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In vitro – in vivo Correlation, a Time Scaling Problem?

Evaluation of mean times

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Dedicated to Professor Dr. D. Palm, Frankfurt/Main, on the occasion of his 60th birthday

Summary: *A new algorithm for the calculation of the mean time and further statistical moments is presented by means of a mathematical deduction which is outlined in brief. The graphical interpretation of the mathematical results demonstrates that the calculation is easy to perform. Furthermore the method is illustrated in detail with data from a sustained release formulation of theophylline. These data were obtained from a clinical study and from an in vitro dissolution test with the same formulation. Statistical analysis revealed that the rate of release in vivo was slower than in vitro by a factor of 0.7, using a commercial dissolution model. Implications of this approach with respect to in vitro – in vivo correlations are discussed.*

Zusammenfassung: *In vitro/in vivo-Korrelation, ein Problem der Zeitbasis? Berechnung von mittleren Verweilzeiten*

Für die Bestimmung der mittleren Verweilzeit wird ein neues Berechnungsverfahren vorgestellt. Das Ergebnis der kurzen mathematischen Herleitung läßt sich graphisch interpretieren. Anhand der graphischen Darstellung kann der Rechengang leicht nachvollzogen werden, der für zwei Datensätze eingehend beschrieben wird. Die Daten stammen aus einer klinischen Studie einer retardierten Formulierung von Theophyllin und aus der In-vitro-Freisetzungsprüfung der gleichen Formulierung. Aus dem Vergleich der mittleren In-vitro- und der mittleren In-vivo-Auflösezeit ergibt sich, daß der In-vivo-Auflöseprozeß um den Faktor 0,7 langsamer abläuft als in einem handelsüblichen Lösemodell, das als Referenz benutzt wurde. Die Bedeutung des theoretischen Ansatzes für die in vitro/in vivo-Korrelation wird diskutiert.

Key words: *In vitro/in vivo correlation · Mean dissolution time · Mean residence time · Statistical moments*

1. Introduction

Statistical moments related to the residence times of drug molecules reflect the overall properties of the time course without the necessity of a pharmacokinetic model [1, 2]. The mean residence time of a drug in the body enables the steady state volume of distribution to be estimated by way of the total clearance [3, 4, 5]. The mean absorption time offers relative values to quantify the "rate of bioavailability" even if a suitable model of the absorption process cannot be formulated [1, 6, 7]. An established correlation between the in vitro and the in vivo dissolution profile obtained via the mean times of both systems is an aid in the developing of galenic dosage forms [6, 8, 9, 10].

Statistical moments define the in vitro release profile of preformulated dissolution models [11]. In the same way, they can be used to describe the blood level profile of predefined pharmacokinetic models [12], since they are related to model parameters as described by Dost in 1958 [13]. The increasing interest in the mean residence time as a quantifying parameter in pharmacokinetics and biopharmaceutics makes an easy procedure for the evaluation of the mean time and further moments from in vitro as well as in vivo data desirable.

2. Theoretical considerations

2.1. Statistical background

The term "mean residence time" of a drug – obviously borrowed from mathematical statistics – requires at least an estimate of the probability density f(t) or the distribution function F(t), since residence times of individual drug molecules cannot be measured. A frequent formulation of the mean residence time in pharmacokinetics assumes that the blood level curve y(t) of a drug gives an estimate of the probability density function f(t):

$$f_{obs}(t) = \frac{y(t)}{AUC} \qquad (1)$$

where $f_{obs}(t)$ is a sample of the true density function f(t) and AUC means the area under the concentration time curve. It is convenient to characterize empirical statistical distribution with its moments, which expressed in terms of density are defined as follows:

$$m_k = \int_0^\infty t^k f(t)\,dt \cong \frac{\int_0^\infty t^k y(t)\,dt}{\int_0^\infty y(t)\,dt} \qquad (2)$$

Substituting the sample $f_{obs}(t)$ for the density f(t) results in estimates of the statistical moments of the residence times. The numerator in equation (2) can be transformed using the method of integration by parts [12]:

$$\int_0^\infty t^k y(t)\,dt = k \int_0^\infty t^{k-1} \int_t^\infty y(t')\,dt'\,dt \qquad (3)$$

The integral $\int_t^\infty y(t')\,dt'$ means the area under the blood level curve from time t onward. It is therefore named the prospective area under the curve $PAUC_o(t)$. It is obvious that the prospective area under the curve from time zero onward $PAUC_o(O)$ is identical to the common expression, AUC.

