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A model for short term drug absorption studies; comparison of sulphadiazine tablets

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The four parameter equation, applicable to drug absorption kinetics over a limited time period, has been used to interpret plasma concentration, time results following oral dosage with three different formulations of sulphadiazine tablets, to each of five subjects. Assessment parameters for the formulations, the time for ten percent absorption, the time interval between 10–90% absorption and the plasma concentration with no disposition (relative availability) have been estimated. Intersubject variation obscured many of the differences between formulations. To blank off this variation, plasma concentrations at each time were averaged and then random variation corresponding to the error of the assay method was applied so as to generate a number of sets of data which including the mean concentrations was equal to the number of sets in the original measurements. Kinetic analysis of these sets indicated a number of significant differences between the formulations.

The 4 parameter model previously described (Saunders & Natunen, 1976) was designed for the study of drug absorption over a limited time period. Most of the plasma samples for the application of this method are taken at times before twice the time at which the maximum plasma concentration occurs.

The mathematical relationship used is

$$C = A.[(1-H).\exp(-k_d t)+H-\exp(-k_a t)] \quad (1)$$

where H and k_d are related to disposition and k_a is the first order absorption rate constant. In the earlier paper it was shown that this equation is capable of representing accurately over a limited time period, C, t data generated by the full five parameter equation.

$$C = A.\exp(-\alpha t) + B.\exp(-\beta t) - (A + B).\exp(-k_a t) \quad (2)$$

The method for finding starting values for the parameters has been modified from that described previously. The earlier method involved the solution of a quadratic equation which unfortunately gave no real roots with much experimental data. In the modified method no attempt is made to find a starting value for H, and the least squares calculation is entered with H = 0. Two calculations are made for A, k_d and k_a and the set of values which gives the better fit to the data is chosen to start the least squares estimations. If both calculations give very small or negative values for any of the parameters, approximate values $k_a = 2/T_m$, $k_d = k_a/2$, $A = 3C_m$, $H = 0$ where T_m, C_m is the maximum point of the C, T curve were used, as before.

The new calculations for starting values are outlined in the Appendix. Use of these methods in place of the one described previously did not alter the final least squares values of the parameters for the data used in the previous paper.

The method is suitable for data which show a well defined maximum indicating that the absorption constant is greater than the disposition rate constants. It is also assumed that the absorption may be adequately represented by a single first order rate constant.

Assessment parameters

The values of A, k_d, k_a and H are of limited use in comparing different formulations of a drug. Assessment parameters with the dimensions of the experimental quantities, concentration and time, have therefore been developed and are estimated from the least squares values of the parameters in the computer program. The three practical assessment parameters used in this paper are

(i) t_{10}, the estimated time for 10% absorption including lag time, t_1 assesses the early stages of absorption
$t_{10} = t_1 - \ln(0{\cdot}9)/k_a$ where t_1 = lag time.

(ii) t_p, the period over which the main absorption occurs taken as the difference between estimated times for 90 and for 10% absorption.
$t_p = [\ln(0{\cdot}9) - \ln(0{\cdot}1)]/k_a$.

(iii) C_0, an estimate of the plasma concentration if no disposition had occurred. C_0 is an estimate of

* Correspondence.

the amount of drug absorbed/apparent volume of distribution and has the units of plasma concentration. This parameter includes the apparent volume of distribution and therefore considerable intersubject variation is to be expected.

C_0 was described as availability, AVL, in the previous paper (Saunders & Natunen, 1976) in which an expression is given for calculating it from the least squares parameters. It is assessed from the area AREA under the C, t curve up to $2t_m$ and the 4 parameters from equation (1).

$$C_0 = \frac{[k_D.(AREA - 2A.H.t_m) + C_2]}{[1 - \exp(-2k_a.t_m)]}$$

where C_2 is the concentration at time $2t_m$.

MATERIALS AND METHODS

Sulphadiazine tablets and pure drug were from May and Baker Ltd., Dagenham, Essex. Other excipients used were Maize Starch B.P. (Macarthys Ltd.), and magnesium stearate (BDH Chemicals Ltd.). *p*-Dimethylaminobenzaldehyde was from Hopkin and Williams.

