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A miniaturized flow-through cell to evaluate skin permeation of endoxifen

Choon Siong Mah [1], Jaspreet Singh Kochhar [1], Pei Shi Ong, Lifeng Kang *

Department of Pharmacy, National University of Singapore, 18 Science Drive 4, 117543, Singapore

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ABSTRACT

Endoxifen, an anti-estrogenic agent, has been recently implicated in the use of breast cancer. Its physicochemical properties make it a good candidate for transdermal delivery. However, as an investigative drug, its limited supply makes it difficult to conduct extensive pre-formulation studies. To address this issue, a miniaturized flow-through diffusion cell has been fabricated that utilized minimal amounts of the drug for *in vitro* skin permeation studies. The novel flow-through cells have been validated against horizontal diffusion cells and shown to cause no noticeable damage to the applied skin, as observed by histological sectioning. The cells were also demonstrated to be useful in search of suitable enhancers for endoxifen. Endoxifen permeation using permeation enhancers was tested by using this new device and limonene was found to achieve highest flux, attaining the requirement for clinical applications. The fabricated cells can thus be useful in carrying out pre-formulation studies for expensive, new drug entities, both in industrial as well as academic research.

1. Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide with about 1.38 million newly diagnosed cases each year (Jemal et al., 2011). Nearly 70% of breast cancer patients are hormone-receptor positive (Del Re et al., 2011). For these patients, tamoxifen has been the most widely used adjuvant endocrine therapy (EBCTCG-Secretariat, 2005). While tamoxifen is effective, it is a pro-drug that requires extensive CYP2D6 metabolism into active metabolites such as endoxifen (ENX) (Fig. 1) (Desta et al., 2004; Holmes and Liticker, 2005; Johnson et al., 2004). Recently, it has been reported that genetic polymorphism in CYP2D6 can impair the biotransformation of tamoxifen into its active metabolites (Hoskins et al., 2009; Singh et al., 2011). To overcome the poor outcomes associated with breast cancer therapy for patients with reduced CYP2D6 activity, direct administration of endoxifen has been advocated (Ahmad et al., 2010a,b; Wu et al., 2009). Clinical trials are currently being conducted on the oral use of endoxifen as the hydrochloride salt form (NIH, 2011a,b).

Apart from the oral route, transdermal drug delivery of the endoxifen has also been explored. However, such studies showed a limited drug flux through skin. Based on the required daily dose of endoxifen, it cannot achieve the therapeutically relevant concentrations (Ahmad et al., 2010a; Lee et al., 2011). Therefore, further studies are needed for its effective delivery through skin. Moreover, a transdermal gel of 4-hydroxy metabolite of tamoxifen is currently under phase 2 clinical trials, indicating the potential for transdermal administration of active metabolites like endoxifen in the management of breast cancer (NIH, 2009).

Conventionally, a variety of transdermal diffusion cells were developed for the evaluation of *in vitro* permeation characteristics of transdermally delivered drugs. In principle, some are based on the static, non flowing cells (Bartosova and Bajgar, 2012) in which the donor and receptor compartments may be placed either vertically (Franz type) (Windheuser et al., 1982) or horizontally (side-by-side) (Bellantone et al., 1986; Tojo et al., 1987) and others are the in-line, flow through cells, that offer the advantage of continual replenishment of receptor fluid and hence aid in maintaining a condition similar to microcirculation in the *in vivo* setting (Bronaugh and Stewart, 1985; Selzer et al., 2012).

Several modified versions of these diffusion cells have also been fabricated and validated against the conventional apparatus. Sanghvi and Collins compared the permeation characteristics of hydrocortisone using the "enhancer cell", which is a modified version of USP type II dissolution apparatus to serve as a diffusion cell (Sanghvi and Collins, 1993). Modified automatic sampling apparatus have been developed (Akazawa et al., 1989; Hanson, 2012; Martin et al., 1989; Permegear, 2012). These static and flow-through cells have been compared and validated (Cordoba-Diaz et al., 2000; Ng et al., 2010; Rapedius and Blanchard, 2001).

However, a major drawback of these cells is the requirement of relatively large amounts of drug owing to their inherent design. Investigational new drug entities, such as endoxifen, are prohibitively expensive for such studies. This motivated us to

* Corresponding author. Tel.: +65 6516 7519; fax: +65 6779 1554.
E-mail address: lkang@nus.edu.sg (L. Kang).
[1] These authors contributed equally.

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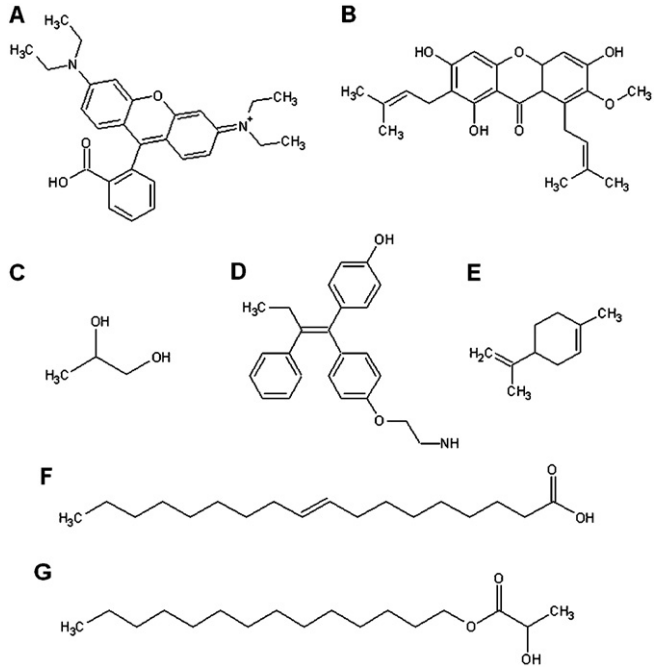

Fig. 1. Chemical structures of (A) mangostin (MW = 410.46, log *P* = 6.64), (B) rhodamine B (MW = 479.02, log *P* = 2.43), (C) propylene glycol (MW = 76.09, log *P* = −1.00), (D) endoxifen (MW = 373.49, log *P* = 4.94), (E) limonene (MW = 136.2, log *P* = 4.83), (F) oleic acid (MW = 282.46, log *P* = 7.42) and (G) myristyl lactate (MW = 286.45, log *P* = 6.08).

develop a miniaturized testing system that utilizes minimum amount of the drug.

