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Assessing vehicle effects on skin absorption using artificial membrane assays

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ABSTRACT

A vast number of variations in drug/vehicle combinations may come into contact with skin. Evaluating the effect of potential drug, vehicle and skin interactions for all possible combinations is a daunting task. A practical solution is a rapid screening technique amenable to high throughput approaches (e.g. 96-well plates). In this study, three artificial membranes (isopropyl myristate (IPM), certramides and Strat-M™) were evaluated for their ability to predict the skin permeability of caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone applied in propylene glycol, water and ethanol as unsaturated and saturated concentrations. Resultant absorption data was compared to porcine skin diffusion cell data. The correlations (r^2) between membrane and diffusion cell data from saturated and unsaturated concentrations were 0.38, 0.47 and 0.56 for the Strat-M™, certramide and IPM membranes, respectively. This relationship improved when only saturated concentrations were evaluated (r^2 = 0.60, 0.63 and 0.66 for the Strat-M™, certramide and IPM membranes, respectively). A correlation between membrane retention and the amount remaining in skin had r^2 values of 0.73 (Strat-M™), 0.67 (certramides), and 0.67 (IPM). Quantitative structure-permeability relationship models for each membrane identified different physicochemical factors influencing the absorption process. Although further investigations exploring complex topical formulations are required, these results suggest potential use as an initial screening approach to assist in narrowing the selection of formulations to be evaluated with a more biologically intact model, thereby assisting in the development of new topical formulations.

1. Introduction

In vivo studies are the optimal approach for assessing the rate and extent of absorption of an exogeneous compound through skin for drug development and risk assessment purposes. However due to ethical and economical considerations associated with in vivo testing, an in vitro approach is often preferred. The in vitro method routinely used is the diffusion cell (Bronaugh and Stewart, 1985; Franz, 1975). Yet when taking into account the vast number of variations in drug/vehicle that may come into contact with the skin, neither established in vitro nor in vivo techniques can be considered as highly efficient or economical for evaluating all possibilities. A high throughput alternative is therefore desirable to avoid the cost and time exhaustive measures of the current testing techniques available.

High throughput technologies have been shown to be advantageous in screening a larger number of chemicals at one time. The focus of a majority of the high throughput research has been on drug candidate selection (Balimane et al., 2006; Kerns, 2001) and not the delivery vehicle. In the field of percutaneous absorption, it is well noted that the delivery vehicle is as important as the drug itself (Flynn and Smith, 1972; Idson, 1972; Ostrenga et al., 1971). Often specific components are added to the vehicle to serve certain purposes such as overcoming the formidable barrier function of the stratum corneum, and/or to promote stability/activity of the active ingredient.

An increase in the complexity of the delivery vehicle (formulation) also increases the potential for interactions to occur between the chemical, vehicle and skin. This consequently affects the absorption process. In vitro studies have shown that the interactions that arise within the chemical–vehicle–skin system synergistically alter a chemical's ability to partition into and diffuse through the skin barrier (Baynes et al., 2001; Cross et al., 2001; Mills et al., 2006; Rosado et al., 2003). Although such phenomena exist, few researchers have attempted to quantify the effects of chemical mixtures on percutaneous absorption (Baynes et al., 2008; Brand and Mueller, 2002; Budsaba et al., 2000; Gregoire et al., 2009; Riviere and Brooks, 2007; Yourick et al., 2008; Wiechers et al., 2012). High throughput methodologies could provide

Abbreviations: PAMPAs, parallel artificial membrane permeability assays; IPM, isopropyl myristate; P_{app}/k_p, apparent permeability coefficient; $K_{o/w}$, octanol–water partition coefficient.

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a feasible solution to identify and quantify these interactions. Such methodologies could also assist in screening formulations and hence provide huge economical and ethical advantages for the development and assessment of novel formulations.

The 96-well plate format has been demonstrated to be suitable for the rapid determination of passive transport (Kansy et al., 1998). Routinely used for the assessment of gastrointestinal absorption, and blood–brain barrier permeability, recent studies have reported the adaption of this parallel artificial membrane permeability assay (PAMPA) for skin (Ottaviani et al., 2006; Sinko et al., 2012, 2009). In the PAMPA method, an artificial membrane imitating skin is coated on a hydrophobic filter of a 96-well filter plate that separates two compartments, the donor and acceptor. The donor compartment typically contains a buffer solution of the test compound, while the acceptor compartment contains the receptor fluid, generally fresh buffer solution at pH 7.4. A schematic illustration of the PAMPA experiment is depicted in Fig. 1.

