The permeability of human eyelid skin to topically applied lidocaine

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1

Abstract

This work investigated the permeability of lidocaine across human eyelid skin and compared this with published data for abdominal skin to understand the characteristics of this type of skin and whether topical anaesthesia for eyelid surgery may be feasible. Eyelid skin is thought to have a relatively high permeability to drugs, however how this compares to other body sites has not been previously quantified. Lidocaine solutions at pH 7.0 and 5.5 were applied to human eyelid skin mounted in Franz diffusion cells. Anatomical features of eyelid skin that may be linked to its increased permeability, superficial corneocyte area and stratum corneum (SC) thickness were measured using light microscopy.

Steady-state fluxes of lidocaine at pH 7.0 and pH 5.5 were 283.9 and 41.0 $\mu g/cm^2/hr$, 2.4 and 3.2 times greater respectively than literature values for abdominal skin. Superficial eyelid corneocyte area (800.5 μm^2) was 35% smaller and the eyelid SC thickness (14.9 μm) was 31% thinner than reported abdominal skin values. These suggest that a shorter diffusional pathlength across the eyelid SC contributes to increased lidocaine permeability. The relatively high permeability of eyelid skin to lidocaine indicates considerable potential for achieving strong topical anaesthetic effects at this site.

Keywords: topical anaesthesia, eyelid skin, topical drug delivery, skin permeation

Introduction

The current method of achieving sufficient anaesthesia for eyelid surgery is by subcutaneous infiltration of local anaesthetic (LA). Delivery of anaesthetics with a hypodermic needle is associated with pain and discomfort, as well as needle phobia in some patients. Furthermore, associated swelling and haemorrhage may cause distortion of surgical landmarks, making surgery more difficult [1, 2]. An alternative to injections is the use of topical anaesthetics, however their use in eyelid surgery is limited as they may provide an insufficient depth of anaesthesia and as a result of how they are formulated may cause chemical injury to the eyes [3-5].

The skin presents a formidable inherent anatomical barrier which is largely a result of the properties of the stratum corneum (SC), the outermost layer of the epidermis. Common topical anaesthetics such as lidocaine are weak bases and have shown pH dependent skin permeation, with greater permeation being observed from formulations with higher pH, in which the drug was mostly unionised [6]. The chemical injuries that have been observed with topical anaesthetics are thought to be caused by the alkalinity (pH 9) of these formulations [3, 4]. In comparison to skin from most other body sites, eyelid skin is believed to have a reduced barrier function suggesting it is a promising site for topical anaesthesia to be able to provide sufficient effect to avoid the need for subcutaneous injections [7, 8]. However, despite topical treatments commonly being applied to the eyelid, the permeability of the human eyelid to drugs has not been previously been reported. Recently application of drugs to eyelid skin has shown promise in a rat model for delivery of drugs to the eye for management of conditions such as glaucoma [9, 10]. However rat skin is known to be considerably more permeable than human skin [11]. A better understanding of the skin barrier of human eyelid skin and knowledge of how its permeability to drugs compares to skin from other body sites such as abdominal skin which is

commonly used for *in vitro* permeation experiments would enable identification of the potential

of eyelid skin as a site for drug delivery in general as well as topical anaesthesia. To achieve

this we have compared permeation of lidocaine from solutions at pH 5.5 and 7.0, where the

drug is in different ionisation states across human eyelid skin to published values for human

abdominal skin and investigated anatomical features of this tissue that are known to affect skin

permeability.

Materials and Methods

Materials

Lidocaine and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich (UK). Milli-Q®

(Millipore, USA) water was used throughout the study. HPLC grade methanol was purchased

from Merck (UK). Phosphate buffer solution (PBS) tablets were obtained from Fisher

Scientific (UK). Materials required for the preparation of histological specimens, hexane,

xylene, ethanol, haematoxylin and eosin, were purchased from Sigma-Aldrich (UK).

