Method of Estimating Relative Absorption of a Drug in a Series of Clinical Studies in Which Blood Levels Are Measured After Single and/or Multiple Doses

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Previously published methods of estimating relative absorption of a drug are primarily applicable to crossover studies in which a panel of subjects is administered one or more test formulations and a readily available form of the drug. The method described provides estimates of average relative absorption of a drug administered to different panels of subjects in a series of clinical trials in which blood levels are measured after either single and/or multiple doses of the drug. The method incorporates corrections for differences in dosage, body weight or surface area, and intra- and inter-subject variation in half-life of the drug. The method is illustrated by results of calculations made from 167 sets of serum concentrations of tetracycline hydrochloride activity including 112 sets obtained following administration of tetracycline to children and 55 sets obtained following administration of tetracycline to adults.

WAGNER AND Nelson (1) summarized seven methods of estimating per cent availability. These methods are primarily applicable to a crossover study in which a panel of subjects are administered one or more test formulations and a readily available form of the drug. Frequently, however, one may wish to compare the relative absorption of a drug in a series of clinical trials performed with different panels of subjects at different times. In such a case one must make corrections for differences in dosage, body weight, and intra- and inter-subject variations in half-life of the drug. The method to be described allows such corrections to be made and also allows comparison of blood levels observed after a single dose and during a dosage interval at the equilibrium state after multiple doses of drug administered at uniform time intervals.

EXPERIMENTAL

Following single doses of drug the basic material balance equation[1] as given by Wagner *et al.* (2) is:

$$F \cdot D = V \cdot K \cdot \int_0^\infty C(t)\,dt \qquad \text{(Eq. 1)}$$

where F is the fraction of the dose which is absorbed, D is the dose, V is the apparent volume of distribution of the drug, K is the first-order rate constant for over-all loss of drug from the body, and the integral represents the area under the blood (serum or plasma) concentration curve from zero to infinite time.

Substitution of $0.693/t^1/_2$ for K, where $t^1/_2$ is the half-life, and rearrangement gives

$$\frac{\int_0^\infty C(t)dt}{D \cdot t^1/_2} = \frac{F}{0.693V} \qquad \text{(Eq. 2)}$$

If $C(t)$ is expressed in micrograms per milliliter, D in milligrams per kilogram of body weight, t and $t^1/_2$ is similar units, then V will be in units of liters per kilogram of body weight which is equivalent to fraction of body weight, and F is dimensionless. The dose, D, could also be expressed in milligrams per square meter of body surface area in which case V will be in units of liters per square meter of body surface area.

Similarly, following multiple doses of a drug one can derive (2) the relationship

$$\frac{\int_{t_1}^{t_2} C_\infty(t)}{D \cdot t^1/_2} = \frac{F}{0.693V} \qquad \text{(Eq. 3)}$$

where the integral represents the area under the blood (serum or plasma) concentration curve during a dosage interval at the steady state after multiple doses of drug given at uniform time intervals, τ, such that $\tau = t_2 - t_1$, and F is the fraction of each dose which is absorbed; the other symbols have the same meanings as indicated above.

The half-lives, $t^1/_2$, may be estimated in the usual manner from the terminal $C(t)$, t values after a single dose of drug or following the last dose of a series of doses given at uniform time intervals; in the latter case, it may or may not be necessary to include values beyond the end of the last dosage interval. The infinite area of Eq. 2 may be estimated from the equation

$$\int_0^\infty C(t)dt = \int_0^T C(t) + \frac{\hat{C}_T}{K} \qquad \text{(Eq. 4)}$$

where $\hat{C}_T$ is the estimated serum concentration at the last sampling time, T, following the single dose of drug, the integral represents the area under the blood (serum or plasma) concentration curve from zero time to time T, and K is defined in Eq. 1. The integrals $\int_0^T C(t)dt$ and $\int_{t_1}^{t_2} C_\infty(t)dt$ may be estimated by trapezoidal rule from observed serum concentrations measured at appropriate time intervals after dosing. The value of $\hat{C}_T$ is obtained by substituting T for t in the equation of the least squares line used to estimate K.

The method of estimating relative absorption of a

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[1] Some data may fit a model for which $\int_0^\infty C(t) \neq \frac{F.D.}{V.K.}$. In these cases, the method proposed is not applicable.

