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A comparative study of the *in vitro* permeation of ibuprofen in mammalian skin, the PAMPA model and silicone membrane

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ABSTRACT

Human skin remains the membrane of choice when conducting *in vitro* studies to determine dermal penetration of active pharmaceutical ingredients or xenobiotics. However there are ethical and safety issues associated with obtaining human tissue. For these reasons synthetic membranes, cell culture models or *in silico* predictive algorithms have been researched intensively as alternative approaches to predict dermal exposure in man. Porcine skin has also been recommended as an acceptable surrogate for topical or transdermal delivery research. Here we examine the *in vitro* permeation of a model active, ibuprofen, using human or porcine skin, as well as the Parallel Artificial Membrane Permeation Assay (PAMPA) model and silicone membrane. Finite dose studies were conducted in all models using commercial ibuprofen formulations and simple volatile ibuprofen solutions. The dose applied in the PAMPA model was also varied in order to determine the amount of applied formulation which best simulates typical amounts of topical products applied by patients or consumers. Permeation studies were conducted up to 6 h for PAMPA and silicone and up to 48 h for human and porcine skin. Cumulative amounts permeated at 6 h were comparable for PAMPA and silicone, ranging from 91 to 136 $\mu g/cm^2$ across the range of formulations studied. At 48 h, maximum ibuprofen permeation in human skin ranged from 11 to 38 $\mu g/cm^2$ and corresponding values in porcine skin were 59–81 $\mu g/cm^2$. A dose of 1 μL was confirmed as appropriate for finite dose studies in the PAMPA model. The formulation which delivered the greatest amount of ibuprofen in human skin was also significantly more efficient than other formulations when evaluated in the PAMPA model. The PAMPA model also discriminated between different formulation types (i.e. gel versus solution) compared with other models. Overall, the results confirm the more permeable nature of the PAMPA, silicone membrane and porcine tissue models to ibuprofen compared with human skin. Further finite dose studies to elucidate the effects of individual excipients on the barrier properties of the PAMPA model are needed to expand the applications of this model. The range of actives that are suitable for study using the model also needs to be delineated.

1. Introduction

Assessment of skin penetration of actives is of critical importance in a number of fields. Effective active pharmaceutical ingredient (API) permeation is required for therapeutic benefits, knowledge of the skin disposition of pesticides is important for human health and quantitation of delivery of cosmetic actives to the skin provides confidence for product claim support. Many different models of human skin have been proposed in order to quantify and predict percutaneous penetration. This reflects the difficulties in sourcing tissue as well as ethical issues and safety concerns associated with biological membranes. Early models of mass transfer in skin focussed on apparatus such as the rotating diffusion cell which employed isopropyl myristate (IPM) impregnated in filter paper as a surrogate skin lipid phase (Albery et al., 1976). Other lipids which have been used to model skin penetration include tetradecane, linoleic acid and dispersions of phospholipids in IPM (Guy and Fleming, 1979). Interestingly, eggshell membranes impregnated with IPM were considered by Washitake et al. (1980); removal of the shell with hydrochloric acid leaves a predominantly keratin rich membrane. With advances in knowledge of the composition of skin lipids more realistic

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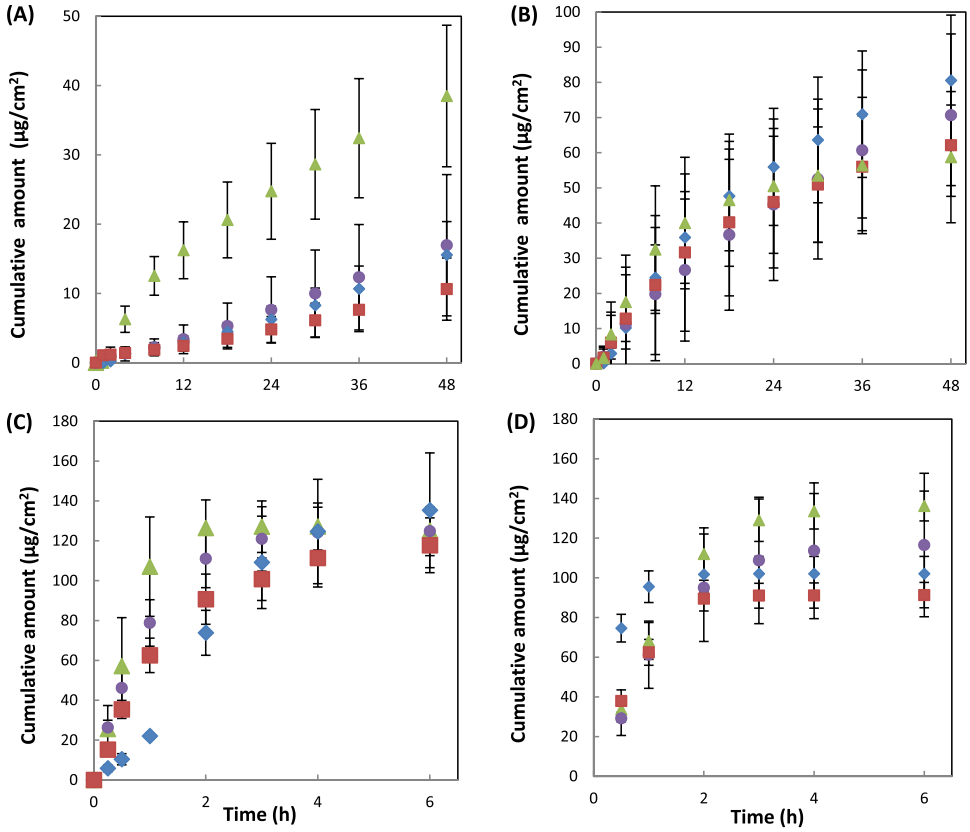