High-performance liquid chromatography (HPLC)

Quantitative analysis of lidocaine was performed using HPLC with an Agilent® 1200 series

HPLC system (Agilent Technologies, USA) and a Phenomenex® Luna 5 µm C18 4.6 x 150

mm column following a methodology similar to that previously reported by Wood et al (2012)

[12]. An isocratic mobile phase of 60:40 water (adjusted to pH 10 with NaOH): methanol was

used. The UV detection wavelength, flow rate and injection volume were 210 nm 1.2 ml/min

and 20 µl, respectively. The retention time of lidocaine under these conditions was 10.3

minutes. The HPLC methods were validated for linearity, precision and accuracy according to

the current ICH guidelines [13, 14].

Human Skin Preparation

Human upper eyelid skin was collected from routine eyelid surgical operations such as blepharoplasty at Moorfields Eye Hospital (Bedford, UK) with ethical approval granted by North Wales Research Ethics Committee (REC reference number 13/WA/0066, 28/02/13). Full thickness skin samples were stored in a freezer at -20 °C until they were ready for use.

Skin Permeation Studies

Measurement of lidocaine transport across eyelid skin was performed using specially designed Franz cells with a small surface area of approximately 0.18 cm^2 (Soham Scientific, UK). Each Franz cell had a receiver volume of approximately 2 ml. Eyelid skin was defrosted prior to use, cut to fit and mounted in each diffusion cell. Typically, each donor provided enough skin for a single Franz cell and the replicate experiments contained human eyelid skin from different donors. PBS (50 mM at pH 7.3) was used as the receiver fluid. The Franz cells were placed on a submersible stirring plate and placed in a water bath at 37°C to provide a skin surface temperature of 32°C. Each Franz cell was allowed to equilibrate for 90 minutes. During this time the receiver fluid was replaced with fresh pre-warmed receiver fluid at 30, 60 and 90 minutes. The receiver fluid used during the equilibration period was analysed by HPLC to confirm the absence of lidocaine in the skin tissue prior to running the experiments. Following the equilibration period, 2 ml of 2.0 % (w/v) lidocaine solution in phosphate buffer (50 mM) at either pH 5.5 or pH 7.0 was placed in the donor compartment and samples (200 µl) were taken for HPLC analysis at regular intervals over a period of 10 hours. The large 2 ml volume of the donor solution was designed to provide 'infinite' dose conditions and allow mathematical modelling of the permeation data with Fick's first law [15]. Eight diffusion cells were dosed with pH 5.5 and eleven cells with pH 7.0 lidocaine solutions. Following removal of each 200

5

µl sample, the same volume of thermostatically equilibrated receiver fluid was added to the receiver compartment.

Measurement of eyelid corneocyte surface area

Eyelid corneocytes were acquired by initially tape-stripping eyelid skin with Scotch TapeTM (3M) to obtain a thin layer of corneocytes. This layer was then flushed with HPLC-grade hexane and sonicated for 20 min to release the corneocytes [7]. The slides were stained with Eosin. Photographs of the corneocytes were taken under a Leica GXM-L3230 light microscope at 40x magnification and the surface areas of corneocytes were measured using the GXCapture7 software. The mean value reported was calculated from 98 individual corneocytes.

Measurement of the SC thickness

Eyelid skin was fixed in formaldehyde following collection from the surgical procedure. The samples were washed in PBS and wrapped in aluminium foil before flash freezing in liquid nitrogen with OCT embedding medium (Thermo scientific, UK). Slices (30 µm) were then prepared with a microtome. The slides were stained with hematoxylin and eosin [16]. Photographs were taken using a Leica GXM-L3230 light microscope at 40x magnification.

Statistical analysis

Data is presented as mean ± standard deviation, and analysed by t-test. Statistical comparison with already published data was conducted using Welch's t-test because of the difference in sample size. Statistical comparison was made using GraphPad Prism 7.0 software. $P < 0.05$ was considered statistically significant.

