Percutaneous Penetration Kinetics of Lidocaine and Prilocaine in Two Local Anesthetic Formulations Assessed by in Vivo Microdialysis in Pigs

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The aim of this study was to characterize and compare the percutaneous penetration kinetics of lidocaine (L) and prilocaine (P) in two local anesthetic formulations by in vivo microdialysis coupled with HPLC. The microdialysis system for studying lidocaine and prilocaine was calibrated by a no-net-flux method in vitro and retrodialysis method in vivo, respectively. A dosage of 0.2 g/cm² of an in-house P–L formulation (2.5% lidocaine and 2.5% prilocaine, methylcellulose-based) and commercially available Eutectic Mixture of Local Anesthesia (EMLA, 2.5% lidocaine and 2.5% prilocaine, carbopol-based) was separately but symmetrically applied in the dorsal region of pigs. Saline (0.9%, w/v) was perfused into the linear microdialysis probe at a flow rate of 1.5 μl/min. Dialysate was collected upon topical application up to 6 h at 20-min intervals and assessed by HPLC. The results demonstrated the area under the concentration-time curve (AUCa-s) of lidocaine and prilocaine in EMLA was 71.95±23.36 μg/ml and 38.01±14.8 μg/ml, respectively, in comparison to 167.11±56.12 μg/ml and 87.02±30.38 μg/ml in the P–L formulation. The maximal concentrations (Cmax) of lidocaine and prilocaine in the dermis were 29.2±9.08 μg/ml and 16.54±5.31 μg/ml in EMLA and 80.93±17.98 μg/ml and 43.69±12.87 μg/ml in the P–L formulation, respectively. This study indicates a well-calibrated microdialysis system can provide vital real-time information on percutaneous drug delivery and specifically a methylcellulose-based P–L formulation can increase percutaneous absorption of both lidocaine and prilocaine in pigs compared to carbopol-based EMLA.

Key words microdialysis; percutaneous; calibration; pharmacokinetics; lidocaine; prilocaine

Percutaneous drug delivery has gained increasing attention in evaluating topically applied drugs as well as screening drug delivery media such as liposomes, emulsions, microemulsions and iontophoresis due to the introduction of cutaneous microdialysis sampling. Currently, 5-fluorouracil,1) tranilast,2) local anesthetics,3) acyclovir,4) and fluconazole 5) have been studied in vivo using cutaneous microdialysis. These studies clearly demonstrated microdialysis sampling is a powerful tool for investigating percutaneous drug delivery in dermatopharmacology.

Due to the free flux of drugs through the semipermeable membrane at steady state, the drug concentration in the probe can be used to measure the real-time drug concentration in the extracellular medium (ECM). However, equilibrium is never reached during the continuous microdialysis process. Thus, relative recovery, the fraction of dialysate concentration over ECM concentration, has been used in the in vivo process. Thus, relative recovery, the fraction of dialysate concentration over ECM concentration, has been used in the in vivo process. Therefore, a no-net-flux method in vitro and retrodialysis method in vivo, respectively. A dosage of 0.2 g/cm² of an in-house P–L formulation (2.5% lidocaine and 2.5% prilocaine, methylcellulose-based) and commercially available Eutectic Mixture of Local Anesthesia (EMLA, 2.5% lidocaine and 2.5% prilocaine, carbopol-based) was separately but symmetrically applied in the dorsal region of pigs. Saline (0.9%, w/v) was perfused into the linear microdialysis probe at a flow rate of 1.5 μl/min. Dialysate was collected upon topical application up to 6 h at 20-min intervals and assessed by HPLC. The results demonstrated the area under the concentration-time curve (AUCa-s) of lidocaine and prilocaine in EMLA was 71.95±23.36 μg/ml and 38.01±14.8 μg/ml, respectively, in comparison to 167.11±56.12 μg/ml and 87.02±30.38 μg/ml in the P–L formulation. The maximal concentrations (Cmax) of lidocaine and prilocaine in the dermis were 29.2±9.08 μg/ml and 16.54±5.31 μg/ml in EMLA and 80.93±17.98 μg/ml and 43.69±12.87 μg/ml in the P–L formulation, respectively. This study indicates a well-calibrated microdialysis system can provide vital real-time information on percutaneous drug delivery and specifically a methylcellulose-based P–L formulation can increase percutaneous absorption of both lidocaine and prilocaine in pigs compared to carbopol-based EMLA.