Fig. 1. Cumulative amounts of ibuprofen permeated from IBUGEL® (◆), IBULEVE® (■), PG (▲) and PEG 300 (●) for: Human skin (A), Porcine skin (B), Silicone membrane (C) and Skin PAMPA dosed at 1 µL/cm² (D). Each data point represents the mean ± SD ($n \geq 5$).

mixtures of phosphatidyl choline, dipalmitoyl phosphatidly choline, ceramides, cholesterol, cholesterol palmitate, linoleic acid and tristearin were later investigated (Firestone and Guy, 1985).

In vitro permeation studies have also been conducted with simple polymeric membranes such as polydimethylsiloxane (Dias et al., 2007; Santos et al., 2009; Oliveira et al., 2012). While these studies are useful to probe thermodynamic activity of actives in specific formulations, they cannot provide any insight into specific excipient interactions with skin. The advent of tissue culture models for research applications stimulated much interest in the development of human skin equivalents (HSEs). Subsequently a number of models have become available including Epiderm™, Episkin™ and Labskin™. Typically these HSEs are based on cultures of normal human keratinocytes and/or fibroblasts and are metabolically and mitotically active. Although HSEs are reported to over-estimate likely permeation in human skin (Schmook et al., 2011; Basketter et al., 2007; Thakoersing et al., 2012; Labouta et al., 2013) they are routinely used for toxicity and/or irritation testing (Spielmann et al., 2007; Alépée et al., 2014).

Recently, the Parallel Artificial Membrane Permeation Assay (PAMPA) has been proposed as a high throughput screening system that may be suitable to study skin permeation (Sinkó et al., 2012). Previously the PAMPA model was investigated for prediction of gastrointestinal absorption (Avdeef and Tsinman, 2006; Avdeef et al., 2007) and as a potential model of the blood-brain barrier (Tsinman et al., 2011). This model consists of a mixture of synthetic ceramides (certamides), cholesterol and free fatty acids mounted in 96-well plates (Sinkó et al., 2012). With respect to skin permeation the number of molecules and formulations evaluated in PAMPA to date remains low. Accordingly we set out to examine the permeation of a model API, ibuprofen, from two commercial preparations and two simple solutions using the PAMPA model. The data are compared with results from studies conducted with an artificial membrane (silicone) as well as with porcine and human skin. A further objective was to identify optimal dosing in the PAMPA model which best simulates typical amounts applied on skin by patients and consumers.

Ibuprofen was a gift from Wyeth (Haversham, Hants., UK). Polyethylene glycol (PEG) 300, propylene glycol (PG), HPLC grade isopropyl alcohol and trifluoroacetic acid (HPLC grade) were supplied by Fisher Scientific (UK). HPLC grade solvents (methanol and water) were provided by Sigma-Aldrich (UK). Phosphate buffered saline (pH 7.4) was prepared using Dulbecco A tablets (Oxoid, UK). Silicone membrane (250 µm) was obtained from Samco (Nuneaton, UK). This grade and thickness of silicone was selected because we have used it previously to examine the effects of a range of hydrophilic and lipophilic vehicles on ibuprofen permeation (Watkinson et al., 2009a, 2009b, 2011). The pre-coated Skin PAMPA Sandwich stirring disks, hydration solution, and Gut-Box™ were obtained from pION Inc. (Billerica, USA). Porcine tissue was obtained from a local abattoir. Excised abdominal human skin was obtained from the UK Human Tissue Bank and was stored in a freezer at −20 °C until required (Research Ethics Committee reference 06/MRE04/37). The commercial formulations selected for evaluation were IBUGEL™ (Ibuprofen 5% w/w) and IBULEVE™ Speed Relief 5% Spray (Dermal Laboratories, Hitchin, Hertfordshire, UK). Two other formulations of ibuprofen were prepared as 5% w/w solutions in isopropyl alcohol and either PEG 300 or PG.