Using this definition, equation (3) reads as follows:

$$\int_0^\infty t^k y(t)\,dt = k \int_0^\infty t^{k-1}\, PAUC_0(t)\,dt \qquad (4)$$

It should be noted that for the first moment t^{k-1} is equal to unity and that the estimation of the first moment, i.e. the mean residence time, needs only two consecutive integration steps.

Equation (4) can be handled in the same way as equation (3) which results in the general definition:

$$PAUC_n(t) = \int_t^\infty PAUC_{n-1}(t')\,dt' \qquad (5)$$

Using the advising formula (5) and equation (2) one comes up with an easily performable algorithm:

$$m_k = k! \frac{PAUC_k(0)}{PAUC_o(0)} \qquad (6)$$

where k! means the algebraic factorial of k and $PAUC_k(0)$ the prospective area value related to time zero.

2.2. Graphical interpretation

The calculation procedure of statistical moments of the residence time based on the method of prospective areas (equ. 5) is illustrated by Fig. 1:

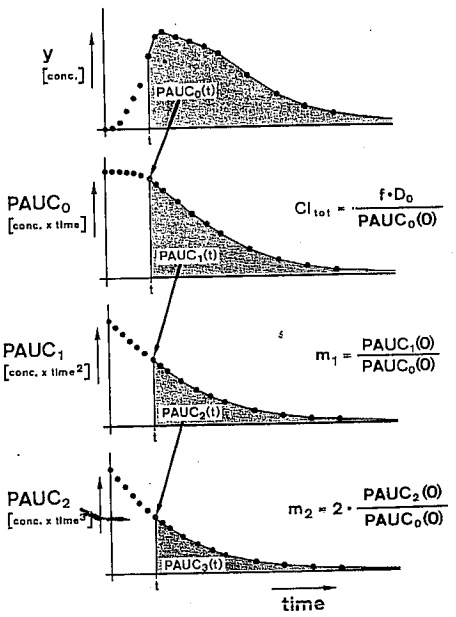

Fig. 1: The evaluation of statistical moments using the method of prospective areas is illustrated. Details are given in the text.

In the top graph blood concentration data y_i are plotted over time t_i as solid points. These data pairs (t_i,y_i) are numerically integrated by a conventional method, e.g. the trapezoidal or the log-trapezoidal rule. But in contrast to the usual way this integration does not start with the first two observations $[(t_1,y_1); (t_2,y_2))]$ but with the last two $[(t_z,y_z); (t_{z-1},y_{z-1})]$ and proceeds in the reverse direction to normal.

With respect to the time indicated "t", this integration gives the prospective area under the curve $PAUC_o(t)$ (shaded region)[1]. In addition, each value of these prospective areas, i.e. $PAUC_o(t_i)$ is recorded. Calculation of the prospective areas under the curve for each time value t_i of the original data produces a new set of data pairs, i.e. $[t_i,PAUC_o(t_i)]$.

The new set is shown in the second graph (solid points); the abscissa is still "time", scaled as in the top graph, but the ordinate is now dimensioned as "concentration × time". This set $[t_i,PAUC_o(t_i)]$ is handled in exactly the same way as the original observation: stepwise integration from the last datum to the first. The shaded area now gives the prospective area under the curve obtained with respect to time "t", i.e. $PAUC_1(t)$.

Calculating the prospective area under these data for each time value t_i results in a third set $[t_i,PAUC_1(t_i)]$, which is depicted in the third graph; the abscissa is scaled as in the top graph, the ordinate is dimensioned as "concentration × (time)[1]".