Key words microdialysis; percutaneous; calibration; pharmacokinetics; lidocaine; prilocaine

In this study, we focused on the calibration of microdialysis in vitro and in vivo prior to characterizing the percutaneous penetration kinetics of lidocaine and prilocaine in EMLA (1% carbopol-based, pH 9.4) and P–L formulation (3% MC-based, pH 8.2). Also, the possibility of using MC to enhance the percutaneous absorption of lidocaine and prilocaine in the P–L formulation was evaluated.

MATERIALS AND METHODS

Chemicals and Reagents Methanol of liquid chromatographic grade was obtained from Hanbang Corp. (Nanjing, China). Triethylamine (TENDA Corp. U.S.A.) was used for pH adjustment. MC was purchased from Yingde Corp. (U.K.). Lidocaine and prilocaine were obtained from Jiulong Fine-chemical Limited Co. (Jinan, China). EMLA was purchased from AstraZeneca (Sweden). NaCl solution (0.9%, w/v) obtained from Xiaoying Pharmaceutical Factory (Nanjing, China) was used for microdialysis perfusion. 3M Tegaderm Transparent Dressings were purchased from 3M Corp. (U.S.A.). Triple distilled water was used for all preparations. Other reagents were of analytical grade.

Instrumentation The microdialysis system (Bioanalytical Systems Inc., U.S.A.) consisted of a microinjection pump controller, a microdialysis syringe pump, gas-tight syringes (1 ml), and linear microdialysis probes (with a semipermeable membrane of 10 mm in length and molecular weight cut-off of 20 kDa). An SA-6000CMT Doppler Scan (Medison Medical Instrument Corporation, Shanghai, China) was used to determine the positions of probes (n=12) in the pig

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dermis.

**HPLC Assay** The chromatographic system was composed of a chromatographic pump and a 2487 ultraviolet detector (Waters, U.S.A.). Lidocaine and prilocaine were separated on a NovaPac-C18 reverse-phase column (4 μm, 3.9×150 mm, Waters, U.S.A.). The mobile phase consisted of water (4.35 mM NH₄H₂PO₄, pH value was adjusted to 7.0 with triethylamine) and methanol at the volume ratio of 40:60. The flow rate was 0.8 ml/min. Twenty milliliters of dialysate was directly injected into the HPLC system for analysis. The elute was monitored at 220 nm for both drugs.

**Method Validation** Six concentration levels (0.1, 0.5, 1, 10, 20, and 50 μg/ml of lidocaine and prilocaine) were prepared for HPLC calibration. The method was fully validated in terms of linearity, limit of quantification (LOQ), precision and accuracy. The inter-day precision and accuracy determinations were carried out on five different days. Intra-day precision and accuracy were evaluated at three different drug concentrations (0.5, 10, 20 μg/ml) by replicate analyses of five samples on the same day.

**Calibration of Relative Recovery in Vitro** by No-Net-Flux Method As the efficacy index of the microdialysis, relative recovery (RR), which varies depending on the experimental conditions, probe characteristics, and physicochemical properties of studied compounds, was evaluated by a no-net-flux method *in vitro* and retrodialysis methods *in vivo* (See Section “Calibration and monitoring of relative recovery *in vivo* by retrodialysis method”).

Two liters of 1.0 μg/ml lidocaine and prilocaine solution were prepared as medium. A beaker containing 200 ml of medium was placed in a water bath (adjusted to 37 °C). The probe was submerged in the medium and then was perfused sequentially with varying drug concentrations of 0.1, 0.2, 0.3, 2, 3, or 4 μg/ml lidocaine and prilocaine in 0.9% NaCl at a flow rate of 1.5 μl/min. There was an equilibration time of 30 min before each perfusion. Dialysate samples were collected in duplicates for evaluation. Before each subsequent concentration, the probe was perfused with 0.9% NaCl solution for 40 min at a flow rate of 1.5 μl/min. Six probes were evaluated and dialysate samples were assessed by HPLC as described above. These probes evaluated were then kept in distilled H₂O that was changed on a daily basis for the *in vivo* study. The RR was calculated according to the function below.

$$RR(\%) = \frac{(C_{\text{dialysate}} - C_{\text{perfusate}})}{(C_{\text{medium}} - C_{\text{perfusate}})} \times 100\%$$ (1)

where $C_{\text{perfusate}}$ is the drug concentration of the perfusate and $C_{\text{dialysate}}$ is the drug concentration in the dialysate.⁴

**Animal Study** Pigs (20—30 d old, weighing 5.5—6.5 kg) were used throughout this study. All animal experiments complied with the requirements of the National Act on the Use of Experimental Animals (People’s Republic of China). Pigs were immobilized in a cage and then anaesthetized with 30% urethane (intraperitoneal injection, 3 ml/kg) 20 min before each experiment. The hair at the dorsum was removed using a razor. A linear probe was then inserted into the superficial dermis, parallel to the skin at the dorsum with the aid of a guide needle. There was an equilibrium period of 90 min to relieve the insertion microtrauma. After the equilibration, the position of the probe in the dermis was determined by measuring the length of the middle of the probe to the skin surface using an SA-6000CMT Doppler Scanner.

