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Transdermal drug delivery in horses: An in vitro comparison of skin structure and permeation of two model drugs at various anatomical sites

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Abstract

Background: Oral and parenteral drug delivery in horses can be difficult. Equine-specific transdermal drug formulations offer improved ease of treatment; development of such formulations requires a deeper understanding of the structural and chemical tissue barrier of horse skin.

Hypothesis/Objectives: To compare the structural composition and barrier properties of equine skin.

Animals: Six warmblood horses (two males, four females) with no skin diseases.

Materials and Methods: Routine histological and microscopic analyses were carried out with image analysis for skin from six different anatomical locations. In vitro drug permeation was analysed using a standard Franz diffusion cell protocol coupled with reversed phase-high-performance liquid chromatography detailing flux, lag times and tissue partitioning ratios of two model drug compounds.

Results: Epidermal and dermal thicknesses varied between sites. The dermal and epidermal thicknesses of the croup were 1764 ± 115 μm and 36 ± 3.6 μm, respectively, and were significantly different ($p < 0.05$) from the inner thigh thicknesses which were 824 ± 35 μm and 49 ± 3.6 μm. Follicular density and size also varied. The highest flux for the model hydrophilic molecule (caffeine) was for the flank (3.22 ± 0.36 μg/cm^2/h), while that for the lipophilic molecule (ibuprofen) was for the inner thigh (0.12 ± 0.02 μg/cm^2/h).

Conclusions and Clinical Relevance: Anatomical location differences in equine skin structure and small molecule permeability were demonstrated. These results can aid in the development of transdermal therapies for horses.

INTRODUCTION

A good understanding of the barrier properties of human skin, and the subsequent protocols delivering a wide range of drugs through skin, has been gained from pharmaceutical and cosmetic sciences.[1–3] By contrast, knowledge of drug delivery through the skin of other species, including horses, is limited. A better understanding of both the physiological and chemical barrier properties of equine skin may lead to improved efficacy of treatment and aid in the design of new horse-specific topical drug formulations. The use of transdermal drug administration in equine medicine offers advantages over standard oral and injectable treatments where dosing can be difficult as a consequence of first-pass drug metabolism.[4,5]

Skin is an effective multilayered barrier that provides mechanical and environmental protection. The epidermis is the outermost nonvascular layer of the skin and is the main barrier to transdermal drug delivery. The thickness of the equine epidermis ranges from 20 to 60 μm depending on anatomical location.[6] The dermis is comparatively thicker (1–6 mm) and contains elastin and collagen which confer flexibility to the skin and

support the epidermis. The dermis contains a suite of secondary structures including hair follicles, sweat glands, blood vessels and nerves.

In combination with regional and species variations of the skin, the physicochemical properties of drug molecules also are important factors to consider for transdermal drug delivery. Molecular weight and log P are most important. Molecular weight is a measure of the size of the molecule; drugs <500 Da are known to permeate the skin. Log P denotes the molecule's partition into an aqueous or lipid-like phase; values >0 denote a favourable partition in lipid environments and <0 denote partition into aqueous environments.[7] The outer layers of the epidermis are classified as highly lipophilic and, thus, favour the partition of molecules with a high (positive) log P, while the dermis has reduced lipophilicity favouring the partition of molecules with a lower (yet still positive) log P. The impact of log P is that poor/slow partitioning of a drug into the tissue surrounding it will cause very slow permeation; hydrophilic molecules permeate slowly, or not at all, through lipophilic tissues and vice versa for lipophilic molecules through hydrophilic tissues, both resulting in poor drug absorption.[8,9]

The aims of this study were twofold. The first aim was to analyse the structure of equine skin from six different anatomical locations to correlate structural features with drug delivery through the tissue. The second aim was to quantify the diffusion of two model drugs, one hydrophilic and one lipophilic, from an aqueous solvent for each anatomical location with the goal of optimising potential administration sites for transdermal drug delivery.

MATERIALS AND METHODS

Animals and ethics

Skin was harvested from six warmblood horses (aged two to six years) within four hours postmortem. Before harvesting, each horse's medical record was reviewed, and skin was inspected by a veterinary surgeon to confirm that no skin disease was evident. The collection of the skin was carried out under approval from the Ethics and Welfare Committee at the University of London Royal Veterinary College (URN 1388 2015, approval date 30 June 2015); no horses were euthanised for the sole purpose of obtaining tissues for this study and permission from the owner for the use of the tissue was granted.

Skin sample collection and sectioning

Skin was dissected from six areas: neck [defined as lateral surface covering the cervical vertebra (C3–C5), 8 × 8 cm], metacarpal region (defined as the area between the carpus and the metacarpophalangeal joint, 10 × 20 cm), flank (defined as the abdominal surface covering ribs 10–14, 12 × 2 cm), croup (defined as the topline of the hindquarters extending proximal to the sacral vertebrae to the dock of the tail, 8 × 8 cm), inner thigh (defined as medial surface of the hind limb proximal to the stifle, 8 × 8 cm) and outer thigh (defined as the lateral surface of the hind limb proximal to the stifle, 10 × 10 cm). Before excision, the hair was carefully trimmed to the skin using clippers, the skin samples were vacuum-packed individually and stored frozen (–20°C) until needed (maximum storage time 90 days).

Skin from each site was analysed for gross structural characteristics. Samples (0.5 × 0.5 cm) were defrosted and the hypodermal adipose tissue was carefully trimmed (defatting procedure) using a scalpel. Samples were embedded in Optimal Cutting Temperature compound (OCT; Sigma Aldrich) using plastic embedding moulds (1 cm^3) and set using dry ice (care was taken to keep samples flat to reduce tangential cutting errors). Samples were removed from moulds, sectioned longitudinally (10 µm, 1° cutting angle) on a cryostat (Leica) and mounted on glass Superfrost plus adhesion microscopy slides (Fisher Scientific); the first five sections of each sample were discarded to eradicate outer surface aberrations. Slides were dried at room temperature for 30 min before staining.

