Just Say No to Postmortem Drug Dose Calculations

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Abstract

For years, a number of professional groups have warned forensic and clinical toxicologists against calculating an administered dose of a drug based on postmortem blood drug concentrations. But to date there has been limited information as to how unreliable these dose calculations may actually be. Using amitriptyline as a model drug, this study used empirically determined pharmacokinetic variables for amitriptyline from clinical studies coupled with clinical overdoses (where the individual survived), and death (ascribed to amitriptyline toxicity) case studies in which the dose of amitriptyline was known. Using these data, standard pharmacokinetic equations and general error propagation it was possible to estimate the accuracy of calculated doses of amitriptyline, compared to the doses that were consumed. As was expected in postmortem cases, depending on the pharmacokinetic equation used, the accuracy (mean +128 to +2347 %) and precision (SD ± 383 to 3698%) were too large to allow reliable estimations of the dose of amitriptyline consumed prior to death based on postmortem blood drug concentrations. This work again reinforces that dose calculations from postmortem blood drug concentrations are unreliable.

KEYWORDS

post-mortem, dosage calculation uncertainty, forensic toxicology, pharmacology, amitriptyline

HIGHLIGHTS

- Estimation of drug dosage from postmortem blood concentration theoretically possible
- Amitriptyline pharmacokinetics information and case studies used to determine uncertainty in dose calculations
- Large differences in estimated dose compared to actual dose identified
- Drug dosage estimations from postmortem blood drug concentrations should not be carried out

It is common for forensic or clinical toxicologists to be asked by attorneys or law enforcement personnel to estimate the dose, or amount of a drug that may have been taken by or administered to a deceased individual prior to their death. The forensic toxicology community has long known that the pharmacokinetic equations used to calculate the dose of a drug from measured drug concentrations in blood collected from a living individual are not able to be performed when the blood is collected from a deceased individual (1). Thus guidelines from professional organisations (e.g. Society of Forensic Toxicologists (SOFT) (2), AAFS Standards Board (ASB) (3) and United Kingdom Association of Forensic Toxicologists (UKIAFT) (4)) recommend against performing these calculations. However, to date there has been no published proof as to how unreliable these calculations may actually be. This study estimated the accuracy and precision that may arise in performing postmortem pharmacokinetic dose calculations by using published clinical and case study data for an example drug, amitriptyline, and general error propagation methodology (5). This work was also able to determine the contribution of each of the variables to the overall uncertainty of the estimate of the dose taken.

Standard Pharmacokinetic Equations

The most common pharmacokinetic equation for the calculation of drug dose (assuming equilibrium and complete absorption) is: -

$$D = Wt \cdot V \cdot C \qquad \text{or} \qquad D = V \cdot C \tag{1}$$

D = dose of drug (at the time of collection of plasma/blood) (mg), Wt = body mass (weight) (kg), C = drug plasma/blood concentration (mg/L) and V = volume of distribution (L/kg) or volume of distribution (L). Although this calculation will only

estimate the drug's dose that corresponds to the drug concentration in the blood sample, it is a snapshot in time and does not give the actual dose of the drug administered.

Calculation of Dose after Intravenous Administration

To determine the actual dose of drug administered, the time elapsed since the dose was administered (t) and the drug's half-life (t½) would also need to be known. For an intravenous bolus dose of the drug, the equation is: -

$$D = Wt \cdot V \cdot C \cdot e^{k.t} \quad (2)$$

k = elimination rate constant (h^{-1}), k = $\frac{0.693}{t½}$, t½ = half-life (h), t = time since administration of dose (h).

However, this equation assumes that the pharmacokinetics of the drug fit a one compartment model. In the one compartment model the drug is assumed to be instantaneously distributed throughout the body into a single theoretical compartment. In reality, it is more common for a drug to follow a two-compartment model where there is an initial (α) distribution of the drug from the "first" theoretical compartment (commonly considered to be the circulation) to a "second" theoretical compartment (commonly considered to be the tissues). The α distribution phase is followed by a second β distribution phase where there is equilibrium between the two compartments and elimination is the predominant factor.