**Calibration and Monitoring of Relative Recovery *in Vivo* by Retrodialysis** Due to the time-consuming process of no-net-flux method, the relative recovery *in vivo* was investigated by retrodialysis instead. Retrodialysis is utilized based on the theoretical assumption that the disappearance rate of compounds from the probe through the semi-permeable membrane is equal to the recovery rate from the extracellular medium and it was successfully employed in many studies. Thus, the disappearance rate was calculated by Eq. 2 and taken as the RR *in vivo*.

$$RR(\%) = \frac{(C_{\text{perfusate}} - C_{\text{dialysate}})}{C_{\text{perfusate}}} \times 100\%$$ (2)

where $C_{\text{perfusate}}$ is the drug concentration of the perfusate and $C_{\text{dialysate}}$ is the drug concentration in the dialysate.⁴

The probes were perfused with 2 μg/ml of lidocaine and prilocaine in 0.9% NaCl at a flow rate of 1.5 μl/min for 40 min. Microdialysis samples at each position were collected in duplicate for calculating the RR *in vivo*. During this study, 48 retrodialysis samples were collected to calculate and monitor the RR of lidocaine or prilocaine before starting topical administration of EMLA or P–L formulation.

**Topical Application of EMLA and P–L Formulation** After performing the retrodialysis at each position, 2 μg/ml of lidocaine and prilocaine was replaced by 0.9% NaCl solution for 40 min to remove the residual lidocaine and prilocaine in the dermis before the topical application of EMLA or P–L formulation. A dosage of 0.2 g/cm² of EMLA or P–L formulation was applied to an area of 1.5 cm×2.0 cm (Fig. 1) and then covered with 3M Tegaderm Transparent Dressing. Formulation was left in place for 2 h. Dialysate samples were collected upon drug application every 20 min up to 6 h. The residual formulation was carefully removed from the local skin with three individual cotton swabs at the end of the application duration.

**Data Analysis** Pharmacokinetic parameters of lidocaine and prilocaine were calculated with 3P97 software (Chinese Pharmacological Society, Professional Committee of Mathematics). Data were analyzed statistically by one-way analysis of variance and Student’s *t*-test using SPSS12.0 software for Windows. A *p* value <0.05 was used for statistical significance.
RESULTS AND DISCUSSION

Validation of Chromatographic Methods In this study, the conditions of HPLC detection of lidocaine and prilocaine were optimized by adjusting the concentration of NH₄H₂PO₄ and the pH value of water in the mobile phase. Results for the calibration curve (n=5) of lidocaine and prilocaine both showed good linearity (r²>0.999) over the range of 0.1—50 μg/ml. The LOQ was established at the concentration of 0.1 μg/ml for two drugs (R.S.D. <20%). The intra- and interday precision and accuracy at low, medium and high concentrations (0.5, 10, 20 μg/ml) of lidocaine and prilocaine were examined. For prilocaine, the intra-day precision (expressed as percent relative standard deviation, R.S.D. %) ranged from 3.48 to 4.39% and the intra-day accuracy (expressed as percent of theoretical values) ranged from 95.44 to 100.47%. For lidocaine, the intra-day precision ranged from 1.27 to 9.23% and the intra-day accuracy ranged from 95.32 to 100.61%. The method showed reproducibility with inter-day precision ranging from 2.43 to 2.95% for prilocaine and 2.37 to 4.26% for lidocaine. The inter-day accuracy ranged from 99.67 to 100.97% for prilocaine and 98.59 to 104.93% for lidocaine. These results indicated that the present method had an acceptable accuracy, precision and reproducibility.

Calibration of Relative Recovery in Vivo by No-Net-Flux Method According to the no-net-flux method, the concentration change between perfusate and dialysate was plotted against perfusate concentration. In Fig. 2, the slopes represent the in vitro relative recovery of lidocaine and prilocaine. The consistent linear correlation of lidocaine (r²=0.9993) and prilocaine (r²=0.9996) over the tested concentrations suggested the free movement of drugs through the probe membrane. Also, this confirmed the feasibility of using in vivo retrodialysis for calibration to calculate the unbound extracellular drug concentrations of both drugs.

