Comparative potency of steroidal neuromuscular blocking drugs and isobolographic analysis of the interaction between rocuronium and other aminosteroids

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Summary
We have determined the relative potency of rocuronium, pancuronium, pipecuronium and vecuronium, and examined the nature of the interaction of rocuronium with the other three steroidal neuromuscular blocking drugs. We studied the dose–response relationships of each drug and their combination with rocuronium in 200 ASA I or II patients during propofol–fentanyl–nitrous oxide–oxygen anaesthesia. Neuromuscular block was recorded as the evoked thenar mechanomyographic response to single twitch stimulation of the ulnar nerve at 10-s intervals. The dose–response curves were determined by probit analysis. Isobolographic and algebraic (fractional) analyses were used to assess the combined effect of equipotent doses of rocuronium and vecuronium, pipecuronium or pancuronium and to define the type of interaction between these drugs. The isobolograms were constructed by plotting single-drug ED50 points on the dose co-ordinates, and a combined ED50 point in the dose field. The calculated doses producing 50% depression (ED50) of the twitch height for rocuronium, pancuronium, pipecuronium and vecuronium were 144.8 (95% confidence intervals 140.4–149.3), 32.4 (31.7–32.9), 27.1 (26.5–27.6) and 23.7 (22.7–24.8) μg kg⁻¹, respectively. Corresponding doses producing 95% depression (ED95) of twitch height were, respectively, 322.1 (307.5–337.3), 58.1 (56.2–60.1), 48.7 (46.9–50.5) and 39.9 (38.4–41.4) μg kg⁻¹. Based on the estimate of ED95, the relative potency was 1 : 4.5 : 5.4 : 6, respectively. The interaction between rocuronium and vecuronium, pipecuronium or pancuronium was found to be additive. (Br. J. Anaesth. 1995; 75: 37–42)

Key words

The currently available steroidal non-depolarizing neuromuscular blocking drugs for clinical use are pancuronium, pipecuronium, vecuronium and rocuronium. Both pancuronium and pipecuronium are long-acting bisquaternary compounds [1–3]. Vecuronium and rocuronium are monoquaternary analogues of pancuronium with intermediate durations of action [4, 5]. Although the dose–response relationships for these compounds have been reported [3, 6–14], comparative data for all agents do not exist. Furthermore, combinations of rocuronium with other steroidal non-depolarizing neuromuscular blocking compounds have not been studied in vivo and the results of animal studies are not always applicable to humans. For example, in the in vitro models, Golpariani and colleagues [15] demonstrated that the interaction between pipecuronium and vecuronium was synergistic. This finding conflicts with our observations in humans [12].

This study was designed to compare the dose-response relationships for pancuronium, pipecuronium, vecuronium and rocuronium and to examine the interaction of rocuronium with the other three steroidal agents in humans by the isobolographic method.

Patients and methods
After obtaining local Ethics Committee approval and informed consent, we studied 200 ASA I or II adult patients of both sexes, aged 18–55 (mean 31) yr and weighing 46–86 (mean 64.3 (sd 9.4)) kg. All patients were undergoing elective procedures; they had no neuromuscular, renal or hepatic disease, and were not receiving any drug known or suspected to interfere with neuromuscular function. All patients were premedicated with lorazepam 2 mg orally approximately 90 min before induction of anaesthesia. An i.v. infusion of lactated Ringer’s solution was commenced before induction of anaesthesia. ECG, pulse oximetry and arterial pressure were monitored. Temperature was monitored by a nasopharyngeal thermistor and maintained at 36.5 ± 0.5 °C.

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Anaesthesia was induced with midazolam 0.03 mg kg\(^{-1}\), propofol 2–2.5 mg kg\(^{-1}\), fentanyl 4–5 \(\mu\)g kg\(^{-1}\) and 70 % nitrous oxide in oxygen, and maintained with a continuous infusion of propofol 8–10 mg kg\(^{-1}\) h\(^{-1}\) and 70 % nitrous oxide in oxygen supplemented with incremental doses of fentanyl. The trachea was sprayed with 4 ml of 4 % lignocaine and intubated without the use of neuromuscular blockers. End-tidal concentrations of nitrous oxide, oxygen and carbon dioxide were measured continuously by a multiple-gas analyser (Capnomac, Datex Instrumentarium, Helsinki, Finland). Ventilation was adjusted to maintain normocapnia (end-tidal carbon dioxide pressure 4.8–5.3 kPa).