The dose calculation for a drug administered by an intravenous bolus that fits a two-compartment pharmacokinetic model is: -

$$D = C \cdot Vd \cdot Wt \cdot (\alpha - \beta) \cdot \left[(\alpha - k_{21}) \cdot e^{-\alpha.t} + (k_{21} - \beta) \cdot e^{-\beta.t}\right]^{-1} \quad (3)$$

When the drug is infused, a revised equation is used:

$$D = C \cdot (((V \cdot Wt) \cdot \beta)/Wt) \cdot Wt \cdot (\alpha - \beta) \cdot \left[(\alpha - k_{21}) \cdot e^{-\alpha.t} + (k_{21} - \beta) \cdot e^{-\beta.t}\right]^{-1} \quad (3a)$$

α = elimination rate constant of "distributive" alpha phase (h^{-1})

β = elimination rate constant of "elimination" beta phase (h^{-1}) [NOTE: This is equivalent to k in equation 1].

K_{21} = first-order transfer rate constant from the peripheral compartment to the central compartment (h^{-1})

Calculation of dose administered after oral administration

If the drug is orally administered, the dose calculation is different as the bioavailability (the amount of drug administered that reaches the systemic circulation) needs to be considered. Assuming the drug has been completely absorbed the equation is: -

$$D = \frac{Wt \cdot V \cdot C \cdot e^{k.t}}{F} \quad (4)$$

F= Bioavailability (no units)

However, the complexity is increased if absorption is not complete. In this case, the rate constant for absorption (K_a) (h^{-1}) is needed giving the equation: -

$$D = \frac{Wt \cdot V \cdot C \cdot (K_a - K)}{F \cdot K_a \cdot (e^{-kt} - e^{-K_a \cdot t})} \quad (5)$$

Determining the precision and accuracy of postmortem dose calculations

To investigate the accuracy and precision of the estimation of the dose of a drug based on a postmortem blood concentration, an example drug, amitriptyline, was selected. There is a large amount of clinical data on amitriptyline's pharmacokinetic parameters. Additionally, there are case studies of the doses that were taken in overdoses or amitriptyline-attributed deaths, along with the relevant blood amitriptyline concentrations in those cases.

Pharmacokinetic Variables of Amitriptyline

A literature search was carried out to identify studies with empirically determined pharmacokinetic parameters of amitriptyline using the search engines PubMed and Scopus (06FEB21 and 07FEB21). Relevant citations within the articles found during the search were also included in the study. Only papers that contained empirically determined amitriptyline pharmacokinetic parameters from human subjects listed in equation 1 - 5 were included. The data obtained from the publications were compiled in Microsoft Excel 2015 (Microsoft Corporation. Redmond. USA). In order to determine if the pharmacokinetic data collected from previously published studies were normally distributed a D'Agostino & Pearson Omnibus Normality test was carried out using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla California USA). A p value of ≤0.05 was considered significant. Only the half-life (t½) and its linked variable, the elimination rate constant (β), were found to not follow normal distribution (data not shown). Table 1 lists the mean, mode, standard deviation (SD), range and coefficient of variance (%CV) that were used for the "average" individual for the calculation of uncertainty and accuracy of the administered dose using equations 1 - 5. In one study (Burch and Hullin (6)) the raw data of the blood concentration versus

time was available and these data were used to determine the variables required for equations 1 - 5 using the Excel plugin PKsolver 2.0 (7).

Uncertainty in dose calculations

The aim of this study was to: a) estimate the overall precision of the calculation of dose from a postmortem blood drug concentration using the various relevant pharmacological equations; b) estimate the accuracy of the calculations of dose using pharmacokinetic equations; and c) estimate the contribution of each of the variables in the pharmacokinetic equations to the overall uncertainty of the calculation of dose from postmortem blood drug concentrations. This was completed using the a) average (mean) pharmacokinetic parameter data from primary literature sources (see Table 1); b) from pharmacological studies where the dose administered was known and c) from case studies (of both overdoses where the individual survived and deaths (attributed to amitriptyline overdose). For case studies to be included in this investigation, it was important that they included a reasonable estimate of the dose of amitriptyline that was taken (Tables 2 and 3).