Methods

(a) *Preparation of tablets.* Sulphadiazine tablets (500 mg) containing 15% w/w extragranular starch and 0·0 and 0·5% w/w magnesium stearate were prepared from $-710\,\mu m + 355\,\mu m$ granules at an applied pressure of 78 MN m^{-2} on an instrumented tablet machine.

(b) *Human volunteer study.* Sulphadiazine blood concentration data were obtained by administering either commercial or formulated tablets containing 0 or 0·5% w/w magnesium stearate to 5 healthy fasting subjects with a gap of one week between each administration according to a 5×3 latin square design. The volunteers were not allowed to eat or drink until 3 h after one of the tablets had been given with 200 ml of water.

Blood samples (10 ml) were collected in heparinized tubes at 1·0, 2·0, 3·0, 4·0, 6·0, 8·0 and 24 h and stored at 4°. Free sulphadiazine in blood was analysed within a week by the method of Werner (1939). The method was automated using a Technicon Auto-analyser. To check the reproducibility of the analytical technique before the analysis of blood samples, several calibration curves were constructed by adding known amounts of the drug to human blood and then subjecting the analytical results to statistical analysis. From this analysis the coefficient of variation showed some dependence on concentration, an average value of 4·14% was estimated over the concentration range studied.

RESULTS

Kinetic analysis of results

Lag times were first determined in each case by fitting a curve to the first three points and extrapolating to C = 0, the intercept on the time axis if positive was then taken as the lag time and subtracted from the experimental times before the kinetic analysis was made.

The 4-parameter equation gave successful calculations for the results from all the three formulations with all the five subjects, statistical calculations could therefore be made without having to use the 9-ordinate method described in the previous paper or to discard any of the data.

The least squares parameter values showed wide intersubject variation. As an example the values of k_a are shown in Table 1.

Table 1. *Values of* k_a *in* h^{-1}. I is no magnesium stearate, II is commercial tablet, III is 0·5% w/w magnesium stearate.

Subject	I	II	III
1	0·442	0·308	0·288
2	0·195	0·194	0·353
3	0·478	0·704	0·191
4	0·360	0·112	0·376
5	0·161	0·250	0·779
mean	0·327	0·314	0·397
s.e.	0·064	0·103	0·101

The intersubject variation masks any significant differences between the mean values of k_a for the different preparations.

Similar wide variations were found with t_p and C_0. Values of t_{10} for I appeared markedly lower than for III. However, due to intersubject variation, the statistic *t* for the difference of values corresponded to a probability less than 0·9. As seen in Table 2, t_{10} for formulation III for each subject was always greater than t_{10} for I and a better statistical analysis was made by taking the ratio of the values for each subject as the variate and testing the significance of the difference of the mean ratio from 1·0; this difference is found to be clearly significant.

The ratio method reduces the effects of intersubject variation and so reveals some significant differences which are masked by this variation. The paired *t*-test gives very similar results.

Table 2. *Values of* t_{10} *in h for I and III.*

Subject	I	III	Ratio, III/I
1	0·238	0·366	1·538
2	0·896	1·977	2·206
3	0·221	0·551	2·493
4	0·293	0·834	2·846
5	0·654	1·056	1·615
mean	0·460	0·957	2·140
s.e.	0·135	0·281	0·252

The statistic t for difference of means = 1·59 (P = 0·9, ø = 8, t = 1·86).
The statistic t for significance of difference of the ratio from 1·0 = 4·52 (P = 0·98, ø = 4, t = 3·75).

Noise method

Another method for eliminating the masking effects of inter-subject variation is to carry out the four parameter kinetic analysis on the arithmetical mean plasma concentrations for all the subjects, at each time. If the object of the calculation is to compare formulations this is a justifiable procedure comparable with the replacement of individual values by their means, commonly used in the analysis of variance. These means do not necessarily represent the separate individual results but are used to indicate the effects of differences of formulation on the group of subjects taken as a whole. In order to give a statistical basis for comparisons, sets of results were generated from the mean plasma concentration/time data by applying random noise with a percentage standard deviation corresponding to that of the assay method. If the total number of sets is equal to the number of sets of results determined experimentally they may be regarded as simulated sets of values which include variations due to experimental errors in the concentration determinations and also variations due to instabilities in the calculation but which are free from the intersubject variation.

