RELATIONSHIP OF THE TRAIN-OF-FOUR RATIO TO PLASMA ATRACURIUM CONCENTRATION

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SUMMARY
The electromyographic response to a short infusion of atracurium 0.25 mg kg\(^{-1}\) was recorded using the train-of-four (TOF) technique, and the plasma atracurium concentration profile measured, in 10 healthy patients. The TOF ratio (T4:T1) was depressed over a time course which did not conform to the predictions of an effect compartment model; the fit of such a model, when possible, was associated with large residual errors. In contrast, the absolute height of the fourth response of the TOF (T4:TO) may be fitted by a standard effect compartment model with smaller errors. This residual error was reduced further on fitting a threshold effect compartment model to the data set T4:TO. The parameter values of such a model were related closely to those for the first response (T1:TO). The kinetics of the effect compartments for the first and fourth response of the TOF were similar, whilst the \(C_{250}\) for the fourth response was approximately 67% that for the first response.

KEY WORDS

Although the train-of-four (TOF) ratio was introduced into clinical practice to facilitate assessment of residual curarization in patients given neostigmine to antagonize the action of tubocurarine [1], it has also been used widely as a tool to investigate mechanisms of drug action. This latter usage was greatly enhanced by the finding that α-bungarotoxin, whilst causing profound neuromuscular block, does not cause fade of the TOF [2]. This and other evidence, reviewed by Bowman [3], was shown to be compatible with the concept that fade of the TOF reflects the action of non-depolarizing neuromuscular blocking drugs at a site other than that responsible for block of the single twitch response, perhaps the prejunctional nerve terminal. This possibility has been advanced as a possible explanation for the fact that, after a bolus of atracurium, fade of the TOF follows a time course different from that of depression of the first response [4].

Despite the success of the effect compartment model, proposed by Hull and others [5] and by Sheiner and others [6], in unifying the time course of neuromuscular block with the temporal profile of the plasma concentration of neuromuscular blocking drugs including atracurium [7-9], there is a paucity of similar reports for the TOF ratio. An attempt was made by Graham and others [10] for pancuronium; they showed that the time course of depression of the TOF ratio could be related to an effect compartment which equilibrated with the plasma with a half-life of 6.1 min, compared with 3.1 min for the first response of the TOF. Unfortunately, the pharmacokinetic and pharmacodynamic data were not obtained from the same subjects and other pharmacodynamic model parameters were not presented.

The present study was an attempt to examine the relationship between the depression of the TOF ratio and plasma atracurium concentration in the context of an effect compartment model. A preliminary report of the findings has been made [11].

PATIENTS AND METHODS
We studied 10 healthy patients (four male) about to undergo minor surgery requiring the use of neuromuscular block. The study was approved by the Ethics Committee of the Royal Liverpool Hospital and informed consent was obtained from each patient. The mean age was 37.3 yr (range 15.3-57.2 yr) and mean weight was 66.8 (sd 14.2) kg.

Premedication was with promethazine 50 mg orally or diazepam 10 mg orally on the evening before surgery, or "Cyclimorph 10" (morphine 10 mg and cyclizine 50 mg) i.m. 1 h before surgery. Anaesthesia was induced with fentanyl 100-200 ug and thiopentone 250-500 mg, and maintained with 66 % nitrous oxide in oxygen supplemented with midazolam 3-10 mg i.v.

After induction of anaesthesia, electromyographic monitoring of the height of the surface compound action potential of the ulnar nerve was begun using the Medelec MS6. TOF stimuli at 2 Hz were repeated every 12.5 s; this pattern of stimuli was continued throughout the study. The ratio of the height of the first response to the control height (T1:TO), and the TOF ratio (T4:T1) were measured.

A cannula was placed in a vein in the antecubital fossa of the arm used for electromyographic moni-
toring, for withdrawal of blood samples; another was placed in a vein in the opposite forearm, for administration of atracurium.

After a period of 20 min when the electromyo-
graphic baseline was established, atracurium 0.25 mg kg\(^{-1}\) was given by constant rate infusion over a period of 10 min. Ventilation of the lungs was controlled and the trachea intubated when appropriate; end-tidal partial pressure of carbon dioxide was maintained in the range 4.0-5.3 kPa (Datex Capnomac).

