LIPO-MICRODIALYSIS: A NEW MICRODIALYSIS METHOD FOR STUDYING THE PHARMACOKINETICS OF LIPOPHILIC SUBSTANCES

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A new microdialysis method (Lipo-MD) using a lipid emulsion as the perfusate instead of Ringer's solution as in conventional microdialysis (MD) was designed. Recovery profiles of the alkylparabens (APBs) dissolved in Ringer's solution by Lipo-MD were compared with those by MD *in vitro*. Recovery of APBs in the perfusate in MD decreased with the increasing lipophilicity of APBs, whereas that in Lipo-MD increased. The enhancement of the relative recovery of APBs by Lipo-MD to MD for methyl-, ethyl-, propyl-, and butylparaben were 2.03, 5.24, 77.0, and 390.7, respectively. The utility of Lipo-MD for determination of lipophilic substances to perform pharmacokinetic study was suggested.

KEY WORDS microdialysis; lipo-microdialysis; lipophilic substances; lipid emulsion; alkylparaben; pharmacokinetics

Microdialysis has been developed and used for continuous sampling of extracellular fluid in most organs.^{1,2)} The method is being applied in the fields of experimental³⁾ and clinical pharmacokinetics.⁴⁾ Recently, transdermal application of immunosuppressive agents has been reported to elicit considerable clinical improvement in patients with psoriasis.⁵⁾ For the application of microdialysis to transdermal drug absorption, the possibility of *in vitro*⁶⁾ and *in vivo*⁷⁾ dermal microdialysis has been reported. However, relative recoveries of lipophilic substances by the conventional microdialysis method (MD) perfused with Ringer's solution are generally too low to determine precise concentration-time profiles of such substances in skin tissues.⁷⁾ In order to develop drug delivery systems providing a higher local therapeutic effect, powerful new tools for pharmacokinetic studies, which will enable us to determine more accurate and precise pharmacokinetic data for lipophilic substances, are required.

In this study, we designed a new microdialysis method (Lipo-MD) perfused with physiological lipid emulsion for the determination of lipophilic substances, and the *in vitro* recovery efficiency of Lipo-MD was compared with that of MD using alkylparabens as the model lipophilic substance.

EXPERIMENTAL DESIGN

Alkylparabens (4-hydroxybenzoic acid alkyl esters, APBs), *i.e.*, methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB), and butylparaben (BPB), were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). A physiological lipid emulsion for parenteral nutrition, Intralipos[®] 20% (The Green Cross Corporation, Osaka, Japan), was used without any pharmaceutical modification. All other chemicals were reagent-grade commercial products.

Each APB exhibiting different lipophilicity was suspended (1 mg/ml) in Ringer's solution (pH 7.4). After sufficient mechanical shaking, the resultant suspension was filtered and the filtrate was used as the donor solution. The microdialysis system consisted of a CMA/102 microinjection pump (Carnegie Medicine, Stockholm, Sweden) and CMA/20 microdialysis probes (ϕ 0.5 mm $\times$ 10 mm, Carnegie Medicine). Two microdialysis probes connected to the microinjection pump were synchro-

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nously perfused with Ringer's solution (pH 7.4) and Intralipos[®] 20% for MD and Lipo-MD, respectively, at the same rate of 1.0 μ l/min during the entire experimental period, and were placed in the test solution (10 ml) stirred at 700 rpm (Fig. 1). The perfusates were collected separately every 60 min by a CMA/142 Microfraction Collector (Carnegie Medicine). A 100- μ l aliquot of the test solution was sampled from the donor at the midpoint of each perfusate collection. An aliquot of the sample solution was used for HPLC assay. The chromatograph was an LC-10AS equipped with a SPD-10A UV-Visible detector (Shimadzu, Kyoto, Japan) operated at 254 nm. The column used was an Inertsil ODS (4.6 mm i.d. $\times$ 150 mm, GL Sciences, Tokyo). The mobile phase of 0.025% phosphoric acid-methanol was supplied at the rate of 1.0 ml/min. The concentrations of the APBs were calculated by peak area measurements.

The relative recovery (R) of APBs from the test solution was calculated by the following equation, where $C_{p,t}$ and $C_{d,t}$ represent the APB concentration in the perfusate and in the donor prepared by Ringer's solution at time t , respectively:

$$R_t (\%) = 100 \times C_{p,t} / C_{d,t}$$

The lipophilic indexes, $\log k'_0$, of APBs at pH 7.4 were determined by the HPLC method.⁶⁾ Statistical analysis was performed by Student's t -test. In all statistical tests, a p value of < 0.05 was considered to be significantly different.

