Influence of Dose Range on Degree of Nonlinearity Detected in Dose-Proportionality Studies for Drugs with Saturable Elimination: Single-Dose and Steady-State Studies

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Deviation from proportionality occurs when the ratio of area under the curve (AUC) values is not equal to the ratio of administered doses. The degree of nonlinearity (f_{NL}) can be quantitated as the ratio of AUCs divided by the ratio of doses. We explore positive deviation from proportionality ($f_{NL} > 1$) using the classical Michaelis–Menten model of nonlinear elimination after a single dose ($n = 1$) or at steady state (ss). The degree of nonlinearity is related to the ratio of the highest dose to the lowest dose ($Rd = D_H/D_L$): $f_{NL}^{n=1} = (2 + Rd \cdot \epsilon)/(2 + \epsilon)$, $f_{NL}^{ss} = (Rd \cdot \Omega - 1)/(Rd \cdot \Omega - Rd)$, where ϵ is the ratio of the initial concentration after the lowest dose to the K_m ($\epsilon = D_L/K_m \cdot V$) and Ω is the ratio of the V_{max} to the average rate of input for the highest dose ($\Omega = V_{max}\tau/F \cdot D_H$). From these relationships, we find that (1) for single-dose studies, K_m is the important Michaelis–Menten parameter, while V_{max} is important at steady state; (2) the degree of nonlinearity cannot exceed the ratio of doses in single-dose studies, and when doses in extreme excess of $K_m \cdot V$ are chosen, the degree of nonlinearity is equal to the dose range; and (3) at steady state, the degree of nonlinearity *can* exceed the ratio of doses and approaches infinity as the average input rate approaches V_{max}. Literature data (phenytoin and ethanol) support these findings. We conclude that the degree of nonlinearity is not a useful measure of nonlinearity in and of itself and propose percentage saturation as being more informative.

KEY WORDS: dose proportionality; Michaelis–Menten; nonlinearity; data analysis; pharmacokinetics.

INTRODUCTION

The purpose of this paper is to draw attention to the relationship between the dose range chosen in dose-proportionality studies and the degree of nonlinearity (i.e., deviation from proportionality) detected. For single-dose studies, deviation from proportionality occurs when the ratio of area under the curve (AUC) values is not equal to the ratio of administered doses. Thus, the degree of nonlinearity ($f_{NL}^{n=1}$) can be quantitated as the ratio of AUCs divided by the ratio of doses:

$$f_{NL}^{n=1} = \frac{AUC_H}{AUC_L} \cdot \frac{D_L}{D_H} \quad (1)$$

where the subscripts H and L refer to the highest and lowest doses, respectively. For example, if a twofold increase in dose results in a fourfold increase in AUC, the degree of nonlinearity is two. For steady-state studies, the average steady-state concentration (C_{ss}) is of primary interest. Similar to the single-dose case, the degree of nonlinearity (f_{NL}^{ss}) can be quantitated as the ratio of C_{ss} values divided by the ratio of dose rates:

$$f_{NL}^{ss} = \frac{C_{ss_H}}{C_{ss_L}} \cdot \frac{D_L/\tau}{D_H/\tau} \quad (2)$$

where τ is the dosing interval. In order to interpret such numbers (i.e., whether large or small), it is necessary to understand how the deviation from proportionality (i.e., $f_{NL} \neq 1$) relates to the underlying kinetic processes and study design. As a first step toward understanding this relationship, we explore positive deviation from proportionality ($f_{NL} > 1$), using the classical Michaelis–Menten model of nonlinear elimination for a drug administered by intravenous bolus (or rapidly absorbed following extravascular dosing in the single dose case) as a single dose or following multiple doses at regular intervals (τ) to steady state. We derive equations for $f_{NL}^{n=1}$ and f_{NL}^{ss} involving the Michaelis–Menten parameters and simulate the influence of these parameters and dose range on the degree of nonlinearity. Finally, we support our theoretical findings with two examples of compounds known to be eliminated by capacity-limited processes.