Results and Discussion

The cumulative amount of lidocaine permeated across eyelid skin over a 10 h period is illustrated in Figure 1 for both the pH 5.5 and pH 7.0, 2% w/v lidocaine solutions and shows typical permeation profiles expected for infinite dose diffusion cell experiments. The variability in the data at each pH is consistent with previous reports of variability in drug permeation across skin from different donors [17]. The steady-state flux of lidocaine across the eyelid skin from solutions at pH 7.0 was approximately 6.9 times greater (p<0.0001) than those at pH 5.5 with values of 283.9 ± 116.4 $\mu g/cm^2/h$ (n=11), and 41.0 ± 28.3 $\mu g/cm^2/h$ (n=8) (Table 1). The pH values of 7.0 and 5.5 used in this study are not believed to directly affect skin barrier function [6]. Increased permeation of lidocaine across skin from the pH 7.0 solution in which it is more unionised in comparison with the more acidic pH 5.5 solution in which it is mostly ionised is in keeping with previously published data on the skin permeability of lidocaine and the pH – partition hypothesis [6]. Supporting this, the lag times for the permeation of lidocaine across eyelid skin from the pH 7.0 and pH 5.5 solutions were not significantly different (Table 1). The lag time of a permeation experiment relates solely to the diffusitivity of the permeating species, therefore the differences in lidocaine permeation at the different pH values can be ascribed to the different partitioning of lidocaine in response to pH [15]. In addition the lidocaine flux values across eyelid skin were found to be significantly greater than those published for lidocaine permeation under the same conditions and lidocaine concentration (2% w/v) across abdominal epidermis (p <0.05), being approximately 2.4 and 3.2 fold greater for the pH 7.0 and pH 5.5 solutions respectively (Table 1) [6]. The rate limiting barrier to drug permeation across the skin is known to be the stratum corneum, the thin, outermost layer of the skin, however other skin layers such as the viable epidermis and dermis may also contribute. Therefore these values which compare data from full thickness eyelid skin with that of abdominal epidermis should be viewed as a conservative estimate of the increased

7

permeability of eyelid skin. Nonetheless the increased permeability of lidocaine across eyelid skin obtained here is comparable to the three-fold increase in transepidermal water loss (TEWL) value at the eyelid in comparison to the abdomen, a measure which is commonly used as an indicator of skin barrier function [18]. Moreover improved permeation of local anaesthetics across the skin is known to correlate with their anaesthetic effect, suggesting that there is considerable potential to develop topical anaesthetics to provide a strong effect at the eyelid [19]. *In vivo* studies would be required to confirm this.

Previously when investigating variation in skin permeability at different body sites an inverse trend has been observed between the size of corneocytes in the SC and the TEWL value obtained at the same site [7, 8]. Example images of superficial corneocytes from the eyelid skin samples used in this study are shown in Figure 2. The average corneocyte area was $800.5 \pm 13.7 \ \mu m^2$ (n= 98). This value is 35 % smaller in size than that reported for abdominal skin ($1239 \pm 95 \ \mu m^2$) [7]. Other factors believed to affect skin permeability include the number of cell layers in the SC and the overall thickness of the SC layer. Figure 3 shows a histological section of full thickness eyelid skin with the stained SC layer being easily visible. The thickness of the eyelid skin SC was measured to be $14.9 \pm 2.9 \ \mu m$ (n= 27), which is 31 % lower than the value reported for abdominal skin ($21.8 \pm 2.2 \ \mu m$) [20]. Previously published work observed fewer cell layers in the eyelid SC (8 ± 2) than abdominal SC (14 ± 4) [18]. As topically applied drugs are thought to predominately traverse the SC via the intercellular route, these anatomical differences suggest that the diffusional pathlength across the SC of eyelid skin would be shorter, contributing to the increased permeability of the skin at this body site. Rougier et al (1988) correlated skin permeability to a pathlength around corneocytes in the SC, equating pathlength to $(n + \sqrt{(A (n - 1)/2)})$, where A is the corneocyte surface area and n is the number of cell layers [21]. Using measured values of eyelid skin corneocyte size, and literature values