Neuromuscular block was not antagonized; neuromuscular monitoring was continued until both the ratios T1:T0 and T4:T1 were 80% or greater. After recovery from neuromuscular block and the end of surgery, anaesthesia was discontinued, spontaneous ventilation re-established and the trachea extubated.

Measurement of plasma atracurium concentration

Blood samples (2.5 ml) were taken before and at 1, 2, 4, 6, 8 and 10 min after the start of the infusion, and at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 75 and 90 min after the end of the infusion. The heparinized blood samples were acidified immediately and cooled, and plasma was separated promptly. The plasma was frozen rapidly in liquid nitrogen and stored at \(-20^\circ\text{C}\) until subsequent analysis. The method for analysis of plasma atracurium concentration has been described in detail previously [12]. The sensitivity was about 5 ng ml\(^{-1}\) and the coefficient of variation was in the range 1.8-6.9%.

Data analysis

In each patient, effect compartment models were fitted to the data sets for drug effect together with the plasma concentration profile. To the depression of the TOF ratio, a standard model [5, 6] was fitted; this was possible in nine of the 10 patients, albeit with large residual errors. The error was not diminished by incorporation of a threshold term to the effect compartment; indeed the threshold model could not be fitted consistently. To the absolute height of the fourth response, both standard and threshold models [12] could be fitted.

The model did not incorporate a specific compartmental description of disposition; rather numerical methods were used to fit the model to the pharmacodynamic data set and the measured plasma concentration profile as described previously [12].

The neuromuscular blocking effect was specified by the model as a fraction, between 0 (no effect), and 1 (complete block of the response). The fit was obtained using the unweighted least squares criterion, with the Gauss–Newton algorithm. Convergence was accepted when each iteration changed the values of all the model parameters by less than 0.1%. Adequacy of fit of the model to the data set was assessed by the magnitude of residual error, supplemented by inspection of the graphs of both model prediction and observation \(\text{vs}\) time. The threshold model was fitted also to the data set T1:T0 as described previously [12].

The relationship between the parameter values fitted to the data sets T4:T0 and T1:T0 was examined using Pearson’s correlation coefficient.
RESULTS

Before discontinuation of the electromyographic recording, mean recovery of T1:T0 was 92.2 %, and of T4:T1, 94.0 %. The plasma atracurium concentration profile and derived pharmacokinetic parameters have been reported previously [13].

Time course of depression of TOF ratio

The temporal features of the depression of the TOF ratio are shown in figure 1, together with the mean depression of the heights of the first and fourth responses of the TOF. It can be seen that the ratio T4:T1 was affected little for the first 3 min of the infusion and then decreased rapidly to around 50 %. In contrast to the first response, there was then further slow progress of the depression to reach a maximum about 25 min after the start of the atracurium infusion. The ratio T4:T1 then re-
TOF RATIO AND ATRACURIUM

1.0 n
0.5 -
0.0 -
-1.0 -
-1.5 -
-2.0 -

A

B

Atracurium concn (ng ml-1)

-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5

Logit depression of the TOF ratio (T4:T1) vs atracurium concentration in the effect compartment of best fit to the data; data for onset and recovery are not superimposed, and there are obvious departures from linearity.

Logit depression of the absolute height of the fourth response of the TOF (T4:T0) in the same patient vs atracurium concentration in the effect compartment of the threshold pharmacodynamic model of best fit to the data set.

○ = During infusion; ■ = after infusion. There is an obvious linear relationship and close superimposition of data from onset and recovery.

Fig. 3. A: Logit depression of the TOF ratio (T4:T1) vs atracurium concentration in the effect compartment of best fit to the data; data for onset and recovery are not superimposed, and there are obvious departures from linearity.

B: Logit depression of the absolute height of the fourth response of the TOF (T4:T0) in the same patient vs atracurium concentration in the effect compartment of the threshold pharmacodynamic model of best fit to the data set.