THEORETICAL

Derivation of $f_{NL}^{n=1}$. The single-dose equation is derived using the classical Michaelis–Menten expression for area under the plasma concentration–time curve (AUC) after intravenous bolus administration or rapid absorption following extravascular dosing (1):[4]

$$AUC = \frac{F \cdot D}{V_{max}}\left(K_m + \frac{F \cdot D}{2V}\right) \quad (3)$$

where F is the fraction of an orally administered dose that reaches the systemic circulation ($F = 1$ for an iv dose), D is the dose, V is the volume of distribution, V_{max} is the maximum rate of elimination, and K_m, the Michaelis constant, is the plasma concentration needed for an elimination rate equal to one-half of V_{max}. Substituting the expression for AUC [Eq. (3)] into that for $f_{NL}^{n=1}$ [Eq. (1)] after iv dosing (or after oral dosing when F is not affected by the nonlinearity):

$$f_{NL}^{n=1} = \frac{2V \cdot K_m + F \cdot D_H}{2V \cdot K_m + F \cdot D_L} \quad (4)$$

Note that the degree of nonlinearity is independent of V_{max}. K_m is the only Michaelis parameter of importance in the single-dose case.

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[4] Equation differs from the original expression in that $C(0)$ has been replaced by FD/V and V_m has been replaced by V_{max}/V, so that V_{max} is the maximum rate of elimination rather than the maximum rate of change of plasma concentrations (V_m).

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If we define two new terms, the ratio of doses ($Rd = D_H/D_L$, $Rd > 1$) and the ratio of the initial concentration after administration of the lowest dose to K_m [$\epsilon = F \cdot D_L/(V \cdot K_m)$, $0 < \epsilon < \infty$], then we can write an equation for the degree of nonlinearity that depends only on these two ratios, and not the actual value of K_m and the doses used:

$$f_{NL}^{n=1} = \frac{2 + Rd \cdot \epsilon}{2 + \epsilon} \quad (5)$$

Derivation of f_{NL}^{ss}. Similarly, the steady-state equation for f_{NL}^{ss} is derived using the classical Michaelis–Menten relationship between average input rate ($F \cdot$ Dose/τ) and average steady-state concentration (2):

$$\frac{F \cdot D}{\tau} = \frac{V_{max} \cdot C_{ss}}{K_m + C_{ss}} \quad (6)$$

An equation for f_{NL}^{ss} is derived by solving the expression above [Eq. (6)] for C_{ss} and substituting the resultant equation into that for f_{NL}^{ss} [Eq. (2)]:

$$f_{NL}^{ss} = \left(\frac{F \cdot D_L}{\tau} - V_{max}\right) \Big/ \left(\frac{F \cdot D_H}{\tau} - V_{max}\right) \quad (7)$$

Note that the degree of nonlinearity is independent of K_m; V_{max} is the only Michaelis parameter of importance in the steady-state case.

If we use the previous definition for the ratio of doses (actually dose rates in this case) and define a new term, the ratio of V_{max} to the average input rate for the highest dose [$\Omega = V_{max} \cdot \tau/F \cdot D_H$, $1 < \Omega < \infty$], then we can write an equation for the degree of nonlinearity that depends only on these two ratios, and not on the actual value of V_{max} and the dose rates used:

$$f_{NL}^{ss} = \frac{Rd \cdot \Omega - 1}{Rd \cdot \Omega - Rd} \quad (8)$$

Note that the lower limit of Ω is unity, since the expression for C_{ss} [Eq. (6)] is valid only when input rates are less than the maximum rate of elimination (V_{max}). As Ω approaches infinity, the dose rates are infinitely small in comparison to V_{max}.

RESULTS AND DISCUSSION

Properties of $f_{NL}^{n=1}$. For single-dose studies, the degree of nonlinearity is dependent only on the dose range used and where the initial concentration for the lowest dose ($F \cdot D_L/V$) lies in relation to the K_m value; it is independent of V_{max}. Using l'Hôpital's rule to evaluate Eq. (5), the limit of $f_{NL}^{n=1}$ as ϵ approaches infinity is

