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Influence of acute normovolaemic haemodilution on the dose–response relationship and time course of action of cisatracurium besylate

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Background. Acute normovolaemic haemodilution (ANH) is an efficacious blood conservation strategy aiming at avoiding allogeneic blood transfusion. ANH was shown to increase the potency of vecuronium, atracurium, and rocuronium. The aim of our study was to investigate whether cisatracurium potency is altered with ANH.

Methods. Using the Relaxometer mechanomyograph, we compared cisatracurium dose–response relationship and time course of action in 60 patients randomly allocated to the ANH or control groups. Patients in each group were randomly allocated to receive one of three cisatracurium doses (30, 40, 50 $\mu g\,kg^{-1}$) followed by a second supplemental dose to reach a total of 100 $\mu g\,kg^{-1}$.

Results. ANH did not result in a significant shift in cisatracurium log dose–probit dose–response curve. There was no significant difference in mean (95% confidence intervals) ED_{50}, ED_{90}, and ED_{95} (effective doses required for 50, 90, and 95% first twitch depression) between the ANH group [29.5 (27–32), 50.4 (47.4–53.4), 58.7 (55.3–62) $\mu g\,kg^{-1}$] and the control group [28.2 (25.3–31), 47.6 (44.9–50.3), 55.3 (52.5–58.1) $\mu g\,kg^{-1}$], whereas there was no difference in mean (SD) Dur_{25} and $Dur_{0.8}$ (time until 25% first twitch and 0.8 train-of-four ratio recoveries) between the ANH group [40.8 (5.9), 64.7 (8.4) min] and the control group [42.2 (7.6), 66.5 (10.7) min].

Conclusions. Our results demonstrated that unlike other previously reported neuromuscular blocking drugs, ANH did not alter cisatracurium potency. Thus, cisatracurium would be the neuromuscular blocking drug of choice in patients who undergo surgery with ANH, as no dose adjustments are required.

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Blood conservation techniques aiming at avoiding or reducing allogeneic blood transfusion during major surgery include preoperative autologous blood donation, intraoperative cell salvage, and acute normovolaemic haemodilution (ANH). ANH autologous blood procurement technique, recommended by the National Institute of Health Consensus Conference,[1] is an efficacious cost-effective[2] blood conservation strategy in procedures with expected blood loss of more than 1 litre.[3] With ANH the amount of red blood cells and other plasma constituents lost during surgical bleeding are reduced through preoperative dilution of the circulating blood volume. Since the first report by Schuh[4] of a significant increase in the potency of succinylcholine and pancuronium with ANH, several publications demonstrated a similar augmentation of vecuronium,[5] atracurium,[6] and rocuronium[7] potencies. Cisatracurium besylate, a benzylisoquinolinium neuromuscular blocking drug, is a purified preparation of 1 of the 10 stereoisomers of atracurium, which retained atracurium's advantage of Hofmann elimination but not atracurium's elimination via the non-specific plasma esterases hydrolysis pathway.[8] The aim of our study was to investigate whether cisatracurium's potency is altered with ANH.

In that regard, a neuromuscular blocking drug that is not influenced by ANH, thus requiring no dose adjustments, could be beneficial in patients undergoing surgery

with ANH. We assessed cisatracurium's dose–response relationship and time course of action in patients undergoing surgery with and without ANH.

Patients and methods

A prospective, controlled, clinical, consecutive study was conducted in conformity with the guidelines of 'Good clinical research practice (GCRP) in pharmacodynamic studies of neuromuscular blocking agents'[9] and the 'Consolidated standards of reporting trials (CONSORT) statement'.[10]

After ethics committee approval, all patients who agreed to participate in the study gave written informed consent. Exclusion criteria were history of neuromuscular disease, small joint arthritis, haemoglobin (Hb) less than 12 g dl^{-1}, body mass index of less than 20 or more than 26 kg m^{-2}, or treatment with drugs thought to interfere with neuromuscular transmission.

Based upon a previous study,[6] our *a priori* power analysis showed that a subgroup size of 10 patients would be required to reveal a statistically significant difference between the two groups. Using a computer-generated randomization list, 60 patients with American Society of Anesthesiologists (ASA) classification I–III undergoing radical cystectomy, radical hysterectomy, or retropubic radical prostatectomy were randomly allocated to the ANH or control groups and were stratified by gender and ASA classification. Patients in each group were randomly allocated to receive one of three initial cisatracurium doses (30, 40, 50 μg kg^{-1}), followed by a second supplemental dose to reach a total dose of 100 μg kg^{-1}.