Tissue staining

The air-dried sections were stained using haematoxylin & eosin (H&E; Brunel Microscopes) following a standard protocol, before dehydration, mounting and sealing with varnish.[10]

Microscopy and image analysis

A Leica 9543 optical microscope was used to prepare digital images, which were analysed using ImageJ software (NIH).[11,12] To determine epidermal and dermal thicknesses, digital photomicrographs from 10 H&E-stained sections were taken from each horse at each anatomical site (×100 magnification for epidermal and ×20 magnification for dermal, 1280 × 960 pixels). Digital zooming of all images was implemented as needed to improve the accuracy of readings. Using ImageJ software, 10 thicknesses per image (randomly assigned using a longitudinal coordinate random number generator) were measured in pixels, and then converted into micrometres (µm) using a conversion factor relevant to each magnification (×20 and ×100).

Mean follicular density was calculated using the same photomicrographs as above by measuring the total area of the tissue sections [measured from stratum corneum (SC) to the top of the basal layer] and dividing by the total number of hair follicles visible in that area. This measurement was repeated for each of the 10 sections from each horse at each anatomical site. Follicular density was calculated as mean ± SD per 1 cm^2 of sectioned tissue.

Follicular area was calculated by measuring the area of the follicular bulb using ImageJ for 50 follicles across five (0.5 × 0.5 cm) samples from each horse at each

anatomical location. To ensure that the maximal bulb area was recorded, sections were viewed in sequence and tracked until the largest area of each individual follicle bulb was obtained. Follicular area was calculated as mean ± SD.

In vitro drug permeation

Experiments were performed using conventional Franz diffusion cells (exposed skin surface area of 1.77 cm^2).[13] Skin samples were defrosted overnight at 4°C and then fatty and connective tissue was carefully removed using a scalpel. The tissues were examined for damage or aberrations and discarded if these were present. All skin studies were performed at 34°C. Franz diffusion cells, with a receptor volume of approximately 20 mL, were filled with phosphate-buffered saline (PBS, pH 7.4; Sigma Aldrich). Skin samples were cut into 2 × 2 cm sections and incubated at 34°C for 15 min in 10 mL PBS, to allow full hydration of the tissue before mounting into the Franz cells, SC side up. The donor chamber was filled with 20 mL PBS and left for a further 30 min at 34°C to equilibrate. Baseline samples (2 mL, replaced with 2 mL of fresh PBS) were taken from the receptor chamber before the start of the experiment as controls. The PBS was removed from the donor chamber and replaced with 20 mL of the analyte to be studied, equilibrated to 34°C. As a positive control, dialysis membrane (DM, 12–14 kDa, mean thickness 45 μm; Sigma Aldrich), soaked for one hour in PBS, was used in place of the skin. Negative controls sampling the receiver solution underneath skin with no drug applied also were run for each of the samples.

To investigate the permeation of both hydrophilic and lipophilic drug molecules, two prototypic molecules were selected: caffeine (log P = −0.07; Sigma Aldrich) and ibuprofen (log P = 3.97; BASF). Saturated solutions of both drugs were prepared in PBS (20 mg/mL caffeine, 1 mg/mL ibuprofen) at 34°C. Samples were withdrawn from the receptor chamber (2 mL) at defined timepoints (17 readings over 24 h) with replacement by 2 mL PBS. Drug content was quantified by high-performance liquid chromatography (HPLC). The donor chambers and spouts were covered with parafilm to prevent evaporative losses. All experiments were carried out in triplicate on skin from each horse at each anatomical site.

HPLC analysis

The receptor medium samples were analysed by ultraviolet-HPLC (UV-HPLC), utilising an HP Agilent 1100 HPLC system (Agilent Hewlett Packard) with the following operational parameters: pump flow rate 1 mL/min, UV–visible detector wavelength 272 and 220 nm for caffeine and ibuprofen, respectively, injection volume 10 μL per sample. A reversed phase protocol [Zorbax HPLC Columnphase C18 (octadecyl), 5 μm particle size, 15 cm long × 4.6 mm inner diameter; Sigma Aldrich] was used at 24°C. The mobile phases consisted of 30% methanol (Fisher Scientific) and 70% water for caffeine, and 50% PBS buffer (pH 7.2) and 50% acetonitrile (Sigma Aldrich) for ibuprofen; all solvents were filtered and degassed via sonication before use.

Permeation data analysis

The cumulative amount of drug permeating across the skin and into the receptor compartment was plotted as a function of time. The lag time was determined via linear interpolation of the first HPLC detection signal to zero concentration. Steady-state flux (i.e. how fast the molecule travels through the skin) was calculated by dividing the rate coefficient (gradient) of the linear section of the cumulative concentration versus time permeation profiles (extracted using the Excel (Microsoft) solver function) divided by skin sample thickness.[14]

The flux lag time (FLT) ratio was calculated to give a prediction of the ideal drug/site combination. Individual FLT ratios were calculated for caffeine and ibuprofen, based on flux and lag times at each of the six skin sites.

Residual drug in tissue extraction and quantification

To quantify the drug remaining in the tissue, an extraction procedure was used with modification.[15] Briefly, the skin samples were removed and rinsed with distilled water, placed in a hand blender (Kenwood Triblade HB681) and pulsed for 5 s, then soaked in 10 mL methanol for 1 h at 50°C with stirring. Samples were centrifuged at 1509*g* for 10 min and the supernatant recovered; then three further extraction/centrifugation cycles with 1 mL of methanol were carried out and supernatants were combined. The extract was filtered and analysed by HPLC, the drug recovery from the skin was calculated to be between 94% and 96% using the following equation:

$$\textit{Drug recovery (from tissue)}\ \% = \frac{(\textit{Final acceptor concentration} + \textit{Extracted tissue concentration})}{\textit{Initial donor concentration}} * 100$$

where the final acceptor concentration was recorded from the last timepoint of the 24-h permeation profile, extracted tissue concentration was the concentration recorded by HPLC after extraction from the skin (method above) and the initial donor concentration is the concentration loaded into the donor chamber. Each experiment was carried out in triplicate on skin from each horse at each site. A drug partition ratio between the skin tissue and acceptor chamber was calculated by dividing extracted and acceptor concentration (after 24 h), with values given as percentage of starting donor chamber drug concentration.

