intervals on the reversal time and recovery index, a factorial experiment[6,7] was designed and conducted. In the factorial experiment, the first factor was the priming interval, which has three levels and the second factor was the different combinations of the antagonists, and had four levels. ANOVA was performed to test the significance for each factor and for the interaction between factors. Tukey and Scheffe methods were used for pairwise and multiple comparisons.

Results

Results are expressed as means ±SEM. A significant increase in T1 and TOF ratio values was seen after priming with edrophonium 0.2 $mg \cdot kg^{-1}$ when compared with priming with neostigmine during the three priming intervals studied (Table II and III).

There was a significant difference in the reversal time between different antagonist combination and between different priming intervals, but no significant interaction was found to influence the reversal time. Pairwise comparison between NE, NN, EE and EN groups showed

TABLE I Study groups and drug dosage ($n = 6$ in each group)

Priming interval					
1 min		*2 min*		*3 min*	
Priming dose $mg \cdot kg^{-1}$	*Second dose* $mg \cdot kg^{-1}$	*Priming dose* $mg \cdot kg^{-1}$	*Second dose* $mg \cdot kg^{-1}$	*Priming dose* $mg \cdot kg^{-1}$	*Second dose* $mg \cdot kg^{-1}$
N 0.012 (Group NE1)	E 0.8	N 0.012 (Group NE2)	E 0.8	N 0.012 (Group NE3)	E 0.8
N 0.012 (Group NN1)	N 0.048	N 0.012 (Group NN2)	N 0.048	N 0.012 (Group NN3)	N 0.048
E 0.2 (Group EE1)	E 0.8	E 0.2 (Group EE2)	E 0.8	E 0.2 (Group EE3)	E 0.8
E 0.2 (Group EN1)	N 0.048	E 0.2 (Group EN2)	N 0.048	E 0.2 (Group EN3)	N 0.048

N – neostigmine.
E – edrophonium.

TABLE II First twitch (T1) height (per cent of control) before the administration of the second antagonist (mean ± SEM)

Priming antagonist $(mg \cdot kg^{-1})$	*Priming interval*		
	1 min	*2 min*	*3 min*
Neostigmine (0.012)	21.2 ± 2.2 (Group NE1)	24.8 ± 1.9 (Group NE2)	44.3 ± 3.8 (Group NE3)
Neostigmine (0.012)	18.7 ± 1.4 (Group NN1)	25.5 ± 2.8 (Group NN2)	39.5 ± 3.8 (Group NN3)
Edrophonium (0.2)	60.5 ± 7.9* (Group EE1)	76 ± 7.8* (Group EE2)	80.8 ± 4.3* (Group EE3)
Edrophonium (0.2)	48.3 ± 4* (Group EN1)	64.2 ± 3.8* (Group EN2)	72.8 ± 5.9* (Group EN3)

*T1 significantly greater ($p < 0.05$) in (EE) and (EN) groups compared to (NE) and (NN) groups after the same priming interval.

that the reversal times were significantly shorter ($p < 0.05$) only in the groups that received the edrophonium-edrophonium (EE) combination, compared with the groups receiving the edrophonium-neostigmine (EN) combination. Reversal times were significantly shorter with a one min priming interval only when compared to a two min priming interval (Table IV). Reversal times with a three min priming interval were not statistically different compared to those with one or two min priming intervals. Multiple comparison showed that reversal times were significantly shorter ($p = 4.7 \times 10^{-4}$) in groups which received edrophonium as the second antagonist (groups NE and EE) when compared to those who received neostigmine as the second antagonist (groups NN and EN).

