Determination of Lidocaine Concentrations in Skin After Transdermal Iontophoresis: Effects of Vasoactive Drugs

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The purpose of this study was to investigate the effect of vasoactive drugs on transdermal lidocaine iontophoresis by measuring the concentrations of radiolabeled lidocaine which has penetrated the skin. Previous studies had demonstrated that coiontophoresis of vasoactive drugs could modulate the transcutaneous flux of lidocaine and suggested that a dermal depot of lidocaine was involved. To address this, lidocaine hydrochloride (^{14}C) was iontophoresed *in vivo* in anesthetized weanling pigs either alone or with the vasodilator tolazoline or the vasoconstrictor norepinephrine. Tissue cores under the active electrode were then collected, quick-frozen, and sectioned on a cryostat, and then the radioactivity was determined in each 40-μm section. Coiontophoresis with norepinephrine resulted in increased concentrations of lidocaine in skin up to a depth of 3 mm. These concentrations decreased to lidocaine-alone levels after a 4-hr washout. Tolazoline decreased tissue concentrations of lidocaine. Concentrations were intermediate when lidocaine alone was administered. These studies support the hypothesis that coiontophoresis of vasoactive drugs modulates the transdermal delivery of lidocaine, in part by altering the cutaneous "depot."

KEY WORDS: lidocaine; iontophoresis; transdermal; percutaneous absorption; skin depot.

INTRODUCTION

Iontophoresis is the process of delivering charged compounds across the skin using an applied electric field. Details of this approach to controlled transdermal drug delivery have been addressed and reviewed elsewhere (1,2). Previous studies in our laboratory *in vivo* in pigs and *in vitro* in the isolated perfused porcine skin flap (IPPSF) model demonstrated that the transdermal flux of lidocaine administered by iontophoresis could be modulated when vasoactive drugs were coiontophoresed (3–5). Specifically, the vasodilator tolazoline increased the transdermal flux of lidocaine as evidenced by an increased venous efflux in the IPPSF and an increase in the lidocaine serum concentrations *in vivo*. In contrast, the vasoconstrictor norepinephrine decreased the venous efflux in the IPPSF. Additionally, tolazoline shortened while norepinephrine prolonged the residence time of lidocaine in the skin flaps as assessed by a fractional absorption index. These data suggested that the primary mechanism behind the action of these vasoactive drugs on lidocaine flux related to modulation of the cutaneous microcirculation and formation of a cutaneous depot or sink.

In order to address this phenomenon from a different experimental perspective, studies were conducted *in vivo* in pigs and the concentration of radiolabeled lidocaine deposited within skin was assessed through serial sectioning of skin samples under the active electrode followed by determination of radioactivity using a method similar to that described by other workers (6,7). The results of this study probe the effects of coiontophoresis of tolazoline or norepinephrine on the profile of lidocaine distribution within skin.

MATERIALS AND METHODS

Twelve female weanling Yorkshire pigs (15–25 kg) were sedated by an intramuscular injection of ketamine (20 mg/kg)/xylazine (2.5 mg/kg) and placed in dorsal recumbency. Hair was carefully clipped from the caudolateral epigastric region of the pig. Iontophoretic electrodes were prepared by bonding a layer of wire mesh (Ag or Ag-Cl) between two layers of Porex (3,5). Overall dimensions of the electrodes were 3.0 cm L × 1.5 cm W × 0.6 cm H, thus providing a 4.5-cm^2 delivery area. The active (positive, Ag) electrode contained 0.75 ml of either 2.6% [^{14}C]lidocaine hydrochloride solution (n = 3), 2.6% [^{14}C]lidocaine hydrochloride/0.04% tolazoline solution (n = 3), or 2.6% [^{14}C]lidocaine hydrochloride/0.04% norepinephrine solution (n = 6). The indifferent (negative, Ag-AgCl) electrode that completed the circuit was attached caudal to the active electrode and contained 10% sodium chloride solution in all studies. These conditions (drug concentrations, buffer, electrode, etc.) were based on the previous experimental studies which suggested a vasoactive effect on transdermal lidocaine fluxes (5). All ^{14}C in the dosing electrode is assumed to be [^{14}C]lidocaine. The saturated electrodes were placed on the skin, covered with a 3.0 × 1.5-cm piece of Parafilm (American Can Co.) to prevent evaporation, and secured with Elasticon tape (Johnson and Johnson). The electrodes were connected to a constant DC power supply (WPI A360R, World Precision Instruments, Hamden, CT) and interfaced to an ammeter (Circuitmate DM25L, Beckman Industrial Corp.) to ensure a constant current of 0.9 mAmp, 200 μAmp/cm^2, for 1 hr.