Statistical analysis

All data were initially processed for homogeneity of variance using a Levene's test (SPSS STATISTICS 25; IBM) with values of $p<0.05$ considered significant. Where Levene's test was not significant, data were processed using ANOVA, followed by a Bonferroni post hoc test (PRISM 6; GraphPad). Where Levene's test was significant, data were processed using Welch's ANOVA, followed by a Games–Howell post hoc test (SPSS STATISTICS 25). A random effect linear ANOVA model was implemented to analyse significant interhorse variation; as no significance ($p>0.05$) was found, interhorse variation was assumed null. Correlations were analysed using Pearson's correlation test (PRISM 6). Values of $r<-0.7665$ or >0.8488 were considered statistically significant.

RESULTS

Equine skin showed a range of thicknesses depending on location (Figure 1a). The croup (1758 ± 93 µm) was significantly thicker than all other sites and the inner thigh was thinnest (814 ± 82 µm). The epidermal layer showed similar variation between anatomical locations with thicknesses ranging from 28 to 62 µm. There was no statistical correlation between full skin thickness and epidermal thickness (Pearson's correlation $r=0.1195$). For ease of comparison, the thickness of the epidermis is expressed as a percentage of the full skin thickness (Figure 1b).

Follicular area and density are shown in Figure 2a,b, respectively. The croup had the largest follicles (0.035 mm^2) and the neck and inner thigh the smallest (0.005 and 0.004 mm^2, respectively). Follicular density did not correlate with follicular area; the highest number of follicles was seen in the neck (38 ± 3 per cm^2) while the fewest in the inner thigh (5 ± 1 per cm^2), which also had the smallest follicles. To more accurately determine the impact of follicular openings for drug delivery, the percentage total area of the follicles was calculated, normalising for differences in skin area (Figure 2c). Follicular coverage ranged from 0.15% to 1.7%.

Figure 3 shows the mean lag times and flux of the two tested molecules. All lag times for caffeine (hydrophilic molecule) were <60 min except for the inner thigh, which was significantly longer at 83.6 min. Flux was highest in the flank and croup (3.12 and 2.62 µg/cm^2/min, respectively), whilst the other sites provided fluxes <1.12 µg/cm^2/min. Ibuprofen (lipophilic molecule) produced the longest times in the flank and croup (225 and 205 min, respectively). The highest flux (0.12 µg/cm^2/min) was produced through inner thigh skin, which had a short lag time. However, the flank and croup not only showed the longest lag times, but also provided the lowest fluxes (0.015 and 0.021 µg/cm^2/min, respectively), showing relatively poor permeation of the molecule. Dialysis membrane was used as an inert control to ensure drug release from the solutions, and recorded lag times <1 min and rapid fluxes >100 µm/cm^2/min. The FLT ratios are shown in Figure 4.

Both drugs showed accumulation in the skin samples (Tables 1 and 2). The percentage caffeine retained in the tissue was higher at all sites compared to ibuprofen, except for the inner thigh. For caffeine, the thickest tissues allowed the greatest proportion of the applied dose to permeate and were the sites with the highest caffeine accumulation within the skin. For ibuprofen, the proportion of the applied dose delivered through the skin was higher for areas with thin dermal sections, and accumulation of the drug was highest in the areas with the thickest epidermal sections.

To better describe the relationships between permeation and drug retention at the different sites, a ratio of the drug remaining within the skin to that recovered in the receiver fluid after 24 h was determined; this was a pseudo 'partition coefficient' between the skin and acceptor fluid after 24 h of dosing. For both caffeine and ibuprofen, the dialysis membrane control (which should offer no restriction to drug molecule movement) showed very high permeation and the percentage total dose that theoretically could be recovered in the acceptor chamber would be 51.8%. For caffeine, higher partitioning was recorded for the metacarpal region and outer thigh skin, illustrating that the drug was significantly retained in these tissues, compared to the flank skin, where the partition coefficient greatly

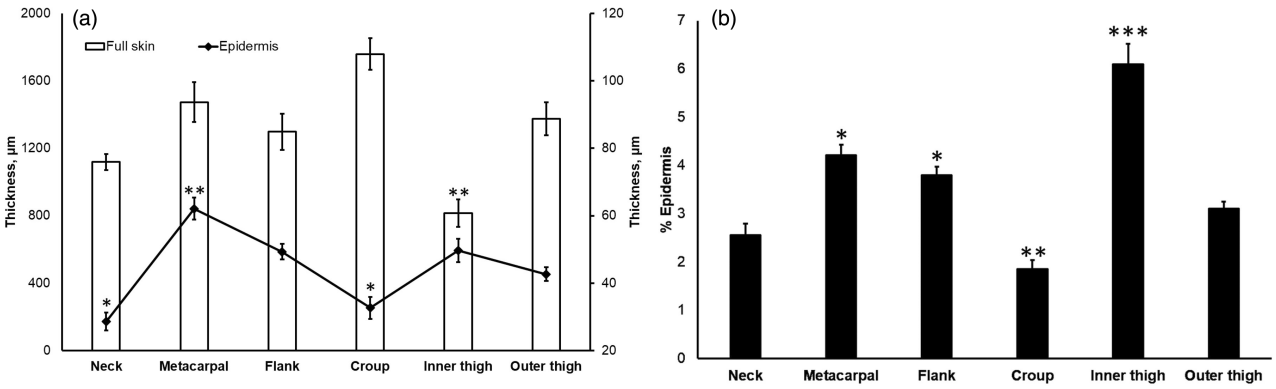

FIGURE 1 (a) Full and epidermal thickness measurements for six equine tissue sites from four horses (full skin thickness on the left y axis and epidermal thickness on the right y axis). (b) Percentage of full thickness skin that is epidermis. $n=40$, data given as mean ± SD. One-way ANOVA with Bonferonni post hoc test was performed on Prism (Graphpad, USA), * or different number of *'s represent groups of data with statistical significance ($p<0.05$).

