Receptor simulation and the shape of the concentration–response curve and its congeners

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WE HAVE INVESTIGATED THE UTILITY OF curves used to represent concentration (or dose) plotted against response (or effect). We applied crude survival data from our laboratory to demonstrate that a known hyperbolic curve compares favourably with the conventionally used sigmoidal regression in robustness, while avoiding problems associated with the use of transforms.

Introduction

We consider the response of a biological system, in which the response (or effect) originates from a set of receptors for which the promoter or agonist is endogenous and the inhibitor or antagonist being studied is exogenous. Plotting response against antagonist concentration gives rise to graphs where the former might be, for instance, viability or survival or some clinical parameter such as blood pressure, any of them decreasing nonlinearly with increased dose or concentration.

Such curves may be approximated by equations so that a descriptive factor can be calculated, such as the EC_{50} (effective concentration for 50% maximal response). In general, it can be shown mathematically that artifacts are less likely the fewer the constants (or transformations) that are used in these regression equations. The *reductio ad absurdum* of ignoring this rule can be demonstrated by using the same number of constants as there are points in a set of data: the fit is perfect, the total variance is zero, but the values of the constants are without practical meaning.

While agonist concentrations may not remain static during experiments, and the preparation of the antagonist, especially if it is a crude plant extract, may contain agonists, partial agonists, more than one antagonist, promoters, antioxidants and so on, it ought still be safer to choose a simple general descriptive equation derived from relevant theory. Gaddum originated such an equation by combining the Law of Mass Action and the Clark hypothesis that relates the effect to the proportion of receptors occupied. He later recommended what has now become the convention, to plot response (between 0% and 100%) against the logarithm of the concentration of antagonist, giving rise to a sigmoidal (S-shaped) curve in reverse.[1] This is visually satisfying (compare two different ways of showing the same data set: Figs 1 and 2) but has led, in part, to the mythical concept of the 'threshold concentration' that is merely a distortion arising from logarithmically transforming the data. The EC_{50} and its attached statistical scatter is calculated using one of a number of semi-empirical methods, of which the best is probably still inspection with the naked eye.

This article presents a more rigorous approach prompted by the needs of students for a sound and robust computer program to fit data obtained by testing herbal extracts on malaria parasites grown *in vitro.*

For simplicity's sake, the equation relating response to $\log_{10}$ (concentration) will be referred to as the 'sigmoid'; the decaying hyperbola [Equation (1)] will be called the 'hyperbola'. Confidence limits is taken to mean 95% confidence limits of the corresponding EC_{50} value throughout.

Method

The first step taken was to write a simulation of the interaction between receptors, agonists and antagonists, molecule by molecule, and to examine the mean response arising from the former to varying concentrations of the latter, using the Clark hypothesis (effect is proportional to the proportion of receptors occupied by agonist molecules), the Avogadro number (for number of molecules in a given volume) and the Einstein-Smoluchowski equation (for root-mean-square distance travelled by molecules in thermal motion). Each receptor is treated independently. Account is taken of the random position, movement and orientation of active molecules in relation to the receptors; thus it was shown that the root-mean-square deviation in response is theoretically independent of concentration, that is, all concentration/effect data should be equally weighted. The program was used to generate 'ideal' data to identify candidate regression equations.

In the event, the Gaddum equation was found to fit data generated by the receptor simulation described. For antagonists, it can be expressed in the form:

$$E = k_1/(k_2 + [Z]) \qquad (1)$$

where E is the effect (however expressed) and $[Z]$ is the concentration of antagonist. This is a falling (decaying) hyperbola with k_1 and k_2 (the EC_{50}) as constants.

Results

Note that Equation (1) is nonparametric and must be solved by repeated iteration until the variance is minimized. The seemingly more obvious approach, to linearize it by conversion to

$$1/E = k_1/k_2 + [Z]/k_2, \qquad (2)$$

then regressing $1/E$ on $[Z]$ for data sets A and D in Table 1, gave rise to EC_{50} values (133 and 45 mg/l, respectively) that were unrealistically high. This is a consequence of distorting the relative weighting of the points by using an hyperbolic transformation.