Pigs (n = 9) dosed with each of the three treatments were sacrificed with T-61 Euthanasia Solution (Hoechst-Roussel, Somerville, NJ) immediately following iontophoresis. Pigs in an additional group (n = 3) dosed with lidocaine/norepinephrine were sacrificed 4 hr after the termination of iontophoresis (4-hr washout).

The skin under the active electrode site was inspected for erythema and petechia, stripped 12 times with cellophane tape to remove some of the stratum corneum, and dissected to the subcutaneous layer. The entire site under the electrode was weighed and placed with the epidermis side down on a cutting board, and the excess was trimmed. A 1-cm^2 core of tissue was obtained from the center of the dosing area using a biopsy punch. The skin (approximately 1 cm^2) was weighed, oriented epidermal side down in an aluminum foil boat, quenched in an isopentane well immersed in liquid nitrogen, embedded in OCT Compound (Miles Scientific),

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and stored at −70°C. A sliver of the trimmed tissue was placed in half-strength Karnovsky's fixative for light microscopy (LM).

Frozen serial sections 40 μm thick were cut through the entire tissue on a cryostat (HistoStat, American Optical) and placed in oxidizing cups. The samples were oxidized (Packard 306 Oxidizer, Hewlett-Packard) and the radioactive content was assayed by liquid scintillation counting (1219 Rackbeta Liquid Scintillation Counter LKB/Wallach).

Samples for LM were fixed overnight at 4°C in half-strength Karnovsky's fixative and routinely processed and infiltrated in a Tissue Tek VIP 1000 Automatic Processor (Miles Laboratories, Inc.). Sections 6 μm thick were cut on a Reichert-Jung 820 rotary microtome and stained with hematoxylin and eosin on a Histomatic Code-on Slide Stainer (Fisher Scientific) for histological evaluation.

Data were analyzed using VMS SAS (version 5.18). The net radioactivity for each tissue section was calculated by subtracting the average background disintegrations per minute (dpm) from the gross dpm and adjusted for relative tissue surface area. The data were filtered by taking a running average over three data points and plotted as mass of lidocaine (mean ± SE, $N = 3$ animals/group) versus depth. Area under the curve (AUC), area under the moment curve (AUMC), and mean penetration depth (MPD), defined as AUC/AUMC, were also calculated for each data set. All data were normalized to the 4.5-cm^2 area of the electrode and thus represent the mass of lidocaine in slices under the electrode.

RESULTS

Transdermal delivery of lidocaine hydrochloride in the pig as assessed by the quantity of lidocaine present in skin after iontophoresis (Table I) can be modulated by coadministration of tolazoline or norepinephrine. Figure 1A compares the mean penetration profiles of each treatment (lidocaine, lidocaine/tolazoline, and lidocaine, norepinephrine) in the skin. Iontophoresis of lidocaine alone (Fig. 1B) shows a high degree of variability in the dermis to a depth of about 800 μm. Coiontophoresis of lidocaine with the vasodilator tolazoline decreases the amount of lidocaine in the skin (Fig. 1C, curve B), while norepinephrine increases lidocaine concentrations (Fig. 1C, curve A), thereby creating a drug reservoir in the dermis at a depth of about 300 μm. The mean mass profile of lidocaine/norepinephrine after a 4-hr washout is relatively flat (Fig. 1D, curve B), indicating depletion of this reservoir over time. Figure 2 is a light micrograph of skin which shows the correlation of depth of penetration to anatomical structures present.