The ulnar nerve was stimulated at the wrist with square-wave supramaximal stimuli of 0.2-ms duration, delivered at a rate of 0.1 Hz, using a Myostet peripheral nerve stimulator (Biometer International, Odense, Denmark). The resultant contraction of the adductor pollicis was recorded using a force displacement transducer and neuromuscular function analyser (Myograph 2000, Biometer International, Odense, Denmark). Preload tension of the thumb was maintained at 300 g throughout the investigation.

The following predetermined doses of drugs were administered: rocuronium 100, 200, 300 or 350 \(\mu\)g kg\(^{-1}\), vecuronium 20, 30 or 40 \(\mu\)g kg\(^{-1}\), pancuronium 20, 30, 40, 50 or 65 \(\mu\)g kg\(^{-1}\) or pipercuronium 20, 30, 40 or 50 \(\mu\)g kg\(^{-1}\). Studies of the single-drug groups were concluded first, so that doses of the combinations could be planned. From the dose–response curves of the neuromuscular agents administered alone, we determined the respective effective doses resulting in a 50 % reduction of twitch tension (ED\(_{50}\)). Subsequently, dose–response curves were obtained by administration of the following drug combinations in a constant dose ratio based on the ED\(_{50}\) values of the single agent.

For the rocuronium–vecuronium combination, the following combinations were administered: 1 ED\(_{50}\) rocuronium + 1 ED\(_{50}\) vecuronium; 1/2 ED\(_{50}\) rocuronium + 1/2 ED\(_{50}\) vecuronium; and 1/4 ED\(_{50}\) rocuronium + 1/4 ED\(_{50}\) vecuronium. Similar dose–ratio combinations were used for both rocuronium–pancuronium and rocuronium–pipercuronium combinations. From the dose–response curve of the combined drugs, the ED\(_{50}\) value of the total dose of the mixture was calculated and, based on the known dose ratio, the single doses of the agents in the combination were obtained for plotting on the isobologram. All drugs were given to subgroups of eight patients and injected over 5 s into a rapidly flowing i.v. infusion. The drug and dose for any individual were chosen randomly. In the combination group, drugs were given simultaneously into two separate i.v. cannulae inserted in one arm. The neuromuscular response was recorded as the maximum depression of twitch tension, expressed as a percentage of the control value. When the maximum effect of the selected dose was reached (that is, when no further decrease in evoked response to three consecutive stimuli occurred), the study was terminated and anaesthesia continued as appropriate for surgery.

### DATA PROCESSING AND ISOBOLOGRAPHIC ANALYSIS

The percentage values for twitch depression in each group were transformed to probits and plotted against the logarithm of the dose using PCNONLIN version 4.2A (Clin Trials, Inc., Lexington, KY, USA) [16]. Regression lines were compared using analysis of covariance. First, we tested the lines to determine if they deviated from parallelism, if they did not, an \(F\) test was applied to see if the elevations differed. If so, a \(t\) test was applied to determine which line differed in elevation [17], using BMDP statistical package, release 7.01 (University of California Press, Berkeley, CA, USA, 1994). The ED\(_{50}\) and ED\(_{95}\) values (doses causing 50 % and 95 % depression of twitch tension, respectively) were calculated from the log–probit regression lines for each group. Using analysis of variance, we compared age and body weight between the different groups. Unless otherwise specified, the results are expressed as mean (95 % confidence intervals (CI)) and were considered significant when \(P < 0.05\).

