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Human Dose–Response Curves for Neuromuscular Blocking Drugs:

A Comparison of Two Methods of Construction and Analysis

John V. Donlon, Jr., M.D., John J. Savarese, M.D.,† Hasan H. Ali, M.D.,‡ Richard S. Teplik, M.D.§*

This study was done to demonstrate the validity of the log-probit method of analyzing dose–response data and to test the accuracy of the incremental dose method for generating dose–response data for pancuronium and *d*-tubocurarine. During balanced general anesthesia, cumulative dose–response data were obtained from ten patients each for pancuronium and *d*-tubocurarine. Dose–response data were also obtained using a single bolus injection method in 46 patients given pancuronium and 27 patients given *d*-tubocurarine. Evoked thumb adduction response was measured using an FT-10 force-displacement transducer and recorded on a Grass®-7 polygraph. Dose–response data were plotted on log-probit paper and analyzed by the Litchfield-Wilcoxon approximation method to determine mean ED_{50} and ED_{95} for each muscle relaxant. The dose–response data were also plotted on arithmetic scales and analyzed by the linear regression method to determine ED_{50} and ED_{95}. These results were compared with those obtained by use of the log-probit method.

The validity of the incremental dose method of obtaining dose–response curves was determined by comparison with the dose–response curves obtained using the conventional single bolus injection method. For each neuromuscular relaxant studied, the mean dose–response curve obtained by the cumulative dose method was not statistically significantly different from the bolus method dose–response curve. Comparing methods of analysis, the log-probit method yields results that are statistically not different from those obtained by use of conventional linear regression analysis.

The log-probit plot is a simple, accurate, appropriate approach for analyzing neuromuscular relaxant dose–response data. The log-probit curve yields results that are not statistically significantly different from those of the linear regression method, and is a better fit to the data at the clinically important extremes (85–99 per cent) of the response scale. For pancuronium and *d*-tubocurarine, the incremental dose method is efficient and accurate for generating dose–response curves. (Key words: Neuromuscular relaxants: pancuronium; *d*-tubocurarine. Pharmacology: dose–response curves. Statistics: linear regression; log-probit analysis.)

VALID clinical statistical comparisons among neuromuscular blocking drugs require the study of large numbers of patients when several drugs are to be evaluated at a variety of dosage levels and when the drugs are administered as a single bolus to each patient. Such a study may be accomplished with fewer subjects if an incremental method of drug administration is used, and if all the dose increments are given within a brief period (10–12 min) relative to the duration of action (45–60 min). Long-acting nondepolarizing neuromuscular blocking drugs are well suited to this type of study.

At the myoneural junction, a muscle action potential is propagated in an all-or-none fashion[1] whenever a motor end plate potential reaches threshold. The motor end plate population may be assumed to have a normal gaussian distribution of threshold sensitivity for propagating a muscle action potential. The twitch strength developed by an entire muscle reflects the sum effort of all muscle fiber units activated by propagated muscle action potentials. The degree of myoneural blockade caused by a neuromuscular blocking drug is related to the percentage of nonfunctioning motor end plates in a muscle. That is, the graded dose–response relationship observed for neuromuscular blockade is based on the sum of muscle fiber units activated by a muscle action potential. The number of all-or-none propagated action potentials depends in turn on how many motor end plate potentials reach threshold.

Thus, the dose–response relationship for neuro-

* Instructor in Anaesthesia, Harvard Medical School, and Assistant Clinical Director of Anesthesia, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

† Associate Professor of Anaesthesia, Harvard Medical School at Massachusetts General Hospital, and Associate Anesthetist, Massachusetts General Hospital, Boston, Massachusetts.

‡ Assistant Professor of Anaesthesia, Harvard Medical School at Massachusetts General Hospital, and Associate Anesthetist, Massachusetts General Hospital.

§ Instructor in Anaesthesia, Harvard Medical School at Massachusetts General Hospital, and Assistant Anesthetist, Massachusetts General Hospital.

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Address reprint requests to Dr. Donlon: Department of Anesthesia, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, Massachusetts 02114.