for abdominal skin and the number of cell layers, provides an estimate of the diffusional pathlength across the SC of eyelid skin being 1.7 times shorter than that of abdominal skin. From Fick's law this would be expected produce a 1.7 fold increase in lidocaine flux across eyelid skin in comparison to that of abdominal skin which is slightly lower than what was measured. Whilst it is likely that the calculated increase in the diffusional pathlength across the SC contributes to the increased permeability of eyelid skin to topically applied drugs reported here, other factors may also contribute. For example, although the SC is known to be the main barrier to drug delivery across the skin, the viable epidermis and dermis may also provide some barrier function [22]. Eyelid epidermis (54.4 ± 9.6 µm) and dermis (469.2 ± 119.7 µm) thickness are reported to be considerably thinner than that of the abdomen, which are 79.4 ± 33.9 µm and 1248.4 ± 262.5 µm respectively and may also influence eyelid skin's increased permeability to lidocaine [23].

The eyelid is a specialised region of skin and its relatively small area, proximity to the eye and contribution to an individual's appearance means that it is unlikely to be suitable for the transdermal administration of drugs for a systemic effect. However a wide array of products designed to produce a local effect are/could be applied to the eyelid, including cosmetics, treatments for skin diseases that affect the eyelid such as eczema and rosacea and potentially novel medicines that use the eyelid as a site to apply anti-glaucoma drugs [9]. It is likely that the insight gained here into the permeability of human eyelid skin to drugs will be of benefit in the development of such formulations.

Conclusions

This study has provided evidence of the high permeability of eyelid skin to topically applied drugs with lidocaine permeation being 2.4-3.2 times greater than literature values for

abdominal skin. This suggests that a strong local anaesthetic effect potentially could be obtained at the eyelid which would reduce the need for subcutaneous injection of local anaesthetics at this site. This information may help with the adaptation of currently available products or encourage the development of new products, specifically designed for application around the eye. The aim would be to provide sufficient anaesthesia for eyelid surgery with a reduced dependence on subcutaneous infiltration.

Acknowledgements

Funding

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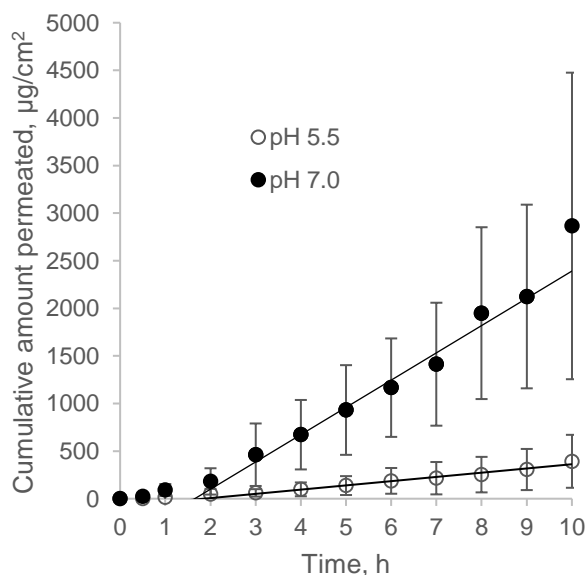