A radial artery cannula was placed under local anaesthetic, before anaesthesia induction. Arterial Hb, haematocrit (Hct), and total plasma proteins were measured before and after ANH. A volume of 15 ml kg^{-1} blood (approximately 20% of blood volume) was procured from the cubital vein and stored in an acid citrate–dextrose reservoir bag to be simultaneously replaced by an equal volume of 6% hydroxyethyl starch (HES) 130/0.4 solution rapidly infused via a cubital vein cannula in the other arm. The patient's blood was ideally re-infused towards the end of the operation after the phase of major blood loss, or sooner if clinically indicated primarily for persistent hypotension or if the Hb less than 7 g dl^{-1} threshold was reached.

After blood procurement and HES fluid replacement, anaesthesia was induced with fentanyl (1.5 μg kg^{-1}) and propofol (2–3 mg kg^{-1}). Patients were ventilated via a facemask, and after lidocaine 2% topical spray of the laryngeal region and when anaesthesia was deep enough, the trachea was intubated without using neuromuscular blocking drugs. Anaesthesia was maintained with propofol (100–150 μg kg^{-1} min^{-1}) and remifentanil (0.1–0.3 μg kg^{-1} min^{-1}) infusions. Patients were ventilated mechanically with 40% oxygen in air and adjusted to maintain 30–40 mm Hg end-tidal carbon dioxide. Patients were warmed using a forced hot-air blanket to maintain core temperature >36°C and skin temperature >32°C.

Neuromuscular block at the adductor pollicis muscle was evaluated using the Relaxometer mechanomyograph (Groningen University, Groningen, Holland).[11] The force transducer of the Relaxometer was attached to the thumb, and the ulnar nerve was stimulated supramaximally at the wrist (pulse width 200 μs, square wave) with train-of-four (TOF) stimuli (2 Hz for 2 s) at 12 s intervals. Data were continuously recorded using the 'AZG-Relaxometer 5.0' program until all patients fully recovered from neuromuscular block. T_1 (first twitch of the TOF) expressed as percentage of control response and TOF ratio (T_4:T_1) were used to evaluate the neuromuscular block.

After T_1(%) baseline stabilization, the designated cisatracurium dose of 30, 40, or 50 μg kg^{-1} was administered into a rapidly running infusion and the maximum neuromuscular block, defined as three consecutive T_1 responses that did not register a decline, was recorded. Cisatracurium dose–response curves were obtained according to Donlon's single-dose method[12] using the ordinary least-squares regression of the probit transformation of T_1(%) maximum twitch suppression against the common logarithm of each dose. Mean regression lines representing the two groups were plotted using the slopes and intercepts, from which the doses required for 50, 90, and 95% T_1 depression (ED_{50}, ED_{90} and ED_{95}, respectively) were calculated.

This was followed by a second supplemental dose to reach a total dose of 100 μg kg^{-1}. Patients were allowed to recover spontaneously from the neuromuscular block. Dur_{25} (time from beginning of cisatracurium first dose administration until 25% T_1 recovery), Dur_{25-75} (time from 25 to 75% T_1 recovery), $Dur_{25-0.8}$ (time from 25% T_1 to 0.8 TOF ratio recovery), and $Dur_{0.8}$ (time from beginning of cisatracurium first dose administration until 0.8 TOF ratio recovery) time course of action variables were calculated.[9]

Statistical analysis

The primary endpoint of our study was to compare cisatracurium ED_{95} in patients who underwent ANH with control subjects. Based upon a previous study, in which atracurium ED_{95} was 208 (37) μg kg^{-1} in patients who underwent ANH and 309 (88) μg kg^{-1} in control subjects, our *a priori* power analysis (α=0.05) with 101 (51) μg kg^{-1} difference between the two groups,[6] showed that a subgroup size of 10 patients would be required to reveal a statistically significant difference between the two groups with >80% power. Analysis of covariance (two-way ANOVA) was used for intergroup analysis. Data were expressed as means (SD or 95% confidence intervals). $P<0.05$ was considered statistically significant.