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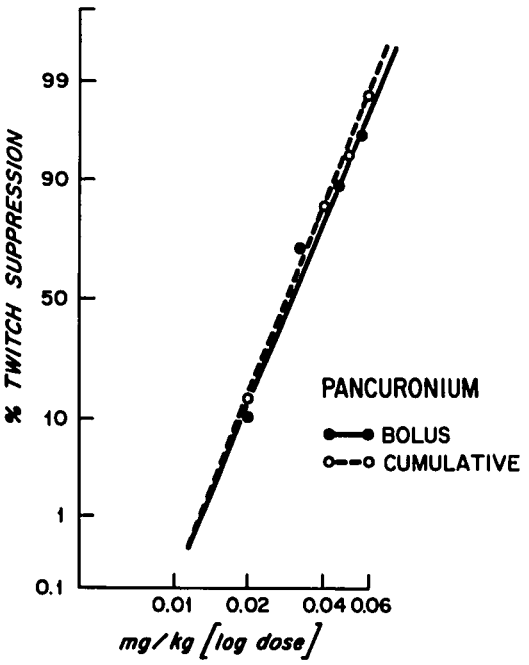

FIG. 1. Log-probit plot comparing cumulative and bolus dose–response curves for pancuronium. The cumulative curve (*open circles*) represents the mean of responses of ten patients (33 data points). The bolus curve (*closed circles*) required a total of 46 patients to determine the four mean data points represented. Notice the straight-line relationship of the dose–response data when plotted on these log-probit scales. The curves are best-fit approximations drawn to the data and analyzed by the Litchfield- Wilcoxon method. The curves are parallel and yield statistically similar ED_{50} and ED_{95} values (see text and table 1).

muscular blockade is based on an all-or-none phenomenon and may be plotted on semilog-normal (probit) scales. The straight-line relationship obtained by this type of plot simplifies comparison of dose–response data[2] and allows an accurate estimation of any effective dose (ED) from a curve constructed from only three or four data points. In addition, a more reliable estimate of points at the extremes of the response scales may be obtained, since the log-probit relationship does not deviate from linearity in these areas.

There were two purposes for this study: first, to demonstrate the efficiency and accuracy of using the log-probit method for analyzing dose–response data; second, to test the validity of constructing dose–response curves by an incremental, cumulative dose method of drug administration as compared with the standard single bolus injection technique.

Methods

Healthy, adult patients of either sex, 18 to 59 years of age, scheduled for elective gynecologic, orthopedic, or general surgical procedures were studied. Informed patient consent was obtained. Subjects were selected at random. In order to establish a somewhat consistent relationship between neuromuscular blocking drug dosage and muscle mass, individuals with markedly excessive adipose tissue were excluded from the study. All patients were free from neuromuscular and endocrine disease. Premedication consisted of morphine, 0.10–0.15 mg/kg, im, and scopolamine, 0.003 mg/kg, im. Anesthesia was induced with thiopental, 3–5 mg/kg, iv. Each patient then received nitrous oxide and oxygen (4:2 l/min mixture) by mask in a semiclosed system. Ventilation was assisted to maintain Pa_{CO_2} in the normal range. Supplemental morphine, 0.1–0.2 mg/kg, or thiopental, 2–4 mg/kg, was given intravenously to maintain general anesthesia during the study period.

The ulnar nerve was stimulated at the wrist through 22-gauge steel needle electrodes at 0.25 Hz with square-wave pulses of 0.2-msec duration. The stimuli were delivered at a supramaximal voltage by a Grass® S88 stimulator through an SIU5 isolation unit. Evoked force of thumb adduction was measured via a precalibrated Grass FT-10 force-displacement transducer and recorded on a Grass model 7 polygraph.

Cumulative dose–response curves for pancuronium and *d*-tubocurarine were determined for two groups of ten patients each. Incremental doses of either pancuronium or *d*-tubocurarine were administered intravenously to each individual until 95 ± 1 per cent block of the twitch response was obtained. Each drug increment was given after the effect of the previous dose had reached a stable response, no greater than a 2.5 per cent change in twitch height having been evident over the course of a minute. It was possible to complete a cumulative dose study, obtaining at least three data points for each patient, within 8–12 min. The mean data from the groups of

TABLE 1. Log-probit Curves for Pancuronium: Two Methods of Construction

	Pancuronium	
	Bolus	Cumulative
n	46	33
χ^2	0.876	0.432
Degrees of freedom	2	2
Slope	1.41 (1.16–1.70)*	1.35 (1.07–1.70)*
ED_{50} (mg/kg)	0.030 (0.024–0.037)*	0.030 (0.023–0.039)*
ED_{95} (mg/kg)	0.053 (0.036–0.076)*	0.050 (0.029–0.085)*

* 95 per cent confidence limits.

ten patients were plotted on log-probit paper, and an average dose–response curve for pancuronium or *d*-tubocurarine was obtained and tested for heterogeneity of data and goodness of fit by the method of Litchfield and Wilcoxon.[2] Heterogeneous data have wide random variations due to the inclusion of unrelated data points. Therefore, nonheterogeneity of data implies that a consistent relationship that can be described by a best-fit curve exists among the data. The individual dose responses were also plotted on arithmetic scales and fitted to a straight line by least-squares regression analysis. The results of these two methods of analysis were evaluated and compared statistically.