$$\lim_{\epsilon \to \infty} f_{NL}^{n=1} = Rd \quad (9)$$

That is, for drugs eliminated by a single Michaelis–Menten pathway, (i) the degree of nonlinearity cannot exceed the ratio of doses (i.e., $1 \leq f_{NL}^{n=1} \leq Rd$); and (ii) when doses in extreme excess of $K_m \cdot V/F$ are chosen, the degree of nonlinearity is equal to the dose range. This trend also becomes apparent in simulations of $f_{NL}^{n=1}$ versus ϵ (logarithmic scale) for various values of the dose range (Fig. 1). When ϵ is small (i.e., concentrations much smaller than K_m), the degree of nonlinearity is 1 (i.e., no deviation from linearity). As ϵ increases (i.e., concentrations approach and exceed K_m), the degree of nonlinearity increases until reaching its maximum value of Rd, which occurs approximately when the initial concentration for the lowest dose exceeds $100 \cdot K_m$. For a given drug at a particular lowest dose (i.e., constant ϵ), the relationship between degree of nonlinearity and dose range is shown in Fig. 2A. The degree of nonlinearity increases linearly as the dose range increases, with a slope of $\epsilon/(2 + \epsilon)$ [from Eq. (5)]. For large values of ϵ, the slope approaches 1. Single-dose data for ethanol (large ϵ) and a phosphate ester prodrug of phenytoin (intermediate ϵ), which have been shown to obey Michaelis–Menten elimination kinetics, illustrate these principles.

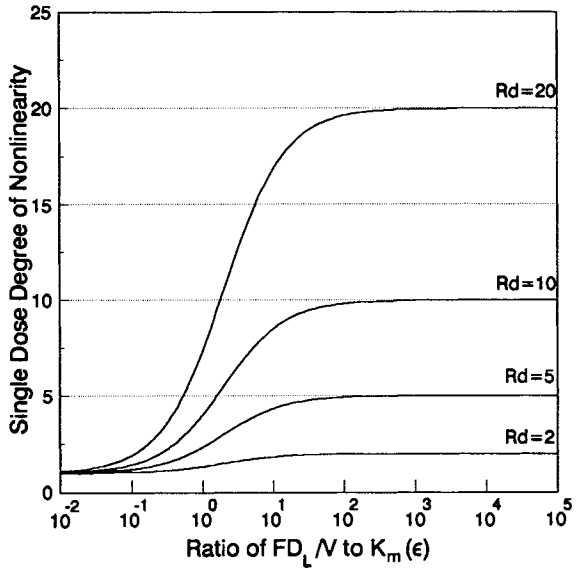

Fig. 1. Relationship between single-dose degree of nonlinearity and ϵ (ratio of initial concentration at lowest dose and K_m) for four values of the dose range, Rd = 2, 5, 10, and 20.

Rangno *et al.* (3) studied the absorption, distribution, and elimination of several relatively large oral and intravenous doses of ethanol. Eight normal volunteers each received three intravenous doses (0.375, 0.5, and 0.75 g/kg) of ethanol at a constant rate over 30 min. The resultant median AUC values were 1.10, 2.13, and 4.55 g · hr/L. When the authors analyzed the concentration–time data using a two-compartment Michaelis–Menten elimination model, they derived a median K_m value of 0.03 g/L. This is approximately 30 times the peak concentration observed after administration of the lowest iv dose (i.e., ϵ is approximately 30 and $f_{NL}^{n=1}$ should approach Rd). Using 0.5 g/kg as the highest dose, Rd = 1.33 and $f_{NL}^{n=1}$ = 1.45. Using 0.75 g/kg as the highest dose, Rd = 2 and $f_{NL}^{n=1}$ = 2.07. Thus, $f_{NL}^{n=1}$ is approximately equal to Rd. These data are shown in Fig. 2B for comparison to the theoretical line generated from Eq. (5) for an ϵ value of 30. It is apparent that ethanol behaves as predicted for a drug with a high ϵ value (i.e., $f_{NL}^{n=1} \approx Rd$). The fact that $f_{NL}^{n=1}$ slightly exceeds Rd may be an artifact of using median data with large interindividual variability.