Microfluidic platforms which are miniaturized fluid flow systems have recently received significant attention in the drug discovery and development horizon, due to their abilities to reduce the amount of reagents necessary for assays and pre-clinical development (Kang et al., 2008). These microscale systems fabricated with biomaterials such as polydimethylsiloxane (PDMS), may provide a useful model to develop miniaturized flow-through cells. We envisaged a PDMS-based, miniaturized flow-through cell to minimize the consumption of candidate drugs. With the economic environment in pharmaceutical firms becoming more tenuous and pharmaceutical cost containment being the main focus, the need to develop pre-formulation testing systems that utilize minimum amount of the drug is the need of the hour.

In this study, we fabricate a miniaturized flow-through cell for *in vitro* skin permeation studies. The system was compared and validated against a static, horizontal diffusion cell (HDC) using two model drugs, namely, rhodamine B and α-mangostin. We also conducted histological sectioning of the skin 24–48 h post-application in both diffusion cells to test for skin damage. Subsequently, the skin permeation of endoxifen was assessed with several skin permeation enhancers (PEs). One of the enhancers was found to be able to deliver enough endoxifen for its clinical applications.

2. Materials and methods

2.1. Materials

Rhodamine B and sodium azide were obtained from Alfa Aesar, UK. Phosphate buffered saline (PBS) (10×) was obtained from Vivantis, Malaysia. Propylene glycol was obtained from Chempure, Singapore. Polydimethylsiloxane (PDMS) (Sylgard 184 Silicone Elastomer Kit) was obtained from Sylgard, USA. Methanol for HPLC was purchased from Tedia, USA. Endoxifen hydrochloride, (R)-(+)-limonene and oleic acid were obtained from Sigma–Aldrich, USA. Myristyl lactate was a gift from Chemic Laboratories, USA. α-mangostin standard was supplied by Dr. Prachya Kongtawelent from Chiang Mai University, Thailand. All PBS solutions used in the permeation experiments contained 0.005% of sodium azide as anti-microbial agent (Sznitowska et al., 2001). Ultrapure water (Millipore, USA) was used in the preparation of aqueous solutions.

2.2. Fabrication of miniaturized flow-through cell (MFtC)

The fabrication process involved two simple PDMS molding steps. Firstly, for fabricating the receptor compartment (16 mm tall, 22 mm wide), a specially designed borosilicate glass mold (16 mm wide) was inserted into a single well of a 12-well plate, (Cellstar, Greiner Bio-One), carrying 0.9 mm poly vinyl chloride tubing, (B. Braun, Germany) bore through its axis. The borosilicate glass sits firmly in a small split created in the tubing. PDMS was then filled into the cavity between the glass mold and the well plate (Fig. 2A) and was subsequently cured at 70 °C for 2 h. The glass mold was then removed to create a hollow cavity for donor compartment to sit in.

The donor compartment (13 mm tall, 16 mm wide) was fabricated with a similar process in a single well of a 24-well plate. A 6 mm hollow lumen was first created with a metal mold (Fig. 2B). The mold was placed in the well of the 24-well plate and PDMS was used to fill the space between the external wall of the mold and the 24-well plate and was similarly cured at 70 °C for 2 h. The metal mold was removed to create a hollow cavity, to serve as the donor liquid compartment. As part of the property of MFtC, donor compartment was designed to hold up to 283 μl of drug solution with an area of 0.283 cm^2. The assembled donor and receptor compartments are shown in Fig. 2C.

2.3. Assembly and operation of MFtC

MFtC was assembled by connecting the tubing of the fabricated cell to an infusion pump system (Terufusion, UK) at one end and sampling tubes at the other end (Fig. 2D). The fabricated diffusion cell was then placed in a water bath maintained at 37 °C using a hot plate. Drug solution is placed in donor compartment. Flow rate of the receptor solution through the fabricated diffusion cell was controlled by the infusion pump that delivers the solution from a syringe (Fig. 2D).

2.4. Validation of MFtC against horizontal diffusion cell

To evaluate the performance characteristics of the MFtC, permeation of model compounds (rhodamine B and mangostin) using a horizontal diffusion cell (TK-6H1, Shanghai Kai Kai Technology, China) and MFtC were compared.

Rat abdominal skins were obtained from National University of Singapore Animal Centre and kept at −80 °C until use. Prior to permeation studies, the skins were thawed and hair was completely removed with an electrical shaver and hair remover cream (Veet) (Varshney et al., 1999). Subcutaneous fat and connective tissues were also lightly trimmed off. All animal experiments were approved by Institutional Animal Care and Use Committee, National University of Singapore.

Rat abdominal skin of 2.0 cm × 2.0 cm was mounted between the donor and receptor compartments of the horizontal diffusion cell, with stratum corneum side facing the donor compartment. The effective diffusion area was 1.13 cm^2. Each donor cell contained 4.5 ml of each model compound in propylene glycol (PG) and the receptor cell contained the same volume of PBS. Mangostin was used at a concentration of 2.3 mg/ml and rhodamine B at concentrations of 1 mg/ml and 5 mg/ml. Both compartments