# Distribution Kinetics of Diazepam, Lidocaine and Antipyrine in the Isolated Perfused Rat Hindlimb

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## INTRODUCTION

Despite recent advances in physiologically based pharmacokinetics, a large gap still exists between our understanding of tissue distribution kinetics in relation to whole body pharmacokinetics. The simplest model of drug distribution kinetics in organs and tissue systems is that of a flow-limited distribution in a one-compartment model. This approach was first used in physiologically based pharmacokinetic modeling. Tissue concentration-time data obtained by destructive sampling are necessary to identify these types of models. Using this technique it has been shown recently that the organ kinetics of fentanyl, alfentanil (1) and the cyclosporine derivative SDZ IMM 125 (2), for example, cannot be described by a simple one-compartment organ model. Besides the problems of interindividual variability in the case of destructive sampling, the underlying assumption of homogenous compartments neglects physiological heterogeneity at the organ level in so far that instantaneous mixing within the intravascular space and the extravascular compartments (e.g. tissue parenchyma) is assumed. An alternative approach is the separate identification of subsystems using the single-pass perfused organ technique. The sensitivity of the latter method allows an investigation of the dynamics of solute mixing and distribution in heterogeneous tissue systems. The isolated perfused rat hindlimb has been proposed as a method to study the kinetics of drug distribution in the body since the corresponding noneliminating tissues (skeletal muscle, skin, adipose and bone) represent more than 70% of body mass in humans (3–5). In these papers data analysis has been restricted to the determination of distribution volumes of indicator substances and drugs calculating the mean transit times with the help of numerical integration methods since various modeling approaches including the dispersion model (6) and Goresky approach (7) failed to describe the outflow concentration-time data after bolus input (3,4).

The objective of this work was to reevaluate kinetics of drug distribution in the rat hindlimb using a stochastic model of organ kinetics (8) utilizing part of previously published data (4). The application of a parametric modeling approach in preference to moment analysis by numerical integration enables a number of specific goals to be achieved. These include: (1) the representation of intravascular mixing in the microcirculatory network by an empirical model, (2) the estimation of parameters of permeation across the capillary wall and diffusion within tissue, (3) the evaluation of the relative contributions of the respective mixing/distribution processes to the observed relative dispersion of transit time distribution, (4) a re-estimation of the distribution volumes and the equilibrium tissue partition coefficients.

## MATERIALS AND METHODS

Out of the series of drugs for which the outflow concentration-time profiles after bolus input into perfused rat hindlimbs have been measured (4) only antipyrine, diazepam and lidocaine as drugs with a high recovery (availability $F > 90\%$) were analyzed in the present study. The details of the experimental method and data acquisition are described elsewhere (3,4). In brief, using a single pass perfusion system the isolated hindlimb of male Wistar rats was perfused with a Krebs Henseleit buffer containing 2% bovine serum albumin. The experiments were performed by bolus injection of markers (Evans blue, $^3$H-water) and the drug ($^3$H-diazepam, $^{14}$C-lidocaine, or $^{14}$C-antipyrine) at two flow rates (4 and 8 ml/min). The outflow concentration was sampled up to 7 minutes and a total of 34 concentration data points was used in the pharmacokinetic modeling of each impulse response (IR) curve data set.

The data were analyzed using the two-phase organ model described in (8). The model assumes drug transport in a random capillary network with permeation across the endothelial barrier and radial intratissue diffusion. Since the model equations are only available in the Laplace domain and the influence of the catheters has to be taken into account the data were fitted using the nonlinear regression programs MINIM (R. Purves, University of Otago Medical School, Dunedin, New Zealand) and SCIENTIST (MicroMath Scientific Software, Salt Lake City, USA) which contain methods of numerical inverse Laplace transformation. Data are analyzed by a sequential procedure: First, the IR outflow concentration-time profiles $C_B(t)$ of the vascular marker are fitted by Eq. (1), which accounts for the influence transit time density (TTD) of catheter ($\hat{f}_{Cath}(s)$)

$$C_B(t) = \frac{Dose}{Q} L^{-1}\{\hat{f}_{Cath}(s)\hat{f}_B(s)\} \quad (1)$$

where the TTD of the vascular marker is given by

$$\hat{f}_B(s) = [p\hat{f}_1(s) + (1 - p)\hat{f}_2(s)] \quad (2)$$

and

$$\hat{f}_i(s) = exp\left\{\frac{1}{CV_i^2} - \left[\frac{MT_i}{CV_i^2/2}\left(s + \frac{1}{2MT_iCV_i^2}\right)\right]^{1/2}\right\} \quad (3)$$

is the inverse Gaussian density function with mean $MT_i$ and relative dispersion $CV_i^2$ ($i = 1, 2$). Second, utilizing this information the IR profile of the pemeating drug, $C(t)$, is analyzed. Eq. (2) accounts for the influence of intravascular mixing (or

