

Comparative Bioavailabilities from Truncated Blood Level Curves

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Abstract □ The period of time after administration over which blood level measurements are required to obtain a reliable bioavailability comparison of two or more formulations of the same drug was considered by the analysis of bioavailability data taken from the literature. The drugs examined, selected to represent a range of absorption and elimination half-lives, were acetaminophen, aminosalicic acid, chloramphenicol, chlordiazepoxide, digoxin, isoniazid, phenylbutazone, sulfamethizole, tetracycline, and warfarin. For most drugs, ratios of areas under the curve changed little between the end of the absorption period and the time when blood sampling was terminated. Reliable bioavailability comparisons among different brands of the drugs apparently could have been made by blood sampling over 24 hr or less.

Keyphrases □ Bioavailability—length of time for blood level measurements, analysis of literature data, 10 drugs considered, truncated blood level curves □ Blood level curves, truncated—comparative bioavailabilities of 10 drugs, analysis of literature data, determination of length of time necessary for blood level measurements □ Sampling, blood—time necessary for bioavailability studies, analysis of literature data, truncated blood level curves

Bioavailability is an important parameter in the comparison of commercial drug formulations. It was defined by Riegelman (1) as the relative rate and extent at which an administered dose reaches the general circulation. However, it is commonly interpreted as only the relative extent of absorption and is expressed as the percent ratio of test to reference formulations absorbed. This ratio is estimated either by the appropriate ratio of total areas under the curves of drug concentration in the blood following administration of the doses (2) or by the ratio of the total cumulative amounts of test and reference drugs excreted in the urine.

Ideally, the areas under the blood concentration curves (*AUC*) should be calculated to infinite time; but in practice, it is usually suggested that areas calculated over three elimination half-lives are sufficient (3). The extrapolation to infinity can frequently be made but often makes no appreciable difference to the final bioavailability estimate. For many commercial formulations, it was observed that the ratios of areas of test to reference formulations approach a limiting value many hours prior to the complete elimination of the drug. In this study, 10 drugs of widely differing kinetic properties were examined to determine how long blood sampling should continue after drug administration to estimate adequately the ultimate test to reference *AUC* ratio.

EXPERIMENTAL

The data used came from both the literature and internal unpublished investigations. Bioavailability studies comparing different formulations of the same drug commonly report blood levels at each sampling time averaged (arithmetic means) over all subjects.

Table I—*AUC* Ratios (Percent) of Acetaminophen Formulations^a

Formulation	Blood Sampling Time, hr						
	0.33	0.67	1.00	1.50	2.00	4.00	6.00
K ₁	120	88	83	86	88	92	93
K ₂	139	105	93	87	86	86	87
K ₃	110	84	78	78	79	78	79
N	121	81	69	71	76	83	85
O	161	113	97	94	96	97	97
P	153	122	111	108	108	106	102
Q	88	75	74	80	86	93	89
S	197	131	109	101	100	97	97

^aCalculated from McGilveray *et al.* (5).

By using these average blood levels, the *AUC* at each sampling time was determined by the trapezoidal rule for the test and reference formulations, and *AUC* ratios were calculated at these sampling times.

Occasionally, blood levels are reported for individual subjects. In these cases, areas from the time of administration to each sampling time were calculated by the trapezoidal rule for each subject. Geometric means of the *AUC*'s at each sampling time were determined for each formulation in the study, and *AUC* ratios of test to reference formulations were calculated. Graphs of *AUC* ratios versus time were tested for parallelism, height, and slope, using the method of profile analysis (4).

RESULTS

Acetaminophen—*AUC* ratios at each sampling time from 20 min to 6 hr were calculated from the individual blood level data of McGilveray *et al.* (5). Tablet R (5) was used as the reference formulation. The curves constructed from the bioavailability-time data (Table I) were found to be approximately parallel ($p > 0.9$) and at the same level ($p > 0.3$). *AUC* ratios did not change significantly after 2 hr postadministration ($p > 0.2$).

Aminosalicic Acid—The individual blood level data reported by Schirmer *et al.* (6) were not in a suitable form for profile analysis. Mean blood level curves were used to calculate the *AUC* ratios at 2, 4, 6, 8, and 12 hr postadministration (Table II). This study included uncoated and enteric-coated tablets, and uncoated Tablet A was used as the reference formulation. Plasma levels of the drug from some enteric-coated tablets were low and erratic and were not included in the analysis. There was little change in the *AUC* ratios of uncoated Tablets B, C, and D after 6 hr. Enteric-coated Tablet E1 showed slowly increasing *AUC* ratios to 12 hr.