There was a significant difference in the recovery index between different antagonist combination and between different priming intervals studied. No significant interaction was found between the different combinations and priming intervals to influence the recovery index. Pairwise comparison between NE, NN, EE, and EN groups showed that the recovery indices were significantly shorter ($p < 0.05$) only in groups that received

TABLE III Train-of-four (TOF) ratio before administration of the second antagonist (mean ± SEM)

Priming antagonist $(mg \cdot kg^{-1})$	*Priming interval*		
	1 min	*2 min*	*3 min*
Neostigmine (0.012)	0 ± 0 (Group NE1)	0.1 ± 0.03 (Group NE2)	0.19 ± 0.04 (Group NE3)
Neostigmine (0.012)	0 ± 0 (Group NN1)	0.06 ± 0.04 (Group NN2)	0.17 ± 0.1 (Group NN3)
Edrophonium (0.2)	0.48 ± 0.04* (Group EE1)	0.42 ± 0.05* (Group EE2)	0.48 ± 0.02* (Group EE3)
Edrophonium (0.2)	0.33 ± 0.02* (Group EN1)	0.32 ± 0.04* (Group EN2)	0.44 ± 0.05* (Group EN3)

*TOF ratio was significantly greater ($p < 0.05$) in (EE) and (EN) groups compared to (NE) and (NN) groups after the same priming interval.

TABLE IV Reversal time in seconds (mean ± SEM)

	Priming interval		
Group	*1 min†*	*2 min*	*3 min*
NE	345.8 ± 53** (Group NE1)	352.2 ± 67.1 (Group NE2)	291.6 ± 21.3 (Group NE3)
NN	354.1 ± 27.8 (Group NN1)	445.8 ± 51.5 (Group NN2)	409.1 ± 33.3 (Group NN3)
EE*	256.6 ± 55.9 (Group EE1)	423.3 ± 71.4 (Group EE2)	240 ± 15.7 (Group EE3)
EN	368.3 ± 24.5** (Group EN1)	440.8 ± 37.8 (Group EN2)	440.8 ± 52.9 (Group EN3)

*Reversal times significantly shorter ($p < 0.05$) in (EE) group when compared to (EN) group (pairwise comparison between groups).
†Reversal times were significantly shorter ($p < 0.05$) at 1 min priming interval when compared to 2 min priming interval (pairwise comparison between the priming intervals).
**Reversal times for NE1 and EN1 groups were significantly shorter ($p < 0.05$) than for groups XIII and XIV (651.6 ± 76.1 and 677.5 ± 70.8 sec respectively) which were given the same dose of neostigmine and edrophonium but as a single bolus dose.

neostigmine-edrophonium (NE) and edrophonium-edrophonium (EE) combinations compared to the other two combinations. Recovery indices were also found to be significantly shorter with one min priming interval only when compared to three min priming interval (Table V). Multiple comparison indicated that recovery indices were significantly shorter ($p = 3.5 \times 10^{-7}$) in the groups which received edrophonium as the second antagonist (groups NE and EE) when compared to those who received neostigmine as the second antagonist (groups NN and EN).

The reversal times following the bolus administration of mixtures of neostigmine and edrophonium to groups XIII and XIV were 651.6 ± 76.1 and 677.5 ± 70.8 seconds, respectively. These times were significantly longer ($p < 0.05$ − t test) than for NE1 and EN1 groups (345.8 ± 53 and 368.3 ± 24.5 seconds respectively) given the same amounts of that drug combination but in divided doses, with a one-minute interval between the doses.

Discussion

There has been great enthusiasm recently for clinical application of the priming principle in order to accelerate the onset of non-depolarizing muscle relaxants. Surprisingly, little attention has been given to the application of this principle for the antagonism of non-depolarizing relaxants. The rationale of the divided administration of non-depolarizing relaxants for facilitation of rapid tracheal intubation is based on the presence of a wide margin of safety of neuromuscular transmission that allows 70–75 per cent occupancy of the cholinergic receptors without any clinically significant effect on neuromuscular activity.[8] Similarly, a "margin of safety" in acetylcholinesterase enzyme inhibition has been described. In 1979, Barber *et al.*[9] reported a study on the kinetics of erythrocyte cholinesterase, the properties of which are very similar to neuromuscular junction cholinesterase.[10] They found that facilitation of twitch height did not occur when erythrocyte cholinesterase was inhibited less than 85 per cent. Between 85 per cent and 98 per cent inhibition, facilitation was linearly related to enzyme inhibition.[9] Therefore, a large proportion of the acetylcholinesterase could be inhibited without effect on neuromuscular junction.[11] This suggests a "margin of safety" in enzyme inhibition.[12] By analogy to the priming principle, the initial relatively small dose of the antagonist will cause partial enzyme inhibition and therefore will decrease the margin of safety of the acetycholinesterase enzyme, allowing a more pronounced effect of the second dose.

