Correction for Non-Ideal Tracer Pharmacokinetic Disposition by Disposition Decomposition Analysis (DDA)

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Purpose. Pharmacokinetic (PK) studies assume that the tracer's PK is equivalent to the parent compound. This assumption is often violated. The aim of this work is to present a method enabling the ideal tracer PK, i.e. the PK of the parent compound, to be predicted from the non-ideal tracer.

Methods. The procedure uses a disposition decomposition-recomposition (DDR) that assumes that the labeling mainly changes the elimination kinetics while the distribution kinetics is not significantly affected. In the DDR procedure an elimination rate constant correction factor (k_{COR}) is determined from a simultaneously fitting to plasma concentration data resulting from an i.v. injection of both the tracer and the parent compound. The correction factor is subsequently used to predict the ideal tracer PK behavior from the disposition function (i.v. bolus response) of the non ideal tracer.

Results. The DDR method when applied to plasma level data of erythropoietin (r-HuEPO) and its iodinated tracer (^{125} I-r-HuEPO) from a high (4000U/kg) and a low (400U/kg) dosing of r-HuEPO in newborn lambs (n = 13) resulted in excellent agreements in the elimination rate corrected dispositions in all cases (r = 0.995, SD = 0.0095). The correction factor did not show a dose dependence ($p > 0.05$). The correction factors were all larger than 1 (k_{COR} = 1.94, SD = 0.519) consistent with a reduction in the EPO elimination by the iodination labeling.

Conclusions. The DDR tracer correction methodology produces a better differentiation of the PK of endogenously produced compounds by correcting for the non-ideal PK behavior of chemically produced tracers.

KEY WORDS: drug tracer; labeling; pharmacokinetics; erythropoietin; iodination.

INTRODUCTION

The pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of biotechnology-produced biopharmaceuticals is confounded by the unknown endogenous production. Tracers obtained by appropriate labeling of the compounds can overcome this problem and enable the disposition kinetics to be determined without being confounded by endogenous production.

Tracer-based PK analysis is based on the assumption that the tracer behaves kinetically identical to the parent compound. The assumption of PK equivalence may be significantly violated as a result of labeling techniques that result in chemical differences between the labeled molecule and the parent molecule (1). Virtually all tracer kinetic studies have either inadequately addressed or ignored the PK equivalence issue, thus jeopardizing the reliability of the tracer kinetic analysis.

The purposes of this communication are: 1.-to briefly discuss the need for a tracer in PK research involving endogenous substances and propose a method for correcting for the non-ideal PK behavior of a tracer, and 2.-to demonstrate the method using as an example the recombinant human hormone erythropoietin (r-HuEPO) and its ^{125}I labeled tracer administered intravenously to newborn lambs.

MATERIALS AND METHODS

Animals

Approval of these studies in newborn lambs was received by the local institutional animal care and review committee. The lambs were cared for alongside their mothers in an indoor, light and temperature controlled environment in which lambs were free to nurse *ad libitum*. All animals were deemed in good health at the time of the study. All lambs were less than 2 weeks of age at the time of the study. The body weight of the group of lamb receiving the 4,000 U/kg dose (n = 7) and the 400 U/kg dosing group (n = 6) were 5.5 ± 0.34 and 6.4 ± 0.76 kg respectively (mean ± *SE*). The same amount of ^{125}I-rhEPO tracer was administered at both dose levels.

Assays

The iodination, radioimmunoassay and immunoprecipitation assays for EPO were done as previously reported (2).

Pharmacokinetic Analysis

Study Protocol in Lambs

Percutaneously placed jugular venous catheters used for sampling and infusion were secured in protective bandages wrapped around the lamb's neck. Doses of 400 or 4,000 U of r-HuEPO was administered by intravenous bolus injection over 15 to 30 seconds followed immediately thereafter by an i.v. bolus injection of 200,000 cpm/kg ^{125}I-r-HuEPO over the similar time period. Each lamb was studied only once. A 6 to 7 hour study period was selected based on our previous pharmacokinetic studies in sheep (3). During this period, 15 to 20 individual blood samples were taken. This provided a sufficient number of data points for accurate determination of the disposition kinetics. The amount of blood sampled and not returned was less than 1% of the estimated blood volume. Plasma was separated by centrifugation and erythrocytes reinfused at frequent intervals.