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heterogeneity of flow) in the blood-tissue exchange model determining the TTD $\hat{f}(s)$ of the permeating drug,

$$\hat{f}(s) = \hat{f}_B\left\{s + k\left[1 - \left(1 + \left(\frac{v}{kd}\right)\sqrt{ds}\ tanh\ \sqrt{ds}\right)^{-1}\right]\right\} \quad (4)$$

which describes the normalized outflow profile

$$C(t) = \frac{Dose}{Q} L^{-1}\{\hat{f}_{Cath}(s)\hat{f}(s)\} \quad (5)$$

The five parameters $MT_i$, $CV_i^2$ ($i$ = 1, 2) and $p$ are used as fixed parameters in fitting Eq. (5) to the outflow curve of the drug with data weighted according to $1/C_i$. The estimated model parameters $v$, $k$, and $d$ represent the tissue steady-state distribution volume $V_T$ and the permeation clearance $CL_{BT}$ as fraction of the vascular volume $V_B$, i.e., $v = V_T/V_B$ and $k = CL_{BT}/V_B$, while $d = L^2/D_{eff}$ is the characteristic relaxation time of the radial intratissue diffusion process which is determined by the effective diffusion coefficient $D_{eff}$ and the diffusion radial length of the tissue region $L$.

The relative dispersion of transit time of drug molecules across the organ $CV^2$ is the sum of the dispersion due to intravascular mixing $CV_B^2$ and extravascular contribution to the dispersion

$$CV^2 = CV_B^2 + \frac{2Q}{CL_{BT}}\frac{v^2}{(1+v)^2}\left(1 + \frac{1}{3}\frac{CL_{BT}d}{V_T}\right) \quad (6)$$

The tissue to plasma (or perfusate) partition coefficient, $K_p$, is defined as the ratio of concentrations in tissue and perfusate at steady-state. If $V_{TW}$ denotes the aqueous volume into which the solute distributes, $K_p$ is given by (4)

$$K_p = \frac{V_T}{V_{TW}} = \frac{V_{T,Drug}}{V_{T,Water}} \quad (7)$$

All data are expressed as means ± SD, except where otherwise indicated. Statistical analyses were performed via the Student's paired $t$ test for comparisons of data obtained for one compound in one preparation (e.g. for different flows). A level of $p < 0.05$ was accepted as the level of significant differences. One way ANOVA was used to compare the parameters of different compounds (Kruskal-Wallis test when normality test failed).

## RESULTS

Typical outflow profiles of the intravascular marker and the simultaneously injected drug diazepam are depicted in Fig. 1. The optimal curve fits for the vascular marker and the permeating drugs are also shown. The range of the approximative coefficients of variations of the individual parameter estimates (<20%) and the lack of significant correlations among the adjustable parameters ($r < 0.9$) can be regarded as an indication of the reliability of the estimation procedure. Note that in all cases the reduced model assuming instantaneous diffusion outside the capillaries ("rapid" intratissue diffusion) failed to describe the data.

The tissue distribution volume of the various substances ($V_T$) together with the intravascular capillary volume ($V_B$) are summarized in Table I. The increase in volumes after doubling flow was statistically significant ($p < 0.01$). The vascular space increased proportional to flow. Equilibrium partition coefficients of $K_p$ = 1.98 ± 1.2 and 1.44 ± 0.27 were calculated for lidocaine and diazepam, respectively, using either the extravascular water (lidocaine) or antipyrine (diazepam) space as reference [Eq. (7)]. With water as reference a value of $K_p$ = 1.01 was obtained for antipyrine from the mean distribution volumes. In contrast to the distribution volumes all partition coefficients were flow independent.

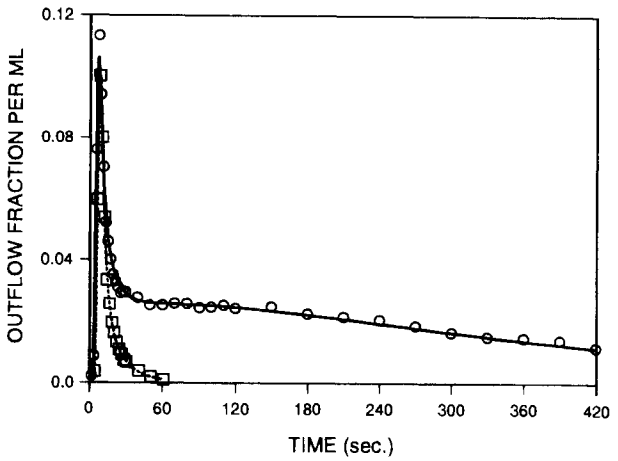


**Fig. 1.** Typical impulse response data for diazepam ( ) and the vascular marker (Evans blue, scale normalized to $C_{max}$ of diazepam) (----) in the perfused rat limb and the curves predicted by the organ model: Eqs. 1, 2 and 3 for Evans blue (----) and Eqs. 5, 4 and 2 for diazepam ( ). Flow rate $Q$ = 8 ml/min.

