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Short communication

Simple high-performance liquid chromatographic assay of propofol in human and rat plasma and various rat tissues

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The use of propofol is becoming increasingly widespread as an intravenous anesthetic agent suitable for the induction and maintenance of anesthesia and for sedation in critically ill patients. The analysis and monitoring of the concentrations of propofol aids in attempts to determine the minimal dose sufficient for an individual patient to maintain anesthesia and help to decrease the risk of drug-related complications and interactions. Recent studies have shown that the concentration of propofol at the effect site, estimated based on the effect-compartment model, was more accurate for predicting the onset of propofol effect than the plasma concentration [1,2]. Furthermore, the redistribution of propofol from various tissues has a more significant impact on the time required for a patient to recover from propofol anesthesia than the elimination clearance of the drug in the awakening phase. To explore the implications of tissue distribution kinetics in the pharmacokinetics and pharmacodynamics of propofol, measurement of the concentrations of propofol in various tissues is indispensable. The objective of the present study was to develop a simple method for the assay of propofol in plasma and tissues.

Propofol (2,6-diisopropylphenol) was purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile and water of high-performance liquid chromatography (HPLC) grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). The HPLC apparatus we used (LC-10AD; Shimadzu, Kyoto, Japan) is comprised of a fluorimetric detector (RF-10AD; Shimadzu), degasser (DGU-14A; Shimadzu), autoinjector (SIL-10AD; Shimadzu), and column oven (CTO-10AS; Shimadzu). A Symmetry C18 column (3.5-μm, 4.6 × 100 mm; Waters, Tokyo, Japan) was selected for the separation of propofol. The temperature of the column oven was set at 40°C. The mobile phase consisted of acetonitrile and water (60:40, vol/vol), which was equilibrated at $1.5\,ml \cdot min^{-1}$. The fluorimetric detector was used for quantifying propofol, and the concentration of propofol was estimated based on the integrated peak area. The excitation and emission wavelengths for the fluorescence detection of propofol were set at 276 and 310 nm, respectively [3].

Various rat tissues (brain, liver, kidney, and adipose and muscle tissues) harvested from rats anesthetized with pentobarbital ($40\,mg \cdot kg^{-1}$ i.p.) were rinsed with chilled saline to remove contamination with blood. The tissues were subdivided into small pieces with a scalpel and placed in homogenization vessels to prepare homogenates. The mobile phase, equal to nine times the tissue weight, was then added, and the tissue was homogenized at 2000 rpm to a uniform slurry (Digital Homogenizer; Iuchi, Osaka, Japan). To 100 μl of plasma or 10% tissue homogenate, 1000 μl of acetonitrile was added and mixed thoroughly in a vortex mixer. The mixture was centrifuged at 15 000 *g* for 10 min at 4°C. Then, 10 μl of the clear supernatant was directly injected onto the HPLC column. The samples for the calibration curves were prepared from an acetonitrile solution containing propofol at various concentrations. The acetonitrile solutions of propofol were diluted 50 times with plasma or 10% tissue homogenate to obtain the final concentrations of 0, 0.05, 0.2, 1.0, 2.0, 5.0, and $10\,\mu g \cdot ml^{-1}$ or per gram of wet tissue. The integrated peak area was plotted against the known concentrations of propofol, and calibration curves were constructed by linear regression (Microsoft Office 97 Excel; Tokyo, Japan). The slopes of calibration curves constructed with human plasma and various rat tissues were compared with one-way analysis of variance. If the analysis of variance

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showed a significant difference, Bonferroni's test was performed to compare the values for various tissues with that for human plasma. Differences were considered to be significant when $P < 0.05$.

Figure 1 illustrates the chromatographs of human blank plasma, human plasma containing propofol $0.2\,\mu g \cdot ml^{-1}$, rat brain blank homogenate, and rat brain homogenate containing propofol $0.2\,\mu g \cdot g^{-1}$ wet tissue, respectively, following the deproteinization with acetonitrile. No interference peaks were observed around the retention time for propofol (4.7 min) for either plasma or tissues. As shown in Fig. 1, the areas (heights) of the propofol peaks were almost equal for human plasma and rat brain homogenate, suggesting that the extent of coprecipitation of propofol with plasma and tissue homogenates was similar. Calibration curves were constructed by increasing the sample concentrations while maintaining the injection volume constant (10 μl) over the range from 0.05 to $10\,\mu g \cdot ml^{-1}$. The linear regression characteristics of the calibration curves for propofol in human plasma and various rat tissue homogenates are summarized in Table 1. The slopes of calibration curves constructed with brain, liver, and kidney tissue homogenates showed significantly different values as compared with that for human plasma (Table 1; $P < 0.05$), while no differences were observed for adipose and muscle tissues. The variations between tissue and plasma samples can be attributed to the additional processes involved in the preparation of tissue homogenates. The correlation coefficients of the calibration curves for all rat tissues investigated were >0.999. The limit of quantification of propofol was $0.05\,\mu g \cdot ml^{-1}$ in plasma when 10 μl of the supernatant was injected.

The intra- and interassay precision coefficients of variation for measuring propofol at a concentration of $0.2\,\mu g \cdot ml^{-1}$ in human plasma were 1.38% and 1.72%, respectively (Table 2). A better intraassay precision (0.84%) was obtained when the concentration of propofol was increased to $10\,\mu g \cdot ml^{-1}$. The interassay variation at $10\,\mu g \cdot ml^{-1}$ (1.89%) was similar to that for $0.2\,\mu g \cdot ml^{-1}$. The corresponding intra- and interassay precision coefficients of variation for propofol measurement in various rat tissues were <4% and are summarized in Table 2.