The in vitro and in vivo relative recoveries of compounds were usually different, as was observed in many studies. Thus the RR obtained in vitro could not predict the actual RR in vivo because of the complex reactions between compounds and local tissue and the clearance by capillary blood flow. However, it would be prudent and beneficial to evaluate the recovery in vitro to optimize the microdialysis conditions.

Calibration and Monitoring of Relative Recovery in Vivo by Retrodialysis In addition to the in vitro calibration of the microdialysis system, the RR of lidocaine and prilocaine was measured and monitored by retrodialysis throughout all the animal experiments. The data collected from the six probes (4 times) showed that the RR of the probe in vivo was 28.99±8.82% for lidocaine and 44.07±8.64% for prilocaine. As shown in Fig. 3, little variation of relative recovery was observed between these six probes for both lidocaine and prilocaine. Additionally, these six probes were randomly chosen at the positions illustrated in Fig. 1, thus, no significant differences in relative recovery were found among these positions. In other words, the positions of topical administration in the dorsum of pigs demonstrated no discernible influence on the relative recovery of lidocaine and prilocaine. Therefore, the relative recovery in vivo remained stable and reliable through the experiments with only slight fluctuation.

In vitro and in vivo recoveries are usually not equal. Generally, the in vivo recovery is lower than that in vitro because the diffusion in a tissue is lower than in a simple liquid used for in vitro studies. The tissue is the rate limiting factor for diffusion of compounds. 3,4,16 In this study, the in vitro recoveries of prilocaine and lidocaine were similar, while the in vivo recoveries were different from each other. As discussed above, the tissue rather than the probe was the key factor to influence the in vivo recovery of compound. The difference may result from different affinity to tissue between two drugs as a result of a difference in liposolubility. Thus, the results showed the importance of calibrating in vivo recovery studies to obtain an actual free drug concentration in the target tissues.

Depth of Probes and Drug Absorption In this study, the AUC₀—₆h (calculated by concentration-versus-time data) of the lidocaine or prilocaine was chosen as the criteria to evaluate the effects of probe depth on cutaneous microdialysis (see Fig. 4).

No discernible relationship between probe depth and the absorption of lidocaine or prilocaine over 6 h was observed in the two formulations. However, the effects of the depth of probes on the cutaneous microdialysis are still under debate. Some studies have suggested that there was no specific relationship between the probe position and the total absorption of penciclovir, 7 salicylic acid 16 or propranolol, 17 whereas another study confirmed a decreasing gradient of nicotine concentration in the dermis with an increase in probe depth. 8

As a multilayered organ, skin consists of dermis, epidermis and stratum corneum which is generally considered as the main permeability barrier of cutaneous absorption. Yet,
the thinness of skin makes it technically difficult to implant the probe at the same depth in the dermis. In this study, the irrelevance of $AUC_{0–6h}$ of lidocaine or prilocaine to the depth of the probes showed that these two drugs could diffuse freely in the dermis. On the other hand, the variation in $AUC_{0–6h}$ values among different trials was also investigated in this study, which meant the difference between individuals was an important factor. More samples should be considered in future studies.

**Percutaneous Pharmacokinetics**

The concentration-versus-time curves of unbound lidocaine and prilocaine in the dermis after administration of EMLA or P–L formulation are shown in Fig. 5. The real-time concentrations of lidocaine and prilocaine in the P–L formulation were significantly higher than those in EMLA during the period of 6h. All data were calibrated by the RR of each probe.

Furthermore, the $AUC_{0–6h}$ was calculated using the trapezoid method. All pharmacokinetic parameters were calculated using a one-compartment open model (without lag-time, with weight $1/C$) by the pharmacokinetic software 3p97 and are summarized in Table 1. In previous studies, the one compartment model was successfully utilized in estimating the pharmacokinetic parameters and these predicted data showed high correlation with actual data.3,18)