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[1] In pharmacokinetics the numerically evaluated area under a set of concentration time data is named area under the curve (AUC). It would be more consistent to distinguish between the area under the data (AUD) and the area under an adjusted curve AUC. In the same way we should then have to distinguish between the prospective area under the curve and under the data.

The procedure may stop here if one is interested in only the mean residence time, since

$$MT = m_1 = 1! \frac{PAUC_1(0)}{PAUC_0(0)} \quad (7)$$

where $PAUC_o(0)$ is the total area under the concentration time data and $PAUC_1(0)$ is the total area under the $PAUC_o(t_i)$ data.

2.3. Extrapolation

If the terminal blood level data can be reasonably interpolated and extrapolated by an exponential function, i.e. by $C_z \cdot e^{-\lambda_z t}$, the prospective areas under the extrapolated parts can be easily calculated:

$$PAUC_k(t_z) = \frac{C_z\, e^{-\lambda_z t_z}}{(\lambda_z)^{k+1}} \quad (8)$$

or

$$PAUK_k(t_z) = \frac{y(t_z)}{(\lambda_z)^{k+1}} \quad (9)$$

where t_z means the time value of the last observation y_z of the concentration time data and $PAUC_k(t_z)$ the areas under the curves from this time onward.

2.4. Excretion data

The second curve in Fig. 1 can also be regarded as "amount of drug not eliminated from the system at time t" if the ordinate is rescaled by multiplication with the total clearance [3, 14]. The intercept of $PAUC_o$ then reads the total amount, i.e. $CL_{tot}\, AUC = CL_{tot}\, PAUC_o(0)$.

Cumulative excretion data or cumulative amounts released from a solid dosage form can always be expressed in this way which means that the above algorithm can be used from the second step onwards [1, 6, 11] (see also Application and results).

3. Application and results

The new approach in calculating statistical moments is demonstrated with data of a sustained release formulation of theophylline (Solosin® retard[2]). The release from this formulation was observed in both an in vivo study and an in vitro dissolution test. The Sartorius® dissolution model[3] was used in the in vitro dissolution test.

The statistical analysis is first demonstrated with the in vitro data: Table 1 gives the time values (column 2) at which a certain amount (column 3) of drug is still held in the formulation, i.e. is not released from the dosage form, in fractional units of the labelled amount.

As already discussed in the theoretical part of this paper the calculation of the $PAUC_o$-values is omitted since the first integration procedure gives the $PAUC_1$-values. This integration starts with the data pairs indexed by 24 and 23 and runs down to index 1. Every two data pairs are integrated by the trapezoidal rule and the result is added to the successive $PAUC_1$-value in column 4.

$$PAUC_1(t_i) = PAUC_1(t_{i+1}) + \frac{M_i + M_{i+1}}{2}\,(t_{i+1} - t_i)$$

An extrapolation is not neccessary since the release was monitored up to complete liberation of the drug. The results of the first reverse integration (column 4) form the basis of the second integration which gives the fifth column of Table 1.

The statistical moments are calculated by means of equation (6), with a simple modification: $PAUC_o(0)$ is substituted by the amount not dissolved at time zero M (0):

Table 1: The observations from an in vitro dissolution test are listed. The liberation from a sustained release formulation of theophylline are examined using a Sartorius dissolution model. The second column gives the time t_i at which a certain percentage of the labelled content (3rd column) is not yet released from the dosage form. For the calculation of the prospective areas under the curve $PAUC_k(t_i)$ see text.

