In Vivo Microdialysis for the Transdermal Absorption of Valproate in Rats

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Received March 24, 1994; accepted June 16, 1994

The suitability of sampling *via* microdialysis for a lipophilic drug, valproate (VPA), was evaluated by the elimination rate constant of VPA solution in an *in vitro* experimental first-order elimination system. The elimination rate constant of VPA in dialysate was found to be $0.43 \pm 0.05 \text{ h}^{-1}$, which was in good agreement with the real elimination rate constant ($0.46 \pm 0.02 \text{ h}^{-1}$). A change in VPA concentration in the solution surrounding a microdialysis probe was well maintained by the microdialysis method, suggesting no adsorption between the membrane of the microdialysis probe and VPA.

On the basis of the *in vitro* experiment, the effect of a penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101), on the transdermal absorption of VPA was examined in rats by the use of microdialysis *in vivo*. An intradermal microdialysis was performed at a flow rate of $1.0 \mu\text{l/min}$ for 7 h after the dermal application of 50 mM VPA solution with or without 3% (w/v) HPE-101. HPE-101 increased the transdermal absorption rate of VPA by 80 times compared with the control. The microdialysis system was found to be quite useful for assessing the *in vivo* transdermal absorption of a lipophilic VPA.

Keywords microdialysis; valproate; HPE-101; *in vivo* transdermal absorption; *in vitro* first-order elimination system; rat

Much attention has been paid to transdermal drug application because it has a number of advantages, *e.g.*, a reduction in side effects caused by excess dosing, minimization of first-pass effects, decreased drug decomposition in the gastrointestinal tract, and increased patient compliance, compared with other administration methods. Transdermal drug absorption has been generally studied *in vitro*, using isolated skin preparations. Recently, the study of *in vivo* transdermal drug absorption has become a matter of great concern.

Microdialysis, as an *in vivo* sampling technique, permits the analysis of test substances with minimal tissue damage from the extracellular space of most body tissues. We have successfully performed the *in vivo* transdermal absorption of methotrexate (MTX) in rats by using microdialysis.¹⁾ MTX is a hydrophilic drug, which permits MTX sampling *via* a microdialysis probe without worrying about the adsorption between the membrane of the microdialysis probe and the drug. However, adsorption becomes a serious problem when a lipophilic drug is tested.

In this study, we selected valproate (VPA) as a lipophilic model drug, and compared the kinetic parameters in the VPA solution collected *via* a microdialysis probe and pipette after the construction of an experimental first-order elimination system, followed by evaluation of the suitability of sampling *via* microdialysis of the lipophilic drug, VPA. On the basis of this experiment, study of the *in vivo* transdermal absorption of VPA was performed by the use of microdialysis, together with a penetration enhancer, 1-[2-(decylthio)ethyl]azacyclopentan-2-one (HPE-101).²⁾

MATERIALS AND METHODS

Materials Sodium valproate was supplied by Kyowa

Hakko Industry Co., Ltd. (Tokyo, Japan). The TDx assay reagent set for the fluorescence polarization immunoassay (FPIA) of VPA was purchased from Abbott Laboratories (Abbott Park, IL, U.S.A.). All other chemicals were of special reagent grade. HPE-101 shown in Fig. 1 was synthesized in our laboratories as described previously.²⁾

Microdialysis System The microdialysis system consisted of a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden) and CMA/10 microdialysis probes with a dialyzing membrane length of 10 mm and an outer diameter of 0.5 mm (Carnegie Medicin). The probe was connected to the microinjection pump and perfused continuously with Ringer's solution (pH 6.5) at a rate of $1.0 \mu\text{l/min}$ during the experimental period.