Table II summarizes the estimated values of the permeation clearance $CL_{BT}$, which decrease according to $CL_{BT,wat} > CL_{BT,ant} > CL_{BT,lido} > CL_{BT,dia}$. (Note that $V_T$ *increases* in the same order.) The increase in $CL_{BT}$ with flow was statistically significant and as in the case of $V_B$ proportionality is suggested by the mean ratio of $[(CL_{BT,8}/CL_{BT,4})/(8/4)]$ = 1.02.

No significant differences between compounds could be found, for the intratissue relaxation time $d$. However, the mean value of $d$ = 124 ± 33 sec observed for $Q$ = 4 ml/min was significantly higher than that of 86 ± 18 sec estimated in experiments with $Q$ = 8 ml/min (n = 17).

**Table I.** Distribution Volumes for the Tracers in Tissue ($V_T$) and for the Vascular Reference (Vascular Volume, $V_B$) in the Isolated Perfused Hind Limb Obtained by Model Analysis of Impulse Response Data

| | | $V_T$ (ml) | | |
|---|---|---|---|---|
| Substance | $n$ | $Q$ = 4 ml/min | $Q$ = 8 ml/min | *Ratio*[a] $R(V_T)/R(Q)$ |
| $^3$H-Water | 10 | 17.45 ± 4.43 | 19.56 ± 5.90 | 0.56 |
| $^3$H-Diazepam | 4 | 20.16 ± 13.15 | 38.73 ± 12.97 | 0.96 |
| $^{14}$C-Lidocaine | 3 | 23.35 ± 9.55 | 34.87 ± 4.76 | 0.75 |
| $^{14}$C-Antipyrine | 4 | 13.88 ± 6.54 | 25.24 ± 5.23 | 0.91 |
| | | | | 0.79 ± 0.18 |
| | | | | 0.71 ± 0.22[b] |
| | | | $V_B$ (ml) | |
| Evans blue | 11 | 0.73 ± 0.31 | 1.35 ± 0.34 | 1.06 ± 0.47 |

[a] $R(V_T) = V_{T,8}/V_{T,4}$, $R(Q)$ = 8/4.
[b] Mean ± SD of all individual ratios.

**Table II.** Permeation Clearances of Transcapillary Exchange in the Isolated Perfused Hind Limb Obtained by Model Analysis of Impulse Response Data

| | | $CL_{BT}$ (ml/min) | | | |
|---|---|---|---|---|---|
| Substance | $n$ | $Q$ = 4 ml/min | $n$ | $Q$ = 8 ml/min | Ratio[a] $R(CL_{BT})/R(Q)$ |
| $^3$H-Water | 10 | 94.5 ± 46.8 | 13 | 197.53 ± 81.0 | 1.05 |
| $^3$H-Diazepam | 4 | 19.6 ± 13.2 | 3 | 43.07 ± 51.0 | 1.10 |
| $^{14}$C-Lidocaine | 3 | 52.3 ± 12.0 | 3 | 82.18 ± 11.4 | 0.79 |
| $^{14}$C-Antipyrine | 4 | 83.3 ± 24.0 | 3 | 135.72 ± 39.0 | 0.81 |
| | | | | | 0.94 ± 0.16 |
| | | | | | 1.02 ± 0.51[b] |

[a] $R(CL_{BT}) = (CL_{BT,8}/CL_{BT,4})$, $R(Q)$ = 8/4.
[b] Mean ± SD of all individual ratios.

The relative dispersion of the intravascular transit time density $CV_B^2$ estimated for each preparation by fitting the outflow curves of Evans blue was independent of perfusion rate: $CV_B^2$ = 1.64 ± 0.57 for $Q$ = 4 ml/min and 1.48 ± 0.94 for $Q$ = 8 ml/min ($n$ = 11). Interestingly, the relative weight $p$ in Eq. (1) changed significantly with flow was changed ($p$ = 0.65 ± 0.14 for $Q$ = 4 ml/min vs. $p$ = 0.54 ± 0.10 for $Q$ = 8 ml/min). From the ratio of $CV_B^2$ to the transit time dispersions of the permeating substances $CV^2$ [Eq. (6)] it follows that the dispersion within the microvascular network accounts on the average for 75% of the total transit time dispersion. (This ratio was flow independent and no statistically significant difference could be found between the various substances.)