Index	Time t_i [h]	Amount M_i not released [%]	$PAUC_1$ (t_i) [% h]	$PAUC_2$ (t_i) [% h²]
1	0.0	100.00	343.94	992.89
2	0.5	84.84	297.72	832.48
3	1.0	76.93	257.28	693.72
4	1.5	67.20	221.25	574.09
5	2.0	58.98	189.71	471.35
6	2.5	51.46	162.09	383.40
7	3.0	46.66	137.56	308.48
8	3.5	42.50	115.27	245.27
9	4.0	36.17	95.61	192.55
10	4.5	31.32	78.73	148.97
11	5.0	26.99	64.15	113.24
12	5.5	23.86	51.44	84.34
13	6.0	19.57	40.59	61.34
14	6.5	16.35	31.61	43.29
15	7.0	14.15	23.98	29.39
16	7.5	11.60	17.54	19.01
17	8.0	9.57	12.25	11.56
18	8.5	7.19	8.06	6.48
19	9.0	5.59	4.87	3.25
20	9.5	3.40	2.62	1.38
21	10.0	2.54	1.13	0.44
22	10.5	0.87	0.28	0.086
23	11.0	0.13	0.03	0.008
24	11.5	0.00	0.00	0.00

Table 2: The observations from an in vivo study are listed. A sustained release formulation of theophylline was administered to healthy volunteers. The second column gives the time t_i at which a certain concentratin of drug y_i (3rd column) is monitored, mean values are listed. For the calculation of the prospective areas under the curve $PAUC_k(t_i)$ see text.

Index	Time t_i [h]	Conc. y_i [µg/ml]	$PAUC_0$ (t_i) [µg h/ml]	$PAUC_1$ (t_i) [µg h²/ml]	$PAUC_2$ (t_i) [µg h³/ml]
1	0.00	0.00	71.13	1094	13 350
2	0.25	0.53	71.06	1077	13 080
3	0.50	0.82	70.89	1059	12 810
4	0.75	1.13	70.65	1041	12 550
5	1.00	1.31	70.35	1023	12 290
6	1.50	1.72	69.59	9.88.5	11 790
7	2.00	2.07	68.64	953.9	11 300
8	4.00	3.56	63.01	822.3	9 528
9	6.00	3.74	55.71	703.6	8 002
10	9.00	3.17	45.35	552.0	6 119
11	12.00	2.63	36.65	429.0	4 647
12	15.00	2.22	29.37	330.0	3 509
13	23.00	1.41	14.85	153.1	1 577
14	24.00	1.31	13.49	138.9	1 431

$$MT_{diss.\,vitro} = m_1 = 1! \frac{343.94}{100}$$

$$= 3.439\ [h]$$

$$m_2 = 2! \frac{992.89}{100}$$

$$= 19{,}858\ [h^2]$$

$$VT_{diss.\,vitro} = \mu_2 = m_2 - (m_1)^2$$

$$= 8.031\ [h^2]$$

The concentration time data of the same formulation when administered to healthy volunteers are listed in Table 2 (columns 2 and 3). In this case an extrapolation from the last datum onward is appropriate. The terminal slope was estimated from concentration time data when a oral solution was administered to the same volunteers ($\lambda_z = 0.0971\ h^{-1}$). (Details on this clinical trial are given in [15].)

The following extrapolations were calculated with equation (9):

$$PAUC_k\,(24\ h) = \frac{1.31}{(0.0971)^{k+1}}$$

These values appear in the 14th row of Table 2 as columns 4 to 6. First the $PAUC_o$ values are calculated (column 4),

[2] Manufacturer: Cassella-Riedel Pharma GmbH, Frankfurt/Main (Federal Republic of Germany).

[3] Fa. Sartorius, Göttingen (Federal Republic of Germany).

once again using the trapezoidal rule, while the extrapolation (14th row) is treated as a reading. From the $PAUC_o(0)$ value it can be read that the extrapolation $PAUC_o(24h)$ amounts to 19% of the total. The reverse integration is then applied to the $PAUC_o$-data generated previously and results in the $PAUC_I$-values listed in the fifth column. The same procedure gives the values in the sixth column.