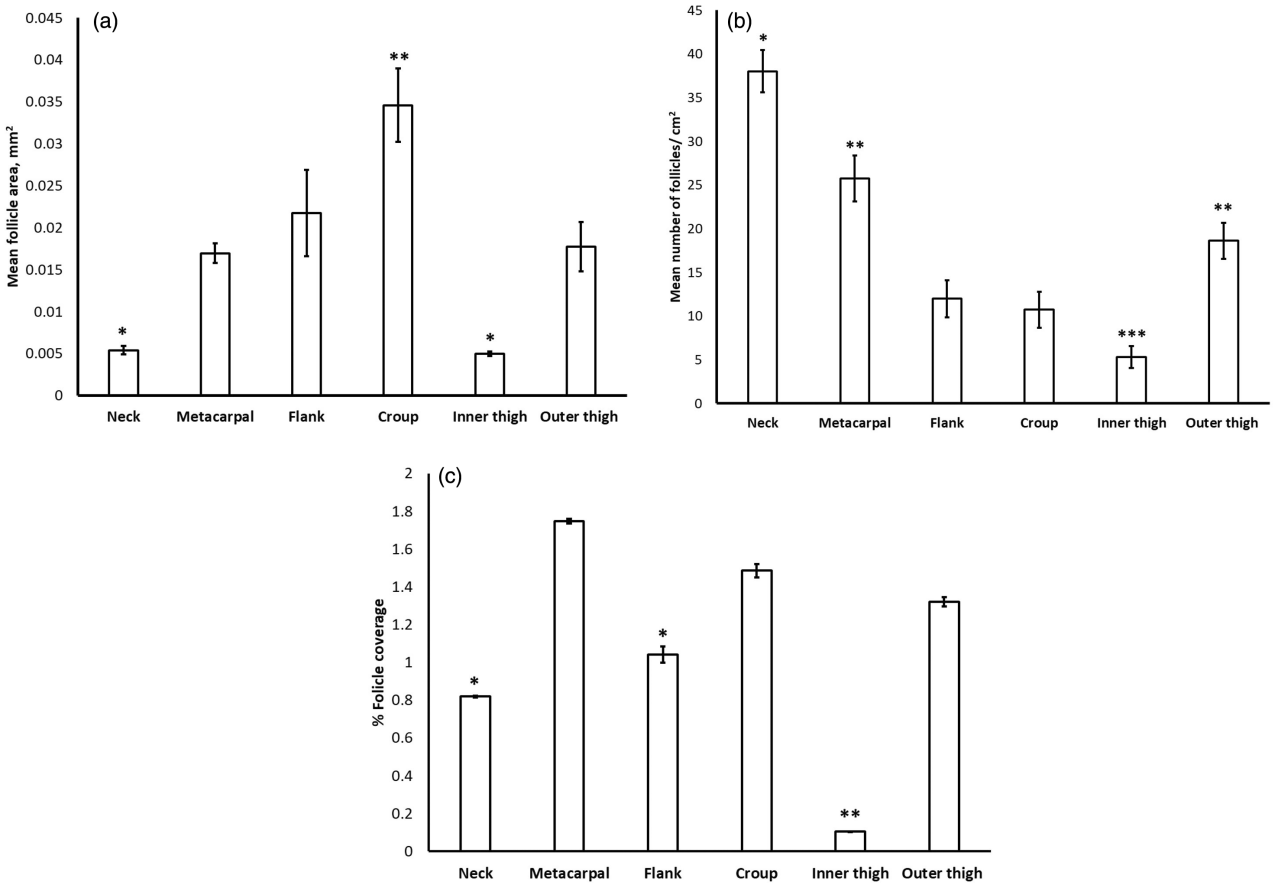

FIGURE 2 (a) Mean hair follicle area for each for six tissue sites from four horses. (b) number of hair follicles at each tissue site per 1 cm of sectioned tissue. (c) Percentage of tissue section covered by the hair follicles for each tissue site. $n = 20$, data given as mean ± standard deviation. One-way ANOVA with Bonferonni post hoc test was performed on Prism (Graphpad, USA), * or different number of *'s represent groups of data with statistical significance ($p < 0.05$).

favoured the acceptor chamber. In comparison, ibuprofen showed relatively high partition coefficients in the flank and metacarpal region tissues. These areas all have relatively thick dermal sections and so transport of the lipophilic drug through this section would be expected to be low. By contrast, for the areas where the dermal layer was thinner (inner thigh and neck) lower amounts of drug were retained in the tissue, compared to that in the acceptor chamber.

DISCUSSION

There was significant site-to-site variation in equine skin thickness, follicular size and density. The variation in the full skin thickness showed some agreement with previous studies where thickness ranged from 1.2 to 7 mm, with variation between sites.[16] Interestingly, our results do show some similarity to human skin thickness (ranging from approximately 1500 μm on the palms and soles to 50 μm on the eyelids).[17] Other species such as dogs have similar skin thickness, ranging from 1480 μm on the distal limb (pad) to 846 μm on the lateral thorax.[18,19]

Transdermal permeation of molecules is limited not only by transport through cells or the intercellular space, and also by the dermal adnexa. For an animal with high follicular density and area, it is likely that this route will have a greater impact on drug delivery. In accordance with another equine study, follicular number was found to be lower in areas of the leg compared to other sites, although this is not always consistent across all sites.[20] In humans, it also has been shown that the volume and number of hair follicles varies significantly with body site.[21] Follicular volume rather than number has been reported to have an impact on transdermal drug flux, with larger volumes leading to an increased rate of permeation.[22–24] In animals, differences in hair thickness (a measure of follicular volume) have been noted both between species and between different anatomical areas, although the impacts on drug permeation were not reported.[25]