The hypothesis that administration of anticholinesterase in divided doses will shorten the reversal time was clearly confirmed in our previous studies.[1,2] It has been found that mixtures of neostigmine and edrophonium did not appear to offer any advantage over either drug alone[13] or even produced less reversal than the same drug alone.[13] In the present study, reversal times following administration of mixtures of neostigmine and edrophonium as a single bolus dose to groups XIII and XIV were significantly prolonged ($p < 0.05$) as compared to groups NE1 and EN1 who received the same total dose but in divided administration with a one min priming interval.

In the present study we tried to identify the effect of different combinations of antagonists and priming intervals on the reversal time. The doses of neostigmine and edrophonium employed in this study for priming and for the second dose were equipotent and calculated based on a report of a recent study.[15] Breen *et al.*[15] reported that the dose–response curves for neostigmine and edrophonium were parallel and that neostigmine was 16 times more potent than edrophonium.

The design of our study (factorial design) permitted a comparison of the effect of one factor at different levels of the other factor. Our results indicated that reversal times were significantly shorter ($p < 0.05$) in groups that received edrophonium-edrophonium (EE) combination when compared to those received edrophonium-neostigmine (EN) combination. Reversal times were also found to be significantly shorter with a one min priming interval than with a two min interval (Table IV).

Several studies have demonstrated that edrophonium in doses between 0.5 – 0.75 $mg \cdot kg^{-1}$ cannot be relied upon

TABLE V Recovery time in seconds (mean ± SEM)

	Priming interval		
Group	*1 min†*	*2 min*	*3 min*
NE*	57.5 ± 10.8 (Group NE1)	75.8 ± 18.7 (Group NE2)	142.5 ± 10.2 (Group NE3)
NN	152.5 ± 24.6 (Group NN1)	170 ± 21.9 (Group NN2)	215.8 ± 12.6 (Group NN3)
EE*	97.1 ± 32 (Group EE1)	85 ± 15.9 (Group EE2)	102.5 ± 18.7 (Group EE3)
EN	155 ± 21.8 (Group EN1)	145.8 ± 28.9 (Group EN2)	154.1 ± 37.3 (Group EN3)

*Recovery indices were significantly shorter ($p < 0.05$) in (NE) and (EE) groups compared to (NN) and (EN) groups respectively (pairwise comparison between groups).
†Recovery indices were significantly shorter ($p < 0.05$) at 1 min priming interval when compared to 3 min priming interval (pairwise comparison between priming intervals).

to antagonize relatively deep blocks produced by pancuronium, atracurium and vecuronium[16–19] and that neostigmine antagonizes a profound neuromuscular block induced by atracurium more rapidly than does edrophonium.[2,17] Our previously reported results with neostigmine[1] demonstrate that following a single bolus administration, mean reversal time to a TOF ratio of 0.75 was significantly shorter following neostigmine 0.05 mg·kg^{-1} than after edrophonium 1 mg·kg^{-1} (7.8 and 10.5 min respectively).[1,2] In the present study, when the twitch height had spontaneously recovered to ten per cent, administration of edrophonium in divided doses with an interval of one min between the doses resulted in a rapid antagonism to a TOF ratio of 0.75 in about four minutes. Following administration of neostigmine in a similar fashion, about six minutes were needed to attain a TOF ratio of 0.75. In clinical practice one should be able to shorten the reversal time by about six min with divided administration of edrophonium, as described in this report.

