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The effects of formulation on the penetration and retention of budesonide in canine skin in vitro

Liisa A. Ahlstrom [a,*], Sheree E. Cross [b], Paul C. Mills [a]

[a] School of Veterinary Science, The University of Queensland, Gatton QLD 4343, Australia
[b] Therapeutics Research Unit, Southern Clinical Division, The University of Queensland, Princess Alexandra Hospital, Brisbane QLD 4102, Australia

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ABSTRACT

This study investigated the effects of formulation on the penetration and retention kinetics of budesonide through canine skin in vitro. Full thickness, thoracic, dog skin was mounted in Franz-type diffusion cells and randomly assigned to receive one of three 0.025% (0.25 mg/mL) budesonide-containing formulations: Barazone (BZ, a novel formulation), isopropyl myristate (IPM) or propylene glycol (PG). At regular intervals over 84 h, the amount of budesonide penetrating or retained within the skin was quantified using high performance liquid chromatography. The restricted (or residual) maximum likelihood mixed model predicted that the flux of budesonide from BZ was 9.2-fold ($P < 0.001$) and 105-fold ($P < 0.001$) greater than from IPM and PG, respectively. Similarly, the skin retention of budesonide from BZ was more than 3-fold ($P < 0.0001$) and nearly 6-fold ($P < 0.0001$) greater than from IPM and PG, respectively. This study has demonstrated that the formulation can greatly affect the skin penetration and retention of budesonide in dogs, and consequently could be selected to maximise drug concentration and retention at the site of action. This has the potential to improve the efficacy and safety of, and owner compliance with, topical glucocorticoid therapy in dogs.

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Introduction

Topically applied glucocorticoids (GCs) are effective at reducing the clinical signs of allergic skin disease in dogs (Bonneau et al., 2009; Nuttall et al., 2009; Ahlstrom et al., 2010), and are preferable to oral or injectable forms as there is direct application of drug to the target organ, thus achieving high local drug concentrations yet reducing the total dose required and the incidence of adverse, systemic effects (Brazzini and Pimpinelli, 2002; Mills and Cross, 2006), such as disruption to the hypothalamic–pituitary–adrenal axis, immunosuppression and disturbances to the gastrointestinal, musculoskeletal, reproductive and urinary systems (Sturgess, 2002). Although low, the risk of systemic side effects with topical GC therapy is likely to increase with the severity of skin disease, as systemic levels of topically applied hydrocortisone (HC) were positively correlated with the severity of atopic dermatitis (AD) in human patients (Turpeinen, 1988). There is evidence to support this correlation in dogs, as the in vitro penetration of HC was greater through the lesional compared to the non-lesional skin of dogs with suspected flea allergy dermatitis (FAD) (Ahlstrom et al., 2011). This is a concern, as it suggests that dogs with allergic skin disease that may require widespread application of topical GCs to alleviate pruritus could be at greater risk of adverse, systemic effects.

To maximise treatment efficacy and reduce the incidence of systemic side effects, a topical GC should penetrate into and be retained within the skin, with minimal entry into the dermal vasculature for systemic distribution (Wiedersberg et al., 2008). Strategies to enhance the skin penetration and retention of drugs involve modification of the physicochemical properties of either the drug or the formulation (vehicle). The vehicle that carries the drug can affect the partitioning into and rate of diffusion through the stratum corneum (SC), which are the rate-limiting steps for the transdermal penetration of GCs (Wiedersberg et al., 2008). This study was performed to determine if there was a difference in the skin retention and penetration kinetics of a GC (budesonide) when applied to normal canine skin in vitro at a concentration of 0.025% from a novel formulation (Barazone) and from two simple vehicles.

Materials and methods

Skin samples

Dog skin was collected from a greyhound (male, entire, approximately 5 years old) that had been presented to the University of Queensland Veterinary School for euthanasia. The dog was euthanased by an intravenous injection of sodium pentobarbital (Lethabarb; Virbac). This protocol was approved by the Animal Ethics Committee of the University of Queensland (SVS/356/06/).

* Corresponding author. Current address: Faculty of Veterinary Science, The University of Sydney, Camperdown NSW 2006, Australia. Tel.: +61 2 9351 6881.
E-mail address: liisa.ahlstrom@sydney.edu.au (L.A. Ahlstrom).