As stated, the main objective of this study was to produce a robust regression

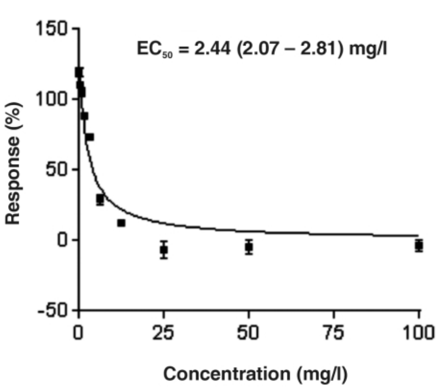

Fig. 1. Hyperbolic regression.

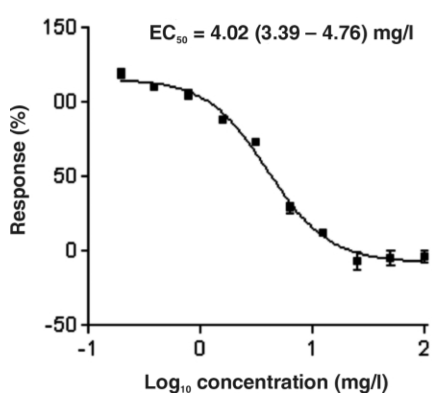

Fig. 2. Sigmoidal regression.

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Table 1. Comparison of fit of concentration-effect data fitted to a hyperbola (hyp) with the same data fitted to a sigmoid (sig).

Data set	Curve form	Mean EC_{50} (mg/l)	95% confidence limits
A	Hyp	72	31–113
	Sig*	58	24–164
B	Hyp	20	12–29
	Sig	20	11–37
C	Hyp	9	5–14
	Sig	10	5–18
D	Hyp	6	3–10
	Sig	7	5–9

*'Sigmoid' incomplete.

equation, solidly founded in theory and providing plausible EC_{50} values with their 95% confidence limits. To this end, 45 sets of crude data were selected from measurements of viability taken using visible light absorptiometry on the conventional 96-well plate used to screen herbal preparations for antimalarial activity. The statistical scatter within each data set was often high. The sets were selected consecutively from a laboratory notebook except that the test sets summarized in Table 1 were included. Each set of data was regressed upon the equations for the 'hyperbola' and for the 'sigmoid' using computer programs minimizing the unweighted total sum-of-squares difference between the points actually measured and those derived by calculation. Plausibility of EC_{50} values is difficult to identify except by visual inspection of the crude data. To be more precise, one can exclude all sets for which no EC_{50} values and/or their confidence limits can be calculated. A confidence range overlapping zero would be included in this class as it implies that there is a chance that the EC_{50} does not exist. A further refinement is to exclude, arbitrarily but realistically, sets for which the EC_{50} values are insufficiently precise because their upper confidence limits are more than twice as large. An example would be the 'sigmoid' regression for data set A in Table 1.

Three sets of data could not be fitted at all, that is, no EC_{50} values nor confidence limits could be calculated using either equation. For the 'hyperbola', the EC_{50} could be calculated for a further seven sets but the confidence limits overlapped zero. For the remaining sets, upper confidence limits were all satisfactorily small. EC_{50} values plausible by inspection and their confidence limits could therefore be calculated for 35/45 sets (78%). For the 'sigmoid', a further three sets could not be fitted at all. EC_{50} values but not their confidence limits could be calculated for 10 sets. For five sets, both EC_{50} and confidence limits could be calculated but the upper confidence limit was too large. Adequate means and confidence limits, then, could only be calculated for 24/45 sets (53%). These results do not apply universally but it seems clear that the regression of concentration-response observations on to the decaying hyperbola of Equation (1) is robust. Observation suggests that confidence limits (where acceptable) are similar for both curves.

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1. Burgen A.S.V. and Mitchell J.F. (eds) (1985). In *Gaddum's Pharmacology*, 9th edn, chap. 1, p. 5. Oxford Medical Publications, Oxford.