Caffeine and ibuprofen were selected as suitable model candidates owing to their similar size (molecular weights, Mw, 194.19 and 206.29, respectively), adequate aqueous solubility and different hydrophilicities.[26,27] The lag time and flux of both caffeine and ibuprofen varied markedly at each of the skin sites, which is in agreement with a number of studies carried out on a variety of animal species. The flux of hydrocortisone (a lipophilic permeant with log P = 1.61) from a 50% ethanolic solution was significantly higher through leg skin (dorsal metacarpal) compared to that through the thorax and groin, which is similar to the finding in this study for the lipophilic molecule ibuprofen.[20] Likewise, the flux of hydrocortisone through greyhound dog leg skin was higher than that for the thorax or groin, although it is notable that drug flux through the canine groin skin

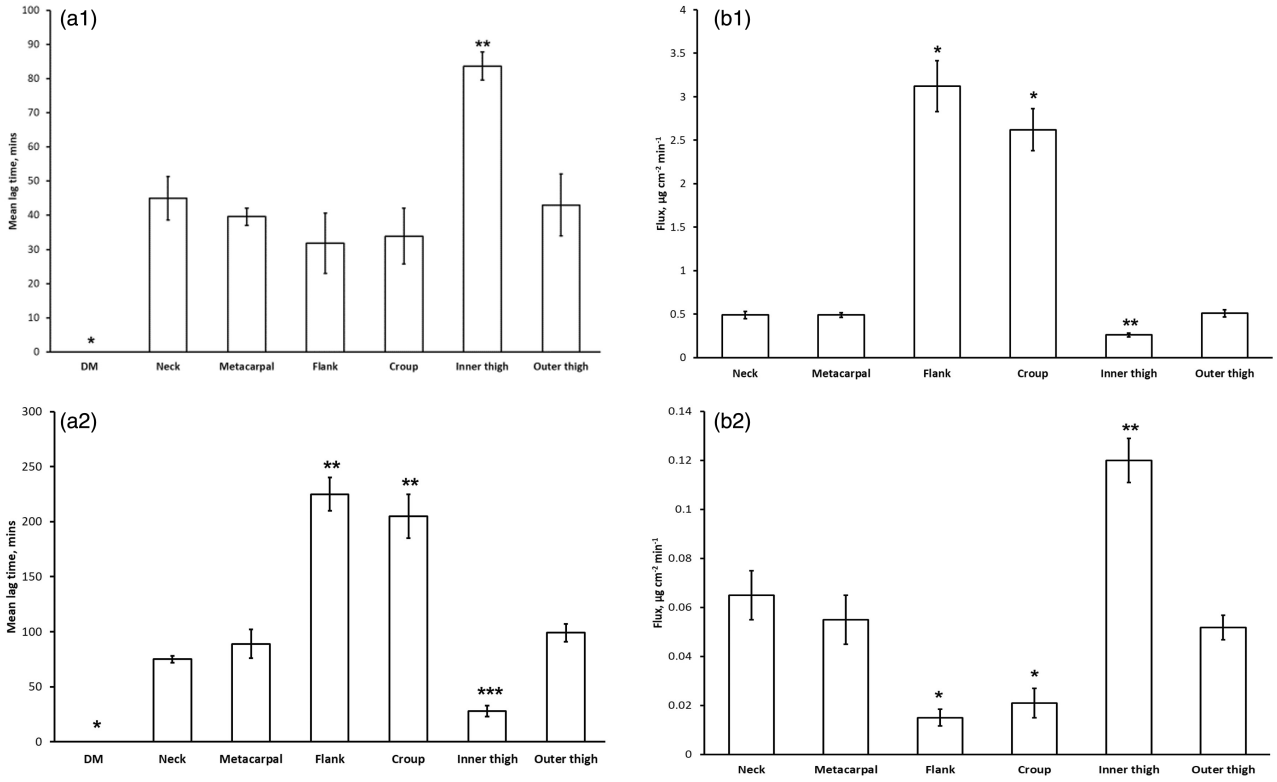

FIGURE 3 Mean lag time and flux for caffeine (A1 and B1) and ibuprofen (A2 and B2) at each of the six tissue sites from six horses and the positive control (dialysis membrane). $n=6$, data given as mean ± standard deviation. Welch's ANOVA, followed by a Games-Howell post hoc test was performed on SPSS (IBM SPSS statistics, USA). Different number of *'s represents groups of data with statistical significance ($p<0.05$).

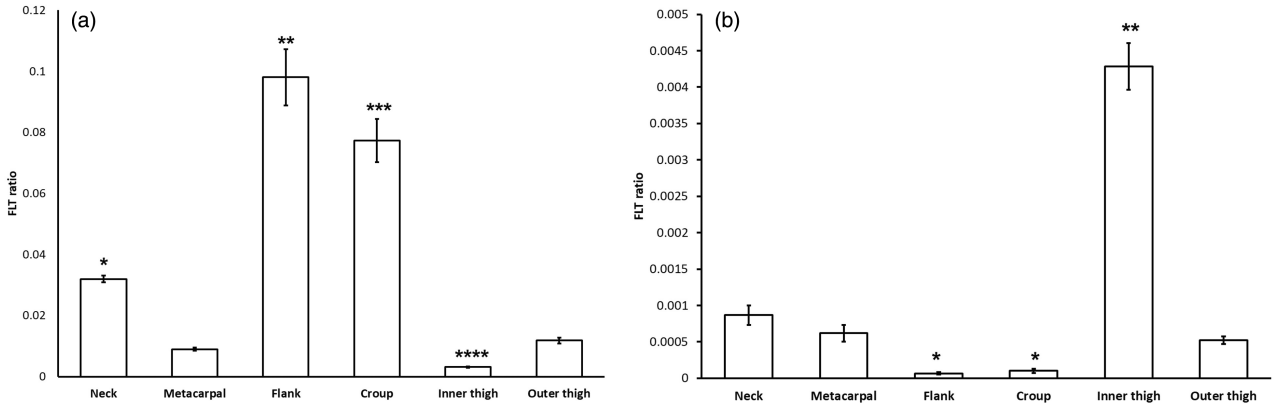

FIGURE 4 Flux lag time ratio of caffeine (a) and ibuprofen (b) at each of the six tissue sites from six horses. $n=6$, data given as mean ± standard deviation. Welch's ANOVA, followed by a Games-Howell post hoc test was performed on SPSS (IBM SPSS statistics, USA), * or different number of *'s represent groups of data with statistical significance ($p<0.05$).