A number of artificial membranes (e.g. dimethlypolysiloxane (silicone), isopropyl myristate) have been proposed as simple lipid-like membrane models to measure fluxes, diffusion and partition coefficients. Ottaviani et al. (2006) reported a simple pure solvent membrane, composed of 70% silicone oil and 30% isopropyl myristate coated on a hydrophobic polyvinylidene fluoride filter. Test compounds were applied in a buffer solution containing 5% dimethylsufoxide, and resultant data was shown to mimic human skin permeation. A positive correlation was also established between the membrane retention of compounds and stratum corneum/water partition coefficients.

More recent advances have seen the synthesis of human ceramide analogues, referred to as certramides, incorporated as a membrane mixture (Sinko et al., 2012; 2009). Other components in this membrane mixture include free fatty acids and cholesterol in order to perform like the stratum corneum. These new certramides are structurally similar to the natural ceramides having comparable molecular size, H-bond donor/acceptor abilities, and high lipophilicity. A high correlation between the evaluated test compounds with three different human skin permeability databases has been demonstrated (Sinko et al., 2012).

Another synthetic membrane (Strat-M™) recently commercially made available was reported by Joshi et al. (2012), demonstrating its use as a surrogate membrane model for human skin diffusion experiments without high lot-to-lot variability, safety and storage limitations. The membrane is composed of multiple layers of polyether sulfone creating morphology similar to human skin, including a very tight surface layer.

Although these in vitro experiments do not completely reproduce in vivo conditions, especially with respect to metabolism, dermal distribution and blood supply, they do offer a major advantage in that experimental conditions can be controlled precisely and they have high throughput capabilities (Ottaviani et al., 2006). Therefore the aim of this experiment was to evaluate three artificial membranes in a 96-well plate format for their ability to predict the percutaneous absorption of six model compounds (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) delivered in three vehicles (propylene glycol, water and ethanol) as saturated and unsaturated concentrations applied in vitro to porcine skin. A secondary aim was to characterize the factors influencing the absorption process for each membrane through the use of quantitative structure-permeability relationships.

2. Materials and methods

2.1. Chemicals

The six compounds, shown in Fig. 2, were selected based on their differing physicochemical properties of molecular weight and log-$K_{o/w}$ (the octanol–water partition coefficient). 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%. Radiolabeled compounds were employed to ensure that total chemical flux was being compared across all systems to minimize any potential effects of metabolism in the skin model.

Non-radiolabeled equivalents of these six compounds, as well as the propylene glycol, isopropyl myristate (IPM), silicone oil, and hexane were purchased from Sigma–Aldrich (St. Louis, MO) at a purity greater than or equal to 98%. 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).

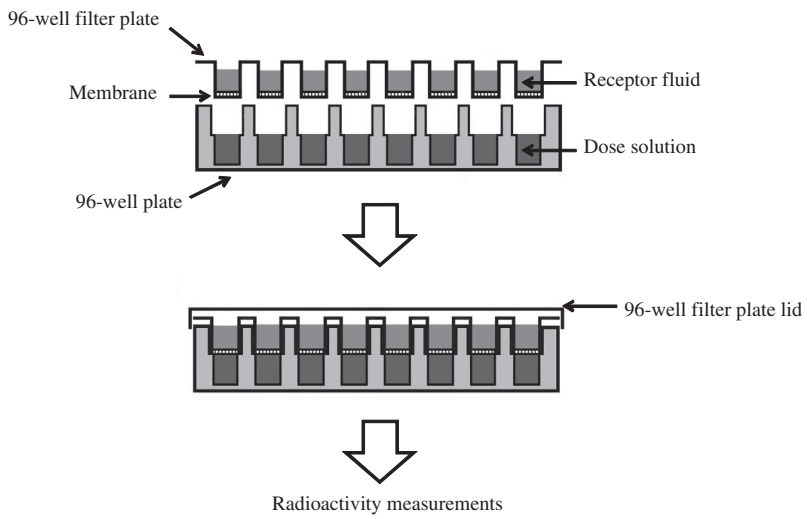

Fig. 1. Schematic representation of PAMPA experiment.