Kinetic Analysis

The disposition decomposition analysis (DDA)(4,5,6,7) differentiates the disposition function (i.v. bolus response, c) according to the following convolution type integrodifferential equation:

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$$c' = -q(c) + h * c \quad (1)$$

where * denotes convolution and $c' = dc/dt$. In eq. 1 the disposition function is decomposed into two fundamental components, namely an elimination function, q(c), and a distribution function, h(t). For drugs with a first order central elimination eq. 1 becomes:

$$c' = -(AUDC + k)c + h * c \quad (2)$$

where AUDC is the total area under the distribution function:

$$AUDC \equiv \int_0^\infty h(t)dt \quad (3)$$

In eq. 2 k is the first order elimination rate constant that is related to the drug clearance, Cl, via the volume of distribution: $Cl = Vk$.

Consider a tracer that is produced as a chemical modification of a drug (biocompound) that has a disposition described by eq. 2. The chemical modification (denoted by CM subscript in the following derivations) may result in a change in the elimination kinetics, i.e. a change in k (eq. 2) from k to k_{CM}. The disposition kinetics of the tracer is then given by:

$$c'_{CM} = -(AUDC + k_{CM})c_{CM} + h * c_{CM} \quad (4)$$

where:

$$h(t) = L^{-1}\{[(s - c'_{CM}(0)/c_{CM}(0))L\{c\} - c_{CM}(0)\,]/L\{c_{CM}\}\} \quad (5)$$

and:

$$k_{CM} = c'_{CM}(0)/c_{CM}(0) - AUDC \quad (6)$$

In eq. 6 $L\{\}$ and $L^{-1}\{\}$ denote the Laplace and inverse Laplace transform operator respectively. If it is assumed that there is no appreciable change in the distribution kinetics (h), the distribution functions (h) in eqs.2 and 4 become identical.

Computational Procedures

The disposition function, c_{CM}, for the drug tracer (^{125}I-r-HuEPO) is estimated by least squares nonlinear regression using a simple two exponential function:

$$c_{CM} = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} \quad (7)$$

According to eq. 4 equation 7 can be decomposed to determine both the distribution function, h, and the elimination rate constant k_{CM} according to eqs. 5 and 6 as previously described (4). Thus, the ideal trace elimination rate constant, k, is related to the perturbed elimination rate constant, k_{CM} of the non ideal tracer through the correction factor k_{COR}:

$$k = k_{COR}\, k_{CM} \quad (8)$$

The distribution function, h, and the first order rate constant, k (treating k_{COR} as an unknown parameter) is inserted into eq. 2 that is then solved via inverse Laplace transformation as previously described (4). This gives the disposition function for the ideal tracer i.e., the disposition function for the non labeled drug, as:

$$c = k_D c_{CM}(0)L^{-1}\{\,1/(s + AUDC + k - L\{h\}\,)\} \quad (9)$$

where:

$$k_D = D / D_{CM} \quad (10)$$

and resulting in:

$$c = B_1e^{-\beta_1 t} + B_2e^{-\beta_2 t} \quad (11)$$

The parameter k_D in eq. 9 is introduced to account for the different doses of the drug (D) and the tracer (D_{CM}). Equations 7 and 11 were simultaneously fitted to the tracer (^{125}I-r-HuEPO) and the drug (r-HuEPO) plasma concentration data respectively. The four B and β parameters in eq. 11 are uniquely calculated from k_{COR} and the four parameters of eq. 7 (4). The calculation of these 4 parameters defining the ideal tracer disposition function was done repeatedly during the simultaneous fitting process as required. Curve fittings were done using the general nonlinear regression program FUNFIT written to run under Windows (Microsoft Corp., Seattle, WA) (8).

