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# A pharmacokinetic study of a topical anesthetic (EMLA®) in mouse soft tissue laceration

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**Abstract** – The use of topical anesthesia instead of injection of local anesthetics for managing soft tissue lacerations in the emergency situations may be a relief for both patients and surgeons. Topical anesthesia in the form of a cream eutectic mixture of local anesthetics (EMLA®) containing 2.5% lidocaine and 2.5% prilocaine has been reported as an efficient anesthetic on skin before venipuncture anesthesia and as an alternative to injection anesthesia in some minor surgery situations. The aim of this study was to compare the pharmacokinetics of EMLA® when applied in a laceration with topical skin application in the mouse. A total of 120 Albino Laboratory-bred strain mouse (BALB-c) male mice were divided into three groups with regard to application mode of EMLA®. Group A: with laceration, 48 mice; Group B: on intact shaved skin, 48 mice; Group C: control group (24 mice) with same procedures but without application of EMLA®. Blood levels were collected at 0, 10, 20, 30, 45, 60, 75, and 90 min post-EMLA® application. Plasma sample analysis was carried out by employing liquid chromatography coupled with tandem mass spectrometric (LC-MS/MS) method, and the pharmacokinetic analysis of the mouse plasma samples was estimated by standard non-compartmental methods. The pharmacokinetic parameters of lidocaine and prilocaine were significantly altered following EMLA® application to lacerated mouse skin in contrast to intact skin. The absorption of lidocaine and prilocaine was rapid following application of EMLA® to lacerated and intact mouse skin. Maximum drug plasma concentration ($C_{max}$) and area under the drug plasma concentration–time curve (AUC) values of lidocaine were significantly increased by 448.6% and 161.5%, respectively, following application of EMLA to lacerated mouse skin in comparison with intact mouse skin. Similarly, prilocaine's $C_{max}$ and AUC values were also increased by 384% and 265.7%, respectively, following EMLA application to lacerated mouse skin, in contrast to intact skin. Further pharmacokinetic studies on different carriers of lidocaine/prilocaine are warranted before any firm conclusions for the clinic can be drawn.

One-third of all traumatic oral injuries are associated with injuries to the lip, gingiva, and oral mucosa (1). Many of these soft tissue injuries require removal of foreign bodies, cleaning, and suturing in the emergency phase during which local anesthesia often must be administered. Many of the emergency patients are scared of local anesthesia because of the injection needle. In clinical practice, attempts have been made to use topical anesthetics, usually benzocaine, to reduce the pain from needle stick. However, the ability of benzocaine to reduce or eliminate pain from needle stick is poor (2, 3). An eutectic mixture of lidocaine 2.5% and prilocaine 2.5% of local anesthetics (EMLA®, AstraZeneca, Karlskoga, Sweden) has been found to be effective in children for the control of pain arising from venipuncture and other minor procedures (4–6). EMLA® has been approved for skin application in adult and the pediatric population. In previous studies, it was shown that EMLA® was efficient in reducing or eliminating pain from needle stick (2, 3, 7). Moreover, EMLA® has been used as an alternative to injection anesthesia for minor clinical procedures such as maxillary sinus puncture, minor gynecological procedures, excision of gingival tissues, gingival probing, scaling, and root planing (8–14).

Patients with facial lacerations visiting emergency services for suturing is a category of injuries where these topical anesthetics could have a potential for clinical use by applying the EMLA® cream directly into the lacerations prior to suturing. By doing this, injection anesthesia can possibly be avoided, and hence, the patient's fear of needle stick can be eliminated. In a recent case report, this application was found to be sufficient in a patient with facial laceration who refused to

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accept injection anesthesia but agreed to try EMLA® cream in the laceration as the only given anesthetic (15). One concern may be that the topical anesthesia could have negative influence on healing of the laceration when applied in the laceration. However, in an earlier study, it was found that topical anesthetics applied in experimental lacerations in rabbits did not interfere with wound healing histologically or clinically (16). Hence, it may be possible that lidocaine–prilocaine cream can be given in lacerations as an alternative to injection anesthesia in adults. However, it is not recommended to be used in infants and very small children owing to the risk of methemoglobinemia following the use of prilocaine in infants (17–21).

As EMLA is used worldwide for topical skin application, the route of administration directly into lacerations must be further evaluated with regard to pharmacokinetics before recommending its use routinely in lacerations. To our knowledge, no studies have examined the absorption and elimination of EMLA® when applied directly into lacerations. The aim of this study was to compare the pharmacokinetics of EMLA® when applied in a laceration as compared with topical skin application in the mouse.