Wagner *et al.* (7) recently reported blood level curves of aminosalicic acid obtained from solutions of the sodium salt, suspension, compressed tablets, and enteric-coated tablets. In all cases the absorptive phases were complete within 3 hr.

Chloramphenicol—Mean plasma levels determined colorimetrically by Glazko *et al.* (8) were used to calculate *AUC* ratios of four brands of chloramphenicol capsules (Table III). Formulation A was used as the reference. Six hours after administration, the *AUC* ratios approached a constant value for Capsules B and C. The *AUC* ratio of Capsule D appeared to increase slightly between 6 and 24 hr but remained very low compared to B and C.

Chlordiazepoxide—*AUC* ratios determined in this laboratory¹ are given in Table IV for from 1 to 54 hr. The ratios were calculated

¹ I. J. McGilveray and G. L. Mattok, unpublished work.

Table II—AUC Ratios (Percent) of Aminosalicylic Acid Formulations^a

Formulation	Blood Sampling Time, hr				
	2	4	6	8	12
B	92	84	78	78	78
C	80	83	85	85	85
D	97	91	85	83	83
E2	—	100	100	100	100
E1	—	78	86	94	98

^aCalculated from Schirmer *et al.* (6).

Table III—AUC Ratios (Percent) of Chloramphenicol Formulations^a

Formulation	Blood Sampling Time, hr							
	0.5	1.0	2.0	4.0	6.0	8.0	12.0	24.0
B	19	22	32	45	52	54	55	53
C	24	32	45	57	62	63	64	62
D	10	12	16	22	26	29	32	35

^aCalculated from Glazko *et al.* (8).

ed on the basis of total drug, *i.e.*, free chlorthalidone and metabolite. With the exception of Formulation 43, the bioavailabilities calculated at 7 hr were within 12% of those calculated at 54 hr. The bioavailability of Formulation 43 increased from 1 to 54 hr, although the increase after 24 hr was only about 10%. AUC ratios calculated from the data of Foldes *et al.* (9) varied erratically between 4 and 24 hr.

Digoxin—Table V gives the AUC ratios of a digoxin formulation calculated from the data of Wagner *et al.* (10); Formulation A was used as the reference. The ratios remain unchanged, within a few percent, from 1.5 to 96 hr. More than half the area under the curve to 96 hr was in the 24–96-hr interval.

After 24 hr, the plasma levels were low and their determination was subject to greater error than when the plasma level was high. Thus, measurement of plasma levels over a period of days apparently gives no additional information about relative availability and, in fact, may increase experimental error.

Isoniazid—AUC ratios were calculated from the individual blood level data of Gelber *et al.* (11), using Formulation 4 as the reference (Table VI). Results of profile analysis indicated that curves of AUC ratios *versus* time were approximately parallel ($p > 0.7$) and at the same level ($p > 0.8$). For each curve, the analysis showed that the slope was not significantly different ($p > 0.1$) from zero in the time interval from 2 to 8 hr.

Phenylbutazone—AUC ratios of nine tablet formulations were calculated from the mean plasma level data of Van Petten *et al.* (12), taking a solution of the drug as the reference formulation.

Table IV—AUC Ratios (Percent) of Chlorthalidone Formulations^a

Formulation	Blood Sampling Time, hr								
	1	3	5	7	24	27	31	48	54
31	100	103	102	102	105	105	103	101	100
32	78	94	102	104	103	103	102	100	100
33	76	95	102	102	101	100	99	96	95
34	99	103	107	107	104	104	102	96	95
35	84	88	90	92	96	96	95	93	92
41	109	104	107	110	118	117	117	116	116
42	88	85	89	92	98	98	98	97	96
43	36	41	49	55	68	68	70	76	77
44	63	74	86	90	95	95	96	99	100
45	47	63	79	85	93	93	92	92	92
51	110	109	105	104	100	99	99	100	100
52	111	107	105	106	104	104	104	106	106
53	120	108	103	104	106	106	105	103	102
54	108	112	113	112	108	108	108	107	107
55	94	93	95	96	94	93	93	94	94

^aCalculated from unpublished data of I. J. McGilveray and G. L. Mattok of this laboratory.

Table V—AUC Ratios of a Digoxin Formulation^a

t, hr	AUC Ratio, %
0.25	27
0.50	36
0.75	43
1.00	49
1.50	54
3.00	54
5.00	52
12.00	51
24.00	54
48.00	54
72.00	57
96.00	57

^aCalculated from Wagner *et al.* (10).

