Use of unbound volumes of drug distribution in pharmacokinetic calculations

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Abstract

Volume of drug distribution is a primary pharmacokinetic parameter. This study assessed effects of drugs’ plasma protein binding and tissue distribution on volume of drug distribution and identified the most appropriate ways for its calculation. Effects of the distribution factors on the unbound and total drug plasma concentrations and on the corresponding volumes of distribution were studied using pharmacokinetic modeling and simulation approach based on in vitro and in vivo concentration vs. time data of diazepam, a model drug with extensive plasma protein binding and tissue distribution. Pharmacokinetics of diazepam were appropriately described by three-compartment pharmacokinetic model that incorporated the processes of plasma protein binding and tissue permeation. According to this model, displacement of the drug from plasma proteins increases the unbound (but not the total) plasma concentrations and induces faster drug elimination from the body. The distribution pattern of the drug in the body and the time course of unbound (pharmacologically active) drug concentrations correlated with the unbound volumes of distribution, but not with the total volumes of distribution. In conclusion, unbound volumes of distribution appropriately describe the drug distribution pattern and the time course of unbound drug concentrations and are recommended for use as primary pharmacokinetic parameters in pharmaceutical research.

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1. Introduction

Binding of drugs to plasma proteins and to the tissue components is one of the major pharmacokinetic (PK) factors that determine the drug’s distribution and elimination. The extent of drug binding affects the time course of drug concentrations in the body following drug administration, and particularly the time course of the unbound drug concentrations that are the basis of the drugs’ pharmacological activities (Berezhkovskiy, 2010a; Hammarlund-Udenaes, 2010; Schmidt et al., 2010). Therefore, drugs’ protein binding and drug distribution patterns and their quantification using volume of drug distribution are among the primary considerations in drug discovery, development, and clinical use.

Volume of drugs’ distribution is a complex parameter that reflects the complexity of the pathways and patterns of drugs’ distribution in the body. Numerous studies have accessed the theoretical and applied aspects related to estimation of volume of distribution and its use in pharmacokinetic calculations (Berezhkovskiy, 2004, 2010c; Poulin and Theil, 2009; Rodgers and Rowland, 2007). Major factors that determine the value of volume of drug distribution are the extent and kinetics of drug distribution into organs and tissues, and the kinetics of drug elimination. For a drug that exhibits mono-exponential kinetics following intravenous administration (i.e., follows one-compartment pharmacokinetic model), volume of distribution can be assumed to be constant and can be readily calculated from the concentration vs. time data.

In other cases, volume of distribution changes with time as a function of tissue penetration and binding of the drug (Wada et al., 1998). In this case the volume of distribution is initially low (\( V_1 \) – volume of the central compartment), increases to the higher value (\( V_{SS} \) – steady-state volume) when the steady-state is achieved, and reaches the maximum value (\( V_p \) – terminal volume of distribution) during the elimination phase (linear terminal phase). For such drugs, \( V_{SS} \) value only is commonly reported in the scientific literature, and the following formula (or its more complex variants, such as Oie–Tozer equation) is used to relate the volume of distribution to the patterns of drug distribution in the body (i.e., plasma and tissue binding of the drug, and its permeation into tissues) (Gibaldi and Perrier, 2007; Rowland and Tozer, 1995a):

\[
V_{SS} = V_P + V_T \frac{f_{up}}{f_{st}}
\]

where \( V_P \) and \( V_T \) are the volumes of plasma and tissues, respectively; and \( f_{up} \) and \( f_{st} \) are the fractions of drug unbound in the plasma and tissues, respectively.
plasma and tissues, respectively. This formula is based on assumption that the value of volume of distribution reflects the relative drug amounts that accumulate in the plasma vs. tissues. Partial displacement of the drug from plasma proteins will lead to a new steady state with increased volume of drug distribution due to reduced ratio of plasma/tissue drug contents (and higher \( f_{DP}/f_{DT} \) ratio) (Aarons, 1981; Aarons and Rowland, 1981; Oie and Tozer, 1979). However, this expectation contradicts the ‘common sense’ reasoning that reduced drug protein binding (either to plasma proteins or to the tissue components) should lead to reduced volume of drug distribution.