Gerber *et al.* (4) studied the disposition of phenytoin after intravenous administration of a phosphate ester of phenytoin over 30 min to four groups of normal volunteers at

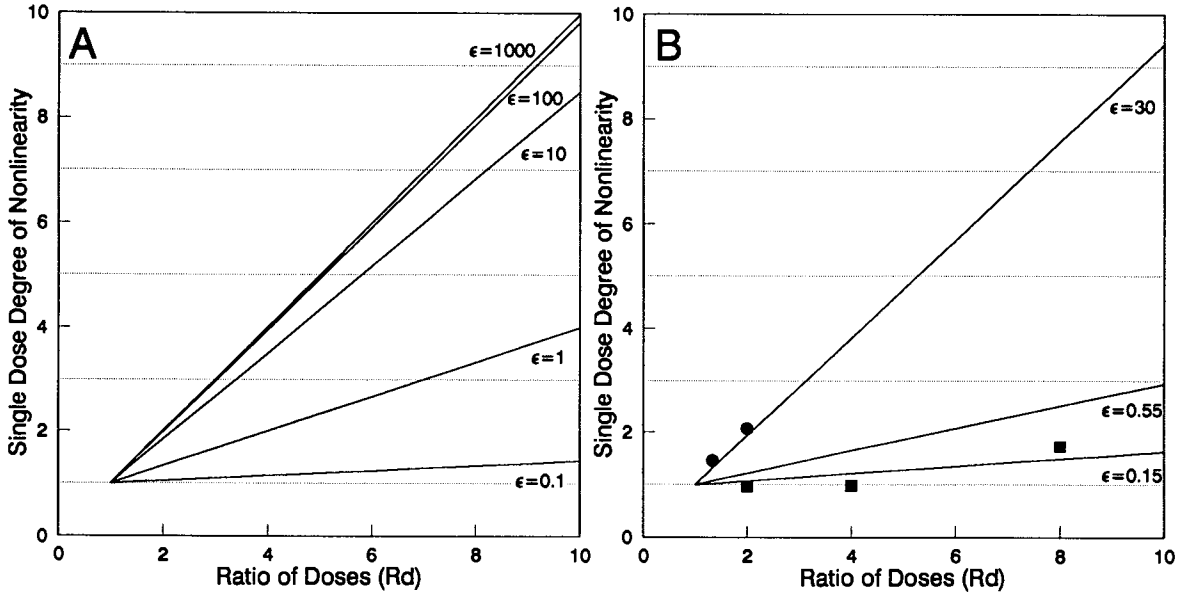

Fig. 2. Relationship between single-dose degree of nonlinearity and ratio of doses (*Rd*) for five values of ϵ (ratio of initial concentration at lowest dose and K_m), 0.1, 1, 10, 100, and 1000 (A). Data for ethanol (•) and phenytoin (■) are shown with the theoretical lines corresponding to their calculated ϵ values (B); ϵ = 30 for ethanol and ϵ = 0.55 for phenytoin. For reference, a line that better describes the phenytoin data (ϵ = 0.15) is also shown.

doses of 150, 300, 600, and 1200 mg. The ester is rapidly and completely hydrolyzed to phenytoin ($t_{1/2}$ = 8 min). The resultant mean phenytoin AUC values were 35, 67, 136, and 481 mg · hr/L. Literature data suggest that K_m ranges from 1 to 15 μg/mL, with an average of approximately 4 μg/mL in epileptic patients (2). Using this literature K_m value and the mean peak phenytoin concentration of 2.2 μg/mL observed after the lowest dose, the estimated ϵ value is 0.55. Thus, the degree of nonlinearity should be much less than the ratio of doses and should increase as the dose range increases. Using 300, 600, and 1200 mg in turn as the highest doses, $f_{NL}{}^{n=1}$ values of 0.96, 0.97, and 1.72 result for *Rd* values of 2, 4, and 8, respectively. These data are shown in Fig. 2B for comparison to the theoretical line generated from Eq. (5) for an ϵ value of 0.55. It is apparent that phenytoin behaves as predicted for a drug with an intermediate ϵ value (i.e., $f_{NL}{}^{n=1} < Rd$). The theoretical line for ϵ = 0.55 does not, however, adequately describe the relationship between degree of nonlinearity and dose range. This is probably due to the uncertainty in estimation of ϵ, since no K_m values were available from the study that reported the AUC values used here. A line generated for ϵ = 0.15 appears to describe the phenytoin data more accurately (Fig. 2B).