Table II. Sum of squared residual error on fitting both standard and threshold models to the depression of the absolute height of the fourth response of the TOF (T4:T0). It was possible to fit both standard and threshold models to this data set, and the residual error was smaller for the threshold model in every case. The small residual errors shown here contrast with the much larger errors on fitting a standard model to the depression of the TOF ratio (see text)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Number of data points</th>
<th>Standard model</th>
<th>Threshold model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td>0.028</td>
<td>0.0021</td>
</tr>
<tr>
<td>2</td>
<td>126</td>
<td>0.025</td>
<td>0.022</td>
</tr>
<tr>
<td>3</td>
<td>117</td>
<td>0.028</td>
<td>0.0059</td>
</tr>
<tr>
<td>4</td>
<td>88</td>
<td>0.058</td>
<td>0.027</td>
</tr>
<tr>
<td>5</td>
<td>102</td>
<td>0.035</td>
<td>0.010</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>0.018</td>
<td>0.0045</td>
</tr>
<tr>
<td>7</td>
<td>133</td>
<td>0.012</td>
<td>0.0087</td>
</tr>
<tr>
<td>8</td>
<td>126</td>
<td>0.0087</td>
<td>0.0060</td>
</tr>
<tr>
<td>9</td>
<td>109</td>
<td>0.030</td>
<td>0.020</td>
</tr>
<tr>
<td>10</td>
<td>107</td>
<td>0.011</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

covered at a rate comparable to that for the ratio T1:T0.

The mean times (sd, range) to maximal depression of the first and fourth responses of the TOF and of the TOF ratio were: T1:T0 15.3 (3.4) min (12.7-23.7 min); T4:T0 16.9 (3.7) min (13.1-26.5 min); T4:T1 23.5 (5.7) min (17-36.2 min).

Biophase model for the TOF ratio

An attempt was made to fit a standard biophase model [5, 6] to the data set T4:T1, together with the plasma atracurium concentration profile in each patient. This was impossible in one patient, in whom a least squares fit could not be obtained for a feasible set of parameter estimates. In the other nine patients the model could be fitted, but with large residual errors (table I).

The poor quality of the fit between the observations and biophase model predictions is illustrated for two patients, whose results are typical, in figure 2. The discrepancy was not ameliorated by an attempt to fit the threshold model.

Concentration–effect relationship for T4:T1

The logit of the depression of T4:T1 is plotted vs the logarithm of the drug concentration in the fitted effect compartment in figure 3A for one patient, whose results are typical. The relationship departs from linearity and the observations in onset and recovery superimpose at only one point. This lack of linearity, and the failure to superimpose observations from onset and recovery implies that to fit a standard model to the data T4:T1 set is inappropriate.

Absolute height of the fourth response

The temporal profile of the fourth response was similar to that of the first response; in particular, mean maximal depression occurred at a time similar to that for the first response, and much earlier than that of the ratio T4:T1.

Concentration–effect relationship for T4:T0

It was possible to fit standard [5, 6] and threshold [12] biophase models to the data set T4:T0 in each patient. The residual error for each model in each patient is shown in table II. In each individual, the
The poor quality of the fit of a simple effect compartment model to the data set T4: T1 is not merely a manifestation of the "noise" inherent in a data set which is the quotient of two measurements, each subject to random error. The lack of fit is a systematic difference in the time course, which has been illustrated graphically (fig. 2). The time course of fade of the TOF ratio presently reported is similar to that reported previously [4]; the maximum mean fade was achieved when the absolute heights of both the first and fourth responses of the TOF had undergone considerable recovery. A single effect compartment model driven by the plasma concentration profile is unable to match this time course.

In contrast, the alternative (and, given the data set T1: T0, tautological) description of the TOF data as the absolute height of the fourth response, T4: T0, gives a data set which is temporally similar to the first response of the TOF. The absolute height of the fourth response is well described by the threshold biophase model of best fit to the data [12], as shown by both the small residual error and the fact that there is a strong linear relationship between the logit effect and the logarithm of the biophase drug concentration.

The parameter values of the threshold model of best fit to the data set T4: T0 bear a close relationship to the values of the model for the data set T1: T0. Whilst the slope of the concentration—response curve is similar, the midpoint is shifted such that the C\textsubscript{50} for T4: T0 is about 67% of the C\textsubscript{50} for T1: T0. Furthermore, whilst k\textsubscript{e} for the data set T4: T0 is in every case slightly less than that for T1: T0, it is also remarkably well correlated with it. It should be stressed that the models were fitted to the data sets T4: T0 and T1: T0 independently, and that the relationships illustrated in figures 4 and 5 must thus reflect the underlying relationships between the phenomena. It is clear that the simplest interpretation for the present observations of the height of the fourth response of the TOF is as a phenomenon showing a parallel but shifted concentration—response relationship within an effect compartment kinetically similar to that within which the depression of the first response is exerted.