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The hair over the lateral thorax was removed using electric clippers, taking care not to damage the skin surface. Full thickness skin from the left and right sides of the thorax (cranial border: dorso-ventral line intersecting the caudal angle of the scapula; dorsal border: transverse processes of the thoracic vertebrae; caudo-ventral border: an arc joining the dorsal border from the last rib, to the cranial border at the level of the costochondral junction) was removed and subcutaneous fat (when present) was carefully excised. Within 1 h of death, the skin was sealed in plastic bags, placed in a −20 °C freezer and used within 3 months of collection (Ahlstrom et al., 2007). On the day of the in vitro experiment, the skin was removed from the freezer and left in its sealed plastic bags at room temperature (25 °C) to defrost for 1–2 h. From each piece of thawed skin, 20 circular discs of 25 mm diameter were cut out using a wad punch, rinsed with tap water and gently dried with gauze swabs. The skin discs were randomly distributed between the three treatment groups (Barazone [BZ], Isopropyl Myristate [IPM] and Propylene Glycol [PG]) resulting in 13 replicates per group.

Chemicals and formulations

Solvent A was prepared as 1:9 tetrahydrofuran in methanol (both HPLC-grade), containing 5 μg/mL HC (Sigma Pharmaceuticals) as the internal standard. To prepare the IPM and PG formulations, budesonide (Crystal Pharma) was added to IPM (Tegosoft M; Evonik Goldschmidt GmbH) and PG (Lyondell Chemie), respectively, to achieve a concentration in each of 0.025% w/v. Both IPM and PG formulations were mixed at 65 °C for 1 h or until the budesonide dissolved in these viscous liquids. BZ (Dermcare-Vet) contained 0.025% w/v budesonide (CAS registry number: 51333-22-3; MW: 430.53; purity: ⩾99%; solubility in unbuffered water, pH 7, 25 °C: 9.5×10^{-3} g/L; $\log P_{(octanol/water)}$: 3.1[1]) in a confidential formulation with a lotion matrix.

In vitro skin penetration study

The skin discs were mounted in Franz-type diffusion cells, with the SC uppermost, as previously described (Mills et al., 2005). The surface area of skin exposed to drug in the diffusion cells was 1.13 cm^2. A measured volume (average volume = 3.2 mL) of receptor solution was added to each receptor compartment with a magnetic stirring bead. The receptor solution was phosphate-buffered saline (PBS) at pH 7.4, containing bovine serum albumin (4% w/v; Trace Biosciences) and sodium azide (0.1% w/v; Sigma Pharmaceuticals). Bovine serum albumin was added, as its inclusion in receptor fluid has been shown to allow a better estimation of the permeability parameters for lipophilic compounds (Cross et al., 2003). Sodium azide was added to prevent bacterial growth (Collier and Bronaugh, 1992). The diffusion cells were placed on a magnetic stirring plate in a water bath kept at 35 °C, resulting in a skin surface temperature of approximately 32 °C during the experiment (Mills et al., 2005). One millilitre of PBS was added to the donor compartment of each diffusion cell to hydrate the skin disc and allow the integrity of the skin and diffusion cell to be visually assessed by closely monitoring for any leakage of saline from the donor compartment. After 1 h, PBS was removed from the donor compartment and 1 g of formulation (either BZ, IPM or PG) was evenly applied to the outer surface of each fully hydrated skin disc via its donor compartment ($t = 0$), taking care to ensure good contact between the formulation and the skin. The donor compartments of the diffusion cells were occluded with glass cover slips to minimise evaporation. For the next 84 h, 150 μL samples were collected at regular intervals (14, 24, 36, 48, 60, 72, 84 h) from the receptor compartments of 24 of the diffusion cells (eight per treatment group) via the sampling ports and immediately replaced with the same volume of fresh receptor solution. Budesonide appeared to be stable in the receptor solution at 32 °C for up to 84 h (unpublished pilot study).

In vitro skin retention study

At regular intervals throughout the 84 h penetration study (5.5, 10, 14, 20, 24, 36, 48, 60, 72, 84 h), diffusion cells (13 per treatment group) were randomly selected to be dismantled and the skin discs removed. Skin discs were rinsed in tap water, gently dried with gauze swabs and then the formulation exposed area was excised and macerated with scissors.