These two examples can be used to illustrate the importance of considering dose range when interpreting degree of nonlinearity detected in single-dose studies. The degree of enzyme saturation has been put forward as a number that quantifies the relationship between the (initial) concentration achieved with a particular dose and the Michaelis constant, K_m (5). This number has been calculated for the two ethanol doses that resulted in an $f_{NL}{}^{n=1}$ value of 1.45 and the two phenytoin doses that resulted in an $f_{NL}{}^{n=1}$ value of 1.72 (Table I). The enzyme responsible for metabolism of ethanol is almost completely saturated (both doses), while that for metabolism of phenytoin is between 35 and 81% saturated, even though the degree of nonlinearity observed was higher for phenytoin than for ethanol. This is solely an artifact of the different dose ranges used (1.33-fold for ethanol and 8-fold for phenytoin) and points out the flaw in interpreting $f_{NL}{}^{n=1}$ values across compounds where different dose ranges were used.

Properties of $f_{NL}{}^{ss}$. For steady-state studies, the degree of nonlinearity is dependent only on the range of dose rates used and where the maximum rate of elimination (V_{max}) lies in relation to the average rate of input for the highest dose ($F \cdot D_H/\tau$); it is independent of K_m. The relationship between the degree of nonlinearity at steady state and the range of dose rates employed is shown in Fig. 3. When the average input rate for the highest dose is much smaller than V_{max} (i.e., Ω is large), the degree of nonlinearity approaches unity (i.e., C_{ss} is proportional to dose rate). As the average input rate for the highest dose approaches V_{max} (i.e., Ω approaches unity), the degree of nonlinearity approaches infinity. That is, during steady-state studies for drugs eliminated by a single Michaelis–Menten pathway, the degree of nonlinearity may approach infinity as the average input rate ap-

Table I. Calculated Degree of Enzyme Saturation for Ethanol and Phenytoin (Following Phosphate Ester Prodrug Administration) over a Range of Doses and the Corresponding Degree of Nonlinearity Observed[a]

Compound	Dose	$f_{NL}{}^{n=1}$	% saturation[b]
Ethanol	0.375 mg/kg	1.45	97
	0.5 mg/kg		98
Phenytoin	250 mg	1.72	35
	1200 mg		81

[a] Ethanol data found in Ref. 3; phenytoin data found in Ref. 4.
[b] Percentage saturation is defined as 100 · $C(0)/[K_m + C(0)]$ (5).

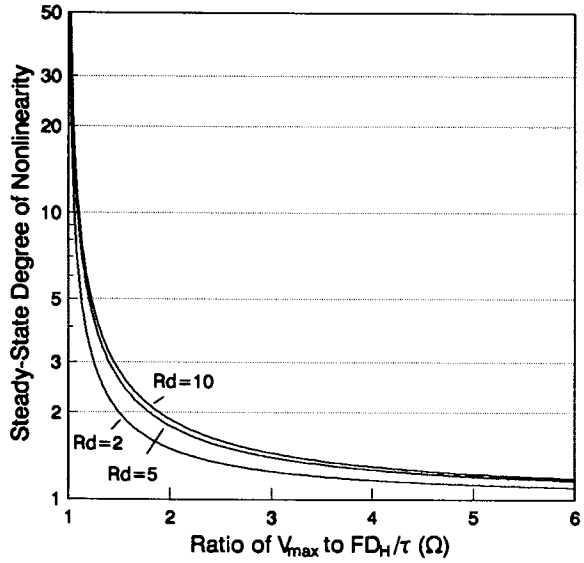

Fig. 3. Relationship between steady-state degree of nonlinearity and Ω (ratio of V_{max} to average input rate for highest dose, $F \cdot D_H/\tau$) for three values of the dose range, Rd = 2, 5, and 10.

proaches the maximum rate of elimination. This is in contrast to the single-dose case, where the degree of nonlinearity is limited by the ratio of doses. This trend is also apparent if the limit of f_{NL}^{ss} [Eq. (8)] as Ω approaches unity is evaluated using l'Hôpital's rule:

$$\lim_{\Omega \to 1} f_{NL}^{ss} = \infty \tag{10}$$

For a given drug at a particular highest dose rate (i.e., constant Ω), the relationship between degree of nonlinearity and range of dose rates employed is shown in Fig. 4. The degree of nonlinearity increases as the ratio of dose rates increases until a plateau is reached. Once the ratio of dose rates is