The objective of the present study was to assess the effect of the individual factors that determine a drug’s volume of distribution (plasma protein binding and tissue distribution) using pharmacokinetic modeling and simulation approach. To this end, a pharmacokinetic model that incorporates the above mentioned factors was used to describe the concentration vs. time curves of diazepam, a model drug that is characterized by extensive plasma protein binding and tissue distribution. Based on the developed model and the values of the estimated parameters, the effect of the individual distribution factors on the drugs’ concentration vs. time curves and values of volumes of distribution was assessed, and recommendations for calculation and usage of volumes of distribution were given.

2. Methods

2.1. Modeling of diazepam in vitro protein binding vs. time data

The time course of in vitro interaction between diazepam and human serum albumin (HSA) was digitalized from Fig. 29 by Drake (2001). In this study circular dichroism measurements of interaction between diazepam and HSA were performed. The studied compounds (2 \( \times 10^{-3} \) M diazepam and 1 \( \times 10^{-4} \) M HSA) exhibited rapid kinetics of interaction: the steady state conditions were achieved within approximately 3 s.

From the digitized data, concentrations of the compounds (free diazepam, free HSA and diazepam–HSA complex) were estimated for each time point. Analysis of the diazepam in vitro protein binding vs. time data assumed existence of a single binding interaction between diazepam and HSA, and was performed for all three data sets simultaneously using Generalized Least Squares algorithm of ADAPT 5 software (D’Argenio et al., 2009). The differential equations of the applied model were as follows:

\[
\frac{dX_1}{dt} = k_d \cdot X_2 - k_a \cdot X_1 \cdot X_2
\]

where \( X_1, X_2, \) and \( X_3 \) are the concentrations of free diazepam, free HSA and diazepam–HSA complex, respectively; and \( k_d \) and \( k_a \) are association and dissociation rate constants, respectively.

The modeling was performed using Generalized Least Squares algorithm, and goodness of fit was determined by comparing the estimated curves to the diazepam concentration vs. time data, values of coefficients of variation of the estimated parameters, and values of Akaike and Schwarz criteria (Gabrielsson and Weiner, 2002).

2.2. Modeling of diazepam concentration vs. time data

The diazepam serum concentration vs. time data following administration of 10 mg IV bolus dose to adult epileptic patients were digitalized from Fig. 2 by Dhillon et al. (1982). Unbound diazepam plasma concentrations were calculated from the total plasma concentrations assuming that the fraction of drug unbound in the plasma \( (f_{DP}) \) is 0.032 (Klotz et al., 1976).

Analysis of the diazepam concentration vs. time data was performed simultaneously for total and unbound concentrations using three-compartment model of drug distribution (Fig. 1) and ADAPT 5 software (D’Argenio et al., 2009). The differential equations of the applied pharmacokinetic model were as follows:

\[
\frac{dX_1}{dt} = k_d \cdot X_2 + k_{cp} \cdot X_3 - (k_{10} + k_{cp})X_1 - k_d \cdot X_1 \cdot X_4
\]

\[
\frac{dX_2}{dt} = k_2 \cdot X_4 - k_d \cdot X_2
\]

\[
\frac{dX_3}{dt} = k_{cp} \cdot X_1 - k_{cp} \cdot X_3
\]

\[
\frac{dX_4}{dt} = k_d \cdot X_3 - k_a \cdot X_1 \cdot X_4
\]

where \( X_1 \) and \( X_2 \) are the amounts of free and protein-bound drug in plasma, respectively; \( X_3 \) is the amount of drug in peripheral tissues; \( X_4 \) is the amount of drug-free protein in plasma. The rate constants are: \( k_2 \), \( k_{10} \), association and dissociation rate constants, respectively; \( k_{cp} \), elimination from the body; \( k_{cp} \), transfer of the unbound drug from plasma to the tissue (central to peripheral compartment); and \( k_{cp} \), transfer of the unbound drug from tissue to the plasma (peripheral to central compartment). The initial volume of distribution \( (V_i) \) was used to transform the plasma drug amounts into concentrations.