Methodology

The accepted method to determine the uncertainty associated with calculations, is that of general error propagation using GUM principles (5). The GUM principles have a sound mathematical basis and have previously been used forensically for alcohol calculations using the Widmark equation (8–10). Detailed information about this method of error calculation can be found in (11, 12). In order to estimate the uncertainty associated with the various dose calculation equations and the proportional contribution of each variable to the total uncertainty GUM Workbench

EDU Version 2.4.1.384 (Metrodata GmbH) was used. It was assumed that all the variables were independent and that each of the variables were normally distributed. The relevant equations (based on the case circumstances (e.g. clinical; overdose or death) were entered into the software along with the relevant data from Table 1, 2 or 3. To calculate the overall uncertainty of these data and the contribution of each of the variables to the uncertainty, a randomly selected individual in each case series was selected. For the intravenous administration of amitriptyline (15 mg) subject AJ was used from (13). For the oral administration of amitriptyline (100 mg) subject DB from (6) was used. As there were only oral overdoses and deaths, the uncertainty estimations focused on those calculations that were suitable for oral administration for these types of cases. Subjects 2 and 3 from study (14) for overdose were used and subjects 9 and 10 from study (15) were used for the death cases. The ratio of amitriptyline to nortriptyline (the major metabolite of amitriptyline) was used to determine if the subject was in the elimination phase or the absorptive phase to allow selection of the relevant pharmacokinetic equation. Based on the work of Bailey & Shaw (16) and Hebb *et al.,* (17) this study considered a ratio of amitriptyline to nortriptyline of < 1.5 to be in the elimination phase and > 1.5 to be in the absorptive phase. The accuracy of the calculated dose was determined using the following equation:

$$\%Accurracy\ (bias) = \left(\frac{Mean\ calculated\ dose - dose\ adminstered}{dose\ administered}\right) \times 100 \qquad (6)$$

Accuracy and precision of dose calculations from drug blood concentrations

As can be seen in Figure 1, on average for clinical and overdose cases, Equation 1 was the most accurate and precise, although there was a mean underestimation of the dose administered (approx. -0.6 to -82 %). As expected, due to the increased complexity of Equations 4 and 5 ((that require additional factors such as half-life (t½), bioavailability (F), time since administration (t) and absorption rate constant (K_a))) on average these equations were less accurate (+23.1 to +549.6%) than Equation 1. Again Fig 1. shows that when the pharmacokinetic Equations 1, 4 and 5 were used to estimate the consumed dose in deaths attributed to amitriptyline toxicity there was a very large overestimation (+127.6 % and +2346.0 %) of the dose that was taken. For the clinical group, the differences between the actual and estimated dose are likely due to individual variation in the pharmacokinetic factors that are influenced by variables such as the person's age, sex, disease state, the physicochemical properties of the specific drug and genetics. For a review see (18). Even with the most investigated and understood forensically relevant drug, ethanol, there are still large contributing uncertainties from specific pharmacokinetic factors (i.e. elimination rate and volume of distribution of ethanol and as in the investigation in this work, may only be generalised to the "average" person. This leads to increased uncertainty of the "true" value, unless specifically measured in the individual which is of course impossible in a deceased individual.

Uncertainty associated with individual factors of the pharmacokinetic equations

As can be seen in Tables 4 - 7, the equation variables that have the most influence the overall uncertainty on the calculation of the consumed dose of a drug are the elimination rate and the volume of distribution of the individual. This is the same as for ethanol (10). However unlike ethanol, where the overall uncertainty for the calculation of results using pharmacokinetic equations is approximately ± 20 % (1 SD) (8–10) in this study (Tables 4 - 7) the calculation of the dose of amitriptyline taken has an uncertainty (precision; 1SD) of +52 to +150 % (clinical); +55 to +68 % (overdose) and +55 to +120 % (death) much larger than those seen with ethanol.