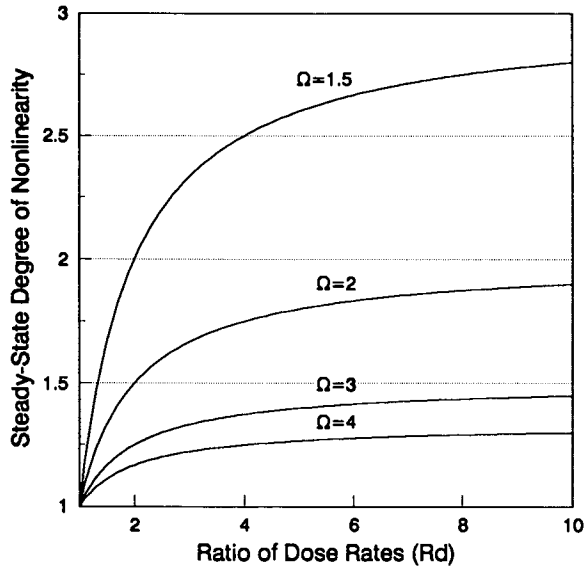

Fig. 4. Relationship between steady-state degree of nonlinearity and ratio of dose rates (Rd) for four values of Ω (ratio of V_{max} to average input rate for highest dose, $F \cdot D_H/\tau$), 1.5, 2, 3, and 4.

within the plateau range and a highest dose rate has been chosen, widening the range of dose rates used (i.e., reducing the lowest dose rate) has no effect on the degree of nonlinearity detected. The plateau is achieved earlier (i.e., at lower values for the ratio of dose rates) when the average input rate for the highest dose is much smaller than V_{max} (i.e., when Ω is large). The plateau value is largest for the average input rate closest to V_{max} (i.e., smallest value of Ω). The plateau value is obtained from the following limit:

$$\lim_{Rd \to \infty} f_{NL}^{ss} = \frac{\Omega}{\Omega - 1} \tag{11}$$

For example, for Ω = 1.5 and Ω = 5, the plateau degrees of nonlinearity are 3 and 1.25, respectively. Multiple-dose data for phenytoin illustrate these principles.

The relationship between daily phenytoin dose (50 to 600 mg) and steady-state plasma concentration was documented in epileptic patients requiring several different dose levels (three to six) to achieve concentrations within the therapeutic range (6). Table II contains the f_{NL}^{ss} and Rd estimates for these data. Notice that in every case f_{NL}^{ss} exceeds Rd. This is in contrast to the trend for $f_{NL}^{n=1}$ values derived from single-dose phenytoin data, where the degree of nonlinearity was always less than Rd.

Percentage saturation, calculated from concentration and K_m, has been proposed as an informative parameter to quantitate nonlinearity. Although appropriate for the single-dose case, the multiple-dose case is better served by a parameter that expresses the relationship between the average input rate ($F \cdot D/\tau$) and the maximum rate of elimination. In a sense, this is also an index of saturation, and we use the term "steady-state percentage saturation." It is inversely related to Ω, calculated as 100/Ω for the highest dose, $100 \cdot Rd/\Omega$ for the lowest dose, and simply $100 \cdot F \cdot D/(\tau \cdot V_{max})$ for any dose in between.

Estimates of Ω for the phenytoin data derived from a rearrangement of Eq. (8) [$\Omega = (f_{NL}^{ss} \cdot Rd - 1)/(f_{NL}^{ss} \cdot Rd - Rd)$] as well as the steady-state percentage saturation at the highest daily dose are also listed in Table II. Values of Ω range from 1.1 to 1.2. Values of Ω close to unity occur as the average input rate for the highest dose rate approaches V_{max}. The corresponding values for the steady-state percentage saturation for the highest daily dose range from 87 to 95%.

Table II. Calculated Values for Ω[a] and Steady-State Degree of Saturation for Phenytoin[b] over a Range of Daily Doses and the Corresponding Degree of Nonlinearity Observed

Subject	D_H/τ (mg/day)	f_{NL}^{ss}	Rd	Ω	% saturation[c]
A	150	5.75	3	1.14	88
B	150	7.33	3	1.11	90
C	300	4.14	1.5	1.11	90
D	450	4.56	2.25	1.16	87
E	600	7.21	1.5	1.05	95

[a] Ratio of $F \cdot D_H/\tau$ to V_{max}.
[b] Phenytoin data found in Ref. 6.
[c] Percentage saturation is defined as $100 \cdot F \cdot D/(\tau \cdot V_{max})$ and calculated for the highest daily dose from Ω (% saturation = 100/Ω).