Figure 1: The cumulative amount per unit area of lidocaine permeation across human eyelid skin over a 10-hour period from the pH5.5 and pH7.0 lidocaine solutions. The data points represent the mean values ± SD (n = 8-11).
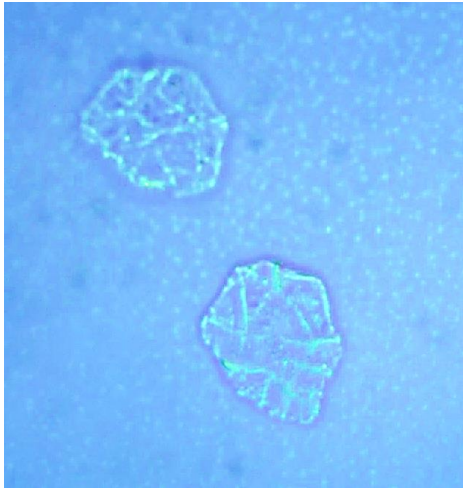
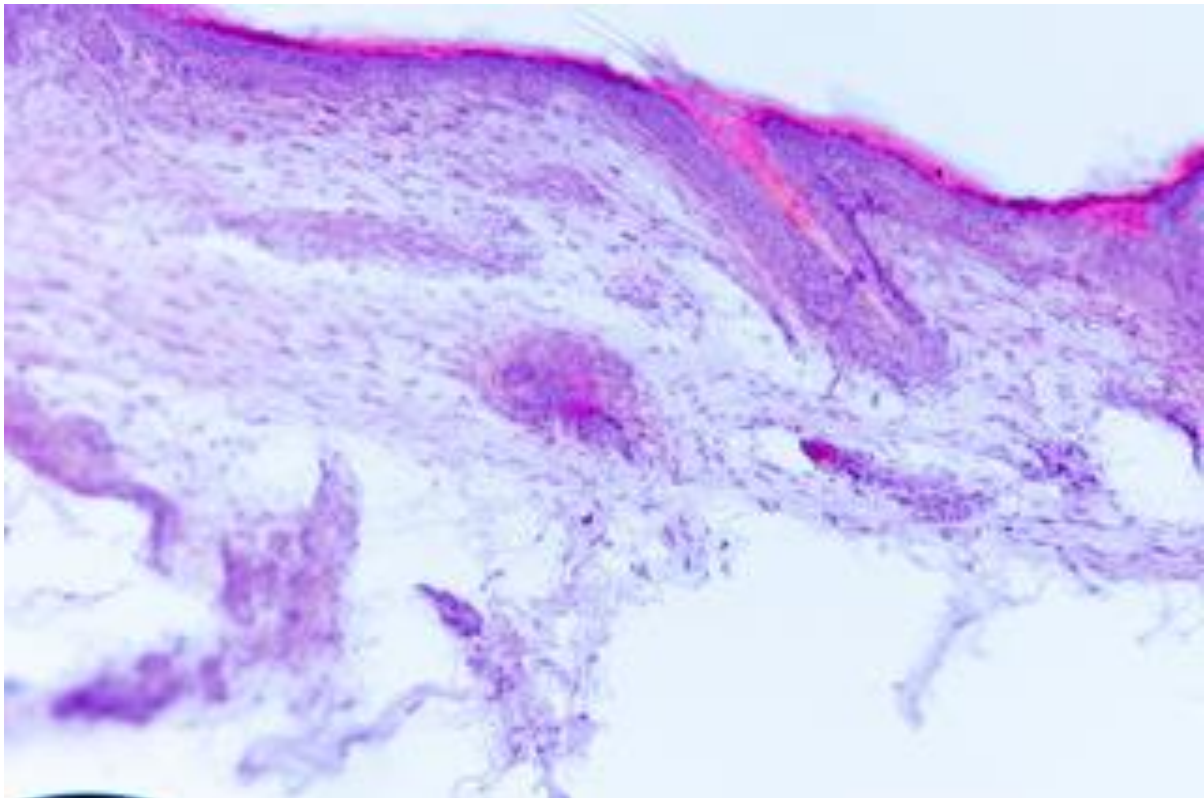Figure 2: A light microscope image of the superficial eyelid skin corneocytes at 40x magnification.

Figure 3: A photographic image of an eyelid skin after frozen sectioning and hematoxylin and eosin staining under a light microscope at 40x magnification.

14