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Topical bioavailability of diclofenac from locally-acting, dermatological formulations

S.F. Cordery[a], A. Pensado[a], W.S. Chiu[a], M.Z. Shehab[a], A.L. Bunge[b], M.B. Delgado-Charro[a], R.H. Guy[a,*]

[a] Department of Pharmacy & Pharmacology, University of Bath, Bath, UK
[b] Chemical and Biological Engineering, Colorado School of Mines, Golden, CO, USA

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ABSTRACT

Assessment of the bioavailability of topically applied drugs designed to act within or beneath the skin is a challenging objective. A number of different, but potentially complementary, techniques are under evaluation. The objective of this work was to evaluate *in vitro* skin penetration and stratum corneum tape-stripping *in vivo* as tools with which to measure topical diclofenac bioavailability from three approved and commercialized products (two gels and one solution). Drug uptake into, and its subsequent clearance from, the stratum corneum of human volunteers was used to estimate the input rate of diclofenac into the viable skin layers. This flux was compared to that measured across excised porcine skin in conventional diffusion cells. Both techniques clearly demonstrated (a) the superiority in terms of drug delivery from the solution, and (b) that the two gels performed similarly. There was qualitative and, importantly, quantitative agreement between the *in vitro* and *in vivo* measurements of drug flux into and beyond the viable skin. Evidence is therefore presented to support an *in vivo* – *in vitro* correlation between methods to assess topical drug bioavailability. The potential value of the stratum corneum tape-stripping technique to quantify drug delivery into (epi)dermal and subcutaneous tissue beneath the barrier is demonstrated.

1. Introduction

The assessment of drug bioavailability following oral administration is a relatively straightforward exercise based on the reasonable assumption that the blood/plasma/serum level profile of the active moiety is a reliable surrogate for that at the site of pharmacological action. As a result, establishing the bioequivalence between oral dosage forms generally involves a standard, validated protocol (involving *in vivo* pharmacokinetic studies) that is recognized by regulatory authorities all over the world.

However, in the case of drug products applied to treat local disease either within, or directly below, the skin, the measurement of bioavailability – and, by extrapolation, bioequivalence – is more complicated (Shah et al., 2015). Here, the relationship between drug concentration at the site of action and that in the systemic compartment is less clear, and the physical measurement of either of those concentrations has proved challenging (if not impossible).

As a result, there is an ongoing effort to develop methodologies with which to evaluate the topical bioavailability and bioequivalence of locally-acting dermatological products (Herkenne et al., 2008; Lehman et al., 2011; Shah et al., 2015). This is particularly important for generic topical products for which, in most cases, the route to regulatory approval is uniquely via expensive, onerous and sometimes quite insensitive clinical outcome studies (Shah et al., 2015). Several approaches for the determination of topical bioavailability and bioequivalence are under investigation,

Abbreviations: A, area of skin; ANOVA, analysis of variance; DMSO, dimethyl sulfoxide; FDA, U.S. Food & Drug Administration; HPLC, high performance liquid chromatography; IVPT, *in vitro* skin permeation test; $J_{invitro}$, average flux of drug into the receptor chamber *in vitro* over the designated time interval; J_{invivo}, average flux of drug from the SC into the underlying tissue *in vivo* over the duration of the clearance Δt; $M_{Cl,23h}$, mass of drug in the SC at 23 h *in vivo* (Clearance for 17 h); M_R, cumulative mass of drug entering the receptor solution from skin *in vitro*; $M_{R,jh}$, cumulative mass of drug entering the receptor solution after j hours *in vitro* ($j = 6$ 8 23 and 24 h as designated); $M_{S,n}$, mass of drug in the n^{th} sample vial; $M_{Up,6h}$, mass of drug in the SC at 6 h *in vivo* (Uptake); P, Pennsaid® solution 2%; Q_R, flow rate of receptor solution *in vitro*; Q1, same qualitative composition; Q2, same quantitative composition; S, Solaraze® gel 3%; SC, stratum corneum; t_n, time between drug application and the end of the n^{th} sampling interval; TEWL, transepidermal water loss; V, Voltaren® gel 1%; V_R, volume of receptor chamber; $V_{S,n}$, volume of solution in the n^{th} sample vial; Δ, tduration of the clearance period.

* Corresponding author.
E-mail address: r.h.guy@bath.ac.uk (R.H. Guy).

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including the use of *in vitro* (human) skin permeation tests, microdialysis (or microperfusion), stratum corneum (SC) tape-stripping, and non-invasive optical/spectroscopic techniques (Yacobi et al., 2014; Raney et al., 2015; Bodenlenz et al., 2017). While it seems unlikely that a single, 'gold-standard' method will be sufficient to uniquely evaluate the bioavailability/bioequivalence of topical products, there is a growing recognition that the rational combination of selected techniques can provide a "weight of evidence" support for such an assessment. The choice of tests would depend, for example, on factors such as the complexity of the drug product (Chang et al., 2013), as well as the drug's potency (and potential for systemic side effects), and site of action. For each potential approach, a robust consideration of practical methodological detail, including the number of replicates/subjects required to power a study and appropriate acceptance criteria, will ultimately be required to inform regulatory decision-making.