That is, the highest daily doses used in this study resulted in average input rates within 87 to 95% of V_{max}.

A possible pitfall in interpretation of f_{NL}^{ss} values can be illustrated using data for subjects B and C. The degrees of nonlinearity are 7.33 for subject B and 4.14 for subject C (Table II). Considering only the degree of nonlinearity, it might be concluded that the phenytoin elimination pathway for subject B was closer to saturation than that for subject C. In actuality, the two subjects are at the same level of saturation at their (different) highest dose rates. The different f_{NL}^{ss} values are solely an artifact of the different range of dose rates used for the two subjects (3-fold for subject B and 1.5-fold for subject C). Thus, f_{NL}^{ss} values (as is true of $f_{NL}^{n=1}$ values) cannot be interpreted without considering the range of dose rates employed in the study.

In summary, the degree of nonlinearity is not an informative measure of nonlinearity in and of itself, as it is dependent not only on the initial concentration achieved after the lowest dose in relation to K_m (or the average rate of input for the highest dose in relation to V_{max}), but also on the dose range (or range of dose rates) employed in the study. Thus, comparisons of the degree of nonlinearity for two different compounds are valid only if the same dose range (or range of dose rates) is used. A more informative parameter for drugs eliminated by a single Michaelis–Menten pathway is percentage saturation (either single dose or steady state).

These findings also have implications for the design of dose-proportionality studies, where intra- and interindividual variation must be considered. First, there may be no point in doing a single-dose dose-proportionality study if the drug is to be administered chronically. The degrees of nonlinearity are likely to bear no resemblance to one another, since one is determined by K_m and the other by V_{max}. Second, there may be no point in doing a single-dose dose-proportionality study with a dose range that is less than the degree of nonlinearity deemed important to detect, as the power to detect this degree of nonlinearity will be (infinitely) small. Third, it should be possible from the relationships developed in this paper to construct guidelines for the dose ratio needed in order to detect the degree of nonlinearity deemed important in single-dose studies when the intra- and interindividual variation is known. This is not as relevant for steady-state studies, since the degree of nonlinearity is less dependent on the range of dose rates. Model dependence of these conclusions (i.e., whether they can be generalized to other types of pharmacokinetic nonlinearity and other modes of input) should be explored in future theoretical studies before application to constructing definitive guidelines for the design of dose-proportionality studies.

REFERENCES

1. M. Gibaldi and D. Perrier. *Pharmacokinetics,* 2nd ed., Marcel Dekker, New York, 1982, p. 294.
2. M. E. Winter and T. N. Tozer. Phenytoin. In W. E. Evans, J. J. Schentag, and W. J. Jusko (eds.), *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring,* 2nd ed., Applied Therapeutics, Spokane, WA, 1986, pp. 493–539.
3. R. E. Rangno, J. H. Kreeft, and D. S. Sitar. Ethanol "dose-dependent" elimination: Michaelis-Menten v classical kinetic analysis. *Br. J. Clin. Pharmacol.* 12:667–673 (1981).
4. N. Gerber, D. C. Mays, K. H. Donn, A. Laddu, R. M. Guthrie, P. Turlapaty, C. Y. Quon, and W. K. Rivenburg. Safety, tolerance and pharmacokinetics of intravenous doses of the phosphate ester of 3-hydroxymethyl-5,5-diphenylhydantoin: A new prodrug of phenytoin. *Clin. Pharmacol.* 28:1023–1032 (1988).
5. N. Gerber and J. G. Wagner. Explanation of the dose-dependent decline of diphenylhydantoin plasma levels by fitting to the integrated form of the Michaelis-Menten equation. *Res. Commun. Chem. Pathol. Pharmacol.* 3:455–466 (1972).
6. A. Richens and A. Dunlop. Serum-phenytoin levels in management of epilepsy. *Lancet* 2:247–248 (1975).