Table 1 Patients' characteristics and blood investigations. Means (SD or range). ANH, acute normovolaemic haemodilution; BMI, body mass index; Hb, haemoglobin; Hct, haematocrit

	Control group	ANH group	*P*-value
Male /female	24/6	24/6	
Age (yr)	53.1 (44.4–62.4)	55.6 (48.7–62.5)	0.0676
Weight (kg)	68.7 (11.6)	70.3 (3.4)	0.4889
Height (cm)	174 (6)	171 (4)	0.0640
BMI (kg m^{-2})	22.8 (4.2)	24.0 (1.6)	0.1692
Blood investigations	**Before ANH**	**After ANH**	***P*-value**
Hb (g dl^{-1})	13.6 (0.9)	8.7 (1.4)	<0.0001
Hct (%)	41.7 (2.5)	28.6 (4.3)	<0.0001
Plasma proteins (g dl^{-1})	6.7 (0.8)	4.4 (0.4)	<0.0001

Results

Patients' characteristics are presented in Table 1. There was no significant blood loss during the neuromuscular monitoring period of the study. Haemoglobin, Hct, and plasma proteins declined significantly with ANH (Table 1). There were no significant differences between the two groups in the T_1 stabilization period, anaesthesia induction period, skin and core temperature, mean arterial pressure, estimated blood loss, fluid replacements, and propofol and remifentanil requirements during the neuromuscular monitoring period of the study.

Our study showed that ANH did not result in a significant shift in the cisatracurium dose–response curve (Fig. 1). There were no significant differences between the two groups in cisatracurium onset time or maximum T_1 block (Table 2). Although cisatracurium ED_{50}, ED_{90}, and ED_{95} were slightly higher in the ANH group indicating a minor decrease in potency, still the differences between the two groups did not reach statistical significance (Table 3). The duration of action (Dur_{25} and $Dur_{0.8}$) and the rate of recovery (Dur_{25-75}, $Dur_{25-0.8}$) parameters were similar between the two groups (Table 4).

Discussion

The principal dose–response relationship finding of our study was that unlike other previously reported neuromuscular blocking drugs, in which ANH was shown to increase their potency thus requiring lower doses,[4–7] ANH did not significantly alter the potency of cisatracurium as evident by the similar ED_{50}, ED_{90}, and ED_{95} in the two groups. Although we did not specifically assess the above-mentioned neuromuscular blocking drugs under the exactly same ANH conditions, we can speculate that the observed differences between cisatracurium and other neuromuscular blocking drugs including atracurium could arise from their significantly different distribution characteristics, because dose–response relationships depend mainly on the initial concentration of a drug rather than the disposition process that follows. Cisatracurium is only 1 of the 10 stereoisomers that constitute atracurium and makes up just 15% of the atracurium mixture.[13] Smith and colleagues[13] demonstrated that cisatracurium possesses a significantly different distribution profile than the other three geometric isomer groups that constitute the atracurium mixture.[13] Furthermore, atracurium's volume of distribution at steady state (Vd_{ss}) and distribution half-life ($t_{1/2}\alpha$) [87.4 (31) ml kg^{-1}, 2.1 (0.4) min][14 15] are different from cisatracurium's Vd_{ss} and $t_{1/2}\alpha$ [168 (44) ml kg^{-1}, 6.4