Isobolographic [18, 19] and algebraic (fractional [20] analyses were used (ED\(_{50}\) level) to define the type of interaction between rocuronium and vecuronium, pancuronium or pipercuronium. Iso-bolographic analysis for drug–drug interactions was conducted according to Tallarida, Porreca and Cowan [18]. This analysis has the advantage of being independent of the slopes of the dose–response curves, that is parallelism does not have to be established. The isobolograms were constructed by plotting single-drug ED\(_{50}\) points on the dose coordinates of the isobologram, and a combined ED\(_{50}\) point in the dose field. A straight line joining the single-drug ED\(_{50}\) points is termed an isobole (isol, equal, bole, effect) or the “additive line”. The entire graph is termed an isobologram. If the ED\(_{50}\) of a combination falls on the theoretical additive line, the effect of the drug mixture is additive. Points to the left of the theoretical additive line would be consistent with a synergistic interaction, whereas points to the right of the line would indicate an antagonistic interaction. CI for each point were calculated from the variances of each component alone and were evaluated for statistical significance using the Student’s \(t\) test.

The algebraic analysis [20] was based on the expression of the component doses of the two agents of the combination as fractions of the doses that produce the same effect when given separately. The sum of the fractional doses, as expressed by the following equation, indicates the type of interaction:

\[
dr/\text{(ED}_{50}\text{)}_r + db/\text{(ED}_{50}\text{)}_b
\]

where (ED\(_{50}\)\(_r\)) = ED\(_{50}\) value of rocuronium given alone, (ED\(_{50}\)\(_b\)) = ED\(_{50}\) value of either vecuronium, pancuronium or pipercuronium administered alone, \(dr\) and \(db\) respectively, the doses of rocuronium and the other drug in the mixture (either vecuronium, pancuronium or pipercuronium) which, when combined, are equipotent with (ED\(_{50}\)\(_r\)) or (ED\(_{50}\)\(_b\)). Values near 1 indicate additive interaction, values greater than 1 imply antagonism and values less than 1 indicate synergism.
Results

The calculated doses for ED$_{50}$ values for twitch depression were 144.8 (95% CI 140.4–149.3), 23.7 (22.7–24.8), 27.1 (26.5–27.6) and 32.4 (31.7–32.9) µg kg$^{-1}$ for rocuronium, vecuronium, pipecuronium and pancuronium, respectively. Corresponding ED$_{50}$ values were 322.1 (307.5–337.3), 39.9 (38.4–41.4), 48.7 (46.9–50.5) and 58.1 (56.2–60.1) µg kg$^{-1}$, respectively. The dose–response curves are displayed in figure 1. Tests for parallelism revealed that these curves have different slopes ($P < 0.05$). The slopes for rocuronium, vecuronium, pipecuronium and pancuronium groups were, respectively, 4.7 (4.4–5), 7.3 (6.7–7.9), 6.5 (6–6.9) and 6.5 (6.1–6.8).

Isobolographic analyses demonstrated additive interactions with respect to the neuromuscular blocking activity of rocuronium with other steroidal agents. The fractional (algebraic) analyses of these interactions also demonstrated additivism (table 1). Figure 2 represents the dose–response curves for rocuronium, vecuronium and their combination, plotted against the logarithm of the dose in ED$_{50}$ units. The ED$_{50}$ isobologram for the rocuronium–vecuronium interaction is also presented in figure 2. The experimentally determined ED$_{50}$ value for the combination was 66.2 (63.7–68.6) µg kg$^{-1}$ for rocuronium and 10.8 (10.4–11.2) µg kg$^{-1}$ for vecuronium. The theoretical additive ED$_{50}$ value was calculated as 72.4 (67.9–76.9) µg kg$^{-1}$ for

![Figure 1](image-url)  
**Figure 1** Log dose–probit plot for twitch height (TH) depression for vecuronium (■), pipecuronium (○), pancuronium (▲) and rocuronium (●). Individual points represent mean (95% confidence intervals) twitch height depression (% control) with each dose.