An AUC-Based Alternative Approach

The total integration (zero to infinity) of eq. 2 results in the well-known noncompartmental expression for the elimination rate constant:

$$k = c(0)/AUC \quad (12)$$

Thus, the correction factor can alternatively be calculated by the "AUC method" according to the following formula:

$$k_{COR} = [c(0)/AUC \text{ for } 125\text{I-r-HuEPO}]/[c(0)/AUC \text{ for r-HuEPO}] \quad (13)$$

RESULTS AND DISCUSSION

Using r-HuEPO as an example, the effect of the labeling can be summarized in simple molecular probability terms as follows: For the same arbitrary concentration of the drug and the tracer the drug's molecular probability of elimination is k_{COR} times larger than that of the tracer. Thus, k_{COR} can be given a very simple interpretation in the tracer *vs.* non tracer comparison.

The simultaneous fitting of eqs. 7 and 11 showed excellent agreement with the tracer and non tracer plasma level data with a mean correlation coefficient of 0.9946, SD = 0.00945 (Table 1). A representative example of a simultaneous fit is presented in Fig. 1. In all 13 lambs the elimination correction factor, k_{cor} was larger than 1 (mean 1.94, SD 0.52), thus indicating that the parent drug (r-HuEPO), i.e. the ideal tracer has a greater elimination rate constant than the non ideal tracer. There was no statistically significant difference in the correction factor between the high (mean 1.84, SD 0.20) and low (mean 2.06, SD 0.79) dosing (t-test, $p > 0.05$). The finding that a correction in the elimination kinetics alone produces excellent fits indicates that the ^{125}I labeling predominantly affects the elimination of the ^{125}I-r-HuEPO tracer and has no significant effect on the distribution kinetics. The results also demonstrate that the correction of the elimination kinetics can accurately be done both at the low (400 U/kg) and the high (4000 U/kg) dosings. Lamb

Table 1. Tracer Kinetics Correction Factor (k_{cor}) and Disposition Function Parameters Determined by Simultaneous Fitting of Eqs. 7 and 10 Using the Disposition Decomposition-Recomposition (DDR) Method

	Correction factor k_{cor}		^{125}I-r-HuEPO					r-HuEPO					
	Method		A_1	A_2	α_1	α_2	k_{CM}[a]	B_1	B_2	β_1	β_2	k[b]	Correlation Coefficient
Subject	DDA	"AUC"[c]	cpm/ml	cpm/ml	1/hr	1/hr	1/hr	mU/ml	mU/ml	1/hr	1/hr	1/hr	r
1	2.02	18.5	37.9	71.0	0.149	1.09	0.341	47.6	61.4	0.00814	0.904	0.0184	0.9992
2	1.76	1.80	33.9	72.7	0.209	1.06	0.462	45.9	60.7	0.135	0.803	0.257	0.9992
3	1.72	1.75	48.9	54.7	0.288	1.52	0.503	57.7	45.8	0.179	1.237	0.288	0.9987
4	1.74	1.77	38.6	65.0	0.189	1.11	0.394	47.9	55.6	0.119	0.896	0.223	0.9971
5	2.20	2.21	29.7	72.5	0.168	1.22	0.433	40.4	61.8	0.0879	0.974	0.195	0.9989
6	1.84	1.88	39.1	63.5	0.24	1.16	0.471	51.1	51.5	0.145	0.895	0.250	0.9987
7	1.60	1.63	43.9	60.2	0.143	0.944	0.281	50.7	53.3	0.0948	0.792	0.173	0.9910
8	2.21	2.21	56.2	87.5	0.481	6.10	1.10	63.7	80.0	0.231	5.52	0.495	0.9922
9	3.45	3.48	8.36	101	0.0371	0.483	0.252	19.5	89.6	0.0160	0.310	0.0724	0.9644
10	2.01	2.01	68.2	72.5	0.487	8.51	0.947	72.4	68.3	0.249	8.03	0.470	0.9962
11	1.44	8.22	43.7	63.3	2.41	3.21	2.83	67.0	40.1	0.229	2.13	0.344	0.9991
12	1.40	1.41	63.9	43.1	0.3	2.43	0.464	67.1	39.9	0.218	2.16	0.328	0.9977
13	1.86	1.10	82.8	59.6	0.488	11.7	0.815	85.1	26.6	0.573	11.3	0.740	0.9972
Mean	1.94	3.69	45.8	68.2	0.430	3.12	0.714	55.1	56.5	0.176	2.77	0.296	0.9946
SD	0.519	4.81	18.9	14.5	0.612	3.49	0.685	16.5	16.9	0.143	3.40	0.190	0.00945