TABLE I—SUMMARY OF HALF-LIVES AND AREA/DOSE × HALF-LIFE RATIOS ESTIMATED FROM SERUM CONCENTRATIONS OF TETRACYCLINE HYDROCHLORIDE ACTIVITY (MICROBIOLOGICAL ASSAY) OBSERVED IN SEVERAL CLINICAL STUDIES AFTER SINGLE ORAL DOSES OF TETRACYCLINE HYDROCHLORIDE

Study	Formulation	Subj.	Type of Subjects	Av. Dose Of Tetracycline HCl, mg./Kg.	Dose of Prepn.	Half-Life, hr. Av.	Half-Life, hr. Range	Ratio of Area 0 → ∞ (mcg. × hr./ml.) / Dose (mg./Kg.) × Half-Life (hr.) Av.	Ratio Range
1	Tetracycline and novobiocin[c] drops (exptl. formula)	19	Children 13–155 lb.	9.5	2.5 ml./15 lb. body wt.	5.24	2.87–10.3	1.12	0.50–1.87
2	Tetracycline and novobiocin[c] half-strength capsules (exptl. formula)	20	Children 37–120 lb.	8.6	1, 2, or 3 capsules	4.76	2.68–8.70	1.19	0.24–1.60
3	Novobiocin and tetracycline[d] suspension	34	Children 19–100 lb.	9.0	5 ml./15 lb. body wt.	5.31	2.06–9.93	1.12	0.59–1.98
4	Novobiocin and tetracycline[d] suspension	39	Children 17–100 lb.	9.0	5 ml./15 lb. body wt.	4.99	2.31–7.32	1.23	0.76–2.76
5 and 6[a] pooled	Tetracycline and novobiocin[c] flavored granules (exptl. formula)	20	Adults 120–204 lb.	7.0	20 ml.	7.08	3.84–18.0	1.13	0.51–2.20
7–12[b] pooled	H.F.C. Tetracycline,[e] 250 mg.	35	Adults 116–220 lb.	7.3	2 capsules	6.28	1.91–13.8	1.09	0.34–2.33

[a] Each study employed 10 subjects. Results of both studies were pooled. [b] In these six studies the same subjects were employed several times. The averages are based on 35 *different* subjects' data. [c] Marketed as Panalba KM by The Upjohn Co., Kalamazoo, Mich. [d] Marketed as Albamycin-T by The Upjohn Co., Kalamazoo, Mich. [e] Marketed as Panmycin by The Upjohn Co., Kalamazoo, Mich. [f] It is the author's experience that similarity in average ratios is not improved when surface area rather than body weight is used as the variable.

drug in a series of clinical studies utilizes calculation of the area/dose × half-life ratios shown in the left-hand side of Eqs. 2 and 3 for each subject administered a given dosage form in each study. The average value of the ratio for the panel of subjects administered a given dosage form of the drug in each study is calculated. If the assumption is made that the average apparent volume of distribution (expressed as a fraction of body weight or liters per $M.^2$ of body surface area) is the same from panel to panel of subjects, then the average ratios are indices of relative absorption efficiency.

RESULTS

The method has been applied by the author to estimate relative absorption efficiencies of several antibiotics administered in different dosage forms to different panels of subjects in dozens of clinical trials. As an example, results are presented of half-lives and area/dose × half-life ratios estimated from serum concentrations of tetracycline hydrochloride activity (in micrograms per milliliter) observed in several clinical trials in which children and adults were administered various novobiocin-tetracycline combinations and tetracycline hydrochloride alone in capsules. Results are summarized in Table I. Although there was wide variation in milligram dosage, body weight, and estimated half-life in these studies, the average area/dose × half-life ratios are amazingly similar. Appropriate statistical tests indicated that the difference between any pair of the averages of the ratios was not significant at the 5% level of confidence. If one assumes that the average volume of distribution expressed as a fraction of body weight was constant, these data indicate that the average efficiency of absorption of tetracycline was not significantly different in the six groups of subjects listed in Table I.

REFERENCES

(1) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **53**, 1392 (1964).

(2) Wagner, J. G., Northam, J. I., Alway, C. D., and Carpenter, O. S., *Nature*, **207**, 1301(1965).