The modeling was performed using Generalized Least Squares algorithm, and goodness of fit was determined by comparing the estimated curves to the diazepam concentration vs. time data, values of coefficients of variation of the estimated parameters, and values of Akaike and Schwarz criteria (Gabrielsson and Weiner, 2002).

2.3. Pharmacokinetic simulations and calculations

The pharmacokinetic model of diazepam (Fig. 1) and the estimated values of the pharmacokinetic parameters (Table 1) were used to predict the time course of plasma (total and protein-bound) and tissue drug concentrations. Several distribution patterns were studied (see Table 2), including: partial displacement of the drug from plasma protein binding, lack of distribution of the drug into the tissue, and combination of both these factors.

For each one of the distribution patterns, predicted time course of drug concentrations following intravenous (IV) bolus administration and IV infusion were plotted and analyzed. The drug doses for these simulations were selected based on the clinically used...
doses of diazepam (Schmidt, 2002): 10 mg IV bolus and 6 mg/h IV infusion. For each of the distribution patterns, based on the IV bolus data set, areas under the concentration vs. time curves (AUC and AUCl) were calculated using trapezoidal rule, clearance values (CL and Cla) were calculated by dividing the dose by appropriate AUC value, and terminal half-life was calculated from the linear terminal slope. The fup values were calculated from the drug amounts accumulated in the X1 and X2 compartments at the steady-state conditions following long-term IV infusion.

The volumes of the central compartment (V1 and V1U) were calculated by dividing the administered dose by the y-axis intercepts of IV bolus concentration vs. time curves (for total or protein-unbound plasma drug concentrations, respectively). The steady state volumes of distribution (VSS and VSSU) were calculated by dividing the total amount of the drug accumulated in the body following long-term IV infusion (sum of X1, X2, and X3) by the total and protein-unbound plasma drug concentrations, respectively:

\[
VSS = \frac{X1 + X2 + X3}{CSS} \quad (9)
\]

\[
VSSU = \frac{X1 + X2 + X3}{CSSU} \quad (10)
\]

The terminal volumes of distribution (Vβ and VβU) were calculated as:

\[
Vβ = \frac{D}{AUC \cdot β} \quad (11)
\]

\[
VβU = \frac{D}{AUClU \cdot β} \quad (12)
\]

where D is the IV bolus dose of the drug; AUC and AUClU are the area under the curve of total or protein-unbound drug concentrations vs. time, respectively; and β is the linear terminal slope of the corresponding graph.

2.4. Verification of the model-based outcomes in a wide range of parameter values

Effect of the distribution patterns (see Table 2) on the time course of drug concentrations and on the values of pharmacokinetic parameters was studied in a wide range of initial parameter values. To this end, simulations of the time course of drug concentrations following IV bolus and IV infusion of the drug according to the developed pharmacokinetic model of diazepam (see Fig. 1) and the studied distribution patterns (see Table 2) were performed using custom-written program in Visual C++ 6.0 (Microsoft Inc.). The simulated doses and parameter values were the same as in

Table 1
Pharmacokinetic parameters estimates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>%CV</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>10.24</td>
<td>11.3</td>
<td>L</td>
</tr>
<tr>
<td>k10</td>
<td>8.68</td>
<td>8.4</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>k1p</td>
<td>22.04</td>
<td>7.1</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>k1c</td>
<td>0.324</td>
<td>12.1</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>k3</td>
<td>1.35 × 10³</td>
<td>0.093</td>
<td>M⁻¹ h⁻¹</td>
</tr>
<tr>
<td>k2</td>
<td>797</td>
<td>4.6</td>
<td>h⁻¹</td>
</tr>
</tbody>
</table>