A variety of HPLC methods for the assay of propofol in blood, plasma, and serum have been developed with

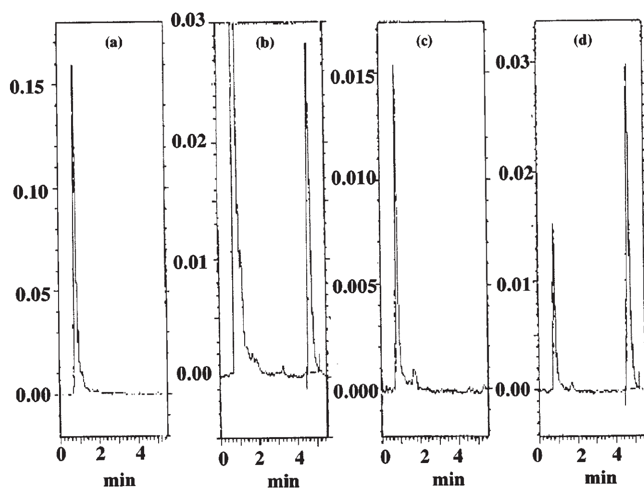

Fig. 1a–d. Chromatographs of human plasma and rat brain tissue homogenate. **a** Human blank plasma; **b** human plasma containing propofol $0.2\,\mu g \cdot ml^{-1}$; **c** rat brain blank homogenate (10%); **d** rat brain homogenate (10%) containing propofol $0.2\,\mu g \cdot g^{-1}$ wet tissue

Table 1. Linear regression characteristics of calibration curves for propofol in human plasma and various rat tissue homogenates

Tissue	Slope	Intercept	r^2
Human plasma	317 346 ± 5 985	−10 038 ± 4 458	0.9998
Brain	297 637 ± 4 695*	−5 005 ± 3 863	0.9998
Liver	301 695 ± 5 158*	−7 042 ± 4 645	0.9998
Kidney	328 885 ± 4 031*	−6 496 ± 4 056	0.9999
Adipose tissue	318 123 ± 5 691	5 822 ± 3 900	0.9998
Muscle tissue	318 604 ± 4 594	7 734 ± 3 797	0.9999

* Significantly different from human plasma; $P < 0.05$

Table 2. Mean percentage inter- and intraassay precision coefficients of variation (%) for propofol measurement in plasma and various rat tissues at concentrations of 0.2 and $10\,\mu g \cdot ml^{-1}$, respectively

Tissue	$0.2\,\mu g \cdot ml^{-1}$ Intraassay	$0.2\,\mu g \cdot ml^{-1}$ Interassay	$10\,\mu g \cdot ml^{-1}$ Intraassay	$10\,\mu g \cdot ml^{-1}$ Interassay
Human plasma	1.38	1.72	0.84	1.89
Brain	2.44	3.46	1.03	1.41
Liver	2.62	1.52	1.93	1.51
Kidney	1.08	2.05	0.80	1.25
Adipose tissue	0.98	1.65	1.03	1.54
Muscle tissue	0.78	2.22	0.75	1.54

UV, fluorescence, or electrochemical detection techniques [3–15]. Most of the methods reported involve either liquid-liquid or solid-phase extraction, and a relatively large sample volume (0.5 to 1.0 ml) is necessary, which is not suitable for mechanism-based pharmacokinetic and pharmacodynamic (PK/PD) studies using small animals such as rats. Few methods for the measurement of propofol reported so far have described the assay of propofol in tissue. Dowrie et al. [14] demonstrated an HPLC-electrochemical detection analytical method for determining the concentrations of propofol in human or rat plasma and a variety of rat tissues utilizing liquid-liquid extraction. In this study, we developed an HPLC method for determining the concentrations of propofol in human plasma and in various rat tissues with fluorescence detection. This assay is very simple and has excellent reproducibility, while no extraction procedure is necessary. The plasma concentration can be measured within 20 min, including sample preparation, and only 10 μl of plasma is necessary for the determination of propofol concentration. Propofol was successfully separated and quantified by the present method, with fluorescence detection; this was done simply by deproteinization with acetonitrile before injection onto the HPLC system. The limitation of quantification was 0.05 $\mu g \cdot ml^{-1}$ in plasma or 10% tissue homogenate for a 10-μl injection volume, and this could be decreased to 0.01 $\mu g \cdot ml^{-1}$ by increasing the injection volume. In comparison with the HPLC-UV detection method for determining the concentrations of propofol in plasma by direct injection of the deproteinized plasma onto HPLC developed by Vree et al. [5] and Pavan et al. [10], the present method with fluorescence detection showed a much higher sensitivity. No internal standard was used in our assay, because we found excellent linear correlation coefficients for the calibration curves by plotting the integrated areas against the known concentrations of propofol (Table 1). Consistent with our observations, Yeganeh and Ramzan [15] also reported that they found no difference for the quantification of propofol using either the peak-area ratio of propofol to an internal standard (4-tert.-octylphenol) or using the area of propofol alone.

The currently developed assay is simple and does not need a procedure for extraction from biological fluids. It is also sufficiently sensitive for the determination of propofol concentrations in plasma and rat tissues for either clinical PK/PD studies or for basic tissue distribution kinetic studies using small volumes of plasma or tissue homogenate. This easy-to-use propofol assay is therefore suitable for routine studies in patients or in small animals.

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