CONTINUOUS MONITORING OF UNBOUND FLOMOXEF LEVELS IN RAT BLOOD USING MICRODIALYSIS AND ITS NEW PHARMACOKINETIC ANALYSIS

Yutaka SAISHO* and Tsuneji UMEDA

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 Japan

Microdialysis sampling method coupled to high-performance liquid chromatography with UV detection was applied to continuous monitoring of *in vivo* unbound flomoxef concentration in rat blood. By comparison with ultrafiltration method, it was demonstrated that it gave reliable results for the unbound drug monitoring in blood. Furthermore, a new method was presented for the calculation of pharmacokinetic parameters from the data obtained by the microdialysis method.

KEYWORDS microdialysis; flomoxef; high-performance liquid chromatography; ultrafiltration; pharmacokinetic parameter

Microdialysis, which was first introduced by Tossman and Ungerstedt in 1981,¹⁾ has been developed to sample the extracellular space of brain²⁾ and other tissues.^{3,4)} In recent years, its several applications to the analysis of exo- and endogenous substances in blood have been reported.^{5,6)} The present investigation was undertaken (a) to monitor the unbound flomoxef concentration in blood of individual rat after i.v. administration of its sodium salt⁷⁾ by the combination of microdialysis and high-performance liquid chromatography (HPLC) with UV detection, (b) to evaluate the reliability for results of the microdialysis method by comparison with those of the established ultrafiltration method, and (c) to propose a new method to calculate pharmacokinetic parameters from the product of concentration and sampling time obtained by the microdialysis method.

As shown in Fig. 1, a microdialysis probe (Bioanalytical Systems, Inc./Carnegie Medicin, West Lafayette, IN) equipped with a dialysis membrane (10×0.5 mm I.D., molecular cut-off 20,000) was introduced inside a 19-gage needle cannula into the jugular vein of 260–320 g male rats under pentobarbital anesthesia, and was perfused with saline at a speed of 2 µl/min by a microinjection pump. After i.v. administration of flomoxef sodium, sample dialysates were collected every 10 minutes, and were directly subjected to HPLC analysis. As illustrated in Fig. 2, the chromatogram was free from interferences. Time courses of the concentrations of unbound flomoxef in blood of individual six rats were monitored continuously until 90 minutes after i.v. administration (20, 40, 80 mg/kg). Unbound flomoxef levels in blood were corrected for the mean of *in vitro* recoveries (ca. 50%) that were tested at 37°C both before and after *in vivo* operation using a well-stirred standard solution of flomoxef sodium.

The ultrafiltration method established for the sampling of unbound fraction in blood was examined to evaluate the reliability for *in vivo* drug monitoring by the microdialysis method. The six blood samples were collected through femoral vein at each time point of 5, 10, 30, 60 and 90 minutes, respectively, after i.v. administration of flomoxef sodium. Unbound fractions of plasma were prepared under centrifugation at 37°C by use of MPS-3 micropartition system (amicon, Lexington, MA). There was no need to adjust the pH of plasma, because the protein binding of flomoxef in plasma was little influenced in the range of pH 6.5–8.0. As shown in Fig. 3 with comparative illustration, good correlations were appeared for the unbound levels of flomoxef between both methods of microdialysis and ultrafiltration. These results would imply that the mass transport resistance⁸⁾ in blood is as low as that in a well-stirred aqueous medium, and that the blood fluid is under ideal surroundings for its microdialysis due to its continuous fast replacement. Therefore, microdialysis of blood would be applicable to the quantitation of individual unbound drug levels in small animals such as rats with satisfactory reliability.

Fig. 1. Microdialysis System

Fig. 2. HPLC of Drug-Free (Lower) and Flomoxef-Containing (Upper) Rat Blood Dialysate. Conditions: column, Nucleosil 5 C₁₈ (150×4.6 mm I.D.); mobile phase, CH₃CN: 50 mM tetra-n-butylammonium bisulfate = 1:3 (v/v); flow rate, 1.0 ml/min; UV detection, 270 nm.