Postmortem changes and their influence on pharmacokinetic parameters

Body changes in the postmortem environment also increase the uncertainty for many of the variables in the pharmacokinetic equations. Postmortem redistribution is the phenomenon known as the “toxicological nightmare” (20) due to changes in drug concentrations at a specific sampling site after death (20). It is more common for drug concentrations to increase after death at specific sampling sites (sometimes up to 10-fold) (21) but they may also decrease (22). Thus there could be a large under, or overestimation of the drug concentration at the time of death, when compared to the measured postmortem drug concentration. This further adds to the uncertainty of the actual blood concentration parameter used in pharmacokinetic equations. Femoral blood is considered to be the “least affected” by postmortem redistribution (23), but recent studies suggest that popliteal blood may be a “better” sample to use than femoral blood (24, 25). However, any postmortem sample is liable to be affected by postmortem redistribution. To date there are no markers to allow determination of the amount of postmortem redistribution that may, or may not have occurred since death

and the time the blood is sampled. Thus, the uncertainty of the true concentration of the drug at death will likely be larger than the uncertainty used in this study (see Table 1). The uncertainty of half-life and volume of distribution of drugs are also likely to be different in fatal drug intoxications compared to those observed in life. For example, Table 1 demonstrates that on average the elimination rate constant (k) decreases by about half in overdose cases when compared to clinical cases (from 0.046 ± 0.031 h^{-1} to 0.023 ± 0.014 h^{-1}). This is likely to further decrease in fatal intoxications, as metabolic enzymes and other process become saturated and drug elimination moves from 1st order to zero-order elimination. The mean volume of distribution measured in living individuals is also likely to be different than that found in fatal intoxication cases, because just as seen in the postmortem environment there are changes in pH (leading to changes in the ionisation of drugs) and drug partitioning (due to loss of cell membrane integrity) along with other changes such as cell lysis (26). Drug absorption is another factor that may be altered in fatal intoxications. The absorption of drugs from the stomach, and thus the bioavailability (F) of a drug, is also assumed to cease in the pharmacokinetic equations. However, this is not always true: especially where tablets (or ethanol) remain in the stomach after death (27, 28). The changes of these variables (i.e. volume of distribution, elimination rate (half-life), drug concentration, and absorption (bioavailability)) in a specific individual leading up to and after death compared to normal healthy individuals is likely to be unknown. As this study demonstrates, these added uncertainties lead to poor accuracy and precision of postmortem dose estimations when compared to the actual doses consumed. Likewise, the changes in the postmortem environment also make other calculations that are commonly carried out with ethanol (e.g. retrograde extrapolation) potentially unreliable. Even in living individuals these calculations are likely to be unreliable due

to the lack of large studies of the elimination rate and volume of distribution of specific drugs.

Conclusions

As expected, both the uncertainty and the accuracy of the dose calculation for a consumed drug from its postmortem blood concentration are too large to allow reliable estimations. This work again reinforces that dose calculations from postmortem blood drug concentrations should not be carried out.

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Table 1: Mean Pharmacokinetic Variables for Amitriptyline from clinical studies. Medians are also given for variables that are not normally distributed.

Variable	Mean Value	Uncertainty (S.D.)	%CV	Median	Range (Min – Max)	Number of Subjects	Ref
α (h^{-1}) (normal)	3.97	1.16	29.24		2.98 – 5.62	4	(13)
α (h^{-1}) (in overdose)	0.364	0.079	21.78		0.224 – 0.462	7	(29)
β (h^{-1})/K (h^{-1}) (normal)	0.046	0.031	67.64	0.0370	0.015 – 0.230	76	(6, 13, 30–37)
β (h^{-1})/K (h^{-1}) (in overdose)	0.023	0.014	59.26		0.008 – 0.059	21	(29, 38, 39)
K_{21} (h^{-1})	0.287	0.067	23.41		0.211 – 0.345	4	(13)
V (L/Kg)	22.77	11.57	50.81		6.40 – 45.50	33	(13, 32–35)
V (L)	1581	852	53.93		459 – 3325	29	(13, 32, 34, 35)
F (no units)	44.1	9.51	21.57		31.00 – 62.00	21	(32–34)
K_a (h^{-1})	1.60	0.88	54.88		0.50 – 3.40	26	(30, 36)
T½ (h)	18.82	7.67	40.76	18.76	3.51 – 47.14	76	(6, 13, 30–37)
Wt (kg)	-	0.4	-		n/a	n/a	(40)
C (mg/L)	<0.1 mg/L 0.1 to 10 mg/L >10 mg/L	- - -	12.50 10.00 7.50		n/a	n/a	(41)