Note: Lamb 1-7 were given 4000U/kg r-HuEPO and lamb 8-13 received 400U/kg.

[a] $k_{CM}=c(0)/AUC=(A_1 + A_2)/(A_1/\alpha_1 + A_2/\alpha_2)$.

[b] $k=c(0)/AUC=(B_1 + B_2)/(B_1/\beta_1 + B_2/\beta_2)$.

[c] k_{cor} is calculated by this method as the ratio: [c(0)/AUC for ^{125}I-r-HuEPO]/[c(0)/AUC for r-HuEPO] defined in (a) and (b).

no. 9 showed an unusually large k_{cor} value that likely is caused by a poor estimation of the disposition as reflected in the low correlation coefficient caused by several abnormal data points early in the study.

The elimination kinetics correction is described simply by a single parameter namely the correction factor, k_{COR}. This factor allows the ideal tracer kinetic behavior to be predicted from the disposition function, c_{CM}, of the non ideal tracer. The procedure for the prediction of the ideal tracer kinetic behavior can be characterized as an elimination correction by a disposition decomposition-recomposition (DDR). In the first step of the DDR the disposition function of the tracer, c_{CM}, (eq. 7), determined by a regular (non simultaneous) curve fitting to plasma data from an i.v. bolus dose of the tracer, is decomposed according to eq. 4. The k_{CM} determined in this way is then replaced by the "ideal" k value, namely k (= k_{COR} k_{CM}),using a correction factor, k_{COR}that has been previously determined as described above. In the second step of the DDR the disposition functions of the ideal tracer i.e., the drug, is predicted following a recomposition according to eqs. 9–10.

The AUC approach (eqs. 12.13) gave a mean k_{cor} value considerably larger than the DDA approach (3.69 vs. 1.94 Table 1). The larger mean value is mainly due to the two large k_{cor} values for subjects 1 and 11 that likely result from a less precise determination by this method, as evident by a larger variability

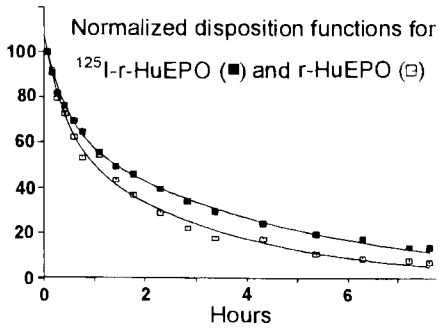

Fig. 1. A representative plot of a simultaneous fitting of eqs. 7 and 9 to plasma level data from an i.v. bolus injection of ^{125}I-r-HuEPO tracer and r-HuEPO (lamb 12). The fits and data have been superimposed to better illustrate the difference in the disposition function of the tracer and the parent drug. This was done by a normalization scaling resulting in the same curve value (100) at the time of the first observation.

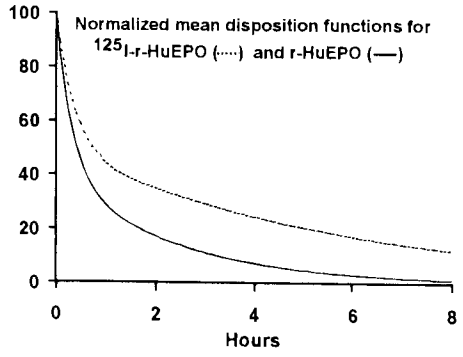

Fig. 2. Normalized mean disposition functions for 125I-r-HuEPO and r-HuEPO calculated from the mean parameters given in Table 1.