Sample analysis

Each receptor solution sample was mixed with 300 μL of solvent A and then vortexed, centrifuged and the supernatant collected and mixed with an equal volume of acetonitrile (HPLC grade; Lab-Scan Analytical Sciences), vortexed, centrifuged and the supernatant filtered (Acrodisc minispike syringe filter, GHP, 13 mm, 0.45 μm; Waters Corporation) and analysed for budesonide concentration by use of a high performance liquid chromatography (HPLC) system consisting of the following: a pumping system (LC20AT; Shimadzu Scientific Instruments), an auto-injector (SIL 20AC; Shimadzu Scientific Instruments), a UV detector (UV–VIS Photodiode array detector SPDM10Avp; 254 nm; Shimadzu Scientific Instruments), a column oven (CTO-20A; Shimadzu Scientific Instruments) and a chromatography software package (Shimadzu VP software). A steel C18 column (Sunfire C18 [5 um; 150 mm × 4.6 mm]; Waters) was used. The mobile phase was acetonitrile that was filtered and degassed through a 0.45 μm hydrophilic membrane filter at a flow rate of 1.0 mL/min. Each macerated skin sample was mixed with 1 mL of solvent A and then homogenised, vortexed, centrifuged and the supernatant collected and mixed with an equal volume of acetonitrile, vortexed, centrifuged and the supernatant filtered and analysed for budesonide concentration as described above for the receptor solution samples. Aliquots of receptor solution and solvent A spiked with known amounts of budesonide were prepared and analysed according to the methods above. The standard curves produced provided evidence of the linearity of the analytical procedure for both receptor solution ($r = 0.9997$) and solvent A ($r = 0.9991$) samples and a workable method was established over a range of 0.08–15.58 μg/mL.

Data analysis

The cumulative amount of budesonide that had penetrated the skin to enter the receptor solution was calculated for each skin replicate at each sampling time. Restricted (or residual) maximum likelihood (REML) mixed model analyses were performed to calculate the mean flux (J) of budesonide through the skin and determine if vehicle had a significant effect on J. In the analyses, treatment group (BZ, IPM or PG) was fitted as a fixed effect, and skin replicate was fitted as a random effect. The mean budesonide J for a treatment group was estimated by the gradient of a regression line through the data points of the cumulative amount of budesonide penetration per skin area versus time profile (permeation profile) (Williams, 2003). Lag time (t_{lag}) was designated as the x-intercept of this regression line. Ninety-five per cent confidence intervals were calculated for each estimate of J. To determine if there was a significant difference in budesonide J from the three vehicles, P-values comparing the mean J from BZ and IPM formulations with the reference group (PG) were calculated using Chi-square tests.

To calculate the amount of budesonide retained in each skin sample following the application of BZ, IPM or PG for variable durations up to 84 h, the amount of budesonide detected in each skin sample was divided by the average fraction of budesonide that was recovered (extraction recovery mean ± standard; 7.3% ± 0.5%) using the same extraction technique, from skin samples taken from the same dog and spiked with known amounts of budesonide. Budesonide retention was described by both the concentration of budesonide (μg/g) and by the fraction of applied budesonide (%) within the skin. The budesonide retention in the skin samples of each treatment group were averaged to determine the unadjusted (crude) means and 95% confidence intervals (CI). REML mixed model analyses were performed to determine if vehicle had a significant effect on skin retention.

In the analyses, treatment group (BZ, IPM or PG) was fitted as a fixed effect, and skin replicate was fitted as a random effect. The skin retention values (fraction or concentration) were log transformed before analyses, to ensure that the assumptions underlying the REML mixed model were met, namely that the residuals were distributed approximately normally and the distribution of residuals was not associated with the fitted values. The β-coefficients derived from the model for skin that received BZ or IPM were then exponentiated, resulting in the estimated ratios of the adjusted geometric means for the skin that received BZ or IPM, relative to the skin that received PG. To determine if there was a significant difference in budesonide retention from the three vehicles, P-values comparing the mean budesonide retention of each treatment group with the reference group (PG) were calculated using Chi-square tests. Statistical significance was set at $\alpha = 0.05$ and SAS version 9.1 (Cary, NC) or Genstat were used for all statistical analyses.

Results

The mean J of budesonide from BZ was 9.2-fold higher ($P < 0.001$) than from IPM, and 105-fold higher ($P < 0.001$) than from PG (see Table 1 and Fig. 1). Similarly, the skin retention of budesonide from BZ was more than 3-fold greater ($P < 0.0001$) than from IPM, and nearly 6-fold greater ($P < 0.0001$) than from PG (see Table 2). There was no difference in the t_{lag} for budesonide penetration between the treatment groups.

Discussion

This study has demonstrated significant effects of formulation on the penetration and retention of a GC, budesonide, in canine skin. Since the same amount of budesonide was applied to skin from each formulation, and the skin used was taken from the same region of one dog, the differences observed in the skin retention and flux of budesonide were attributable to differences between

[1] Value calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 for Solaris (1994–2011 ACD/Labs).

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