Using equation (6) the moments are calculated as follows:

$$MT_{sys} = m_1 = 1! \frac{1094.}{71.13}$$
$$= 15.38 \text{ [h]}$$
$$m_2 = 2! \frac{13350.}{71.13}$$
$$= 375.37 \text{ [h}^2\text{]}$$
$$VT_{sys} = \mu_2 = m_2 - (m_1)^2$$
$$= 138.82 \text{ [h}^2\text{]}$$

where MT_{sys} is the mean and VT_{sys} is the variance of the residence times in the system, including the liberation of the drug from the formulation, possible transport, the absorption, the distribution within and elimination from the body.

The total mean transit time of the system is the sum of the mean in vivo dissolution time $MT_{diss.vivo}$ and the mean residence time including transport and the absorption process MT_{oral}. The mean residence time of the solution MT_{oral} was calculated as 10.36 [h] ($\cong$ 1/0.0971 [h^{-1}]), and is hardly influenced by the absorption process. The difference between the mean time, when giving theophylline sustained release formulation, MT_{sys}, and the mean time when given as a solution, MT_{oral}, is the mean in vivo dissolution time.

$$MT_{diss.vivo} = MT_{sys} - MT_{oral}$$
$$= 15.38 - 10.36 \text{ [h]} = 5.02 \text{ [h]}$$

The mean in vivo dissolution time is 1.46 times the mean in vitro dissolution time $MT_{diss.vitro}$.

4. Discussion

The formulation of statistical moments related to the residence times of drug molecules based on the total amount released from a system is consistent with this term as defined by mathematical statistics, regardless of wether the system has linear or nonlinear characteristics [11, 12]. In contrast, the formulation of moments by means of the concentration profile is restricted to linear kinetics with elimination occurring from the compartment monitored [12].

The proposed algorithm is always applicable to in vitro dissolution data. It can be used for urinary excretion data even if the drug is eliminated via other routes at the same time provided that the kinetics of the drug is linear with dose [1, 3, 12].

The numerical differentiation of urinary data before evaluation of the statistical moments, as proposed by Yamaoka et al. [2] is redundant.

Provided that the concentration time course is a sample of the probability density, the new algorithm using the numerically developed PAUC data is an easily performable and consequently easily programmable approach to the estimation of statistical moments of residence times. If extrapolation – generally by a single exponential – seems neccessary to improve the estimates of the moments, the slope of the extrapolation remains valid for all PAUC data of any order "k". This facilitates fast computation of the extrapolations (see equ. 8 and 9). Whether such extrapolation is valid has to be judged carefully, as indeed is always the case when adjusting models to data. In contrast, neither the first moment curve nor any higher moment curve as defined or used by other authors demonstrates a log-linear phase. As a consequence the use of the log-trapezoidal rule for the numerical integration of these curves as suggested by Benet and Galeazzi [5] and recently by Gouyette [16] is – to be theoretically correct – not adequate. Furthermore the extrapolation formula of these curves are of increasing complexity (see [2, 16, 17]). Both disadvantages are avoided with the algorithm introduced by this paper.

As outlined by von Hattingberg et al. [1], Cutler [7] and Yamaoka et al. [2] the mean invasion time – encompassing release and absorption – is a reasonable variable for the measurement of the "rate of bioavailability". Since the absorption of theophylline is fast the mean in vivo dissolution time (5.02 h) hardly differs from the mean invasion time (5.32 h) [15]. The statistical approach allows common handling of in vivo and in vitro data based on a common interpretation [12]. This leads to new insight into the connection of in vitro and in vivo dissolution [9, 10, 12, 18, 19]. The time scaling of in vitro dissolution profiles toward the in vivo process is one of the basic steps in the prediction of concentration time profiles, i.e. continuous in vivo in vitro correlation. A similar approach i.e. the calibration of in vitro dissolution experiments with respect to the in vivo liberation process was discussed by Levy in 1967 [20].

The prediction can be performed by one of the recently proposed convolution algorithms (for details and further references see [21, 22]).

The facilitated evaluation of statistical moments – which are used to obtain the above-mentioned calibration [18, 19] – has been outlined in detail by this paper to encourage the analysis of in vitro dissolution data with respect to their in vivo significance. These data are readily available and do not require additional studies.

5. References

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