Section 2.3, with exception of the values of k3, k10, and k1p that varied in the range of 1.35 × 10⁴ to 1.35 × 10⁸, 8.68 × 10⁻² to 868, and 0.2204–2204 h⁻¹, respectively (i.e., corresponding to 0.001–10, 0.01–100, and 0.01–100-fold range as compared to the values of k3, k10, and k1p of diazepam, see Table 1). Thus, effect of the distribution patterns on the pharmacokinetics of drugs that follow the developed model (see Fig. 1) was studied for a wide range of pharmacokinetic patterns (125 combinations of k3, k10, and k1p values × 4 distribution patterns = 500 simulated data sets). The pharmacokinetic parameters for the individual data sets were calculated using the same approach as in Section 2.3. The fup values and the ratios of VSS and VSSU values (VSS for pattern B/VSS for pattern A, and VSSU for pattern B/VSSU for pattern A, respectively) were calculated for each one of the studied pharmacokinetic patterns, and were plotted using SigmaPlot 9.0 software (Systat Software Inc.).

3. Results

Kinetics of in vitro interaction between diazepam and human serum albumin (HSA) was appropriately described by the applied model (data not shown). The estimated values of diazepam–HSA association and dissociation rate constants (4.22 × 10⁷ M⁻¹ h⁻¹ and 768 h⁻¹, respectively) were used as initial values for modeling the diazepam concentration vs. time data. The three-compartment pharmacokinetic model (Fig. 1) appropriately described the concentration vs. time profiles of diazepam following IV bolus administration. The observed vs. predicted results are presented in Fig. 2, and the calculated PK parameters are summarized in Table 1. High quality of fits can be seen from the observed vs. predicted data plots, low coefficients of variation of the estimated parameters, and low values of the Akaike and Schwartz criteria.

The predicted IV bolus plasma concentration vs. time curves for different distribution patterns are shown in Fig. 3. As expected, patterns A and B had bi-phasic curves, while lack of tissue binding resulted in straight lines (patterns C and D). Change of the distribution pattern significantly affected the major pharmacokinet-
Fig. 3. The time course of drug plasma concentrations predicted for IV bolus of the drug from the three-compartment PK model. The letters indicate the individual drug distribution patterns.

Table 3
The pharmacokinetic parameters of the studied distribution patterns.

<table>
<thead>
<tr>
<th>Distribution pattern</th>
<th>fP</th>
<th>t1/2,ß (h)</th>
<th>AUC (mol h/L)</th>
<th>AUCu (mol h/L)</th>
<th>CL (L/h)</th>
<th>CLu (L/h)</th>
<th>V1 (L)</th>
<th>V1u (L)</th>
<th>VSS (L)</th>
<th>VSSu (L)</th>
<th>Vß (L)</th>
<th>Vµu (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.032</td>
<td>9.51</td>
<td>1.24 x 10^-9</td>
<td>4.23 x 10^-9</td>
<td>2.84</td>
<td>82.9</td>
<td>10.3</td>
<td>317</td>
<td>33.2</td>
<td>1005</td>
<td>38.9</td>
<td>1138</td>
</tr>
<tr>
<td>B</td>
<td>0.064</td>
<td>8.54</td>
<td>6.40 x 10^-6</td>
<td>4.23 x 10^-7</td>
<td>5.48</td>
<td>82.9</td>
<td>10.3</td>
<td>164</td>
<td>54.0</td>
<td>858</td>
<td>67.5</td>
<td>1021</td>
</tr>
<tr>
<td>C</td>
<td>0.032</td>
<td>2.50</td>
<td>1.24 x 10^-9</td>
<td>4.27 x 10^-9</td>
<td>2.83</td>
<td>82.3</td>
<td>10.3</td>
<td>316</td>
<td>10.2</td>
<td>310</td>
<td>10.2</td>
<td>297</td>
</tr>
<tr>
<td>D</td>
<td>0.062</td>
<td>1.29</td>
<td>6.40 x 10^-6</td>
<td>4.24 x 10^-7</td>
<td>5.48</td>
<td>82.8</td>
<td>10.3</td>
<td>163</td>
<td>10.2</td>
<td>163</td>
<td>10.2</td>
<td>155</td>
</tr>
</tbody>
</table>