## DISCUSSION

In denervated limbs with minimal basal vascular tone, an increase in flow is expected to be accompanied by an opening of capillary beds. Thus, the increase in vascular volume $V_B$ and distribution clearance $CL_{BT}$ can be interpreted as an recruitment effect leading to an increase in the amount of tissue perfused. For $V_B$ and $CL_{BT}$ a proportional increase with flow was observed, while our results suggest a less than proportional increase of the tissue distribution volume (Tables I and II) in accordance with similar findings for cefixime in rat liver (6). The flow-independence of the partition coefficients $K_p$, on the other hand, reflects the validity of the underlying modeling concept. The interpretation of the flow dependence of $CL_{BT}$ in rat hindlimb as recruitment of capillary surface area is in accordance with observations in skeletal muscle (9) and is also supported by the fact that the recruitment factor did not differ between water and the lipophilic drugs.

In contrast to the traditional method of $K_p$ estimation using destructive sampling at steady-state, which assumes tissue homogeneity and is further complicated by the difficulties in determining drug concentration in tissue samples, the present approach does not require measurement of drug concentration within tissue and can be also applied to heterogeneous systems. For antipyrine the estimated $K_p$ = 1.0 was near to the value of 0.9 in rat skeletal muscle *in vivo* (10) and the $V_T$ of 0.8 l/kg is in perfect agreement with the whole body distribution volume of 0.80 in the rat (11). The water volume of 0.62 l/kg was comparable to that of 57% of total body weight in man (12) and is nearly identical to that of 0.65 in the rat *in vivo* (5). The observed good agreement with the *in vivo* distribution volumes underlines the conjecture that a flow of 8 ml/min reflects the *in vivo* situation more realistically than a flow rate of 4 ml/min (3,4). Note that for protein-bound drugs $V_T = V_{TW}(f_{uB}/f_{uT})$ is determined by the fractions unbound in "blood" and tissue phase, $f_{uB}$ and $f_{uT}$, respectively. Thus, $V_T$ decreases with increasing albumin percentage in perfusate, i.e. decreasing $f_{uB}$, and only the unbound tissue volume $V_{uT} = V_{TW}/f_{uT} = V_T/f_{uB}$ can be calculated. The values of 204 and 97 ml obtained for diazepam ($f_{uB}$ = 0.19) and lidocaine ($f_{uB}$ = 0.36), respectively, are in agreement with the mean values estimated for three different albumin concentrations in (4).

The *intrinsic* distribution clearance $CL_{BT}$ is determined by the permeability-surface area product, $PS$, and the unbound fraction of drug $f_{uB}$ in perfusate ($CL_{BT} = f_{uB}PS$) and should be distinguished from the organ distribution clearance, $CL_d = Q(1 - e^{-PS/Q})$, defined by the simple parallel tube (single capillary) model (13,14), which does not account for vascular heterogeneity. The ratio $CL_{BT}/Q$ varies from 24.7 (water) to 5.4 (diazepam) indicating barrier limited distribution even for water. However, this is not in contradiction to its apparent flow-limited distribution in whole body pharmacokinetics, since in the context of physiological pharmacokinetic modeling organ distribution appears already flow-limited for $CL_{BT}/Q \approx 0.5$ ($CL_d/Q \approx 0.4$) as shown for fentanyl in the rat (14). This can be explained by the non-impulse character of the drug disposition curve playing the role of the input function *in vivo*. Thus one has always to distinguish between the practical (or "apparent") flow-limitation observed under *in vivo* conditions and the barrier-limited distribution detectable with the more sensitive IR experiments in isolated perfused organs.

Interestingly, very similar permeability-surface area products are obtained for water ($PS$ = 198 ml/min), diazepam (227 ml/min) and lidocaine (227 ml/min) from the $CL_{BT}$ values and fractions unbound $f_{uB}$ of the drugs.

In contrast to the *a priori* assumption of rapid intratissue diffusion conventionally made in drug and tracer kinetics the relaxation time $d$ accounts for the "slow" equilibration process within the tissue phase. Given the present results for water and antipyrine an interpretation based on tissue binding (8) appears not convincing. The relatively long relaxation time $d$ thus simply points to the fact that the tissue phase is not well-mixed.

Since the convective dispersion accounts for 75% of $CV^2$ value attempts to estimate distribution parameters by the moment method using Eq. (6) may prove less successful. The flow-independence of $CV_B^2$ is in accordance with the theoretical prediction for geometrical dispersion determined by the morphology of the microvascular network (15).

The results obtained in the present study suggest that the pharmacokinetic organ model (8) is a useful approach to analyze the kinetic events and the extent of tissue sequestration of drugs in the single pass, isolated hindlimb preparation.

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