Table I. Lidocaine Penetration into Skin Immediately After Iontophoretic Delivery (Mean ± SE)

	Dose penetrated* (μg)	Mean penetration depth (μm)	% dose
Lidocaine	634 ± 90	1345 ± 99	3.4 ± 1.0
Lidocaine/tolazoline	456 ± 72	1191 ± 78	3.1 ± 0.6
Lidocaine/norepinephrine	942 ± 134	1210 ± 69	5.8 ± 0.7
Lidocaine/norepinephrine (4 hr postdosing)	443 ± 14	1504 ± 23	3.2 ± 0.3

* Estimated from AUC of penetration profile.

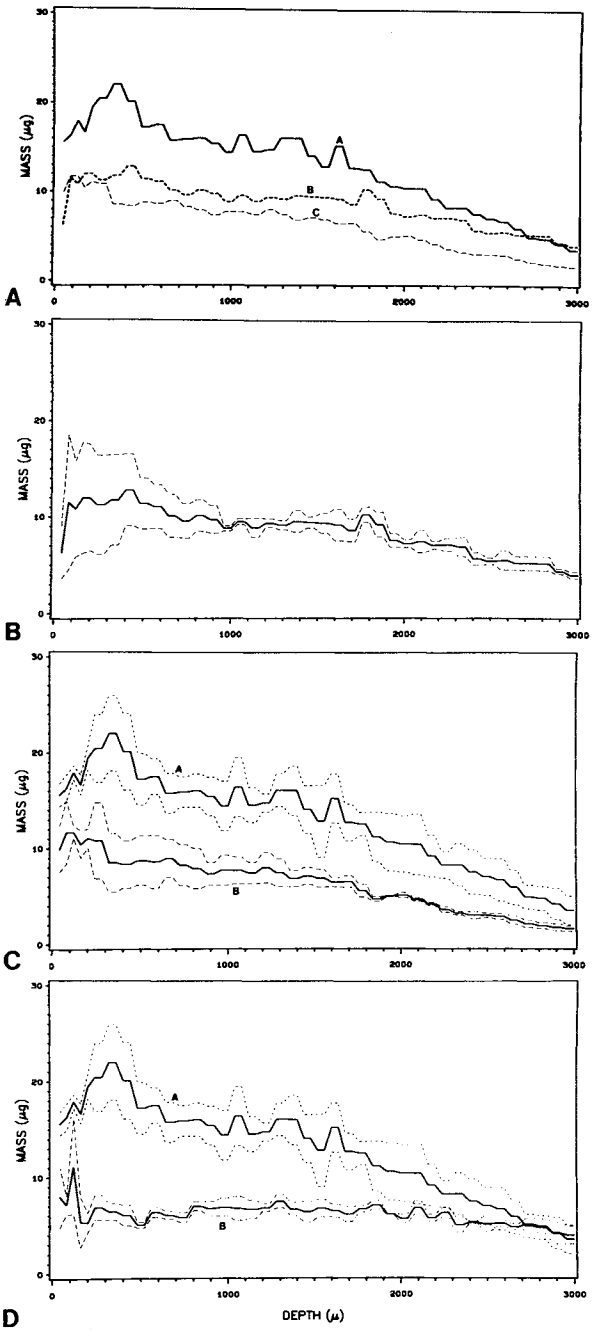

Fig. 1. Depth of penetration of lidocaine in skin delivered by iontophoresis. A depicts mean depth profiles for lidocaine/norepinephrine (A), lidocaine alone (B), and lidocaine/tolazoline (C). B shows the mean lidocaine profile, while C shows the mean profile when norepinephrine (A) or tolazoline (B) is coiontophoresed. Finally, D shows the mean profile for lidocaine/norepinephrine treatment immediately after (A) or 4 hr after (B) conclusion of iontophoresis. All curves are mean ± SE.