was approximately fivefold higher than that of the same equine skin site, illustrating the difficulties in correlating drug permeation data between species.[28] In addition, comparisons of formulation effects also have been recorded. Five human diclofenac formulations applied to equine skin produced fluxes that were not equivalent to, or interchangeable with, those through human or rat skin, highlighting the need for species-specific formulations.[29] These results illustrate that trends may be found when using different tissue sites between species, and that large differences in drug fluxes are apparent when comparing a given site between species.

Caffeine showed the shortest lag times in those areas that either had comparatively thinner epidermal layers compared to the dermis, or a higher coverage of larger follicles. This is similar to a study on feline skin using methimazole (a hydrophilic drug with log P = −0.38) that showed significantly higher permeation through the skin of the ear, compared to leg, thorax and groin skin.[30] The epidermis (or more specifically its outermost layer, the SC) is usually regarded as the predominant barrier to permeation of a hydrophilic drug, and so if this is thinner or disrupted by the presence of hair it is reasonable to expect a shorter lag time. This is supported by the increased lag time of the inner thigh skin, where a thicker epidermal layer is coupled with sparse coverage of small hair follicles. The flux of caffeine inversely correlates to the lag times, with short

TABLE 1 Percentages of the applied caffeine dose permeating full thickness skin at each of the six tissue sites from six horses and control after 24 h, and % of the original dose recovered from the tissue post methanol extraction. Data are shown as means of triplicate experiments.

Site	% of total dose in acceptor chamber after 24 h (A)	% total dose remaining in tissue (B)	Drug partition between tissue and acceptor (B/A)
Dialysis membrane	41.45	0.02	0.0005
Neck	0.56	0.97	1.73
Metacarpal region	0.44	2.78	6.32
Flank	5.44	3.05	0.56
Croup	4.84	3.31	0.68
Inner thigh	0.25	0.71	2.84
Outer thigh	0.48	2.57	5.35

Note: Data given as means of triplicate experiments.

TABLE 2 Percentages of the applied ibuprofen dose permeating full thickness skin at each of the six tissue sites from six horses and control after 24 h, and % of the original dose recovered from the tissue post methanol extraction, data given as mean of triplicate experiments.

Site	% of total dose in acceptor chamber after 24 h (A)	% total dose remaining in tissue (B)	Drug partition between tissue and acceptor (B/A)
Dialysis membrane	39.52	0.56	0.014
Neck	1.09	0.15	0.13
Metacarpal region	0.93	1.41	1.51
Flank	0.15	0.46	3.06
Croup	0.36	0.21	0.58
Inner thigh	3.23	1.18	0.36
Outer thigh	0.94	0.57	0.61

Note: Data given as means of triplicate experiments.

lag times showing higher fluxes as would be expected from the skin thickness.

In contrast to caffeine, ibuprofen showed the longest lag times at sites that had the thickest dermal layers because the hydrophilic nature of the dermis can slow or prevent passage of lipophilic molecules. This is further illustrated by areas with thinner dermal layers showing shorter lag times. Transdermal fluxes of ibuprofen also are inversely correlated, with lag times in agreement with the caffeine data.

The interrelationship between flux and lag times is further demonstrated by the FLT ratio, which highlights the optimal sites of the administration for the two drugs. A higher ratio represents sites with a relatively high flux and short lag time, a combination which is advantageous for rapid drug delivery for systemic action. The ratio shows that for a hydrophilic molecule (caffeine), application to the flank would result in the highest fluxes through the tissue with a fast onset of action, while application to the inner thigh would be a poor choice as a result of relatively low fluxes and delayed onset of action. The highest ratio obtained for the lipophilic molecule (ibuprofen) was for the inner thigh and neck, showing that these would be preferable administration sites for this drug, while the flank and croup would be poor options. Correlating the FLT ratio to the physiology of the skin, sites with a thinner epidermis were a less effective barrier to the hydrophilic molecule (caffeine), while for the lipophilic molecule (ibuprofen) highest fluxes were in areas where the epidermis was potentially more 'intact' (lower follicular density) with a thinner dermal section.

Measurements for both the lag time and flux require the molecules to traverse the entire tissue, and thus do not account for the substances accumulating and subsequent partitioning in the tissue. Both drugs were successfully recovered from the skin samples and showed significant uptake into the tissue 24 h postdosing. This shows that the skin could act as a reservoir for certain drug molecules at particular anatomical locations. Ibuprofen had a higher retention at all sites compared to caffeine, with the site-to-site variation attributed to the thick dermal layers present in all of the tissues reducing the transport of lipophilic molecules or ibuprofen through this layer. These findings highlight that more research is required to investigate this potential reservoir effect (based on differential accumulation) and the impact that this may have on dosage and frequency strategies.

A potential criticism of this experimental protocol used herein is that the skin was frozen and then defrosted as required. It is generally acknowledged that frozen skin has reduced metabolic and biochemical function. However, freezing is unlikely to affect the structure of the barrier layers of the skin and hence transdermal permeation.[31–33] To minimise skin changes, it was not stored for >90 days. Samples from the same animal were tested within a 30-day window as this has been shown to minimise variation.[34,35] It also should be noted that the effect of the animal's age on transdermal

permeation was not investigated. In humans, it has been shown that permeation of lipophilic compounds remains relatively unaffected by age, yet permeation of hydrophilic molecules decreases with age.[36] Further research is required to explore this variation in horses.