The predicted IV infusion plasma concentration vs. time curves for different distribution patterns are shown in Fig. 4. Partial displacement of the drug from plasma proteins induced proportional decrease (2-fold, patterns B and D) in the total steady-state plasma concentrations, but did not affect the unbound steady-state plasma concentrations. Time required for reaching the steady-state conditions was in the following order: A > B > C > D (see Fig. 4B), i.e., the elimination half-life values for the individual distribution patterns followed the same order as for IV bolus administration (see the previous paragraph, Table 3, and Fig. 6D).

The predicted peripheral tissue concentration vs. time curves for different distribution patterns are shown in Fig. 5. Partial displacement of the drug from plasma proteins resulted in somewhat higher amounts of the drug that accumulated in the peripheral compartment during the first 9 h after IV bolus drug administration (pattern B vs. A, by up to ~17%, see Fig. 5A). The differences between peripheral tissue concentrations in patterns A and B during IV infusion of the drug were much less pronounced (see Fig. 5B). Pattern B had shorter elimination half-life as compared to pattern A, as can be seen from more rapid drug elimination following IV bolus, and faster accumulation during IV infusion. Due to lack of tissue penetration in patterns C and D, the drug is not reaching peripheral compartment, and peripheral tissue concentrations in these patterns are equal to zero.

The calculated values of the unbound plasma factors, terminal half-lives, and volumes of distribution for the different distribution patterns are summarized in Table 3 and Figs. 6 and 7.
Fig. 5. The time course of drug concentrations in the peripheral tissues predicted for IV bolus and infusion of the drug from the three-compartment PK model. The letters indicate the individual drug distribution patterns.

can be seen that the initial total volume of distribution was the same for all the distribution patterns, and the order of the total steady-state and terminal volumes of distribution was $B > A > C = D$ (Table 3 and Fig. 7A). The unbound initial volume of distribution was lower for the patterns with partial drug displacement from plasma proteins ($B$ and $D$, Fig. 7B). The order of the unbound steady-state and terminal volumes of distribution was the same as the order of the corresponding elimination half-lives $A > B > C > D$ (Figs. 6D and 7B).

Similar outcomes were obtained in simulation-based validation of the developed pharmacokinetic model. In these simulations, different combinations of $k_a$, $k_{10}$, and $k_{cp}$ parameter values were studied, covering very different pharmacokinetic patterns: extensive vs. negligible plasma protein binding of the drug, rapid vs. slow drug elimination, extensive vs. negligible distribution to the peripheral tissues, etc. The range of the pharmacokinetic parameters in the studied patterns was approximately 0.3–97% for $f_{up}$ (see Fig. 8), 10–67,000 L and 17–72,000 L for $V_{SS}$ and $V_{SSU}$ values, respectively (data not shown). In all the studied patterns, partial displacement of the drug from plasma proteins increased the total steady-state volume of distribution (the ratio of $V_{SS}$ values for pattern $B$/pattern $A > 1$, see Fig. 9) and decreased the unbound steady-state volume of distribution (the ratio of $V_{SSU}$ values for pattern $B$/pattern $A < 1$, see Fig. 9), consistent with changes in $V_{SS}$ and $V_{SSU}$ for diazepam (see Fig. 7). The magnitude of the changes was dependent on the values of $k_a$, $k_{10}$, and $k_{cp}$ parameters that were used in the simulations (see Fig. 9).