RESULTS & DISCUSSION

The entire microdialysis system worked satisfactorily. Figure 1 shows the relative recovery profiles of APBs. The microdialysis reached steady states readily in both systems. The *in vitro* relative recovery decreased in the order of MPB > EPB > PPB > BPB in MD with the increase in lipophilicity (Fig. 1a). On the other hand, in Lipo-MD, it increased in the order of MPB < EPB < PPB < BPB (Fig. 1b). The steady-state recovery (R^*) estimated by the mean of the R_t values at 3 to 5 hours increased significantly in each APB, *i.e.*, from $62.8 \pm 6.0\%$ to $127.4 \pm 23.3\%$, from $42.2 \pm 7.0\%$ to $221.3 \pm 30.1\%$, from $4.6 \pm 1.8\%$ to $354.3 \pm 54.9\%$, and from $1.7 \pm 1.0\%$ to $738.0 \pm 109.2\%$ for MPB,

Fig. 1. Relative Recovery Profiles of APBs by MD (a) and Lipo-MD (b)

MPB (Δ); EPB (∇); PPB ($\square$); BPB ($\circ$)
Results are expressed as the mean $\pm$ S.D. of four determinations.

Fig. 2. Plot of Relative Recovery of APBs at the Steady State (R^*) against Lipophilic Index ($\log k'_0$)

MPB (Δ , $\blacktriangle$); EPB (∇ , $\blacktriangledown$); PPB ($\square$, $\blacksquare$); BPB ($\circ$, $\bullet$); MD: open symbols; Lipo-MD: closed symbols
Results are expressed as the mean $\pm$ S.D. of four determinations.

EPB, PPB, and BPB, respectively, by the application of Lipo-MD instead of MD. The R^* values of each APB were independent of the donor concentration in both systems, suggesting that the dialysis is linear (data not shown). The R^* values were plotted against the lipophilicities of the APBs (Fig. 2). Significant linear relations (r values for MD and Lipo-MD were 0.963 and 0.972, respectively) were recognized between the logarithms of the R^* values and the $\log k'_0$ values of the APBs in both dialysis systems.

Enhancement of the relative recovery by Lipo-MD was estimated by the ratio of the R^* values of Lipo-MD and MD. The degree of enhancement was extremely large in relatively lipophilic APBs. The enhancement ratios by Lipo-MD for MPB, EPB, PPB, and BPB were 2.03, 5.24, 77.0, and 390.7, respectively, and exhibited an exponential increase with the lipophilicities of the APBs

(Fig. 3). The enhancement is assumed to be derived from the significant increase in the partition of APBs to the perfusate through the microdialysis probe by the use of lipid emulsion as the perfusate in Lipo-MD. The R^* values of APBs from human plasma by Lipo-MD were comparable to those from Ringer's solution by the correction of plasma protein binding in the preliminary *in vitro* investigation.

Recently, transdermal application of highly lipophilic immunosuppressive agent such as cyclosporin A or tacrolimus has been reported to improve psoriasis considerably.⁵⁾ However, the utilization of the conventional MD perfused with Ringer's solution to study dermal and systemic pharmacokinetics of these highly lipophilic substances is practically impossible⁷⁾ because the relative recoveries of such substances are generally too low to determine precise concentration-time profiles. The direct effect of Lipo-MD on transdermal permeability is assumed to be minimal because the leakage of lipid emulsion, the perfusate, from the probe during the experiments was negligible.

In this report, we clarified the striking characteristics of newly designed Lipo-MD which enables several hundred-fold improvement in the recovery of BPB used as a model lipophilic substance. Lipo-MD is suggested to be a powerful tool for pharmacokinetic studies, providing more accurate and precise pharmacokinetic data for lipophilic substances, including relatively high molecular weight substances.⁸⁾ The application of Lipo-MD to free moving dialysis systems⁹⁾ or to protein binding study^{8,10)} will also become possible.

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Fig. 3. Plot of Enhancement Ratio in Relative Recovery of APBs at the Steady State (R^*) by Lipo-MD against Lipophilic Index ($\log k'_0$)

MPB (Δ); EPB (∇); PPB ($\square$); BPB ($\circ$)
Enhancement ratio was expressed as the ratio of R^* values determined by Lipo-MD to that by MD.