Statistical analysis was performed using general linear models procedure [Tukey's studentized range (HSD) test] for mean lidocaine mass at each 40-μm depth. The lidocaine/tolazoline and lidocaine/norepinephrine treatments were significantly different ($P < 0.05$) between 800 and 1800 μm. In addition, the lidocaine/norepinephrine and lidocaine/

Fig. 2. Light micrograph of skin correlating depth to structure. Bars represent skin depth (1 = 1000 μm, 2 = 2000 μm, 3 = 3000 μm). E, epidermis; D, dermis; N, nerve; HF, hair follicle; A, apocrine gland; SC, subcutaneous. H&E, 68×; reduced 10% for reproduction.

norepinephrine (4-hr washout) showed differences ($P < 0.05$) between 320 and 1800 μm.

Analysis of variance procedure was performed using Tukey's studentized range (HSD) test for variables listed in Table I. The analysis showed a significant difference ($P < 0.05$) in the mean AUC values (dose penetrated) between the lidocaine/norepinephrine treatment and the lidocaine/tolazoline and lidocaine/norepinephrine (4 hr postdosing) treatments, confirming the results seen from the analysis of the penetration profiles themselves. The percentage dose deposited in the skin was also significantly increased in the lidocaine/norepinephrine group ($P < 0.05$). Table I also shows that very little change in the MPD was seen with the cotreatments except in the 4-hr lidocaine/norepinephrine group, which had the greatest MPD.

DISCUSSION

These studies demonstrate that the transdermal delivery of lidocaine hydrochloride may be modulated by coiontophoresis of vasoactive compounds. Previous studies monitoring the transdermal flux of lidocaine (4,5) indicated that tolazoline increased and norepinephrine decreased the quantity of lidocaine delivered to the cutaneous microcirculation (e.g., venous flux). The changes in shapes of these efflux profiles also suggested that norepinephrine increased and tolazoline decreased the amount of lidocaine delivered into a putative cutaneous depot. The results reported in the present study clearly support this depot concept, where a depot is operationally defined as a treatment or condition which results in increased lidocaine concentrations within the skin. Depot formation with norepinephrine coiontophoresis was demonstrated by an elevated lidocaine mass–depth profile, by increased intracutaneous AUC, and by an increased percentage dose delivered. In the norepinephrine studies, the reversibility of depot formation is also clearly supported since the lidocaine penetration profiles decreased after 4 hr, indicating dissipation of the depot.

There are several important points which can be obtained from these studies. First, modulation of the cutaneous microcirculation significantly alters the transdermal flux of lidocaine and the magnitude of its cutaneous depot. Second, depending on the purpose of lidocaine delivery, coiontophoresis with vasoactive compounds would result in a more reproducible and controlled delivery of lidocaine. If a local skin effect (e.g., cutaneous anesthesia) were desired, norepinephrine would result in higher skin concentrations. It is important to note that, as shown in Fig. 2, nerves are located at the depth of lidocaine penetration. This is similar to the use of norepinephrine in lidocaine injectable employed for local anesthesia. Another point to note about these profiles is that, unlike in passive studies (6), with iontophoresis the epidermal and corneal layers do not contain the higher drug concentrations since the motive force for drug penetration is apparently derived from the transcutaneous electrical potential rather than from a surface drug concentration gradient.

These studies also further demonstrate the feasibility of lidocaine delivery by coiontophoresis. If transdermal delivery of a drug such as lidocaine is desired for systemic effects, then the use of a vasodilator such as tolazoline would minimize cutaneous depot formation and maximize transdermal flux. Finally, if *in vivo* predictions are to be made, studies must be conducted in model systems containing a functional microcirculation such as *in vivo* or isolated perfused skin preparations if modulation of the vasculature is intended.

In conclusion, coiontophoresis of vasoactive drugs significantly modulates the transdermal delivery of lidocaine into skin *in vivo* in swine. The technique employed appears

to provide a sensitive tool for quantitatively assessing the delivery of drug into different depths of skin. Depending on whether a compound's intended effect is for topical or systemic therapy, the use of vasoactive drugs may further increase the ability to regulate drug delivery by iontophoresis.

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