In Vitro Experiments As illustrated in Fig. 2, an experimental first-order elimination system for VPA solution was constructed according to the method described by Goto *et al.*³⁾ Ringer's solution was added continuously into well-stirred VPA solution (0.6 mM) kept at 37°C in a side-arm beaker at a flow rate of 2.5 ml/min *via* a peristaltic pump (Koe Science Co., Tokyo, Japan). Microdialysis was applied to the VPA solution at a rate of $1.0 \mu\text{l/min}$. The dialysate samples were collected into a small sample tube every 30 min for 3 h. Simultaneously, $30 \mu\text{l}$ of the VPA solution was pipetted from the side-arm beaker at the midpoint of each sampling period.

Animal Experiments Male Wistar rats (SPF), weighing 280–320 g, were anaesthetized with urethane (1.5 g/kg as 300 mg/ml , *i.p.*), and anaesthesia was continued through-

Fig. 1. Chemical Structure of HPE-101

Fig. 2. Scheme of the Apparatus Used for the *in Vitro* Experimental First-Order Elimination System

out the experimental period.

For intradermal microdialysis, the abdominal fur of the rats was shaved using a Thrive Model 900 animal clipper (Daitoh Electric Co., Tokyo, Japan), with care taken not to damage the skin. The skin was incised over the dermis in the shaved abdominal region, followed by the intradermal insertion of an introducer assembled by inserting the L-shaped needle into the black tubing (Fig. 3A). After setting the black tubing under the skin, the L-shaped needle was withdrawn (Fig. 3B), followed by insertion of the microdialysis probe (Fig. 3C). The black tubing was torn off by pulling upward and outward (Fig. 3D). After completion of the probe implantation, a hemispherical glass reservoir with an inner diameter of 20 mm was placed over the shaved abdominal region. At 1 h after the probe implantation, 2 ml of VPA solution (50 mM) composed of 60% (v/v) ethanol and 40% (v/v) phosphate buffer (50 mM, pH 5.0) with or without 3% (w/v) HPE-101 was applied to the reservoir (Fig. 3E). The dialysate samples were collected into a small sample tube every 1 h for 7 h (Fig. 3F).

Analysis of VPA Free VPA was analyzed by the Abbott TDx automated fluorescence polarization analyzer (Abbott Laboratories, Irving, TX, U.S.A.) based on FPIA technology.⁴⁾

Data Analysis The *in vitro* relative recovery was calculated from the VPA concentration in the dialysate divided by the VPA concentration in the side-arm beaker at each sampling period. The absorption profile was constructed by plotting the cumulative amount of VPA collected from the dermis against time. The steady-state rate of transdermal absorption was calculated from the linear slope of the absorption profile.⁵⁾ Statistical analysis was carried out using the Mann-Whitney *U*-test.

RESULTS AND DISCUSSION

***In Vitro* Relative Recovery of VPA** Using an *in vitro* experimental first-order elimination system, the elimination of the VPA solution in the beaker and the dialysate were comparatively examined.

The concentration-time profiles of VPA in the beaker

and the dialysate are shown in Fig. 4. The elimination rate constants of VPA in the beaker and the dialysate were 0.46 ± 0.02 and $0.43 \pm 0.05 \text{ h}^{-1}$ ($n=4$), respectively. There was no significant difference between them. A change in VPA concentration in the solution surrounding the microdialysis probe could be detected by microdialysis, suggesting no adsorption between the membrane of the microdialysis probe and VPA *in vitro*. It was also clarified that the *in vitro* relative recovery of VPA through the microdialysis probe used in this study was $80.2 \pm 3.1\%$ throughout the experimental period (Fig. 4).

***In Vivo* Transdermal Absorption of VPA** On the basis of the *in vitro* experiment described above, the effect of a penetration enhancer, HPE-101, on the *in vivo* transdermal absorption of VPA was examined in rats by the use of microdialysis.

