A New Physiologically Based Pharmacokinetic Analysis for In Vivo Organ Disposition of Nitroxyl Spin Probes in Mice as Measured by Multisite Detection L-band ESR (MSD-LESR)

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We developed a physiologically based pharmacokinetic (PB-PK) model for spin-labeled drugs in animals and applied it for analyzing the decay curves of spin probes in the organs of mice as measured by multisite detection L-band ESR (MSD-LESR). The proposed method will be useful for not only understanding the PK features of nitroxyl radicals as spin probes but also developing spin-labeled drugs as a substitute for radioisotope-labeled drugs.

In vivo L-band electron spin resonance (ESR) spectroscopy using a surface-coil-type resonator enabled us to measure the ESR spectra from spin probes; further, the local concentrations of these probes could be determined in not only blood vessels but also the surface of organs in animals treated with the spin probes.[1] A classical compartment model has been widely used for the pharmacokinetic (PK) analysis of drug disposition in the blood of animals and humans administered drugs through the oral and intravenous routes.[2] A physiologically based PK (PB-PK) model in which the distribution and metabolism of a drug in multiple organs are connected to the blood flow rate has also been developed to predict the profiles of drug concentrations in organs from in vitro data.[3,4] However, to the best of our knowledge, no reports have analyzed the multiple curves of drug concentration in the organs of animals on the basis of a PB-PK model combined with the simultaneous curve fitting method.

In this study, in order to evaluate the distribution and elimination of spin probes in the organs of a live mouse, we developed a new PB-PK model for describing the multisite ESR signal decays due to spin probes observed in the organs of a mouse. The theoretical equations for a PB-PK model were represented by simultaneous differential equations of mass balance with all linear processes in each organ, and these equations can be solved by the Laplace transform.

Two spin probe nitroxyl radicals (Sigma-Aldrich, St. Louis, MO, U.S.A.)—3-carbamoyl-PROXYL (CMP) with a neutral charge and 3-carboxy-PROXYL (CXP) with a negative charge at physiological pH 7.4—that exhibited different PK profiles from each other[5,6] were used. Flexible (FSCR) and semirigid (SRSCR) surface-coil-type resonators consisting of a single-turn coil with diameters of 5 and 11 mm, respectively, were prepared in our laboratory.[7] Male ddy mice (6-week-old, approximately 30 g) were used. Animal and analytical experiments were performed as previously reported.[1] Briefly, mice were anesthetized using an intraperitoneal injection of sodium pentobarbital (Abbott Laboratories, Abbott Park, Ill, U.S.A.). After the abdomen was opened through a middle incision, the FSCR was tied with the branch vessel of the inferior vena cava by using a surgical silk thread, while the SRSCR was fixed tightly on the surface of the liver or kidney. After the abdomen was sutured, mice with FSCR or SRSCR were fixed on a handmade Teflon holder and inserted into the cavity of the L-band ESR spectrometer JES-RE-3L (JEOL, Japan). Each spin probe dissolved in saline was intravenously injected through the tail vein at a dose of 130 μmol/kg body weight, and in vivo L-band ESR spectra from nitroxyl radical were measured in one organ per each mouse immediately after the injection at every 30 s by using the spectrometer.[1,8]

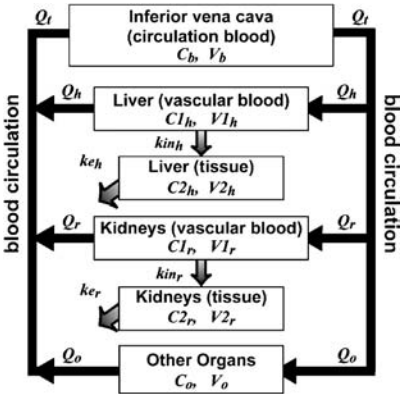

Figure 1. Proposed physiologically based pharmacokinetic model for the multisite disposition of spin probes in the organs of a mouse.