## Methodology

### Materials

Lidocaine-HCl and prilocaine-HCl were purchased from Sigma-Aldrich company (St. Louis, MO, USA). The internal standard (IS), [$^2H_{10}$]-lidocaine, was purchased from Alsachim company (I.G., France). EMLA® cream, the commercial formulation of lidocaine and prilocaine (AstraZeneca) containing 2.5% each was locally purchased from a drug store. All the solvents used in the analysis of lidocaine and prilocaine plasma samples were of HPLC grade, whereas other chemicals and reagents were of analytical grade.

### Experimental animals

A total of 120, Albino Laboratory-bred strain mouse (BALB-c) male mice were used in this study. The animals were acquired from the Health Sciences Center (HSC) Animal Resource Center. All experiments with these mice were in compliance with international guidelines for the handling and treatment of experimental animals and were approved by the local HSC animal resource center that has oversight on animal use.

The animals were divided into three broad groups:

*Group A:* A total of 48 mice were divided into subgroups of six, each containing six mice for the following times: 0, 10, 20, 30, 45, 60, 75, and 90 min post-treatment with EMLA® application through a laceration. Subgroup 0 was treated with castor oil and immediately bled for analysis. Subgroups 10–90 min were bled after each time period following EMLA® application. In this group, EMLA® was applied through a laceration made on the back of the animal after shaving. Each animal was lightly anesthetized with isoflurane in a modified gas chamber. The animals were then shaved on the back, and a 1.0-cm-long laceration was made using a #15 blade scalpel. At the designated time, the animals were bled using a transcardiac needle, and the blood samples were collected in heparinized centrifuge tubes. The blood samples were then centrifuged at 2000 x g for 10 min, and the resulting plasma samples were collected and kept frozen at $-80^{\circ}$C pending liquid chromatographic (LC) analysis.

*Group B:* A total of 48 mice were also divided into subgroups of six, each containing six mice for the following times: 0, 10, 20, 30, 45, 60, 75, and 90 min post-treatment with EMLA® application directly on the shaved skin. All other protocols described for Group A were followed for group B as well.

*Group C:* This group consisted of 24 mice and served as the sham group for both laceration and direct skin application groups. Groups A and B internal controls consisted of animals which were subjected to the same handling and surgical procedures but without EMLA® application.

### Plasma samples analysis

Lidocaine and prilocaine were measured in mouse plasma samples using liquid chromatography coupled to tandem mass spectrometric (LC-MS/MS) method. Briefly, a 20 µl of IS was added to a 200 µl mouse plasma, extracted with ether, and then, 10 µl was injected on MS/MS system using a positive electrospray ionization (ESI+) of a tandem triple-quadrupole mass spectrometer under multiple reaction monitoring (MRM) mode (Micromass, Manchester, UK). The mass detector was set to monitor the transitions of the precursors to the product ions as follows: $m/z$ 235 > 86 for lidocaine; $m/z$ 221.4 > 86 for prilocaine; and $m/z$ 245.5 > 96 for the IS. The compounds were analyzed on Symmetry® $C_{18}$ column (5 µm, 3.9 mm × 50 mm) using a mobile phase of methanol–water–formic acid (50:50:0.1, v/v/v) at a flow rate of 0.2 ml min$^{-1}$. The method was fully validated and it was linear over the concentration range of 20–2000 ng ml$^{-1}$ for both compounds ($r > 0.99$) with a limit of quantification of 20 ng ml$^{-1}$. Intra- and inter-run precision of the assay was below 15%.

### Pharmacokinetic analysis

The pharmacokinetic parameters for lidocaine and prilocaine in mouse plasma were estimated by standard non-compartmental methods. The maximum drug plasma concentration ($C_{max}$) and the time needed to attain this concentration ($T_{max}$) were directly obtained from the plasma profiles for each drug; the elimination half-life ($t_{1/2}$) values were calculated from $Ln2/k_{el}$. The area under the drug plasma concentration–time curve ($AUC_{0-t}$) was calculated from the measured data points from time zero to time of last quantifiable concentration by the linear trapezoidal rule, and the AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated

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using the equation: $AUC_{0-\infty} = AUC_{0-t} + C^*/k_{el}$, where $C^*$ is the last quantifiable drug plasma concentration. The drug clearance (CL/F) was calculated as the ratio of Dose/$AUC_{0-\infty}$. The volume of distribution ($V_d/F$) was calculated as $V_d/F = (CL/F)/k_{el}$ (22).

