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Transbuccal delivery of doxepin: Studies on permeation and histological investigation

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ABSTRACT

According to previous studies reporting the anesthetic/analgesic action of oral topical doxepin administration, this study evaluated a model of buccal permeation to determine the depth of delivery of doxepin into excised porcine buccal mucosa following topical application of a saturated aqueous doxepin solution. Buccal mucosa permeation studies were performed using Franz diffusion cells. Cumulative amounts of doxepin permeated were plotted as a function of time. Kinetic permeation parameters as flux (J_s), lag time (T_l) and permeability coefficient (K_p) were calculated. Theoretical human plasmatic steady-state doxepin concentration and drug retained in the tissue were also determined in order to evaluate its potential therapeutic use, central or peripheral. Finally, a histological evaluation of the buccal mucosa was performed to test potential damage due to the permeation phenomenon. Obtained results showed a poor aqueous doxepin permeation through buccal mucosa membrane (median parameters J_s = 34.79 μg/h, $K_p = 0.49 \times 10^{-3}$ cm/h and T_l = 2.8 h). Predicted doxepin plasma concentrations would reach 46 ng/mL, far from the required to have central nervous system activity as tricyclic agent. However, median doxepin amount remaining in the mucosa membrane was 0.24 μg/cm^2/μg tissue, which evidenced a reservoir function of the buccal mucosa. Histologically, no structural damage was observed in the tissues. This study lays the foundation for further research within this area with a view to potentially adopting alternative strategies for enhanced buccal absorption of doxepin in clinical practice.

1. Introduction

The buccal cavity provides a potential site for oral administration of drugs owing to the low cost, ease administration and high level of patient compliance for both, mucosal and transmucosal actions (Patel et al., 2011). In the first case, the aim is to achieve a site-specific release of the drug, whereas the second case involves drug absorption to reach the systemic circulation, since oral mucosa is highly vascularized. Any drug diffusing across oral mucosa membranes has direct access to the systemic circulation via capillaries and venous drainage and will bypass hepatic metabolism (Rossi et al., 2005). A key issue for buccal absorption is the permeability of the drugs through the buccal mucosa (Holm et al., 2013). The buccal mucosa appears to be better in terms of permeability, surface area, compliance, etc., when compared to the other mucosal and transdermal routes of delivery (Kulkarni et al., 2009). Continuous research for the improvement of the oral cavity drug delivery has resulted in the development of several dosage forms which can be broadly classified into liquid, semi-solid, solid or spray formulations (Sudhakar et al., 2006). Furthermore, several drugs highly administered by other routes have been assayed through buccal mucosa (Birudaraj et al., 2005; Mashru et al., 2005; Giannola et al., 2007). Within this context, buccal administration could be an alternative, non-invasive delivery route also for doxepin hydrochloride.

Some drugs without being properly direct analgesics were observed to provide an effective analgesic activity in different painful situations (Mico et al., 2006). The role of antidepressant in the management of different pain syndromes has elicited considerable interest (Sandig et al., 2013). Among them, doxepin belongs to the tricyclic antidepressant family. This therapeutic group is also known for its analgesic effect (Sudoh et al., 2003). Clinical trials have proved the anesthetic and analgesic effect of a

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topical doxepin suspension (0.5%) when administered as a mouth wash (Epstein et al., 2003, 2006; Epstein, 2008). In the Biopharmaceutics Classification System, doxepin HCl, is designated as a class one compound, with high solubility and high permeability (Wu and Benet, 2005).

Porcine buccal mucosa has been suggested as a suitable model membrane because its morphology and permeability properties are similar to that of human buccal mucosa in ultra-structure (non-keratinised) and enzyme activity (Diaz Del Consuelo et al., 2005a). Percutaneous studies can be used as a quality control procedure to characterize the dosage form; to study the *ex vivo* release and permeation rate characteristics of drug products during development phase; to establish the drug delivery characteristics of topical formulations as one means of assuring lot to lot bioavailability equivalence; and to enable minor reformulations of topical products on which the bioavailability/bioequivalence has already been defined (Skelly et al., 1987). It is essential to identify the contribution of different variables in the *ex vivo* permeability conditions in order to obtain reliable and consistent permeability values. *Ex vivo* studies allow the control of experimental conditions such as temperature, pH, and osmolality, which can be used to identify or isolate a single factor that is responsible for variation, as well as, the variability associated with the use of different mucosal regions, tissue storage conditions and tissue processing methods (Kulkarni et al., 2009).

Based on these considerations, the major aim of the present study was the study of the permeation of doxepin through buccal mucosa, and for this purpose porcine buccal mucosa was employed as an *ex vivo* model.