Figure 1 shows the PB-PK model where the mass balances of the spin probes in the blood circulation, liver, kidney, and other organs are described by the simultaneous differential equations. These equations at each measurement site (blood, liver, and kidney) and other organs are solved by the Laplace transform in which Q is the blood flow rate (mL/min/kg),[9] V is the distribution volume (mL/kg), $\tilde{C}$ is the Laplace transform of the concentration of a spin probe (mM), k_{in} (1/min) is the rate constant of uptake into the tissue, k_e (1/min) is the elimination constant from the tissue, and D (μmol/kg) is the dose; the subscripts b, h, r, and o represent the blood, liver (hepatic), kidney (renal), and other organs, respectively. The Laplace-transformed equations for the concentration–time profiles in each organ are as follows:

$$(sV_b + Q_h + Q_r + Q_o)\tilde{C}_b - D = Q_h\tilde{C}1_h + Q_r\tilde{C}1_r + Q_o\tilde{C}_o \quad (1)$$

$$\tilde{C}_h = \frac{Q_h/V_h}{s + k_{inh} + Q_h/V1_h} \cdot \frac{s + k_{inh} + k_{eh}}{s + k_{eh}} \cdot \tilde{C}_b \quad (2)$$

Table 1. PK parameters of CMP and CXP in the organs of mice as calculated by MULTI(FILT) based on the proposed PB-PK model[a]

probes	Blood	Liver (hepatic)			Kidney (renal)			Other organs
	V_b /mL kg^{-1}	V_h /mL kg^{-1}	k_{inh} /10^3 min^{-1}	k_{eh} /10^3 min^{-1}	V_r /mL kg^{-1}	k_{inr} /10^3 min^{-1}	k_{er} /10^3 min^{-1}	V_o /mL kg^{-1}
CMP	68 ± 7	100 ± 3	5.4 ± 1.1	5.9 ± 1.4	22 ± 1	8.5 ± 1.0	8.5 ± 1.0	490 ± 10
CXP	93 ± 8	100 ± 2	0.3 ± 0.2	13 ± 7	23 ± 1	9.0 ± 0.4	36 ± 11	190 ± 10

[a]Total distribution volume (V_t) was defined as the sum of V_b, V_h, V_r, and V_o.

$$\tilde{C}_r = \frac{Q_r/V_r}{s + k_{inr} + Q_r/V1_r} \cdot \frac{s + k_{inr} + k_{er}}{s + k_{er}} \cdot \tilde{C}_b \quad (3)$$

$$\tilde{C}_o = \frac{Q_o/V_o}{s + Q_o/V_o} \cdot \tilde{C}_b \quad (4)$$

The concentration in the blood (eq 5) can be derived from these eqs 1–4, where s is the Laplace operator, and Q_t is the sum of Q_h, Q_r, and Q_o.

$$\tilde{C}_b = \frac{D}{sV_b + Q_t - \dfrac{Q_h(Q_h/V1_h)}{s + k_{inh} + Q_h/V1_h} - \dfrac{Q_r(Q_r/V1_r)}{s + k_{inr} + Q_r/V1_r} - \dfrac{Q_o(Q_o/V_o)}{s + Q_o/V_o}} \quad (5)$$

From the eqs 2, 3, and 5, the parameters V, k_{in}, and k_e were calculated simultaneously by the multiline fitting program, MULTI(FILT); this program was combined with the fast inverse Laplace transform (FILT) and nonlinear least squares regression.[10] MULTI(FILT) was written in Microsoft FORTRAN and run on a personal DOS-V computer.[11]

The results of curve fitting on the basis of the PB-PK model are shown in Figure 2. After the injection of spin probes, the concentration of CMP was more rapidly decreased in each organ compared to that of CXP. Theoretical curves were well fitted with the observed data except for those of CXP in the liver, where the reoxidation might lead to a biphase profile; the estimated PK parameters are summarized in Table 1.