A separate series of patients was studied under similar circumstances except that each patient was given only one preselected bolus dose of either pancuronium or *d*-tubocurarine. Forty-six patients received pancuronium, and 27 received *d*-tubocurarine. At least four dose levels of each drug were studied. Mean data points were calculated, plotted on log-probit paper, and analyzed, as described above, by the method of Litchfield and Wilcoxon. The data obtained using the single bolus injection method were also plotted on arithmetic scales and fitted to a straight line by the least-squares regression method. The ED_{50} and ED_{95} were determined and compared with those determined by log-probit plot.

As a further check on the accuracy of the Litchfield-Wilcoxon approximation method, a Fortran program was written for an IBM 370/168 computer to calculate a log-probit regression line using the method of Finney.[3] The method of Finney is an iterative nonlinear least-squares technique for fitting a probit equation to percentile data. This log-probit regression line was tested for significance at 95 per cent confidence limits and compared with the log-probit dose–response curves estimated by the Litchfield-Wilcoxon method.

Finally, the accuracy of the incremental dose method for generating dose–response data for pancuronium and *d*-tubocurarine was evaluated by comparing the resultant dose–response curves with those generated by the conventional single-dose-per-patient approach. These curves were compared graphically as a log-probit plot, and analyzed statistically for parallelism, potency, and comparable ED_{50} and ED_{95} results.

TABLE 2. Linear Regression Curves for Pancuronium: Two Methods of Construction

	Pancuronium	
	Bolus	Cumulative
n	46	33
r	0.905	0.935
Slope	23.9	25.3
ED_{50} (mg/kg)	0.0326	0.0325
ED_{95} (mg/kg)	0.0516	0.0503
Y_{Int}	−0.28	−0.32

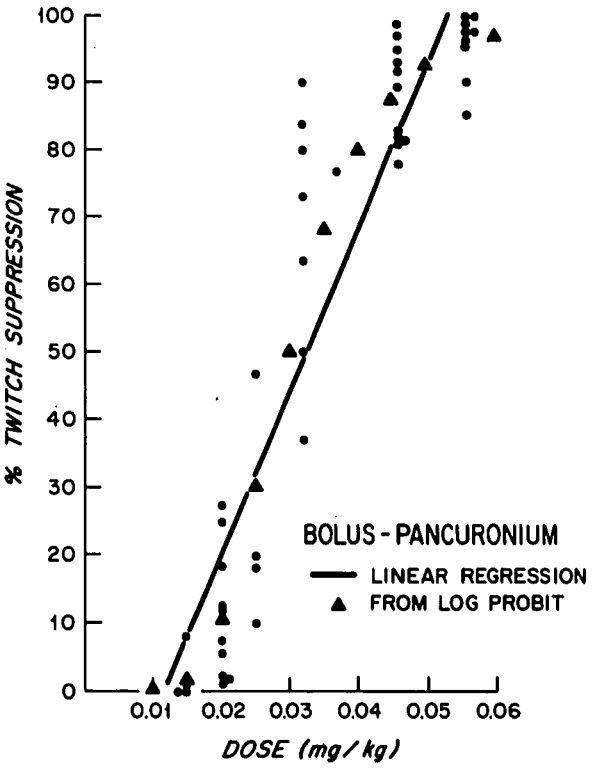

FIG. 2. Bolus pancuronium data (*solid dots*, n = 46) plotted arithmetically and analyzed by linear regression (*solid straight line*). The log-probit curve (*solid triangles*) from figure 1 has been superimposed for comparison. Notice that the straight-line log-probit relationship in figure 1 becomes a sigmoid-shaped curve when transposed to arithmetic scales. This sigmoid curve (*solid triangles*) represents the dose–response data more accurately than does the linear regression line. This is especially true at the extremes of the response range (0–10 and 90–99 per cent twitch suppression).