4. Discussion

Drug distribution within the body and its relationship to the volume of distribution are among the most complex topics in the pharmacokinetics. This complexity originates from the profound differences in the patterns of tissue permeation and extent of the plasma and tissue binding between individual drugs. Due to these factors, different tissues and body fluids may contain different unbound and total drug concentrations. Moreover, both unbound and total drug concentrations at these locations, as well as their ratio (i.e., fraction of drug unbound) may change with time (e.g., during the distribution vs. elimination phase). Therefore, it is dif-
Fig. 7. The values of total and unbound volumes of drug distribution. The letters indicate the individual drug distribution patterns.

It is difficult to capture these patterns of drug distribution with a single parameter, formula, or ‘rule of thumb’.

For the majority of the drugs, unbound drug concentrations in the plasma or within the target organ/tissue are responsible for the obtained pharmacological effects (i.e., represent the active drug concentrations) (Berezkovskiy, 2010a; Hammarlund-Udenaes, 2010; Hoffman and Stepensky, 1999; Schmidt et al., 2010). However, in the majority of preclinical and clinical studies, only the total plasma (or serum) drug concentrations (protein-bound and unbound) are measured, despite general availability of methods for measurement of unbound drug concentrations (Dasgupta, 2007). As a result, relationship between total drug concentrations and drug amount in the body at different time points is quantified using volume of distribution parameters ($V_1$, $V_{SS}$, and $V_\beta$). This study assessed whether this commonly used approach describes correctly the distribution pattern of the drug in the body and the time course of active (unbound) drug concentrations. This assessment was performed using the pharmacokinetic modeling and simulation approach. A distribution mechanism-based PK model was developed for a model drug (diazepam) based on the available in vitro protein binding and in vivo plasma concentration vs. time data. From this model, relationships between the distribution factors, the time course of drug concentrations, and the volume of distribution values were assessed in a systematic fashion.

It is commonly accepted that values of volume of distribution parameters reflect the relative amount of the drug that accumulates in the tissues vs. the plasma. It is assumed that displacement of the drug from plasma proteins has no effect on the value of volume of distribution, but leads to increased drug clearance (see Figs. 6E and 7A, pattern $D$ vs. $C$). Only if displacement from the plasma proteins induces drug redistribution from the plasma to the peripheral tissues, then volume of distribution increases along with increase in drug clearance and decrease in AUC (see Figs. 6B, E and 7A, pattern $B$ vs. $A$).

I am suggesting to use an alternative approach and to assume that the value of drug’s volume of distribution is increased by the following factors: (a) binding of the drug to the plasma proteins, (b) permeation of the drug to the peripheral tissues, and (c) binding of the drug to the tissue components. This approach applies calculation of unbound volumes of distribution ($V_{1U}$, $V_{SSU}$, $V_{\beta U}$) based on unbound drug concentrations in the plasma. Unbound volumes of drug distribution decrease upon displacement of the drug from plasma proteins (see Fig. 7B, pattern $B$ vs. $A$ and $D$ vs. $C$). For instance, initial unbound volume of distribution ($V_{1U}$) increases, leading to increased unbound plasma drug concentrations, while the unbound drug’s clearance is not affected (see Fig. 6F). Subsequently, reduced unbound volume of distribution results in more rapid drug elimination from the body during the distribution and post-distribution phases (compare Figs. 6D and 7B).

Use of unbound volumes of distributions may be preferable due to following reasons: (1) this approach focuses on the unbound, pharmacologically active, rather than total plasma drug concentrations, (2) unbound volumes of distribution appropriately describe the pharmacodynamic behavior of the drug, and (3) unbound volumes of distribution may be more directly related to the drug concentration in the tissues where the active drug is localized.
the distribution patterns of the drug in the body and correlate with
the time course of active drug concentrations, (3) differences in
the drugs’ pharmacokinetics upon displacement from the plasma
proteins are attributed to the changes in drug distribution rather
than elimination, which is more mechanistically correct. Hence, I
recommend the use of unbound volumes of distribution, instead of
the total volumes of distribution, for pharmacokinetic calculations
in pharmaceutical research.