Table 2: Collation of Amitriptyline fatal overdose case studies in which the dose taken was known and death was attributed to amitriptyline overdose,

Subject Number	Estimated amitriptyline dose taken (mg)	Subject Weight (kg)	Amitriptyline Concentration at autopsy (mg/L)	Blood Sample Site	Amitriptyline: nortriptyline Ratio	Estimated phase (absorption/elimination)	Time since administration (h)	Reference
1	2000	Not given	0.82	Not given	0.72	elimination	Not given	(42)
2	2500	67	6.00	Not given	1.2	elimination	Not given	(43)
3	≤ 2500	54	18.00	Not given	∞	absorption	Not given	(43)
4	≤ 5000	55	3.00	Not given	1.5	elimination	Not given	(43)
5	≤ 2500	55	5.00	Not given	∞	absorption	Not given	(43)
6	≤ 2500	70	9.00	Not given	1	elimination	Not given	(43)
7	≤ 1250	57	9.00	Not given	0.5	elimination	Not given	(43)
8	2000	Not given	3.00	Not given	3	absorption	2 to 4	(15)
9	4500	Not given	10.00	Not given	3.33	absorption	60 to 70	(15)
10	1200	Not given	1.00	Not given	0.5	elimination	< 12	(15)
11	7500	Not given	3.15	Not given	13.1	absorption	1	(14)
12	2500	Not given	3.00	Not given	Not given	?	7 to 8	(44)

Table 3: Collation of Amitriptyline non-fatal overdose cases studies in which the dose taken was known.

Subject Number	Estimated amitriptyline dose taken (mg)	Subject Weight (kg)	Amitriptyline Concentration (mg/L)	Blood Sample Site	Amitriptyline: nortriptyline Ratio	Estimated phase (absorption/elimination)	Time since administration (h)	Reference
1	2475	Not given	0.78	Serum	1.01	elimination	~ 148 h	(45)
2	1875	Not given	0.65	Serum	1.46	elimination	24	(14)
3	3600	Not given	0.81	Serum	2.52	absorption	3	(14)
4	675	Not given	0.45	Serum	3.03	absorption	10	(14)
5	750	Not given	0.84	Serum	16.20	absorption	3	(14)
6	2500	Not given	1.45	Serum	4.57	absorption	11	(14)
7	350	Not given	0.49	Serum	1.57	elimination	2	(14)
8	1500	Not given	0.94	Serum	5.53	absorption	2	(14)
9	375	Not given	0.55	Serum	5.58	absorption	4	(14)
10	1050	Not given	0.39	Serum	1.66	elimination	4	(14)
11	3000	Not given	1.28	Serum	16.7	absorption	1	(14)
12	450	Not given	0.46	Serum	2.36	absorption	2	(14)
13	1750	Not given	0.72	Serum	5.23	absorption	9	(14)
14	2000	Not given	0.38	Serum	3.31	absorption	2	(14)
15	4500	Not given	0.50	Serum	1.79	elimination	3	(14)
16	1500	Not given	0.41	Serum	0.61	elimination	9	(14)
17	3000	Not given	1.52	Serum	6.49	absorption	1	(14)
18	700	Not given	0.21	Serum	2.30	absorption	13	(14)
19	4000	Not given	0.77	Serum	1.80	elimination	15	(14)

Table 4: The proportion (as a percentage) that each variable in the dose calculation contributes to the total uncertainty in the various pharmacokinetic equations for clinical cases. Data from subject AJ (13)**. The blood concentration of amitriptyline was 0.02 ± 0.002 mg/L 12 hours after the dose was administered. The subject had a body mass of 73 ± 0.4 kg. Actual administered dose 15 mg (intravenous infusion). The uncertainty on the calculated dose was expressed as 1 SD.**