This interpretation is consistent with the early observations of Preston and van Maanen [16] who showed that, in the rat sciatic—gastrocnemius nerve—muscle preparation, the dose—response curve for tubocurarine could be shifted to the left by increases in the stimulus frequency between 0.067 and 5 Hz. Similar observations were made in the anaesthetized human by Shank, Ramzan and Triggs [17]: the ED\textsubscript{50} of tubocurarine was shown to be reduced by around 35% on increasing the stimulus rate from 0.02 to 2 Hz, whilst the slope of the dose—response curve was little altered by changes in the stimulus frequency.

One might suppose that the fact that the value of k\textsubscript{e} for the model fitted to the data set T4: T0 is consistently smaller than that for the model fitted to the data set T1: T0 implies that the depression of the fourth response is mediated at a site less accessible to the plasma and thus, perhaps, different from the site at which the action of atracurium on the first response is mediated. The difference between the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T4: T0</th>
<th>T1: T0</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{50}</td>
<td>368.3 (71.4)</td>
<td>523.7 (116.3)</td>
<td>0.983</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gamma</td>
<td>2.79 (0.32)</td>
<td>2.44 (0.35)</td>
<td>0.527</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>k\textsubscript{e} (min\textsuperscript{-1})</td>
<td>0.105 (0.019)</td>
<td>0.120 (0.023)</td>
<td>0.988</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>C\textsubscript{50}</td>
<td>151.6 (72.8)</td>
<td>225.4 (91.4)</td>
<td>0.904</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The concept that fade of the TOF is an effect of non-depolarizing neuromuscular blocking drugs which depends upon action at a site distinct from the postjunctional receptor has been widely invoked to explain several discrepancies between the time course and drug specificity of neuromuscular block and fade [3, 4, 14]. This concept has powerful support from experiments on the voltage clamped end-plate in vitro which show that, after tubocurarine, fade of the end-plate current in response to a train of iontophoretic pulses of acetylcholine is much less marked than fade of the neurally evoked response [15]; the implication of this finding is that fade results from a drug action at the prejunctional site where these drugs are non-depolarizing.
values of $k_{m}$ for the two effects, although consistent, is, however, less striking than the correlation between them. If the depression of the first and fourth responses of the TOF were in fact a consequence of drug action at two independent sites, then the values of $k_{m}$ for the models of the two effects would also be independent and hence poorly correlated.

The fitted value of $k_{m}$ is a single number which summarizes the access of the drug to its sites of action at all the end-plates throughout the muscle. Some end-plates might be expected to have greater access to drug than others, by virtue either of their position in an arteriovenous gradient, or their proximity to a greater number of capillaries. The consequences of such heterogeneity have been explored by Storella [18]. A muscle was simulated with three compartments having different rates of access to a non-depolarizing neuromuscular blocking drug; each compartment contained similar concentration–response curves for $T1: T0$ and for $T4: T0$. For the whole muscle, the TOF ratio $T4: T1$ was relatively more depressed during recovery than during onset; thus it is possible to account for the differences between the time courses of $T4: T1$ and $T1: T0$ without recourse to the postulate of a separate site of action.

The clinical work reported here cannot define the site of action of the non-depolarizing neuromuscular blocking drug, atracurium, which must ultimately depend upon in vitro studies; it cannot be used to confirm or refute the concept that fade results from an action at a prejunctional site, and it does not

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**Fig. 4.** $C_{p\text{a}0}$ for the fourth response of the TOF ($T4: T0$) vs value for the first response ($T1: T0$) ($n = 10$). There is a strong positive linear correlation ($r = +0.983$). ——— Regression line; ——— line of identity.

**Fig. 5.** $k_{m}$ for the fourth response of the TOF ($T4: T0$) vs value for the first response ($T1: T0$). There is a strong positive linear correlation ($r = +0.988$). ——— Regression line; ——— line of identity.
challenge the utility of the TOF ratio as a tool in the practice of anaesthesia. It does, however, show that two fully independent sites of action are not necessary to account for the pattern of depression of the TOF observed clinically. Depression of the TOF ratio is not related closely to the plasma atracurium concentration profile, but the depression of the fourth response appears to be mediated at a site which is not kinetically independent from that at which the depression of the first response occurs.

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REFERENCES