CONCLUSION

This study found transdermal permeation differences between equine skin from different anatomical locations. These findings could be used as a basis for future studies, including in vivo studies, on transdermal drug delivery.

AUTHOR CONTRIBUTIONS

Samuel Bizley: Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. **Jayesh Dudhia:** Conceptualization; data curation; funding acquisition; investigation; methodology; resources; supervision; writing – review and editing. **Roger KW Smith:** Conceptualization; data curation; funding acquisition; investigation; methodology; resources; supervision; writing – review and editing. **Adrian C Williams:** Conceptualization; formal analysis; funding acquisition; methodology; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

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Résumé

Contexte: L'administration de médicaments par voie orale et parentérale chez les chevaux peut être difficile. Les formulations de médicaments transdermiques spécifiques aux équidés offrent une meilleure facilité de traitement; le développement de telles formulations nécessite une compréhension plus approfondie de la structure de la barrière tissulaire physique et chimique de la peau de cheval.

Hypothèses/Objectifs: Comparer la composition structurale et les propriétés barrières de la peau équine.

Animaux: Six chevaux à sang chaud (deux mâles, quatre femelles) sans affection cutanée

Matériels et méthodes: Une analyse histologique et microscopique de routine est effectuée pour six emplacements cutanés différents. La perméation in vitro du médicament a été analysée à l'aide d'un protocole standard de cellule de diffusion de Franz couplé à une chromatographie liquide à haute performance en phase inversée (RP-HPLC) détaillant le flux, les temps de latence et les taux de partitionnement tissulaire de deux médicaments modèles.

Résultats: L'épaisseur de l'épiderme et du derme varie d'un site à l'autre. L'épaisseur du derme et de l'épiderme de la croupe est respectivement de $1.764 \pm 115\,\mu m$ et de $36 \pm 3.6\,\mu m$ et elle diffère significativement ($p < 0.05$) de celle de l'intérieur de la cuisse variant de $824 \pm 35\,\mu m$ et de $49 \pm 3.6\,\mu m$. La densité et la taille des follicules varient également. Le flux le plus élevé pour la molécule hydrophile modèle (caféine) est observé sur le flanc ($3.22 \pm 0.36\,\mu g/cm^2/h$), tandis que celui de la molécule lipophile (ibuprofène) est à l'intérieur de la cuisse ($0.12 \pm 0.02\,\mu g/cm^2/h$).

Conclusions et pertinence clinique: Des différences dans la structure de la peau équine et la perméabilité aux petites molécules sont obsercées selon la localisation anatomique. Ces résultats peuvent aider au développement de thérapies transdermiques destinées aux chevaux.

Resumen

Introducción: la administración de fármacos por vía oral y parenteral en caballos puede ser difícil. Las formulaciones de fármacos transdérmicos específicos para equinos ofrecen una mayor facilidad de tratamiento; el desarrollo de tales formulaciones requiere una comprensión más profunda de la barrera estructural y química del tejido de la piel del caballo.

Hipótesis/Objetivos: Comparar la composición estructural y propiedades de barrera de la piel equina.

Animales: Seis caballos de sangre caliente (warmblood) (dos machos, cuatro hembras) sin enfermedades de la piel.

Materiales y Métodos: Se realizaron análisis histológicos y microscópicos de rutina con análisis de imagen para piel de seis localizaciones anatómicas diferentes. La permeación del fármaco *in vitro* se analizó utilizando un protocolo estándar de Franz de difusión en celdilla junto con cromatografía líquida de alto rendimiento de fase inversa (RP-HPLC) que detalla el flujo, tiempos de retraso y las proporciones de partición en tejidos de dos compuestos farmacológicos modelo.

Resultados: Los grosores epidérmico y dérmico variaron entre sitios. Los grosores dérmico y epidérmico de la grupa fueron $1764 \pm 115\,\mu m$ y $36 \pm 3.6\,\mu m$, respectivamente, y fueron significativamente diferentes ($p < 0.05$) de los

grosores de la cara interna del muslo, que fueron 824 ± 35 μm y 49 ± 3.6 μm. La densidad y el tamaño folicular también variaron. El mayor flujo para la molécula hidrofílica modelo (cafeína) fue para el flanco (3.22 ± 0.36 μg/cm^2/h), mientras que para la molécula lipofílica (ibuprofeno) fue para la cara interna del muslo (0.12 ± 0.02 μg/cm^2/h).
Conclusiones y relevancia clínica: Se demostraron diferencias de ubicación anatómica en la estructura de la piel equina y la permeabilidad de moléculas pequeñas. Estos resultados pueden ayudar en el desarrollo de terapias transdérmicas para caballos.

Zusammenfassung
Hintergrund: Orale und parenterale Medikamentenverabreichung kann bei Pferden schwierig sein. Pferdespezifische transdermale Wirkstoff-Formulierungen ermöglichen eine einfachere Behandlung; für die Entwicklung dieser Formulierungen ist ein tieferes Verständnis der Strukturen und der chemischen Gewebebarrieren der Pferdehaut nötig.
Hypothese/Ziele: Ein Vergleich der strukturellen Zusammensetzung und der Eigenschaften der Hautbarriere der Pferdehaut.
Tiere: Sechs Warmblüter (zwei männlich, vier weiblich) ohne Hauterkrankungen.
Materialien und Methoden: Es wurde eine histologische und mikroskopische Analyse mittels Bildanalyse der Haut in sechs unterschiedlichen Lokalisationen durchgeführt. Die *in vitro* Durchlässigkeit der Wirkstoffe wurde mittels Standard Franz Diffusionsprotokoll gekoppelt mit Umkehrphasen Hochleistungsflüssigkeitschromatografie (RP-HPLC) analysiert, wobei Fluss, Verzögerungszeiten und der Anteil der Gewebsverteilung zweier Wirkstoffkomponenten als Modell detailliert dargestellt wurden.
Ergebnisse: Die epidermale und dermale Dicke variierte zwischen den Körperstellen. Die dermale und epidermale Dicke der Krupp betrug 1,764 ± 115 μm bzw 36 ± 3.6 μm und war signifikant verschieden ($p < 0.05$) von der Dicke der inneren Schenkel, die 824 ± 35 μm bzw 49 ± 3.6 μm betrug. Die Follikeldichte und Größe variierten ebenfalls. Der höchste Fluss für das hydrophile Modellmolekül (Koffein) war im Bereich der Flanke (322 ± 0.36 μg/cm^2/h) zu finden, während jenes für das lipophile Molekül (Ibuprofen) im Bereich der Innenschenkel am höchsten war (0.12 ± 0.02 μg/cm^2/h).
Schlussfolgerungen und klinische Bedeutung: Es wurden die Unterschiede der anatomischen Lokalisationen der Struktur der Pferdehaut und der Durchlässigkeit kleiner Moleküle gezeigt. Diese Ergebnisse können bei der Entwicklung transdermaler Therapien für Pferde helfen.