## Results

The plasma concentration–time profiles for lidocaine and prilocaine following EMLA® (18 mg/25 g) application to intact and lacerated mouse skin are illustrated in Figs 1 and 2, respectively. The computed pharmacokinetic parameters for lidocaine and prilocaine in mice following EMLA® are shown in Tables 1 and 2, respectively. The absorption of lidocaine and prilocaine was very rapid following EMLA® application to lacerated and intact mouse skin, attaining a peak plasma concentration in 30–45 min after EMLA® application (Tables 1 and 2). The pharmacokinetic parameters of lidocaine and prilocaine were significantly altered following EMLA® application to lacerated mouse skin in contrast to intact skin. The AUC of lidocaine was significantly increased from 14 831.8 to 38 785.2 ng min $ml^{-1}$ (161.5%) following application of EMLA to an intact mouse skin in comparison with lacerated skin, respectively (Fig. 1 and Table 1). Similarly, prilocaine's AUC values were also increased from 8279.4 to 30 278.3 ng min $ml^{-1}$ (265.7%) following application of EMLA® to an intact mouse skin in comparison with lacerated skin, respectively (Fig. 2 and Table 2). Furthermore, the maximum plasma concentration ($C_{max}$) of lidocaine was also significantly higher, 165.7 compared to 909.2 ng $ml^{-1}$ (increased by 448.6%) following application of EMLA® to the intact mouse skin and to lacerated skin, respectively (Fig. 1 and Table 1). Similar to lidocaine, prilocaine's $C_{max}$ values were also increased from 118.3 to 572.6 ng $ml^{-1}$ (by 384%) following application of EMLA® to intact mouse skin in comparison with lacerated skin, respectively (Fig. 2 and Table 2).

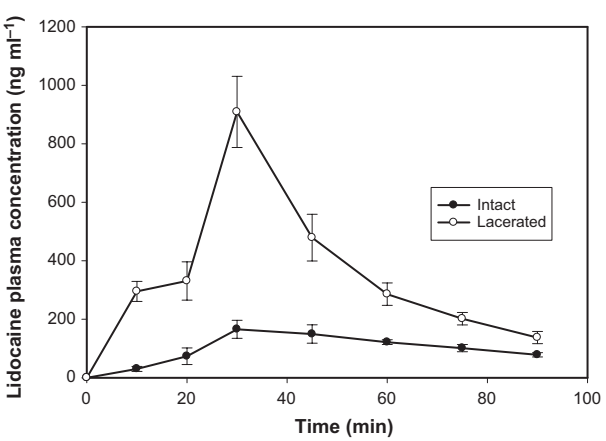


*Fig. 1.* Mean (±SEM) plasma concentration of lidocaine in six mice following eutectic mixture of local anesthetics (EMLA®) (18 mg/25 g) application to intact and lacerated mouse skin.

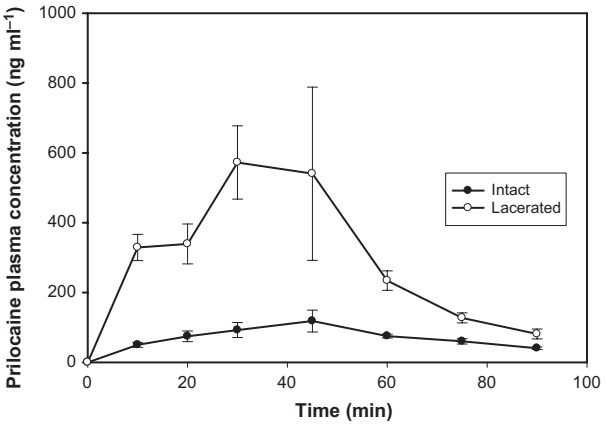


*Fig. 2.* Mean (±SEM) plasma concentration of prilocaine in six mice following eutectic mixture of local anesthetics (EMLA®) (18 mg/25 g) application to intact and lacerated mouse skin.