The aim of the work presented here is to demonstrate a proof-of-concept for the use of complementary methods in topical bioavailability/bioequivalence assessment. Specifically, the SC tape-stripping approach *in vivo* has been used together with *in vitro* skin permeation to compare three marketed diclofenac products, which are approved for different therapeutic indications and are not considered bioequivalent. One formulation, Solaraze® (diclofenac topical gel 3%), is used to treat actinic keratosis, while the other two, Voltaren® (diclofenac topical gel 1%) and Pennsaid® (diclofenac topical solution 2%), are for pain relief in particular forms of arthritis.

SC tape-stripping was the subject of a (now withdrawn) U.S. Food & Drug Administration (FDA) guidance (US FDA, 1998) and involves collecting the outermost skin layer (i.e., the SC) using adhesive tapes post-application of a drug-containing formulation; subsequently, the drug in the SC can be extracted and quantified. Recently, tape-stripping results, from experiments using modified (Parfitt et al., 2011) and improved (N'Dri-Stempfer et al., 2009) protocols, correctly mirrored the established bioequivalence of topical anti-fungal creams (the site of action of which, naturally, is the SC itself) using clinical end-point studies. However, there remains an open question as to whether SC tape-stripping is a useful (or even meaningful) method to assess the bioavailability and bioequivalence of topical drug products which are designed to elicit their effects either within the viable epidermis/dermis or, in the case of pain relief induced by diclofenac, for example, in the subcutaneous tissue beneath the site of application.

Consequently, it was decided to compare the results from SC tape-stripping *in vivo* with data from *in vitro* skin permeation experiments. Specifically, using the three diclofenac products, measurements from the optimized "uptake and clearance" SC tape-stripping protocol (as reported in a study with econazole nitrate creams (N'Dri-Stempfer et al., 2009)) were correlated with percutaneous fluxes determined in conventional *in vitro* Franz diffusion cell experiments. The hypothesis tested, therefore, was that drug "clearance" from the SC must reflect 'input' into the viable skin tissue and beyond, assuming that the SC is the rate-limiting barrier; i.e., into the subcutaneous space (or, in the case of an *in vitro* skin permeation test, the receptor solution of the diffusion cell).

2. Materials & methods

2.1. Materials

The formulations tested (Table 1) were Pennsaid® (diclofenac topical solution 2%) (Mallinckrodt Brand Pharmaceuticals, Inc., Staines-upon-Thames, UK), Voltaren® (diclofenac topical gel 1%) (Novartis, Basel, Switzerland) and Solaraze® (diclofenac topical gel 3%) (PharmaDerm, Princeton, NJ, USA). Diclofenac sodium, solvents and HPLC reagents were from Sigma (Gillingham, UK).

Abdominal pig skin was obtained from a local abattoir, dermatomed (Zimmer®, Hudson, OH, USA), to a nominal thickness of 750 μm, frozen within 24 h of slaughter, and thawed before use.

2.2. Stratum corneum (SC) tape-stripping experiments

The protocol was approved by both the Research Ethics Approval Committee for Health at the University of Bath, and the FDA's Research Involving Human Subjects Committee. The approach closely followed a previous *in vivo* study using econazole (N'Dri-Stempfer et al., 2009); specifically, the mass of drug in the SC at one 'uptake' and at one 'clearance' time point was measured. Fourteen healthy volunteers (8 female, 6 male, mean age 28 ± 8 years), who met the study inclusion criteria (Table 2), participated in the study having given their informed consent. The test site was the volar forearm, at least 5 cm above the wrist and a minimum of 0.5 cm below the bend in the arm at the elbow; for volunteers with significant hair growth in the test region, the skin was shaved using a new disposable razor at least 24 h before the study began.

On the first day of the experiment, both arms were washed (Carex Complete, Cussons, Manchester, UK) and dried then left for 1 h to allow skin hydration to return to normal. This procedure ensured that the starting skin condition was, as close as possible, the same for all volunteers. Immediately before application of formulations, at a forearm site away from those to be treated, two tape-strips were taken to provide drug-free samples of SC to act as controls for the analytical method. In addition, at the skin sites to be treated, the baseline (i.e., intact, unstripped skin) trans-epidermal water loss (TEWL) rate was measured (AquaFlux, Biox Systems Ltd., London, UK).

Six self-adhesive, foam padding frames (Pressure Point Foam Padding, Scholl, Slough, UK), with internal dimensions of 1.5 × 5.5 cm, were applied to each arm; as the width of the frame was 0.8 cm, the minimum distance between the edges of the treated skin sites was 1.6 cm. There were duplicate application sites for each product on both arms of each volunteer. Using a cotton bud (Johnson & Johnson, Berkshire, UK) to spread the formulation over the treatment area, products P and V were applied at 10 mg/cm^2, while product S was applied at 20 mg/cm^2, reflecting the respective recommended use levels. The exact amount applied was determined gravimetrically.

Table 1
Diclofenac formulations tested.

Product	Code	Indication	Components
Pennsaid 2% solution	P	Osteoarthritis of the knee	Diclofenac sodium, dimethyl sulfoxide, ethanol, water, propylene glycol, hydroxypropyl cellulose
Voltaren 1% gel	V	Joint pain	Diclofenac sodium, carbomer homopolymer Type C, cocoyl caprylocaprate, fragrance, isopropyl alcohol, mineral oil, polyoxyl 20 cetostearyl ether, propylene glycol, water, strong ammonia solution
Solaraze 3% gel	S	Actinic keratoses	Diclofenac sodium, hyaluronate sodium, benzyl alcohol, polyethylene glycol monomethyl ether, water