Fig. 3. Mean Concentrations of Unbound Flomoxef in Rat Blood Treated with Microdialysis and Ultrafiltration Methods after i.v. Administration of 20, 40 and 80 mg/kg of Flomoxef Sodium. Dialysate values are corrected for *in vitro* recovery.

Since microdialysis method needs a time period to collect the blood dialysate, it is unable to give the concentration at a time point. Instead of the measured concentration itself, the product of it and the sampling time is more likely to be a significant response variable, first proposed by us, for pharmacokinetic analysis of *in vivo* drug level monitoring by the microdialysis method. The product that is the each column area shown in Fig. 3, is corresponding to the area, in a time period, under the concentration-time curve which is ordinarily obtained by the discontinuous sampling method to withdraw blood. By use of the non-linear curve-fitting program NONLIN,⁹ pharmacokinetic analysis was undertaken according to the one-compartment model to which the product was fitted as a function described by the following equation;

$$P = A \int_{t_1}^{t_2} e^{-k_e \cdot t} dt$$

where P is the product of concentration and sampling time during the interval t_1 to t_2 , A is initial unbound drug concentration and k_e is first-order elimination rate constant. Comparative pharmacokinetic analysis was likewise carried out for the concentration-time data of the ultrafiltration method. As shown in Table I, the analyzed pharmacokinetic parameters gave good agreements between both the methods.

Table I. Pharmacokinetic Parameters for Unbound Flomoxef

Parameter		Microdialysis	Ultrafiltration
k_e	(min^{-1})	0.0648 ± 0.0082	0.0756
A	($\mu\text{g}\cdot\text{ml}^{-1}$)	66.0 ± 12.0	65.2
$t_{1/2}$	(min)	10.9 ± 1.7	9.2
V_d	($\text{l}\cdot\text{kg}^{-1}$)	314 ± 62	307
AUC_∞	($\mu\text{g}\cdot\text{ml}^{-1}\cdot\text{min}$)	1020 ± 150	860
CL	($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	20.0 ± 3.2	23.2

Dose: 20 mg/kg. Microdialysis data express mean $\pm$ S.D. (n = 6).

In the present study, microdialysis has been shown to be reliably applied to *in vivo* determination of unbound drug levels in blood. Furthermore, we have presented a new method for pharmacokinetic analysis from the data obtained by the microdialysis method. In conclusion, microdialysis of blood has been also evaluated to be a unique and useful sampling method for continuous drug monitoring in individual rats. Further studies are in progress on the application of the method to monitoring other drugs.

ACKNOWLEDGEMENT The authors express their sincere thanks to Dr. K. Matsunaga for giving his technical advices on operation of rat and to Dr. T. Tasaki and Mr. M. Zaizen for the pharmacokinetic analysis of the data.

REFERENCES AND NOTES

- 1) U. Tossman and U. Ungerstedt, *Neurosci. Lett. Suppl.*, **7**, S479 (1981).
- 2) H. Benveniste, *J. Neurochem.*, **52**, 1667 (1989).
- 3) O. Meirieu, M. Pairet, J. F. Suruta and M. Ruckebusch, *Life Sci.*, **38**, 827 (1986).
- 4) H. Jarry, M. Dietrich, A. Barthel, A. Giesler and W. Wuttke, *Endocrinology*, **125**, 624 (1989).
- 5) R. M. Caprioli and Shen-Nan Lin, *Proc. Natl. Acad. Sci., USA*, **87**, 240 (1990).
- 6) D. O. Scott, L. R. Sorensen and C. E. Lunte, *J. Chromatogr.*, **506**, 461 (1990).
- 7) A new parenteral oxacephem antibiotic which was synthesized in our laboratories; T. Tsuji, H. Satoh, M. Narisada, Y. Hamashima and T. Yoshida, *J. Antibiot.*, **38**, 466 (1985).
- 8) P. M. Bungay, P. F. Morrison, R. L. Dedrick, *Life Sci.*, **46**, 105 (1990).
- 9) C. M. Metzler, G. R. Elfring and A. J. McEwen, *Biometrics*, **130**, 560 (1974).

(Received January 10, 1991)