*Table 1.* Mean pharmacokinetic parameters of lidocaine in mouse plasma following EMLA® (18 mg/25 g) application to intact and lacerated mouse skin ($n = 6$)

| Parameter | Intact | Lacerated |
|---|---|---|
| $T_{max}$ (min) | 30 | 30 |
| $C_{max}$ (ng $ml^{-1}$) | 165.73 | 909.17 |
| $t_{1/2}$ (min) | 49.06 | 28.38 |
| $AUC_{0-\infty}$ (ng min $ml^{-1}$) | 14 831.81 | 38 785.2 |
| CL/F (l $min^{-1}$) | 1.21 | 0.46 |
| $V_d/F$ (l) | 85.9 | 19 |

AUC, area under the drug plasma concentration–time curve; CL/F, oral drug clearance; $C_{max}$, Maximum drug plasma concentration; EMLA, eutectic mixture of local anesthetics; $T_{max}$, time needed for the drug to attain maximum plasma concentration; $V_d/F$, volume of distribution.

*Table 2.* Mean pharmacokinetic parameters of prilocaine in mouse plasma following EMLA® (18 mg/25 g) application to intact and lacerated mouse skin ($n = 6$)

| Parameter | Intact | Lacerated |
|---|---|---|
| $T_{max}$ (min) | 45 | 30 |
| $C_{max}$ (ng $ml^{-1}$) | 118.3 | 572.55 |
| $t_{1/2}$ (min) | 30.21 | 19.65 |
| $AUC_{0-\infty}$ (ng min $ml^{-1}$) | 8279.43 | 30 278.34 |
| CL/F (l $min^{-1}$) | 2.17 | 0.59 |
| $V_d/F$ (l) | 94.7 | 16.85 |

AUC, area under the drug plasma concentration–time curve; CL/F, oral drug clearance; $C_{max}$, maximum drug plasma concentration; EMLA, eutectic mixture of local anesthetics; $T_{max}$, time needed for the drug to attain maximum plasma concentration; $V_d/F$, volume of distribution.

## Discussion

The present study reveals that application of EMLA® to lacerated mouse skin significantly enhanced the bioavailability of both lidocaine and prilocaine as compared with application to intact skin (Fig. 3). The

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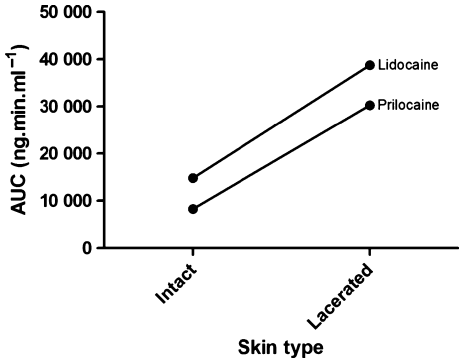


*Fig. 3*. Area under the plasma concentration–time curve ($AUC_{0-\infty}$) for lidocaine and prilocaine following eutectic mixture of local anesthetics (EMLA®) (18 mg/25 g) application to intact and lacerated mouse skin.

enhancement of bioavailability seen was most probably due to an increase in the extent of absorption of lidocaine and prilocaine following EMLA® application to lacerated mouse skin. Moreover, the high bioavailability of the two drugs could also be caused by a decrease in first-pass metabolism leading to a significant increase in $C_{max}$, AUC, as well as a decrease in oral clearance (CL/F).

The clinical relevance of this finding with regard to safety of using EMLA® in lacerations remains unclear. Another preparation of 2.5% lidocaine/2.5% prilocaine, Oraqix® (Dentsply Pharmaceutical, York, PA, USA) topical anesthetic agent in a thermosetting gel, has been approved by the FDA for use in periodontal pockets, that is, for reducing pain from gingival pocket curettage (13). The use of this substance showed high safety margins in blood after curettage in gingival pockets in adult patients with periodontitis, as reported earlier (14). The investigators found that the levels were far from those levels reported to cause initial signs of CNS toxicity. They concluded that there is a large safety margin with respect to systemic effects following the application of up to 3.5 g Oraqix® thermosetting gel in periodontal pockets. Curettage in the gingival pockets is very much comparable to an open wound. Some studies indicate that the total wound surface area of the periodontal pocket is equivalent to that of the palm of the hand, 50–75 $cm^2$, or that of the ventral surface of the forearm, 200 $cm^2$ (23, 24), which is considerably larger than laceration wounds. Moreover, the application as cream vs thermosetting gel may have influence, as well on the uptake. It would therefore, as a next step, be interesting to undertake a study comparing plasma levels of lidocaine and prilocaine after direct application of topical anesthetics of different carriers in lacerations in this animal model and also as injection anesthesia. Such studies, now employing a larger number of animals to overcome the main limitation of the current study, are in progress in our center.

## Conclusion

We recommend further pharmacokinetic studies on various carriers of lidocaine/prilocaine before any conclusions for the clinic can be drawn.

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