Results

PANCURONIUM

The incremental cumulative dose technique was used in ten patients to obtain 33 data points. Single bolus injections of pancuronium were given to 46 patients. The mean dose–response data obtained for pancuronium using these two methods (cumulative, bolus) are presented and compared in figure 1. These data are plotted on log-probit scale paper and have

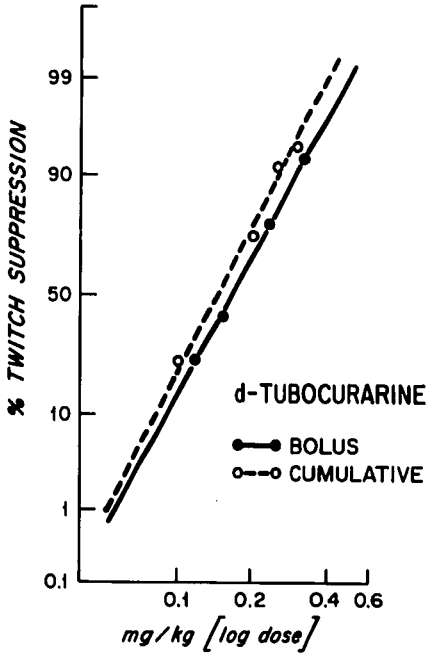

FIG. 3. Log-probit plot comparing cumulative and bolus dose–response curves for *d*-tubocurarine. The cumulative curve (*open circles*) represents the mean of responses of ten patients (34 data points). The bolus curve (*closed circles*) required a total of 27 patients to determine the four mean data points represented. As in figure 1, these straight-line curves are parallel and yield statistically similar values (see text and table 3).

best-fit curves drawn and analyzed by the Litchfield-Wilcoxon approximation method. A comparison of the log-probit dose–response curves shown in figure 1 is summarized in table 1. Analysis of both log-probit curves in figure 1 by the Litchfield-Wilcoxon method indicates that there is no significant heterogeneity ($\chi^2 \ll 5.99$) of data, and the goodness of curve fit to the data is significant ($P < 0.05$). These two curves compared by the Litchfield-Wilcoxon method do not deviate significantly from parallelism ($P < 0.05$). There is no statistically significant difference ($P < 0.05$) between ED_{50} values or between ED_{95} values as determined by log-probit analysis of either the cumulative or the bolus injection dose–response data.

Both the cumulative and the bolus injection dose–response data for pancuronium were also plotted arithmetically and analyzed by least-squares regression. The resulting linear regression lines are compared in table 2. These curves showed good data correlation (r = 0.935, 0.905) and produced values for ED_{50} (0.0325 mg/kg, 0.326 mg/kg) and ED_{95} (0.503 mg/kg, 0.0516 mg/kg) that were not significantly different ($P < 0.05$) from each other or from those determined by the log-probit plot method.

In figure 2, the log-probit curve for bolus injection of pancuronium has been transposed onto arithmetic scales and superimposed onto the dose–response data and linear regression line for bolus injection of pancuronium as a further comparison of how well each method of plotting approximates the dose–response data.

d-Tubocurarine

The study was repeated with *d*-tubocurarine and the results were not different from those obtained with pancuronium. The mean dose–response curves generated for *d*-tubocurarine by the two methods (cumulative, bolus) were comparable (fig. 3). Both log-probit analysis (table 3) and linear regression analysis (table 4) of the data indicated no significant difference between the results of the cumulative dose and bolus injection methods. The values for ED_{50} and ED_{95} determined by log-probit analysis (table 3) were not significantly different ($P < 0.05$) from those produced by linear regression analysis (table 4).

Computer Analysis

To evaluate the accuracy of the Litchfield-Wilcoxon approximation, a point-by-point log-probit analysis was made by computer of the data from both the bolus injection and the cumulative dose pancuronium studies. The results of these computer-calculated log-probit regression curves are summarized in table 5, and compared with the results of the Litchfield-Wilcoxon approximation technique.

Discussion

The results show that, under the given conditions, it is possible to use an incremental dose method to ob-

TABLE 3. Log-probit Curves for *d*-Tubocurarine: Two Methods of Construction

	d-Tubocurarine	
	Bolus	Cumulative
n	27	34
χ^2	0.93	0.55
Degrees of freedom	2	2
Slope	1.50 (1.04–2.20)*	1.56 (1.24–1.93)*
ED_{50} (mg/kg)	0.17 (0.12–0.23)*	0.14 (0.09–0.20)*
ED_{95} (mg/kg)	0.32 (0.15–0.66)*	0.29 (0.17–0.48)*

* 95 per cent confidence limits.

tain data and generate dose–response curves that are not statistically significantly different when compared with curves obtained using conventional single bolus injection methods. The validity of the cumulative dose method was demonstrated for two different neuromuscular blocking agents. The cumulative dose–response curve method is commonly used in pharmacology and has been used to study neuromuscular relaxants such as pancuronium, *d*-tubocurarine, metocurine, and gallamine.[4] It is an efficient, valid method of generating dose–response data without loss of accuracy. The cumulative dose method usually took 8–12 min to complete, and is therefore not appropriate for use to study short-acting drugs. The effect of the initial dose of the drug studied must remain stable throughout the 12-min study period for the technique to be valid.