<table>
<thead>
<tr>
<th>Group</th>
<th>Recombined component</th>
<th>Fraction of ED$_{50}$</th>
<th>Pipercuronium component</th>
<th>Fraction of ED$_{50}$</th>
<th>Pancuronium component</th>
<th>Fraction of ED$_{50}$</th>
<th>Dose (µg kg$^{-1}$)</th>
<th>Sum of fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single drug study</td>
<td>Rocuronium</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>144.8 (140.4–149.3)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Vecuronium</td>
<td>—</td>
<td>1.00</td>
<td>23.7 (22.7–24.8)</td>
<td>—</td>
<td>—</td>
<td>10.8 (10.4–11.2)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Pipecuronium</td>
<td>0.46</td>
<td>—</td>
<td>66.2 (63.7–68.9)</td>
<td>—</td>
<td>—</td>
<td>12.2 (11.8–13)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Pancuronium</td>
<td>—</td>
<td>0.45</td>
<td>65.2 (62.8–68.4)</td>
<td>0.45</td>
<td>68.2 (65.9–71)</td>
<td>0.47</td>
<td>15.2 (14.7–15.8)</td>
</tr>
<tr>
<td>Interaction studies</td>
<td>Rocuronium + Vecuronium</td>
<td>0.45</td>
<td>—</td>
<td>68.2 (65.9–71)</td>
<td>—</td>
<td>—</td>
<td>12.2 (11.8–13)</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Pipecuronium</td>
<td>—</td>
<td>—</td>
<td>68.2 (65.9–71)</td>
<td>—</td>
<td>—</td>
<td>15.2 (14.7–15.8)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
rocuronium and 12 (11–13) μg kg⁻¹ for vecuronium. The confidence intervals of these points overlapped and the result of Student’s t test for potency ratio was not significant.

For the rocuronium–pipecuronium interaction (fig. 3), the experimentally determined ED₅₀ value for the combination was 65.2 (62.8–68.2) μg kg⁻¹ for rocuronium and 12.2 (11.8–13) μg kg⁻¹ for pipecuronium. The theoretical additive ED₅₀ value was calculated as 72.4 (68–76) μg kg⁻¹ for rocuronium and 13.5 (12.8–14.3) μg kg⁻¹ for pipecuronium. The confidence intervals of these points overlapped and the result of Student’s t test for potency ratio was not significant.

Similar observations were noted for the rocuronium–pancuronium interaction (fig. 4). The experimentally determined ED₅₀ value for the combination was 68.2 (65.9–71) μg kg⁻¹ for rocuronium and 15.2 (14.7–15.8) μg kg⁻¹ for pancuronium. The theoretical additive ED₅₀ value was calculated as 72.4 (69.1–75.6) μg kg⁻¹ for rocuronium and 16.2 (15.5–17) μg kg⁻¹ for pancuronium. The confidence intervals of these points overlapped and the result of Student’s t test for potency ratio was not significant.

**Discussion**

We have found that the dose–response curves for rocuronium, pancuronium, pipecuronium and vecuronium were not parallel. Based on the estimate of ED₅₀, the relative potency was 1 : 4.5 : 5.4 : 6, respectively. In addition, isobolographic analysis demonstrated that combinations of rocuronium with the other three steroidal neuromuscular blocking drugs were additive. The fractional (algebraic) analyses of these interactions also demonstrated zero (additive) interaction (table 1).

Combinations of vecuronium and pancuronium [10] or vecuronium and pipecuronium [12] have been reported to be additive. In contrast, Golpariani and colleagues [15] demonstrated in the in vitro phrenic nerve–hemidiaphragm preparations a synergistic interaction between ORG 9426 (rocuronium) and vecuronium, pipecuronium or pancuronium. However, in the same study the authors did
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not observe potentiation of the effect of pancuronium–vecuronium or pancuronium–pipercuronium combinations [15].

There are differences between in vitro and in vivo studies. Riker and Wescoe [21] showed an additive effect of tubocurarine and gallamine in cat tibiales anterior. In contrast, Ghoneim and colleagues [22] found that a mixture of tubocurarine and gallamine resulted in a block that was greater than that expected by simple addition. Pollard and Jones [23] showed a greater than additive effect with pancuronium–tubocurarine and tubocurarine–alcuronium in the rat phrenic nerve–hemidiaphragm preparation. Schuh [24], on the other hand, described only an additive interaction with pancuronium–tubocurarine, pancuronium–gallamine or tubocurarine–gallamine combinations in patients. Similarly, Golparian and colleagues [15] demonstrated that the interaction between pipercuronium and vecuronium was synergistic in vitro in contrast with our observations in humans [12].