Table VI—AUC Ratios (Percent) of Isoniazid Formulations^a

Formulation	Blood Sampling Time, hr								
	0.25	0.50	0.75	1.00	1.50	2.00	4.00	6.00	8.00
1	31	34	54	71	90	100	109	109	108
2	47	77	94	104	111	114	116	115	113
3	73	75	83	91	102	109	112	111	112
5	40	48	73	83	94	100	110	111	112
6	59	84	91	97	105	107	105	103	102

^aCalculated from Gelber *et al.* (11).

Van Petten *et al.* divided their study into two groups (Table VII). Except for Formulation D, the ratios varied little after 12 hr but varied considerably before that time. The extended time required to reach constant AUC ratios may be due to the fact that many phenylbutazone tablets are coated.

Sulfamethizole—AUC ratios were calculated from the individual blood level data of Mattok and McGilveray (13), using Tablet B as the reference (Table VIII). The AUC ratio curves were approximately parallel ($p > 0.7$), at the same level ($p > 0.6$), and constant in slope ($p > 0.3$) over the profile.

Table VII—AUC Ratios (Percent) of Phenylbutazone Formulations^a

Formulation	Blood Sampling Time, hr						
	2	4	6	8	12	24	48
Group I							
D	24	34	45	42	61	72	79
C	87	83	83	84	86	87	87
A	104	100	99	99	99	99	99
F	58	63	68	73	78	82	84
E	18	28	37	44	50	57	62
Group II							
H	61	70	79	85	89	95	101
J	152	131	120	115	111	108	108
I	104	98	95	95	95	98	98
G	152	131	121	118	113	107	104

^aCalculated from Van Petten *et al.* (12).

Table VIII—AUC Ratios (Percent) of Sulfamethizole Formulations^a

Formulation	Blood Sampling Time, hr				
	1.5	2.5	4.0	6.0	8.0
C	92	95	105	112	114
D	61	80	90	93	93
E	58	65	85	98	100

^aCalculated from Mattok and McGilveray (13).

Table IX—AUC Ratios (Percent) of Tetracycline Formulations^a

Formulation	Blood Sampling Time, hr					
	2	3	4	6	9	24
A	68	74	78	81	81	78
B	68	71	73	74	74	72
C	81	84	83	82	83	82
D	86	90	92	93	94	95
E	81	79	78	78	77	71
F	29	32	35	36	34	29
G	26	27	27	26	25	22
H	71	76	79	81	81	78
K	76	79	81	83	83	83

^aCalculated from Lovering *et al.* (14).

Table X—AUC Ratios (Percent) of Warfarin Formulations^a

Formulation	Blood Sampling Time, hr							
	1	4	8	12	24	48	72	96
C	125	112	108	108	107	105	104	103
D	82	95	98	98	97	95	93	92

^aCalculated from Wagner *et al.* (15).

Tetracycline—Lovering *et al.* (14) reported individual blood level data for nine tablet formulations, and these data were used to calculate AUC ratios relative to a reference solution. Results of the profile analysis (Table IX) showed that the AUC ratios of Tablets F and G differed significantly ($p > 0.05$) from the other tablets. The mean AUC ratio of F and G decreased between 6 and 24 hr, but the mean ratio for the remaining formulations was level from 6 to 9 hr and then decreased to 24 hr. The decrease for both groups was slight.

Warfarin—Individual plasma level data from Wagner *et al.* (15) were analyzed for three 5-mg tablets, using Formulation A as the reference (Table X). Results of profile analysis indicated that curves were approximately parallel ($p > 0.8$) and at approximately the same level ($p > 0.07$). The hypothesis of equal AUC ratios across all sampling times was not rejected ($p > 0.6$).

Based on a one-compartment model, approximate first-order absorption and elimination rate constants, k_1 and k_2 , respectively, were taken from the papers cited or were calculated from:

$$C_t = \frac{fD}{V} \frac{k_1}{k_1 - k_2} (e^{-k_2 t} - e^{-k_1 t}) \quad (\text{Eq. 1})$$

where C_t is the concentration of drug in the blood at time t ; f is the fraction of dose, D , absorbed; and V is the apparent volume of distribution (16) (Table XI). Elimination rate constants were calculated using the final points on the mean blood level-time curves. Absorption rate constants were calculated from the first derivative of Eq. 1 and the mean peak blood level time, t^* , when $dC_t/dt = 0$:

$$t^*k_2 - \ln k_2 = k_1 t^* - \ln k_1 \quad (\text{Eq. 2})$$

The time to 99% absorption was calculated by assuming the expo-

ponential disappearance of drug from the GI tract at a rate controlled by k_1 (Table XI).