As shown in Table 2, the $AUC_{0–6h}$ of lidocaine and prilocaine in the P–L formulation was significantly higher than those in EMLA with 2.32 times for lidocaine and 2.29 times for prilocaine, indicating the higher degree of absorption of the P–L formulation. Notably, the $C_{max}$ of lidocaine and prilocaine in the P–L formulation was 2.77 times and 2.64 times higher than those in EMLA. Also, the absorption rates (assessed by $T_{1/2a}$) of lidocaine and prilocaine in the P–L formulation were both higher than those in EMLA. In other words, the percutaneous penetration of lidocaine and prilocaine was more efficient from the P–L formulation than that from EMLA. Though the P–L formulation showed slightly shorter $T_{max}$ in comparison to EMLA, the P–L formulation demonstrated its advantage of reaching the effective concentration over EMLA. For example, the $C_{max}$ of lidocaine and prilocaine in the dermis was detected 2.55 h and 2.47 h after EMLA was given topically, while the same drug concentra-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prilocaine</th>
<th>Lidocaine</th>
</tr>
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<tbody>
<tr>
<td>$T_{max}$ (h)</td>
<td>2.47±0.44</td>
<td>2.25±0.25</td>
</tr>
<tr>
<td>$C_{max}$ (µg ml$^{-1}$)</td>
<td>16.54±5.31</td>
<td>43.69±12.87</td>
</tr>
<tr>
<td>$T_{1/2a}$ (h)*</td>
<td>1.05±0.38</td>
<td>0.68±0.21</td>
</tr>
<tr>
<td>$T_{1/2b}$ (h)*</td>
<td>1.75±0.73</td>
<td>1.07±0.56</td>
</tr>
<tr>
<td>$AUC_{0–∞}$</td>
<td>46.34±19.59</td>
<td>93.27±28.68</td>
</tr>
<tr>
<td>$AUC_{0–6h}$ (µg h ml$^{-1}$)*</td>
<td>38.01±14.8</td>
<td>87.02±30.38</td>
</tr>
</tbody>
</table>

Data are presented as the mean±S.D. ($n=12$). $C_{max}$ indicates maximal concentration; $T_{max}$, time of maximal concentration (both $C_{max}$ and $T_{max}$ were detected data and drug concentration was calibrated by RR); $T_{1/2a}$, absorption half time; and $T_{1/2b}$, elimination half time. Data were analyzed statistically by one-way analysis of variance and Student’s $t$-test. A $p$-value $<0.05$ was used for statistical significance. * $p<0.05$. 

![Fig. 4. The Relationship between the Depth of Probes and the $AUC_{0–6h}$ of Drugs Following Topical Application of EMLA (a) or P–L Formulation (b)](image1)

![Fig. 5. (a) Average Time–Concentration Profile of Lidocaine in the Dermis Following the Topical Application of EMLA or P–L Formulation and (b) Average Time–Concentration Profile of Prilocaine in the Dermis Following the Topical Application of EMLA or P–L Formulation](image2)

A dose of 0.2 g/cm$^2$ was applied to the backs of the pigs. Application period was 2 h. Data were calibrated by RR and expressed as mean±H1000/h1000 S.D. ($n=12$).
tion was detected in less than 1 h after the P–L formulation was administered. A comparison of the bioequivalence of these two anesthetic formulations strongly suggested that the P–L formulation could provide higher drug concentrations in a shorter period compared to EMLA. This indicates the superiority of the P–L formulation, from a clinical point of view, over EMLA, which needed at least 90 min or even longer to achieve full local anesthesia.19) The concentration of lidocaine in dermis was higher than prilocaine for two formulations in this study. This phenomenon was accordance with another in vitro study, which reported that the skin permeability of lidocaine was higher than prilocaine.20) In Table 1, the values of $T_{1/2}$ were significantly different between EMLA and the P–L formulation, which might be explained as different reserve effects of skin for the two formulations since EMLA had higher affinity to skin than the hydrophilic P–L formulation. The residual drug released form the P–L formulation, even after its removal, were higher than that from EMLA, resulting in different drug elimination rates in the dermis.

It should be also noted that pigs rather than rats were selected as model animals to investigate the percutaneous kinetic process of EMLA and P–L formulation. Bouclier et al.21) investigated the histology of the dorsal skin of human beings, minipigs, rabbits, mice, rats and guinea pigs and found that minipigs had the most similar skin to human beings. Some studies22,23) compared the skin permeability of mice, rats, rabbits, pigs, snakes, frogs and loaches in vitro, suggesting that pig skin had comparable permeability to human beings. In addition, Bond and Barry24) reported that mouse skin was not a proper in vitro model for evaluating enhancers for human beings. Thus, studies using minipigs as an animal model to evaluate percutaneous drug delivery would minimize the bias caused by species differences and provide more skin areas to perform tests.

CONCLUSIONS

In conclusion, the percutaneous penetration kinetics of lidocaine and prilocaine in EMLA have been investigated by in vivo microdialysis coupled with HPLC in pigs. The calibration of the microdialysis system was performed in vitro and in vivo. A methylcellulose-based P–L formulation could boost the percutaneous absorption of lidocaine and prilocaine, showing the potential of increasing the local anesthesia within a reduced application time in comparison to carbopol-based EMLA. This study demonstrates that well-calibrated microdialysis sampling combined with HPLC in pigs could be used to assess the percutaneous penetration kinetics and bioequivalence of topical formulations.

REFERENCES