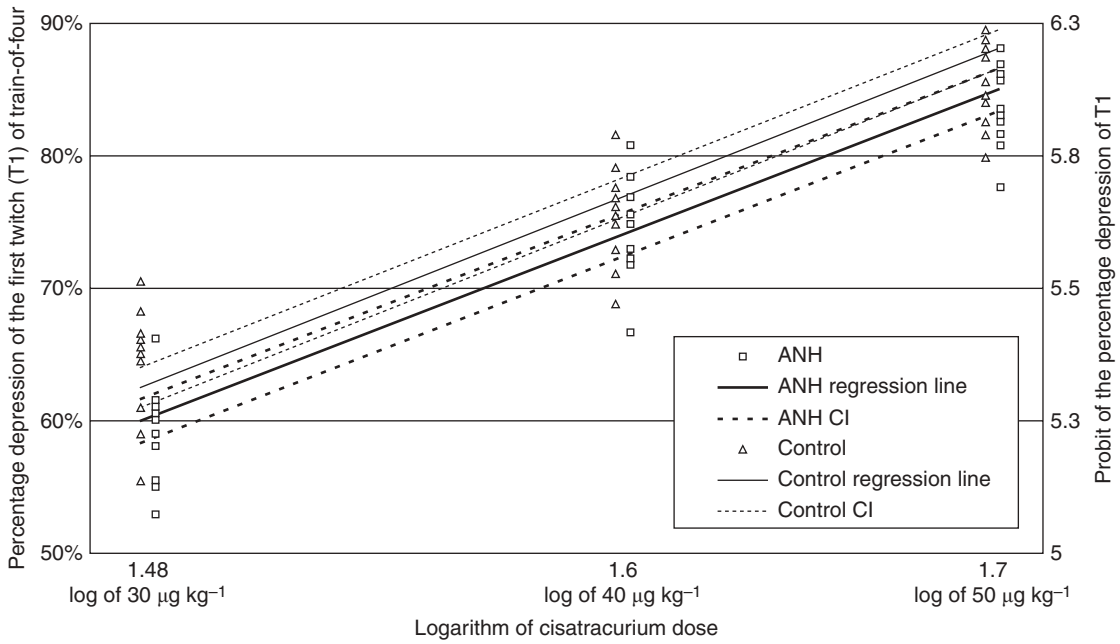

Fig 1 Cisatracurium log dose–probit curves (upper and lower 95% confidence intervals) in the acute normovolaemic haemodilution (ANH) and control groups.

Table 2 Time to T_1 baseline stabilization, anaesthesia induction, cisatracurium onset, and maximum T_1 block. Means (SD), n=30. ANH, acute normovolaemic haemodilution; onset time, time from beginning of cisatracurium first dose administration until first response of train-of-four (T_1) maximum suppression

	Control group	ANH group	*P*-value
Time (min)			
T_1 baseline stabilization	2 (1)	2 (1)	
Anaesthesia induction	3 (2)	3 (1)	
Onset time (min)			
30 μg kg^{-1}	7.0 (1.5)	6.6 (2.0)	0.6270
40 μg kg^{-1}	7.2 (2.4)	7.0 (2.1)	0.8478
50 μg kg^{-1}	6.3 (2.1)	6.0 (1.8)	0.7391
Maximum T_1 block (%)			
30 μg kg^{-1}	60.8 (7.4)	59.6 (7.9)	0.7299
40 μg kg^{-1}	78.0 (6.0)	74.5 (7.1)	0.2495
50 μg kg^{-1}	88.9 (4.8)	85.1 (3.8)	0.0655

(0.5) min].[8 13] Whereas, the previously reported succinylcholine,[4] pancuronium,[4] vecuronium,[5] and rocuronium[7] were either depolarizing[4] or aminosteroid neuromuscular blocking drug,[4 5 7] all with distribution features completely different from cisatracurium.

In our study, Hct declined to 28.6 (−31%), indicating significant haemodilution. A recent study in patients who underwent ANH with HES demonstrated that haemodilution fluids increased the extracellular fluid volume by 600–800 ml;[16] this would consequently result in cisatracurium dilution as cisatracurium is rapidly distributed into the extracellular space.[15] Unlike other neuromuscular blocking drugs in which ANH was shown to significantly increase their potency and shift their dose–response curves to the left,[4–7] in our study, cisatracurium's dose–response curve was actually shifted to the right, indicting a decrease, albeit not statistically significant, in its potency (Fig. 1, Table 3). This could be attributed to the fact that because ANH did not significantly influence the potency of cisatracurium, our dose–response curve just reflected the initial dilution of cisatracurium plasma concentrations.