DISCUSSION

Ten formulations (Tables I-X) exhibited changes in AUC ratios of more than 15% after the estimated absorption period. The AUC ratios of an enteric-coated aminosalicic acid tablet measured against an uncoated reference tablet increased from 78 to 98% between 4 and 12 hr. The increase may be the result of slow drug release from the enteric-coated tablet. The AUC ratio of chlordiazepoxide Formulation 43 increased 57% between 5 and 54 hr, but the increase was only 10% after 24 hr. Formulations 44 and 45 showed some increase between 5 and 24 hr but were relatively constant thereafter.

Three phenylbutazone formulations showed AUC ratio changes of more than 15% between 6 and 48 hr, but only slight changes occurred after 24 hr. The results may indicate that the time for complete absorption lies between 6 and 24 hr for a number of phenylbutazone formulations. Chloramphenicol Formulations B and D (Table III) and Formulation F (Table IX) showed large changes in the AUC ratio in the postabsorption period, but these formulations were of low bioavailability.

The constancy of the AUC ratios in these studies, a few hours after administration of the drug, suggests that it may not be necessary to follow blood levels to complete elimination of the drug, or even over two to three elimination half-lives, to obtain AUC ratios that are approximately equal to the bioavailability (3). The time over which samples should be taken depends upon the relative values of the test and reference absorption rate constants, k_1 and k_1^* , respectively, and the elimination rate constant, k_2 .

The AUC ratios also depend upon the time available for absorption. Formulation comparison studies are usually carried out in starved, healthy subjects. Under these conditions, the rate at which the drug, whether in solution or not, flows through those regions of the GI tract that favor dissolution and absorption may be uniform; for certain regions of the GI tract, the rate may be rapid.

Consider a one-compartment model with first-order absorption and elimination, in which absorption is allowed to proceed from time zero to time T , where T is the end of the absorption period. The concentration of drug in the blood at time t is given by Eq. 1 if $t \leq T$. If $t > T$:

$$C_t = C_T e^{-k_2(t-T)} \quad (\text{Eq. 3})$$

where C_T is the concentration of drug in the blood at time T . If $t \leq T$ (16), the corresponding AUC's to time t are:

$$A_t = \frac{fD}{V} \frac{1}{k_2(k_1 - k_2)} (k_2 e^{-k_2 t} - k_1 e^{-k_1 t} + k_1 - k_2) \quad (\text{Eq. 4a})$$

If $t > T$:

$$A_t = A_T + \frac{C_T}{k_2} (1 - e^{-k_2(t-T)}) \quad (\text{Eq. 4b})$$

where A is the AUC at time T .

The AUC ratios of test to reference formulations follow. Quantities marked by an asterisk refer to the reference formulation. If it

Table XI—Approximate First-Order Appearance and Elimination Rate Parameters

Drug	Reference	Appearance Constant (k_1), hr ⁻¹	Elimination Constant (k_2), hr ⁻¹	Time to Peak, hr	Time to 99% Absorption, hr
Acetaminophen	5	1.80	0.23	1.3	2.5
Aminosalicic acid	6	2.00	0.80	2.0	2.3
Chloramphenicol	8	1.20	0.30	2.0	3.7
Chlordiazepoxide	— ^a	1.70	0.02	3.0	3.3
Digoxin	10	4.00	0.07	1.0	1.2
Isoniazid	11	2.90	0.20	1.0	1.6
Phenylbutazone	12	0.75	0.01	5.0	6.0
Sulfamethizole	13	0.90	0.56	1.5	5.0
Tetracycline	14	0.85	0.09	3.0	5.0
Warfarin	15	1.40	0.02	<4.0	3.3

^aUnpublished data of I. J. McGilveray and G. L. Mattok.

Table XII—Calculated AUC Ratios for $k_2 = 0.1 \text{ hr}^{-1}$

T, hr	for $k_1^* = 0.5 \text{ hr}^{-1}$ and $k_1 = 0.125 \text{ hr}^{-1}$							for $k_1^* = 4.0 \text{ hr}^{-1}$ and $k_1 = 1.0 \text{ hr}^{-1}$						
	2 hr	4 hr	8 hr	12 hr	24 hr	∞	0.5 hr	1.0 hr	2.0 hr	4.0 hr	8.0 hr	12.0 hr	24.0 hr	∞
0.5	0.27	0.27	0.27	0.27	0.27	0.27	0.38	0.43	0.44	0.45	0.45	0.45	0.45	0.46
1.0	0.29	0.30	0.30	0.30	0.30	0.30	0.38	0.49	0.58	0.62	0.63	0.64	0.64	0.64
2.0	0.31	0.34	0.35	0.35	0.35	0.35	0.38	0.49	0.66	0.78	0.83	0.85	0.86	0.87
4.0	0.31	0.38	0.43	0.44	0.45	0.45	0.38	0.49	0.66	0.82	0.92	0.95	0.97	0.98
8.0	0.31	0.38	0.51	0.58	0.63	0.64	0.38	0.49	0.66	0.82	0.92	0.96	0.99	1.00
24	0.31	0.38	0.51	0.62	0.84	0.95	0.38	0.49	0.66	0.82	0.93	0.96	0.99	1.00