	Equation			
Variable	**1**	**2**	**3**	**3a**
α (h^{-1})	-	-	12.2 %	4.2 %
β (h^{-1})/K (h^{-1})	-	35.1 %	37.5 %	78.6 %
K_{21} (h^{-1})	-	-	12.7 %	4.3 %
V (L/Kg)	96.3 %	62.5 %	36.1 %	12.4 %
Wt (kg)	0.0 %	0.0 %	0.0 %	0.0 %
t (h)	-	0.0 %	0.0 %	0.0 %
C (mg/L)	3.7 %	2.4 %	1.4 %	0.5 %
Calculated Dose ± SD (mg) (% error)	33.0 ± 17 (52 %)	58 ± 37 (64 %)	940 ± 790 (85 %)	43 ± 63 (140 %)

Table 5: The proportion (as a percentage) that each variable in in the dose calculation contributes to the total uncertainty in the various pharmacokinetic equations for clinical cases. Data from subject DB (6)**. For the absorption the blood concentration of amitriptyline was taken at 3 h (0.058 ± 0.00725 mg/L) for the elimination phase 36 h after dose was administered (0.0087 ± 0.001088 mg/L). The subject weight was not given so volume of distribution (L) used. Administered dose was 100 mg. The uncertainty on the calculated dose was expressed as 1 SD.**

	Equation			
Variable	**1 (absorbing)**	**1 (post-absorption)**	**4**	**5**
F	-	-	2.3 %	14.1 %
β (h^{-1})/K (h^{-1})	-	-	83.9 %	0.8 %
V (L)	94.9 %	94.9 %	13.1 %	80.1 %
K_a (h^{-1})	-	-	-	0.7 %
t (h)	-	-	0.0 %	0.0 %
C (mg/L)	5.1 %	5.1 %	0.7 %	4.3 %
Calculated Dose ± SD (mg)	92 ± 51	13.8 ± 7.6	160 ± 240	230 ± 140
(% error)	(55 %)	(55 %)	(150 %)	(60 %)

Table 6: The proportion (as a percentage) that each variable in in the dose calculation contributes to the total uncertainty in the various pharmacokinetic equations in cases where there was an overdose of amitriptyline. Data for equation 1 (absorbing) and 4 from subject 2 (table 3). Data for equation 1 (post-absorption) and for equation 5 from subject 3 (table 3). The subject weight was not given so volume of distribution (L) used. The uncertainty on the calculated dose was expressed as 1 SD.

	Equation			
Variable	**1 (absorbing)**	**1 (post-absorption)**	**4**	**5**
β (h^{-1})/K (h^{-1})	-	-	25.0 %	0.3 %
V (L)	96.7	96.7	62.0 %	81.6 %
F (no units)	-	-	10.9 %	14.4 %
K_a (h^{-1})	-	-	-	0.9 %
t (h)	-	-	0.0 %	0.0 %
C (mg/L)	3.3	3.3	2.1 %	2.8 %
Calculated Dose ± SD (mg) (% error)	1030 ± 560 (55 %)	1280 ± 700 (55 %)	4000 ± 2800 (68 %)	3100 ± 1800 (60 %)

Table 7: The proportion (as a percentage) that each variable in in the dose calculation contributes to the total uncertainty in the various pharmacokinetic equations in cases where death was attributed to amitriptyline overdose. Data for equation 1 (absorbing) and 4 from subject 9 (table 2). Data for equation 1 (post-absorption) and for equation 5 from subject 10 (table 2). The subject weight was not given so volume of distribution (L) used. The uncertainty on the calculated dose was expressed as 1 SD.

	Equation			
Variable	**1 (absorbing)**	**1 (post-absorption)**	**4**	**5**
β (h^{-1})/K (h^{-1})	-	-	76.0 %	5.7 %
V (L)	96.7 %	96.7 %	20.4 %	77.9 %
F (no units)	-	-	3.6 %	13.7 %
K_a (h^{-1})	-	-	-	0.0 %
t (h)	-	-	0.0 %	0.0 %
C (mg/L)	3.3 %	3.3 %	0.0 %	2.7 %
Calculated Dose ± SD (mg) (% error)	15800 ± 8700 (55 %)	1580 ± 870 (55 %)	160000 ± 190000 (120 %)	4600 ± 2800 (61 %)