The modeling and simulation approach that was applied in this
study allowed a thorough and extensive analysis of quantitative
relationships between the individual pharmacokinetic processes,
the time course of unbound and total drug concentrations, and the
values of pharmacokinetic parameters. Based on this approach it
was clarified that partial displacement of the drug from plasma
proteins increases the total steady-state volume of distribution
and decreases the unbound steady-state volume of distribution
(see Table 3 and Fig. 9) not only for diazepam, but also appar-
ently for all the drugs that follow the developed pharmacokinetic
model (Fig. 1). For diazepam, the relative differences in the values
of unbound and total volumes of distribution between the studied
patterns (see Table 3) originate from the relative kinetics of
diazepam plasma protein binding (very rapid), permeation to the
tissues (slower), and elimination (the slowest). Similar changes, but
of different magnitude, take place in drugs with different kinetics
of these processes, as shown for VSS and VSSU parameters in Fig. 9.

In this study, the modeling and simulation approach served as
a powerful tool to analyze the complex relationships between the
pharmacokinetic parameters in the studied model and to assess validity of the model for drugs with different kinetics of
pharmacokinetic processes. Due to experimental limitations and
complexity of the studied system, it would be virtually impossi-
ble to perform such analysis in in vitro or in vivo settings. Based
on the thorough and extensive analysis of the studied model using
modeling and simulation approach, it appears that use of unbound
volumes of distribution (and not total volumes of distribution) is
preferable for wide range of drugs with very different pharmacoki-
netic properties. Therefore, the general conclusions of this study
regarding the use of unbound vs. total volumes of distribution are
valid irrespectively of the quality of the original pharmacokinetic
data for diazepam or precision of its protein-binding parameters,
e.g., even if the models with two diazepam-binding sites on albumin
may be more mechanistically correct (Noctor et al., 1992;
Zhivkova and Russeva, 1998).

It should be stressed that the pharmacokinetic analysis in this
study was based on several assumptions, including: (1) linearity of
the drugs’ pharmacokinetic behavior (i.e., first-order pharmacoki-
netics of all the processes, except interaction between diazepam
and plasma proteins), (2) elimination from the central compart-
ment only, and (3) that the drugs’ pharmacological effects derive
from the unbound plasma concentrations (i.e., the site of action
is located in the central compartment, or there is rapid equilibrium
between unbound drug concentrations in the plasma and at the site
of action). All these assumptions are indeed valid for diazepam
that was used as a model drug in this study. Diazepam has linear phar-
cokinetics at the clinically applied doses, and it is eliminated by
hepatic metabolism (Mandelli et al., 1978). The plasma and brain
(CSF) diazepam concentrations equilibrate rapidly, as was seen in
animal (Arendt et al., 1983; Greenblatt et al., 1989b; Kaur and
Kim, 2008) and human studies (t1/2 equilibrium = 1.6 min) (Buhrer
et al., 1990). Moreover, the pharmacological effects of diazepam
are directly related (via sigmoid fmax model) to the plasma diazepam
concentrations (Greenblatt et al., 1989a; Sunzel et al., 1988).

Predictions of the changes in the drug distribution, however,
may be partially incorrect if the above-mentioned assumptions
are not valid. Indeed, some of the drugs’ distribution or elimi-
nation processes are not linear (saturable) in the clinically used
concentrations range (Hoffman et al., 2001; Roberts and Buckley,
2007; Rowland and Tozer, 1995b). Specifically, plasma protein
binding can be non-linear (e.g., due to self-induction of drug-
protein binding (Berezhkovskiy, 2010b)) leading to significant,
clinically relevant changes in plasma protein binding in patients
with decreased plasma protein concentrations (e.g., due to uremia,
hepatic diseases, or epilepsy). Hence, effect of the pharmacokinetic
non-linearity on the model predictions requires further investiga-
tion. The predictions may be partially incorrect also for drugs whose
distribution is governed to a high extent by transporter-mediated
processes (which may be insensitive to the changes in drug protein
binding), and for some biopharmaceuticals that can undergo sub-
stantial metabolism in the peripheral organs and tissues (Mahmood
and Green, 2005; Wang et al., 2008).