(SD 4.81 vs. 0.519 Table 1) that is statistically significant (F-test, $p < 0.01$). The larger variability is to be expected by the AUC method because the extrapolation required to determine the AUC can be quite substantial. In contrast the DDA method does not make use of extrapolations beyond the last data points and thus avoid this error prone problem. The mean k_{COR} value for the AUC method obtained by excluding the two large observations from subjects 1 and 11 is 1.93 which is very close to the DDA derived valued (Table 1) consistent with the fact that mathematically the DDA and AUC derived parameters are identical.

The tracer correction can also be done by compartmental means by fitting the same compatmental model to the tracer and non tracer data assuming only a difference in the central elimination kinetics (k). The DDA method was chosen in this work because it is based on fewer assumptions and is a more general approach (it covers compartmental systems as a special case). For example, the DDA approach is not restricted to disposition functions where all exponential terms are positive (characteristic of linear compatmental kinetics), physiologically relevant circulatory disposition models not covered by classical linear compartmental models are also considered.

Bauer et.al. (1) clearly demonstrated that iodination of proteins significantly alter their PK behavior. The effect was found to be greatest for small proteins i.e. <60kDa. The size of r-HuEPO is 34kDa (9). It was also found that the oxidant itself used in the iodination process (Chloramine-T) induces changes in the PK. By using the less harder iodinated Bolton-Hunter iodination reagent (which avoids exposing the protein to oxidant), the change in the PK may be reduced. The PK effect of iodination increases with increased degree of oxidation. Chemical labeling with agents such as iodine may change the elimination and/or the distribution kinetics.

The distribution kinetics is primarily determined by binding, partitioning and dynamic drug transport. Small changes in the binding of a poorly bound drug will not have much effect on its distribution kinetics. This appears to be the case for EPO since it has not been shown to have significant plasma protein binding (9). Accordingly a mild degree of iodination of EPO should not affect its PK very much through changes in binding.

The exact reason why the iodination process reduces EPO's plasma elimination (Fig. 1) is unclear. The addition of large iodine atoms, or changes resulting from the preparations steps (e.g. oxygenation) may have a protective effect against enzymatic degradation and accordingly reduce the elimination. This decrease in EPO elimination appears best explained by reduced receptor affinity caused by molecular changes that result in a poorer fit or linkage of the tracer molecules to the EPO receptors. There is support of this speculation from several lines of evidence that indicate that endocytosis via EPO receptors on erythroid progenitor cells is an important route of elimination for EPO (10).

The tracer correction method using the DDA approach can be applied to drugs with a linear or nonlinear elimination kinetics. In the nonlinear case the correction becomes more complex since the disposition difference will then typically involve two parameters. Two parameters are required to describe the difference in the kinetics. The nonlinear case is more complex to deal with since a nonlinear elimination process typically involve a modeling with two parameters (e.g. v_m and k_m for a Michaelis-Menten process) requiring two correction/conversion factors in contrast to a single one in the linear case. In the nonlinear case it may be very difficult to differentiate the kinetic difference into two parameters especially when, as in the present case, only a single dose is given to each subject. We have shown that EPO exhibit nonlinear elimination kinetics, but were unable to differentiate the tracer difference beyond a single correction factor. This is not a limitation of the method but rather due to a less than optimal experimental design. Thus, the present EPO analysis is a linear approximation to a nonlinear case, but appears to be an excellent approximation judged from the high correlation coefficients. More optimal experiments involving multiple, different dosings within the *same* individuals is the subject of future investigations. The two different doses in the present case were of little use in resolving the nonlinearity because each subject received only one dose and inter-individual differences masked the nonlinear dose effect.

It is possible that the tracer and non tracer may differ also in respect to the volume of distribution. For drugs such as EPO which is not highly bound such differences may be small in comparison to the difference in the elimination kinetics. No significant difference in the volume was observed between the tracer and non tracer, accordingly the simultaneous fits were done using the same volume of distribution for the tracer and non-tracer. In any case a correction for difference in the volume of distribution is trivial and is readily done through a volume correction/conversion factor in the simultaneous curve fitting.

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