The choice of log-probit paper to construct dose–response curves for neuromuscular blockade has been discussed elsewhere.[5] We[4–7] and several others[8,9] have used semilog-probit for semilog-logit[10] plots to describe dose–response relationships. Semilog-probit plots are validly used in pharmacology to describe dose–response relationships that are based on all-or-none events. For reasons outlined in the introduction, we believe that the clinically observed graded neuromuscular blockade response may be understood as a summation of all-or-none responses occurring in a population of motor end plates (muscle fibers) of varying sensitivities within a muscle. Thus, the log-probit plot should be a valid approach for analyzing the dose–response phenomenon of nondepolarizing muscle relaxants. This dose–response relationship can most effectively be represented as a cumulative frequency distribution, which, when plotted arithmetically, gives the familiar sigmoid curve.[11] This indicates a nonlinear relationship between dose and pharmacologic response. Stanski and Sheiner[12] suggest that a somewhat simplified approach would be to ignore the end asymptotes of the sigmoid curve and use only the linear 20–80 per cent response range, where linear regression can be used to relate log dose to response to obtain sensitivity estimates of ED_{50}. For some drugs, such as neuromuscular blocking drugs, however, the clinically important response is in the ED_{95} range. We have shown in figure 2 that using the log-probit method of analysis will more accurately represent the extremes of the dose–response relationship. The use of log-probit scales conveniently transforms the unwiedly sigmoid curve to an easily handled straight line. When the linear bolus pancuronium log-probit curve is transposed to arithmetic scales (fig. 2), it becomes a sigmoid-shaped curve and is seen to be an excellent fit to the data, especially at the extremes (1–15 per cent and 85–99 per cent) of the response scale.

Since clinical neuromuscular blockade may be viewed as reflecting an all-or-none event at the neuromuscular junction, log-probit analysis provides a simple, accurate method for evaluation of dose–response data. In particular, the simplified Litchfield-Wilcoxon method has been applied to log-probit-plotted data to calculate slope, ED_{50} and ED_{95} results with 95 per cent confidence limits. With this method, log-probit curves may also be compared statistically, as to slope, parallelism and potency ratios. Chi-square values may be obtained and used to determine heterogeneity of data and goodness of fit of each log-probit curve. The validity of the Litchfield-Wilcoxon approximation method of log-probit analysis was substantiated by comparison with a rigorous point-by-point computer-calculated log-probit regression. This

TABLE 4. Linear Regression Curves for *d*-Tubocurarine: Two Methods of Construction

	d-Tubocurarine	
	Bolus	Cumulative
n	27	34
r	0.890	0.865
Slope	3.52	2.86
ED_{50} (mg/kg)	0.192	0.137
ED_{95} (mg/kg)	0.320	0.293
Y_{int}	−0.17	−0.12

TABLE 5. Log-probit Analysis for Pancuronium: Comparison of Litchfield-Wilcoxon Approximation and a Computer Regression Analysis

	Litchfield-Wilcoxon Approximation	Computer Analysis
Bolus (n = 46)		
Slope	1.41 (1.16–1.70)*	1.51 (1.13–2.02)*
ED_{50} (mg/kg)	0.030 (0.024–0.037)*	0.029 (0.024–0.035)*
ED_{95} (mg/kg)	0.053 (0.036–0.076)*	0.057 (0.045–0.100)*
Cumulative (n = 33)		
Slope	1.35 (1.07–1.70)*	1.45 (1.05–2.00)*
ED_{50} (mg/kg)	0.030 (0.023–0.039)*	0.030 (0.022–0.036)*
ED_{95} (mg/kg)	0.050 (0.029–0.085)*	0.055 (0.043–0.100)*

* 95 per cent confidence limits.

computer analysis was performed after the results of the Litchfield-Wilcoxon method had been determined. The Litchfield-Wilcoxon method produced results that not only were within the 95 per cent confidence limits of the computer-calculated log-probit regression curve, but were virtually identical to the computer-generated results (table 5).

The log-probit analysis results also compare favorably with the results of conventional data analysis using the linear regression method of least squares.

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