2. Materials and methods

2.1. Materials

Doxepin hydrochloride; 3-(dibenz[b,e]oxepin-11(6-H)-ylidene)-*N*,*N*-dimethyl-1-propylanaminehydrochloride and phosphate buffer saline (PBS) (pH 7.4), were obtained from Sigma–Aldrich (Madrid, Spain). Albumin solution 4% was obtained from Laboratorios Grifols (Barcelona, Spain). Dimethyl sulfoxide (DMSO) was supplied by Merck Lab. (Madrid, Spain). All other chemicals and reagents used were of analytical grade. Double-distilled water was obtained from a Milli-Q® Gradinet A10 system apparatus (Millipore Iberica S.A.U.; Madrid, Spain).

2.2. Tissue samples

The studies were conducted under a protocol approved by the Animal Experimentation Ethics Committee of the University of Barcelona (Spain) and the Committee of Animal Experimentation of the regional autonomous government of Catalonia (Spain). 3–4-month-old female pigs were used. The porcine buccal mucosa from the cheek region was obtained immediately after the pigs had been sacrificed in the animal facility at Bellvitge Campus (University of Barcelona, Spain) using an overdose of sodium thiopental anesthesia. The fresh buccal tissues were transferred from the hospital to the laboratory in containers filled with Hank's liquid. The remaining specimens were stored at −80 °C in containers with a PBS mixture containing 4% albumin and 10% DMSO as cryoprotective agents (Amores et al., 2014).

2.3. Ex vivo *permeation of doxepin throughout porcine buccal mucosa*

For the permeation studies, the porcine buccal mucosa was cut to 500 ± 50 μm thick sheets, which contributes to the diffusional barrier (Sudhakar et al., 2006), using an electric dermatome (GA 630, Aesculap, Tuttlingen, Germany) and trimmed with surgical scissors in adequate pieces. The majority of the underlying connective tissue was removed with a scalpel.

Membranes were then mounted in specially designed membrane holders with a permeation orifice diameter of 9 mm (diffusion area 0.636 cm^2). Using the membrane holder, each porcine buccal membrane was mounted between the donor (1.5 mL) and the receptor (6 mL) compartments with the epithelium side facing the donor chamber and the connective tissue region facing the receiver of static Franz-type diffusion cells (Vidra Foc Barcelona, Spain) avoiding bubbles formation.

Infinite dose conditions were ensured by applying 100 μL as donor solution of a saturated doxepin solution into the receptor chamber and sealed by Parafilm® immediately to prevent water evaporation. Prior to conducting the experiments, the diffusion cells were incubated for 1 h in a water bath to equalize the temperature in all cells (37 ± 1 °C). Each cell contained a small Teflon® coated magnetic stir bar which was used to ensure that the fluid in the receptor compartment remained homogenous during the experiments.

Sink conditions were ensured in all experiments by initial testing of doxepin saturation concentration in the receptor medium. Samples (300 μL) were drawn via syringe from the center of the receptor compartment at pre-selected time intervals (0.1, 0.2, 0.3, 0.7, 1, 2, 3, 4, 5 and 6 h) for 6 h. The removed sample volume was immediately replaced with the same volume of fresh receptor medium (PBS; pH 7.4) with great care to avoid trapping air beneath the membrane.

2.4. Drug assay

The cumulative doxepin amount through the mucosa membrane from the acceptor compartment was monitored by a validated HPLC methodology. Results are reported as means ± SD and median (min–max) of six different experiments. The system consisted of a Waters 515 pump (Waters, Milford, MA, USA) with UV–vis 2487 detector (Waters, Milford, MA, USA) set at 235 nm. Doxepin was detected with a retention time of 4.6 min with a reverse-phase C18 Sunfire® column (4.6 mm × 150 mm, Waters Associates, Milford, MA, USA) using as mobile phase methanol/(acetate buffer pH 6.0/water (36/64, v/v)) (72/28, v/v), pumped at 1 mL/min flux rate (Sandig et al., 2013).

2.5. Data analysis

The cumulative amount of doxepin permeated (Q_t) through porcine buccal mucosa was calculated, at each time point, from the concentration of doxepin in the receiving medium and plotted as function of time. Doxepin flux (J_s, μg/h) across the buccal mucosa was calculated at the steady state by linear regression analysis (GraphPad Prism®, v. 3.00 software; GraphPad Software Inc., San Diego, CA, USA) of permeation data.

Apparent permeability coefficient (K_p) was calculated by dividing the J_s by the initial drug concentration (C_0) in the donor phase:

$$K_p = \frac{J_s}{C_0} \quad (1)$$

It is assumed that under sink conditions the drug concentration in the receiver compartment is negligible compared to that in the donor compartment.

2.5.1. Theoretical systemic concentration of drug

The potential systemic capacity after buccal mucosa administration can be predicted by the theoretical human plasmatic steady-state concentration (C_{ss}), using the following equation: