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Experimental factors affecting in vitro absorption of six model compounds across porcine skin

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ABSTRACT

This comparative study evaluated the effect of several experimental variables on the absorption of six model [^{14}C]-labeled compounds (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid, and testosterone) through porcine skin. Using static and flow-through diffusion cells, finite or infinite, saturated or unsaturated doses were applied in one of three vehicles: propylene glycol, water, and ethanol following a full factorial experimental design. The flux of each compound into the receptor phase, with or without bovine serum albumin (BSA), was monitored over 24 h. Levels of radioactivity were also determined in the stratum corneum by tape stripping and in the remaining skin. Apparent permeability coefficients (*Kp*) and dose absorbed were calculated and compared. The overall results emphasize the importance of experimental design and confirm previous findings that identified dose volume, saturation level and vehicle as the main sources of variation in the in vitro assessment of dermal absorption, whilst diffusion cell model and the presence/absence of BSA in the receptor phase had minimal effect. Although the acquired data do not directly reveal new mechanistic information on dermal absorption, the unique and complete study design has provided a suitable data source for the development of dermal absorption prediction models.

1. Introduction

Assessing the extent and rate of passive drug absorption through skin is of great interest in both pharmaceutical and toxicological research. Such research has shown the stratum corneum, the outermost epidermal layer, to be the primary limiting factor to passive drug absorption for most compounds. In addition, a number of other factors such as the drug's physicochemical properties, including size, lipophilicity, and solubility, the vehicle in which it contacts the skin, the anatomy of the skin and the duration and extent of exposure, also contribute to the absorption process (Guy, 2010; Roberts et al., 2008; OECD, 2004).

Typically, the evaluation of the effect that some of these experimental factors exert on dermal absorption is assessed through the use of one of two in vitro diffusion cell systems; the (Franz) static diffusion cell (Franz, 1975) and the (Bronaugh) flow-through diffusion cell (Bronaugh and Stewart, 1985). Both cell types are widely accepted and routinely used, as the principle behind both techniques is similar. That is, a membrane or dermatomed skin, is placed between two chambers, a donor and a receptor. The compound of interest is applied to the surface of the skin and over time, diffuses from the donor phase to the receptor phase. In the static diffusion cell, samples are withdrawn periodically from the receptor phase and replaced with the same volume. The withdrawn sample is analyzed to measure the penetration flux. In the flow-through diffusion cell, the compound passing through the membrane is carried away by the receptor fluid which is collected for analysis (Addicks et al., 1987). Both steady-state (infinite dosing) and pseudo steady-state (finite dosing) conditions have been used to estimate the *Kp* (apparent permeability coefficient) values. The experimentally determined *Kp* is the most widely used parameter in the prediction of skin penetration, and is defined as:

$$Kp = J_{ss}/\Delta C$$

where J_{ss} is the steady state flux and ΔC is the concentration gradient across the skin.

A review of the literature highlights a vast variation in the combination of experimental variables assessed. Examples include dosing solutions (infinite versus finite and saturated versus unsaturated), vehicle, and receptor fluid. Such variations make the direct comparison of data difficult. Therefore, the purpose of the following study was to standardize several experimental variables to identify their impact on the permeability of six compounds

Abbreviations: BSA, bovine serum albumin; PG, propylene glycol.

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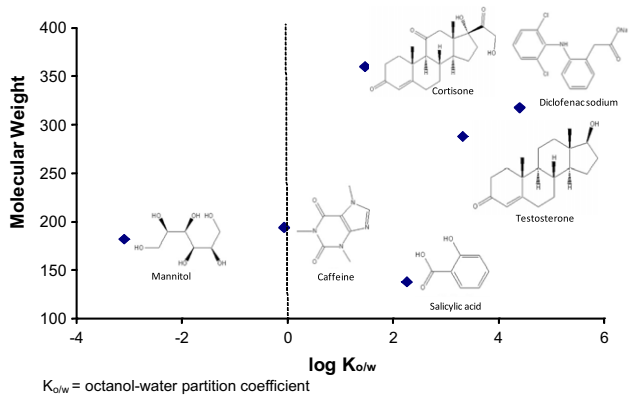

Fig. 1. Compound selection based on molecular weight, and $\log K_{o/w}$. The chemical structure of the compound is located to the immediate right of its respective data point.

(caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) through porcine skin. The six compounds were selected on the basis of their molecular weight, $\log K_{o/w}$ (octanol–water partition coefficient) (Fig. 1) and commercial availability of [^{14}C]-label. Porcine skin is frequently used as a surrogate model for human skin (Simon and Maibach, 2000) based on morphological (Kong and Bhargava, 2011; Monteiro-Riviere et al., 2008; Monteiro-Riviere, 1991) and functional data showing similar penetration for topically applied compounds (Schmook et al., 2001; Schaefer and Redelmeier, 1996).

Both static and flow-through in vitro diffusion cell systems were used for this evaluation. The six compounds were applied as varying combinations of finite, infinite, saturated and unsaturated doses in one of three vehicles (propylene glycol, water, and ethanol). In addition, the effect of the presence or absence of bovine serum albumin (BSA) in the receptor phase was also evaluated. This unique full factorial study design allowed for a comprehensive evaluation of several commonly used experimental variables, permitting direct comparison of results, and generating data that is considered appropriate for the development of dermal absorption prediction models.