The principal time course of action finding of our study was that cisatracurium's duration of action (Dur_{25} and $Dur_{0.8}$) and recovery rate parameters (Dur_{25-75} and $Dur_{25-0.8}$) were not prolonged in the ANH group compared with the control group. However, Xue and colleagues[6] showed that ANH prolonged atracurium's time course of action. In addition to the fact that recovery parameters are crude measurements of the elimination process and might not detect minor differences between groups, still the differences between cisatracurium and atracurium could be attributed to the differences in the disposition process. Fisher and colleagues[14] found that more than 60% of atracurium clearance was organ-based through pathways other than Hofmann degradation and ester hydrolysis.[14] Although, cisatracurium retained atracurium's advantage of spontaneous organ-independent, base catalysed, temperature-dependent Hofmann degradation, but unlike atracurium, cisatracurium is not eliminated via the non-specific plasma esterases hydrolysis pathway.[8] The clinical implications of our results are that no adjustment in cisatracurium's initial and repeat doses is necessary in patients who undergo surgery with ANH.

Table 3 Cisatracurium effective doses 50, 90, and 95%. Means (95% confidence intervals), n=30. ANH, acute normovolaemic haemodilution; ED_{50}, ED_{90}, ED_{95}, effective dose 50, 90, 95%

	Control group	ANH group	*P*-value
Slope	5.68 (5.08–6.28)	5.55 (5.04–6.07)	0.7253
ED_{50} (μg kg^{-1})	28.2 (25.3–31)	29.5 (27–32)	0.4390
ED_{90} (μg kg^{-1})	47.6 (44.9–50.3)	50.4 (47.4–53.4)	0.1391
ED_{95} (μg kg^{-1})	55.3 (52.5–58.1)	58.7 (55.3–62)	0.0997

Table 4 Cisatracurium time course of action. Means (SD), n=30. ANH, acute normovolaemic haemodilution; Dur_{25}, time from beginning of cisatracurium first dose administration until first response of train-of-four (T_1) 25% recovery; Dur_{25-75}, time of T_1 recovery from 25 to 75%; $Dur_{0.8}$, time from beginning of cisatracurium first dose administration until 0.8 train-of-four ratio recovery; $Dur_{25-0.8}$, time from T_1 25% until 0.8 train-of-four ratio recovery

	Control group	ANH group	*P*-value
Dur_{25} (min)	42.2 (7.6)	40.8 (5.9)	0.1758
Dur_{25-75} (min)	12.3 (2.6)	11.9 (2.2)	0.9170
$Dur_{25-0.8}$ (min)	24.3 (7.6)	23.9 (8.1)	0.6269
$Dur_{0.8}$ (min)	66.5 (10.7)	64.7 (8.4)	0.7669

The protein bound fraction of cisatracurium was recently reported to be 38%.[17] In our study, there was a significant decline in total plasma proteins with ANH. The decreased protein-binding capacity could have purportedly resulted in higher concentrations of cisatracurium's pharmacologically active free fraction. However, plasma protein-binding changes were recently shown to have little clinical relevance.[18] Benet and Hoener[18] demonstrated that except drugs with very high extraction ratio, changes in plasma protein binding do not influence the clinical exposure of a patient to parenterally administered drugs such as cisatracurium. They found that the concept that effective concentrations of drugs are dependent on protein binding is incorrect, as the effective concentrations of drugs are mainly dependent on the disposition process.[18]

The ED_{50} (28.2 μg kg^{-1}) of our control group closely matched the previously reported 29,[19] 30,[20] and 31.1 μg kg^{-1} [21] ED_{50} using the single-dose method. Similarly, the ED_{95} of our control group (55.3 μg kg^{-1}) was comparable with the previously reported 48,[19] 53,[20] and 57.6 μg kg^{-1} [22] ED_{95}.

Our study has limitations, because our study design did not enable us to explore the effect of the diverse ANH conditions used in other studies that could have influenced the outcome. First, whether there is a critical threshold for ANH volume to alter the potency of cisatracurium, as we only used the recommended 15 ml kg^{-1} volume of blood (20% of blood volume).[3] Second, does the type of replacement fluid make a difference: we used HES as opposed to a mixture of Ringer's lactate and dextran in Xue and

colleagues' studies.[5 6] Furthermore, if blood was procured before the induction of anaesthesia as opposed to ANH after induction of anaesthesia and administration of cisatracurium intubating dose, obviously ANH would only influence cisatracurium's repeat dose administrations.

In conclusion, our results demonstrated that unlike other previously reported neuromuscular blocking drugs, cisatracurium dose–response relationship and time course of action were not influenced by ANH, thus requiring no dosing adjustments. This would render cisatracurium as the neuromuscular blocking drug of choice in patients who undergo surgery with ANH.

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