The decay curves of spin probes in the blood can be affected by several rate factors such as distribution, elimination (metabolism and excretion), and redox (reduction and reoxidation) rates. Because the reduction and reoxidation rates, which were equilibrated within 5 min in the body, were found to affect only the initial decay,[6] the decay curve in the slower terminal phase primarily depended on both the distribution and elimination rates.[5,6] Neutral CMP exhibited the larger distribution volume in the organs (V_o) and its smaller distribution volume in the blood (V_b) than those of anionic CXP, respectively. Although the clearance rates of anionic CXP in both the liver (k_{eh}) and kidney (k_{er}) were faster than those of neutral CMP, respectively, the uptake of CXP into the hepatic tissue (k_{inh}) was very small. The PB-PK analyses indicated that the difference in the blood disposition values between spin probes was caused by differences in their distribution volume in whole organs; total distribution volume (V_t) of CMP and CXP corresponded to 680 and 406 mL/kg, respectively, and the renal clearance rate (k_{er}) of CXP was fourfold faster than that of CMP. These results closely agreed with those of previous studies of spin probe disposition in single organ sites in animals.[5,6]

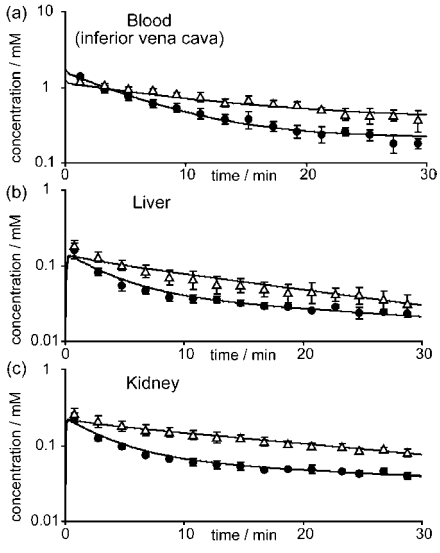

Figure 2. The theoretical curves (solid curve) based on the PB-PK model vs. time and plots of the observed concentrations of the spin probes (●) CMP and (△) CXP in the (a) blood, (b) liver, and (c) kidney of mice; these probes were administered at a dose of 130 μmol/kg body weight.

In conclusion, the proposed method will be useful for not only understanding the comprehensive PK features of nitroxyl radicals as redox probes but also developing new spin-labeled drugs based on multisite detection L-band ESR (MSD-LESR) and PB-PK analysis.

References and Notes

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8 The radical concentrations were determined as follows. The intensities of the central signal in the triplet spectrum from the nitroxyl radical were measured. To prepare the calibration lines, 5 concentrations of spin probes were added to the fresh blood (500 μL) of mice, and each resulting solution (0.1–2 mM in blood) was transferred to a 600-μL polyethylene tube that was fixed with SCRs. The radical amounts (nmol) in the SCRs were obtained by multiplying their concentration (mM) within the polyethylene tube and the volumes of FSCR (3.1 μL) and SRSCR (14.1 μL). In in vivo ESR measurements, the signal intensities were calibrated to the corresponding radical amounts (nmol), and the radical concentrations (mM) in the blood and organs of mice were obtained by dividing the determined radical amounts (nmol) by the volume of the measurable blood vessel (1.6 μL) or organ (47.5 μL).
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11 We defined $C_h = (V1_hC1_h + V2_hC2_h)/V_h$ and $C_r = (V1_rC1_r + V2_rC2_r)/V_r$. $V1_h$ and $V1_r$ were assumed to be the volume of the blood in the liver and kidney; this corresponded to 12% of 65 mL/kg in the liver and 16% of 15 mL/kg in the kidney, respectively.[9] Blood flow rates in the cardiac output ($Q_t = 400$ mL/min/kg), liver ($Q_h = 90$ mL/min/kg) and kidney ($Q_r = 65$ mL/min/kg) were obtained from the literature.[9] The data expressed as the means ± standard deviations for 4–5 mice are shown in Figure 2 and Table 1.

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