The use of HPE-101 gave rise to a marked increase in VPA concentration in the dermal dialysate as shown in Fig. 5. The concentration of VPA in the dermal dialysate which had received 3% HPE-101 increased gradually for 4 h, and thereafter reached a plateau. The highest concentration of VPA in the dermal dialysate was observed in the fraction from 6 to 7 h. It increased from $3.60 \pm 0.32 \mu\text{g/ml}$ in the control to $285 \pm 42 \mu\text{g/ml}$ by co-application of 3% HPE-101 ($p < 0.02$). The transdermal absorption profiles for VPA with or without 3% HPE-101 are shown in Fig. 6. HPE-101 significantly increased the transdermal absorption rate of VPA, from $0.2 (\mu\text{g/dialysate}) \cdot \text{h}^{-1}$ in the control to $16.1 (\mu\text{g/dialysate}) \cdot \text{h}^{-1}$. About an 80 times increase was observed. Our previous study showed that HPE-101 produced a continuous increase in enhancing the activity of dermal penetration of indomethacin at concentrations up to 3% (w/v), above which the enhancing activity reached a plateau.²⁾ Therefore, we adopted the 3% concentration of HPE-101 in this study.

The concentration of MTX in a dermal dialysate could not reach a plateau for 12 h when applied with 3% HPE-101.¹⁾ However, VPA could attain a plateau for shorter time compared with MTX by co-application of HPE-101. Considering the fact that the drug solubility in both the stratum corneum and viable skin gradually

Fig. 3. Illustrations of the Technique Used for the Intradermal Insertion of a Microdialysis Probe

The skin was incised over the dermis in the shaved abdominal region, followed by intradermal insertion of an introducer assembled by inserting an L-shaped needle into the black tubing (A). The L-shaped needle was withdrawn (B), followed by inserting the microdialysis probe into the tubing (C). The tubing was torn off by pulling upward and outward (D). After completion of the probe implantation, a hemispherical glass reservoir was placed over the shaved abdominal region, and 1 h later a drug solution was applied to the reservoir (E), followed by collecting the dialysate samples (F).

increased with increasing drug lipophilicity,⁵⁾ lipophilic VPA thus permeates through the skin faster than MTX and attains a plateau for a shorter time than MTX when applied with HPE-101.

Regarding the easy penetration of VPA through skin, Ogiso *et al.*⁶⁾ reported that the transdermal application of 5% (w/w) VPA ointment in rabbit skin resulted in a sufficient absorption of VPA into the systemic circulation with approximately 100% bioavailability. However, they observed that the time lag of transdermal penetration of VPA *in vitro* in the application of 5% (w/v) VPA solution was approximately 7 h.⁶⁾ In this study using HPE-101, the time lag, which was defined as the intercept on the time

axis extrapolated from the absorption profile, was approximately 2.5 h, which was almost the same as the control (Fig. 6). The difference in the time lag obtained by Ogiso *et al.* and us might be attributable to a difference in experimental methods. It has been reported that *in vitro* permeation methods using excised skin are influenced by hydration⁷⁾ and microbial growth.⁸⁾ By contrast, an *in vivo* method using microdialysis is independent of these problems.

Considering the fact that the microdialysis system enables us to detect a change in concentration of lipophilic VPA without adsorption, the system is thought to be an easily available method for assessing the *in vivo* trans-

Fig. 4. Concentration-Time Profiles of VPA in a Beaker (■) and in Dialysate (□) (A). *In Vitro* Relative Recovery of VPA (●) at Each Fraction (B). Points represent the mean of 4 experiments.

Fig. 5. VPA Concentration-Time Profiles in the Dialysate Collected from the Dermis with (■) or without (□) 3% HPE-101 in Rats.

Data represent the mean $\pm$ S.D. of 4 rats. a) Significantly different from the control at $p < 0.02$ by Mann-Whitney *U*-test.

Fig. 6. Absorption Profiles of VPA across the Skin with (●) or without (○) 3% HPE-101 in Rats.

Points represent the mean of 4 rats.

dermal absorption of VPA.

Acknowledgments The authors would like to thank Miss Yoshie Ichinose for her technical assistance. This study was performed at the Laboratory of the Animal Center for Biomedical Research, Nagasaki University School of Medicine.

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