2. Materials and methods

2.1. Study chemicals

Radiolabeled [^{14}C]-Caffeine (specific activity = 10.8 mCi/mmol), [^{14}C]-Cortisone (specific activity = 10.7 mCi/mmol), [^{14}C]-Diclofenac sodium (specific activity = 13.6 mCi/mmol), [^{14}C]-Mannitol (specific activity = 11.0 mCi/mmol), [^{14}C]-Salicylic acid (specific activity = 15.0 mCi/mmol) and [^{14}C]-Testosterone (specific activity = 9.37 mCi/mmol) were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). All compounds were determined by the manufacturer to have a radiochemical purity of 99%.

The analytical reagents (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) as well as the propylene glycol were purchased from Sigma–Aldrich (St. Louis, MO) and were all greater than or equal to 98% purity. Absolute ethyl alcohol (200 proof) was obtained from Pharmco-Aaper Chemical Co. (Shelbyville, KY). Ultrapure water was obtained from the in-house laboratory water purification system (Pure Water Solutions, Hillsborough, NC).

2.2. Experimental design

In accordance with an approved Institutional Animal Care and Use Committee protocol, porcine skin was obtained from the dorsal area of weanling female Landrace/Yorkshire cross pigs. Following euthanasia, the hair-clipped skin was dermatomed with a Padgett Dermatome (Kansas City, MO) to a thickness ranging from 500–990 μm measured by calipers (Mitutoyo Corporation, Kanagawa, Japan). Each circular section was punched out to provide a dose area of 0.64 cm^2, and placed into a two-compartment Teflon flow-through diffusion cell or onto a glass static diffusion cell. The dermal side of the skin sections were perfused using the receptor fluid. The receptor fluid was a Krebs–Ringer bicarbonate buffer spiked with dextrose (Sigma–Aldrich, St. Louis, MO) and with or without BSA (4.5%, w/v; Fisher Scientific, Fair Lawn, NJ). The temperature of the perfusate and the diffusion cells was maintained at 37 °C using a Brinkman circulator (Westbury, NY). The pH of the receptor solution was maintained between 7.3 and 7.5. The flow rate of the flow-through receptor solution was 4 ml/h. The receptor solution was kept homogenous in concentration and temperature by a magnetic stirring bar. After careful release of any air bubbles underneath the skin in the receptor compartments, blank samples were collected before dose application.

The porcine skin harvested for the experiments was used within an hour of collection and therefore the tissue was considered viable, so no additional skin integrity evaluations were conducted.

The six compounds (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) were topically applied either in propylene glycol, water, or ethanol at a concentration of 1.28 μg/μl as finite (20 μl) and infinite (1000 μl) volumes to an area of 0.64 cm^2. Saturated doses of caffeine, diclofenac sodium and salicylic acid were experimentally determined in triplicate by dissolving an excess quantity of compound into each vehicle and vortex-mixing. This solution was centrifuged and an aliquot of the supernatant was analyzed by high performance liquid chromatography (Waters Alliance 2695 separations module) with ultra-violet detection (Waters 996 photo-diode array) using a reverse phase column (Waters Atlantis © T3 5 μm, 4.6 × 150 mm) and acetonitrile (Fisher Scientific, Fair Lawn, NJ)/water mix mobile phase. Saturated doses of cortisone, mannitol and testosterone were also experimentally estimated in triplicate, by dissolving small quantities of compound into each vehicle, vortex-mixing after each addition, until a precipitate was seen. Saturated doses were also administered as finite and infinite volumes. Table 1 lists the experimentally estimated solubility values used to represent saturated doses. All doses were occluded following application. Fig. 1 highlights the differences in physicochemical properties between the six selected compounds.

Samples of the receptor fluid were collected at the following predetermined intervals post dose application: 0, 15, 30, 45, 60, 75, 90, 105, 120 min and then 3, 4, 5, 6, 7, 8, 12, 16, 20 and 24 h. Flux of each compound into the receptor phase was monitored over 24 h.

At the end of the 24 h, the dose was removed in the case of infinite dosing and the dose area was swabbed with cotton swabs containing a soap solution (1% Ivory Liquid, Procter and Gamble, Cincinnati, OH), then tape-stripped six times (Scotch Magic Tape,

Table 1
Experimentally determined compound solubilities.

Compounds	Experimentally determined solubility (μg/ml)		
	PG	Water	Ethanol
Caffeine	11,753 ± 319	38,456 ± 3,194	5,917 ± 140
Cortisone	1,000 ± 0.00	242 ± 6.88	1,540 ± 30.5
Diclofenac sodium	28,143 ± 1,547	21,607 ± 1,413	76,937 ± 2,615
Mannitol	803 ± 40.9	134,651 ± 1,837	435 ± 22.0
Salicylic acid	113,127 ± 12,063	4,655 ± 315	337,090 ± 3,843
Testosterone	14,896 ± 21.2	24.0 ± 0.44	133,000 ± 408

PG = propylene glycol; values expressed as mean ± standard error.