要約
背景: 馬の経口および非経口薬物送達は困難である。馬に特化した経皮吸収型製剤により、治療が容易になる。このような製剤の開発には、馬の皮膚の構造的および化学的な組織バリアに関する深い理解が必要である。
仮説/目的: 本研究の目的は、馬の皮膚の構造組成およびバリア性を比較することであった。
対象動物: 皮膚疾患のないウォームブラッド6頭(雄2頭、雌4頭)。
材料と方法: 6つの解剖学的部位から採取した皮膚について、画像解析を用いて通常の組織学的および顕微鏡的解析を実施した。In vitroにおける薬物透過性を、標準的なフランツ型拡散セルプロトコルおよび逆相高速液体クロマトグラフィ(RP-HPLC)を用いて、2種類のモデル化合物のフラックス、ラグタイム、組織分配比を詳細に解析した。
結果: 表皮および真皮の厚さは部位によって異なる。臀部の真皮および表皮の厚さは、それぞれ1,764 ± 115 μmおよび36 ± 3.6 μmであり、内腿の真皮および表皮の厚さは824 ± 35 μmおよび49 ± 3.6 μmであり、有意差($p < 0.05$) があった。毛包密度およびサイズも変化した。親水性分子(カフェイン)のフラックスが最も高かったのは脇腹(3.22 ± 0.36 μg/cm^2/h)であり、親油性分子(イブプロフェン)のフラックスが最も高かったのは内腿(0.12 ± 0.02 μg/cm^2/h)であった。
結論と臨床的関連性: 馬の皮膚構造と低分子化合物の透過性に解剖学的部位の違いがあることが示された。これらの結果は、馬の経皮治療薬の開発に役立つと考えられた。

摘要
背景: 马的口服和非肠道给药可能很困难。马特异性透皮药物制剂提高了治疗的简易性 ; 这种配方的开发需要对马皮的结构和化学组织屏障有更深入的了解。
假设/目的: 比较马皮肤的结构组成和屏障功能。
动物: 六匹没有皮肤病的温血马(两只雄性 , 四只雌性)。
材料和方法: 对来自六个不同解剖位置的皮肤进行常规组织学和显微镜分析 , 并进行图像分析。使用标准Franz扩散池方案结合反相高效液相色谱(RP-HPLC)分析体外药物渗透 , 详细说明两种模型药物化合物的通透量、滞后时间和组织分配比。
结果: 不同部位的表皮和真皮厚度不同。臀部的真皮和表皮厚度分别为1764 ± 115 μm和36 ± 3.6 μm , 与大腿内侧824 ± 35 μm和49 ± 3.6 μm.毛囊密度和大小也不同 , 差异显著($p < 0.05$)。体侧的亲水分子模型(咖啡因)通透量最高(3.22 ± 0.36 μg/cm^2/h) , 而亲脂性分子(布洛芬)的最高通透量为大腿内侧(0.12 ± 0.02 μg/cm^2/h)。
结论和临床相关性: 马的皮肤结构和小分子通透性在解剖学位置上存在差异。这些结果有助于开发马匹的透皮疗法。

Resumo

Contexto: Administração oral e parenteral de medicações para cavalos pode ser difícil. Formulações transdermais específicas para equinos facilitam o tratamento; o desenvolvimento destas formulações requer maior conhecimento na barreira tecidual química e estrutural da pele equina.

Hipótese/Objetivos: Comparar a composição estrutural e as propriedades da barreira cutânea de equinos.

Materiais e métodos: Realizou-se análise histológica e microscópica de rotina a partir de imagens da pele seis locais anatômicos diferentes. A permeabilidade *in vitro* foi avaliada utilizando o protocolo de difusão celular de Franz padrão pareado com fluxo detalhado de cromatografia líquida de fase reversa de alta performance (RP-HPLC), tempos de atraso e taxas de partição tecidual de dois compostos de fármacos modelo.

Resultados: As espessuras epidérmica e dérmica variaram entre os locais. As espessuras dérmica e epidérmica da garupa foram $1.764 \pm 115\,\mu m$ e $36 \pm 3.6\,\mu m$, respectivamente, e foram significativamente diferentes ($p < 0.05$) das espessuras da parte interna da coxa que foram $824 \pm 35\,\mu m$ e $49 \pm 3.6\,\mu m$. A densidade e o tamanho foliculares também variaram. O maior fluxo para o modelo de molécula hidrofílica (cafeína) foi para o flanco ($3.22 \pm 0.36\,\mu g/cm^2/h$), enquanto para o modelo de molécula lipofílica (ibuprofeno) foi para a parte interna da coxa ($0.12 \pm 0.02.\mu g/cm^2/h$).

Conclusões e relevância clínica: Foram demonstradas diferenças regionais anatômicas na estrutura e permeabilidade a pequenas moléculas na pele equina. Estes resultados podem auxiliar no desenvolvimento de terapias transdermais para equinos.