In this case four sets of noise were applied for each preparation to the mean plasma concentration set and for each preparation the resulting five sets of results were analysed for significant differences. For each mean parameter value the number of degrees of freedom was taken as 3, one of the original five being used to give mean plasma concentrations and another used to calculate the mean of the set.

With this procedure a number of significant differences between the three formulations appeared as shown in Table 5.

The detailed results of the three different methods for examining the values are shown in Tables 3 for the direct comparison, 4 for the comparison of ratios and 5 for the noise method.

DISCUSSION

In Table 3 only one clearly significant difference occurs indicating that the early stage of absorption for preparation II is delayed relative to that for I.

Table 3. k_a *and assessment parameters calculated directly from the experimental results.*

	I	II	III
k_a	0·33	0·31	0·40
s.e.	0·06	0·10	0·10
t_{10}	0·46*	0·99*	0·96
s.e.	0·13	0·04	0·28
t_p	8·1	10·0	6·8
s.e.	1·8	2·8	1·4
C_0	20·7	15·4	16·4
s.e.	1·9	3·0	2·1

* The statistic t for difference of means = —3·7, $P > 0{\cdot}99$.

Table 4 indicates that this difference is only marginally significant but indicates two significant differences between preparations I and III. The early stage of absorption, t_{10}, for III is significantly delayed relative to I while the value of C_0, of III is significantly less than for I.

Table 4. *Mean ratios of parameters for each subject.*

	II/I	III/I	III/II
k_a	1·006	1·749	1·900
s.e.	0·234	0·808	0·600
t_{10}	2·606‡	2·140*	1·790
s.e.	1·058	0·252	0·764
t_p	1·397	1·150	1·184
s.e.	0·479	0·403	0·640
C_0	0·763	0·790†	1·191
s.e.	0·167	0·078	0·220

* The statistic t for difference from 1·0 = 4·52, $P > 0{\cdot}95$.
† The statistic t for difference from 1·0 = 2·67, $P > 0{\cdot}95$.
‡ The statistic t for difference from 1·0 = 1·5, $P > 0{\cdot}7$.

Table 5 shows the results when intersubject variations are completely eliminated by pooling the plasma concentrations for all the subjects at each time. Four sets of noise with standard deviation 4·14% corresponding to the mean standard deviation of the plasma concentration assay have been applied to give five sets of results for each

Table 5. *Mean parameters for each preparation for sets of results generated by the noise method.*

	I	II	III
k_a	0·287[a]	0·201[a,b]	0·258[b]
s.e.	0·010	0·007	0·013
t_{10}	0·367[c,d]	0·571[d]	0·703[c]
s.e.	0·014	0·026	0·106
t_p	7·69[e]	10·95[e,f]	8·80[f]
s.e.	0·311	0·393	0·377
C_o	23·4[g,h]	14·3[g,i]	17·0[h,i]
s.e.	0·52	0·28	0·86

Values of the statistic t for the pairs of mean values indicated by the superscripts are as follows:

[a] $t = 6·71$ $P > 0·99$ [f] $t = 3·95$ $P > 0·95$
[b] $t = 3·80$ $P > 0·98$ [g] $t = 15·3$ $P > 0·99$
[c] $t = 3·12$ $P > 0·95$ [h] $t = 6·33$ $P > 0·99$
[d] $t = 6·76$ $P > 0·99$ [i] $t = 2·97$ $P > 0·95$
[e] $t = 6·50$ $P > 0·99$

preparation with three degrees of freedom. With these data a considerable number of differences between the three formulations appear.