The results of this study, therefore, support the contention that combinations of structurally similar neuromuscular blocking drugs produce an additive response in humans. On the other hand, combinations of structurally dissimilar neuromuscular blocking drugs resulted in a potentiating effect [14, 25]. Lebowitz and colleagues [25] reported a greater than additive effect in patients with pancuronium–dimethyltubocurarine and pancuronium–tubocurarine combinations but not with a dimethyltubocurarine–tubocurarine combination. Similarly, Naguib [14], Meretoja and colleagues [26] and Mirakhur, Gibson and Ferres [27] showed, respectively, that rocuronium–mivacurium, vecuronium–atracurium and vecuronium–tubocurarine combinations produced more than additive (synergistic) effects.

Hypotheses that have been proposed to explain the synergistic interaction include: (1) the existence of multiple binding sites at the neuromuscular junction (pre- and postsynaptic receptors) [23, 25, 28]; (2) non-equivalence of binding sites in the regions of the α chain responsible for ligand recognition, resulting from the asymmetric azimuthal orientation of the five subunits in the acetylcholine pentamer which determines different contacts for the α1 and α2 chains [29–31]; (3) alteration in the pharmacokinetic behaviour of one drug by the other, a hypothesis disputed by Martyn, Leibel and Matteo [32]; and (4) the existence of different sites for the antagonist and the agonist [33] (the antagonist site may be distinct from, but tightly coupled allosterically to, the agonist site). Standaert [34] suggested that this mechanism could underline the synergism between steroid and bis-isoquinoline antagonists.

Consistent with our data, the slopes of dose–response curves for pipercuronium and pancuronium or pipercuronium and vecuronium have been reported to differ significantly [9, 12]. However, the curves for pancuronium and vecuronium did not differ in slope [10]. The difference in the slopes observed in this study could be attributed to the effect of different neuromuscular blocking drugs at different sites at the neuromuscular junction [35, 36].

The ED50 value of 144.8 (95 % CI 140.4–149.3) µg kg⁻¹ for rocuronium calculated in the current study is in close agreement with 147 (130–165) µg kg⁻¹ reported by Cooper and colleagues [13] who used a single-twitch mode of stimulation and mechanomyography during thio- pentone–fentanyl–nitrous oxide–oxygen anaesthesia. In this study, the calculated ED50 value (95 % CI) for vecuronium during propofol–fentanyl–nitrous oxide–oxygen anaesthesia was 23.7 (22.7–24.8) µg kg⁻¹ similar ED50 values were reported by Ørding and colleagues [7] (28 (95 % CI 25.9–29.8) µg kg⁻¹) who used a single-switch mode of stimulation and mechanomyography during neurolept anaesthesia. For pipercuronium, the ED50 value of 27.1 (95 % CI 26.5–27.6) µg kg⁻¹ calculated in the present study is in keeping with values of 31.7 (SEM 2.9) and 24.96 (95 % CI 22.32–27.6) µg kg⁻¹ reported by Pittet and colleagues [8] and Stanley and Mirakhur [9], respectively, who used a single-twitch mode of stimulation and electromyography [8] or...
mechanomyography [9] during fentanyl–nitrous oxide–oxygen anaesthesia. The calculated ED50 value for pancuronium in this study was 32.4 (31.7–32.9) μg kg⁻¹. Similar ED50 values were reported by Stanley and Mirakhr [9] (30.42 (95% CI 27.45–33.30 μg kg⁻¹)) and Donlon and colleagues [37] (30 (24–37) μg kg⁻¹) who used a single-twitch mode of stimulation and mechanomyography during thiopentone–opioid–nitrous oxide–oxygen anaesthesia.

References