In this study the differences between the individual distinct dis-
tribution patterns have been assessed and compared. It will be
interesting to use the applied PK model and its extensions that
include non-linear processes (see above) and to investigate the
kinetics of transit from one distribution pattern to another. This
approach will be useful for recapitulating events that take place
during drug interactions, e.g., due to competition between two
drugs for protein binding. In this way, the effect of the individ-
ual distribution factors on the magnitude of the interaction and
on its clinical consequences can be clarified. For instance, partial
displacement of diazepam from plasma proteins is expected to pro-
duce a temporary increase in the unbound plasma concentrations
followed by rapid decline to their initial values. Part of the drug
released from the protein can reach peripheral tissues and lead
to transient increase in free drug tissue concentrations. Therefore, it
is expected that partial displacement of diazepam from plasma pro-
teins would result in temporary increase in the magnitude of its
pharmacological effects, as described previously for diazepam
and other drugs by Benet and Hoener (2002). In general, the magnitude
and duration of changes in the pharmacological effects and their
clinical relevance for diazepam and for other drugs depend on: (1)
the relative kinetics of their plasma protein binding, permeation to
the tissues, and elimination, (2) the location of sites of action and
kinetics of their equilibrium with unbound plasma or tissue drug
concentrations, and (3) the pharmacokinetic–pharmacodynamic
relationships at the site of action. Relationship between the extent
of drug displacement from plasma proteins and the time course of
pharmacological effects is complex and warrants detailed investi-
gation.

In conclusion, in this study pharmacokinetic modeling and
simulation approach was applied to investigate the effects of
the individual distribution factors (plasma protein binding and
tissue distribution of the drug) on the values of unbound and
total volumes of distribution. It was found that unbound vol-
umes of distribution appropriately described the pattern of drug
distribution in the body and the time course of active drug concen-
trations. Therefore, unbound volumes of distribution, not the
total volumes of distribution, are recommended for use as primary
pharmacokinetic parameters in pharmacokinetic calculations and
in pharmaceutical research.

In this study the unbound volumes of distribution were cal-
culated from the simulations based on the developed PK model.
Formulas that can be applied for calculation of the unbound vol-
umes of distribution in pre-clinical and clinical studies are as
follows:

\[
V_{1U} = \frac{D}{C_{0U}} = \frac{D}{C_{0} \cdot f_{DP}} \quad (13)
\]

\[
V_{1U} = \frac{V_{1}}{f_{DP}} \quad (14)
\]
\[ V_{\text{SS}} = \frac{V_P}{f_{\text{up}}} + \frac{V_T}{f_{\text{GT}}} \]  \hspace{1cm} (15)

\[ V_{\text{SS}} = \frac{V_{\text{USS}}}{f_{\text{up}}} \]  \hspace{1cm} (16)

\[ V_P = \frac{F \cdot D}{A U C U \cdot \beta} \]  \hspace{1cm} (17)

\[ V_P = \frac{V_{\text{up}}}{f_{\text{GT}}} \]  \hspace{1cm} (18)

The unbound volumes of distribution can be calculated from the corresponding total volumes of distribution by dividing them by the fraction of the drug unbound in plasma (Eqs. (13), (15) and (17)). Eq. (14) is a special case of Eq. (13) that is valid for IV bolus drug administration. Eq. (16) is a special case of Eq. (15) that is valid for the two-compartment pharmacokinetic model with rapid equilibrium between the unbound and bound drug in the individual compartments. As opposed to Eq. (1) (an analogous equation for \( V_{\text{USS}} \) calculation), Eq. (11) is also valid for drugs that do not bind tissue components \( f_{\text{GT}} = 1 \) or do not permeate into peripheral tissues \( (V_P = 0) \), and is suitable for \( V_{\text{USS}} \) Calculation for drugs with different distribution patterns, including all the patterns that were assessed in this study and others.

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References