The values of the absorption constant, k_a, indicate a slower absorption for II relative to I and III, these last two being indistinguishable. Since t_p is inversely proportional to k_a the same differences appear, the period for the main absorption of II is significantly greater than those for I and III.

t_{10}, the estimate of the early stages of absorption is governed by lag time as well as by k_a. I shows a more rapid onset than either of the other two.

The values of C_o, show significant differences between all the three preparations with I>III>II.

It therefore appears that the commercial tablets have the smallest absorption rate constant giving the longest period for the main absorption, but having the lowest relative availability as assessed by C_o. The presence of magnesium stearate (III relative to I) has no clear effect on absorption rate constant and on the period of absorption, but it does delay the onset of absorption and it reduces the availability relative to I.

APPENDIX

Starting values for k_d, k_a, A
H is taken as zero so that

$$C = A\,[\exp(-k_d t) - \exp(-k_a t)]$$

From the data lag time is estimated and subtracted from all experimental times. The maximum t_m, C_m is located. Ordinates C_{23}, C_{43} and C_2 at $2t_m/3$, $4t_m/3$, $2t_m$ are interpolated and the area under the C, T curve, I, up to $2t_m$ is assessed.

Quadratic equation method

Let $R_a = \exp(-2k_a t_m/3)$ $R_d = \exp(-2k_d t_m/3)$
$C_{23} = A(R_d - R_a)$ $C_{43} = A(R_d^2 - R_a^2)$
$C_2 = A(R_d^3 - R_a^3)$
$F_1 = C_{43}/C_{23} = R_a + R_d$
$F_2 = C_2/C_{23} = R_a^2 + R_a R_d + R_d^2$
eliminating R_d gives

$$R_a^2 - F_1 R_a + F_1^2 - F_2 = 0$$

The smaller root of this equation is then R_a and the larger, R_d.

A is assessed from I_2 so as to give an averaged value over the whole time period.

$$I_2 = \int_0^{2t_m} C\,dt = A\left[\frac{1-\exp(-2k_d t_m)}{k_d} - \frac{1-\exp(-2k_a t_m)}{k_a}\right]$$

The calculation fails if the quadratic equation gives no real roots.

Q Method

This calculation is based on the equation for the slope at the maximum, $\frac{dC}{dt} = 0$

$$k_d \exp(-k_d t_m) = k_a \exp(-k_a t_m)$$

calling the exponential terms E_d and E_a

$$\frac{k_a}{k_d} = \frac{E_d}{E_a} = Q.$$

i.e. $k_d = k_a/Q$ and $E_d = Q\,E_a$
The ordinate at the maximum, C_m is

$$C_m = A(E_d - E_a) = A\,E_a(Q - 1)$$

If the area under the curve up to t_m is I_m

$$I_m = \int_0^{t_m} C\,dt = A\left[\frac{1-E_d}{k_d} - \frac{1-E_a}{k_a}\right]$$

eliminating E_d and k_d in terms of Q gives

$$I_m = A.\,\frac{(Q-1)}{k_a}\,[1 - (Q+1)E_a]$$

The ordinates C_2 at $2t_m$ and C_m at t_m are

$$C_2 = A(E_d^2 - E_a^2) = A\,E_a^2(Q^2 - 1)$$
$$C_m = A(E_d - E_a) = A\,E_a(Q - 1)$$
$$\frac{C_2}{C_m} = E_a(Q+1)$$
$$\frac{I_m}{C_m} = \frac{1}{k_a\ E_a}\,[1 - (Q+1)E_a]$$
$$= \frac{1}{k_a\ E_a}\left[1 - \frac{C_2}{C_m}\right]$$

Rearranging this equation

$$I_m\,k_a\,E_a - (C_m - C_2) = 0$$

I_m, C_m, C_2 are estimated from the data. A starting value for k_a of $2·0/t_m$ is taken and Newton's method is used to solve the above equation for k_a.

Q and therefore k_d are calculated from C_2/C_m and A is assessed as in the quadratic method from I_2.

This method breaks down if the Newton solution diverges but it is much more rugged than the quadratic equation method. When both methods work the Q values usually give a better fit to the data than the values from the quadratic equation method.

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