It is known that inhibition of the cholinesterase enzyme by edrophonium occurs by a mechanism different from that for neostigmine.[20] Neostigmine contains a carbamate group which is transferred and chemically bonded to the esteratic sub-unit on the acetylcholinesterase. Edrophonium, on the other hand, binds electrostatically to the anionic sub-unit and by hydrogen binding to the esteratic site on the enzyme.[20] The equilibrium constant of such a reaction is small.[21] Therefore, it can be predicted that *in vivo* activity of edrophonium should be rapid in onset, and this could explain the greater and more rapid recovery of TOF ratios and T1 following the administration of edrophonium 0.2 mg·kg^{-1} as the priming antagonist (EE and EN Groups) (Tables II and III).

Nevertheless, the relatively prolonged reversal times observed with two min priming intervals compared with other intervals can not be explained. One might speculate that this delay is probably due to unknown pharmacodynamic effect related to acetylcholinesterase enzyme inhibition following divided administration of the antagonists with two min priming interval.

In conclusion, in this study we have shown that different combinations of neostigmine and edrophonium and different priming intervals between the doses can result in different recovery rates following antagonism of atracurium-induced neuromuscular blockade. Although we did not evaluate all possible combinations of anticholinesterases and all possible priming intervals, this study demonstrated that a one minute priming interval and the administration of edrophonium 0.2 mg·kg^{-1} followed by edrophonium 0.8 mg·kg^{-1} were the best of those studied. With this technique of administration, about four minutes were necessary to obtain a TOF ratio of 0.75 when antagonism of atracurium paralysis was attempted at 90 per cent depression of twitch height in healthy adults during halothane anaesthesia who had received atracurium 0.5 mg·kg^{-1}.

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Résumé

La présente étude a été conçue afin d'investiguer les effets de différentes combinaisons de néostigmine et d'édrophonium lorsqu'administrés en doses fractionnées ainsi que l'effet des différents intervals (interval d'amorce) entre les doses. Soixante-douze patients divisés en 12 groupes ($n = 6$ pour chaque groupe) ont été inclus dans l'étude. Une dose initiale de néostigmine 0.012 $mg \cdot kg^{-1}$ ou d'édrophonium 0.2 $mg \cdot kg^{-1}$ a été administrée suivie, à des intervals d'amorce différents (1, 2 ou 3 minutes), par soit l'édrophonium 0.8 $mg \cdot kg^{-1}$ ou la néostigmine 0.048 $mg \cdot kg^{-1}$ afin d'antagoniser le blocage neuromusculaire induit par l'atracurium. La décurarisation a été tentée après une récupération spontanée de dix pour cent de la hauteur du twitch. Un antagonisme adéquat du bloc neuromusculaire (rapport de l'ondée-de-quatre (TOF) de 0.75) a été obtenu chez tous les patients. Une accélération significative ($p < 0.05$) de l'indice de recouvrement (temps pris pour que la hauteur du twitch retourne de 25 à 75 pour cent du contrôle) et le temps d'antagonisme (temps pris à partir de la fin de l'injection de la dose d'amorce jusqu'à l'obtention d'un rapport TOF de 0.75) ont été obtenus dans les groupes avant reçu la combinaison d'édrophonium-édrophonium. Les indices de récupération et les temps d'antagonisme ont été trouvés significativement plus courts ($p < 0.05$) après une minute d'inverval d'amorce.

Dans deux groupes additionnels de patients prémédiqués et anesthésiés comme les autres, des mélanges équipotents d'antagonistes ont été administrés en une dose unique. Les temps d'antagonisme ont été trouvés significativement plus longs ($p < 0.05$) en comparaison à ceux qui ont reçu la même quantité de mélanges en doses fractionnées avec un interval d'amorce de une minute.

Il est conclut que l'administration d'édrophonium à doses fractionnées (0.2 $mg \cdot kg^{-1}$ suivi une minute plus tard par 0.8 $mg \cdot kg^{-1}$) est la meilleure combinaison pour le groupe étudié afin d'antagoniser un bloc neuromusculaire profond (une dépression de 90 pour cent de T1) induit par l'atracurium. Avec cette technique d'administration un rapport TOF de 0.75 peut être obtenu dans approximativement quatre minutes.