Table XIII—Calculated AUC Ratios for $k_2 = 0.01 \text{ hr}^{-1}$

T, hr	for $k_1^* = 0.5 \text{ hr}^{-1}$ and $k_1 = 0.125 \text{ hr}^{-1}$							for $k_1^* = 4.0 \text{ hr}^{-1}$ and $k_1 = 1.0 \text{ hr}^{-1}$							
	2 hr	4 hr	6 hr	8 hr	12 hr	24 hr	∞	0.5 hr	1.0 hr	2.0 hr	4.0 hr	8.0 hr	12 hr	24 hr	∞
0.5	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.38	0.42	0.44	0.45	0.45	0.45	0.45	0.45
1.0	0.29	0.30	0.30	0.30	0.30	0.30	0.30	0.38	0.49	0.58	0.61	0.62	0.63	0.64	0.64
2.0	0.31	0.34	0.34	0.34	0.35	0.35	0.35	0.38	0.49	0.65	0.77	0.82	0.83	0.85	0.87
4.0	0.31	0.38	0.41	0.42	0.44	0.45	0.45	0.38	0.49	0.65	0.81	0.90	0.93	0.96	0.98
8.0	0.31	0.38	0.44	0.49	0.55	0.61	0.64	0.38	0.49	0.65	0.81	0.91	0.94	0.97	1.00
24	0.31	0.38	0.44	0.49	0.58	0.76	0.95	0.38	0.49	0.65	0.81	0.91	0.94	0.97	1.00

is assumed that k_2 , D , and V are the same for the test and reference formulations administered to a given subject, then, if $t \leq T$:

$$\frac{A_t}{A_t^*} = \frac{f(k_1^* - k_2)(k_2 e^{-k_1 t} - k_1 e^{-k_2 t} + k_1 - k_2)}{f^*(k_1 - k_2)(k_2 e^{-k_1^* t} - k_1^* e^{-k_2 t} + k_1^* - k_2)} \quad (\text{Eq. 5a})$$

If $t > T$:

$$\frac{A_t}{A_t^*} = \frac{A_T + \frac{C_T}{k_2}(1 - e^{-k_2(t-T)})}{A_T^* + \frac{C_T^*}{k_2}(1 - e^{-k_2^*(t-T)})} \quad (\text{Eq. 5b})$$

Tables of AUC ratios were constructed from Eqs. 1 and 3, taking typical values of k_1^* and k_2 from Table XI. The value of k_1 was taken as $0.25k_1^*$ throughout to represent formulations from which drug is released slowly. The time to which absorption was allowed to proceed, T , varied from 0.5 to 24 hr, and the AUC ratio was calculated over appropriate time periods. The AUC's in Tables XII and XIII at the end of the period available for absorption, $t = T$, are usually within 10–20% of the AUC ratio when t is infinite. The AUC ratios at $t = 2T$ are, in most cases, within a few percentage points of the AUC ratio at infinite time and experimentally indistinguishable from it. Thus, for slowly eliminated drugs, a limited period during which absorption occurs can account for the approach to constant values of AUC ratios long before two or three elimination half-lives have elapsed.

The duration of the absorption period may vary considerably among drugs. If a drug dissolves only at gastric pH and is administered with water on an empty stomach, the dissolution period will be fixed by the gastric emptying time and may be less than 1 hr. If absorption occurs only over a short proximal segment of the GI tract, the absorption period also may be very short. In any case, barring adhesion of drug crystals to the intestinal mucus, the absorption period is probably limited by the time required for the intestinal contents to reach solid matter in the lower intestine and certainly by the total intestinal transit time (about 30 hr).

In conclusion, analysis of blood level profiles obtained in several bioavailability studies indicated that "partial" AUC ratios at the end of the absorption period often agree with the total area ratios. Careful consideration of the rate constants and the general behavior observed during experimental work, which must precede any bioavailability trial, may permit shorter blood sampling schedules.

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