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A comparative ex vivo drug permeation study of beta-blockers through porcine buccal mucosa

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ABSTRACT

Apparent permeability coefficients (k_p) of a series of beta-blockers: acebutolol, atenolol, labetalol, metoprolol, oxprenolol and propranolol, through porcine buccal mucosa were determined. The aim of the study was to determine the permeation parameters (apparent permeability coefficient, k_p; flux, J; and lag time, T_L) as a measure of the intrinsic permeability of porcine buccal mucosa to these drugs, in order to predict the efficacy of their possible administration through human buccal mucosa. A positive linear correlation was observed between the apparent permeability coefficient, $k_p$and the partition coefficient, *P*. Oxprenolol and propranolol are the drugs that presented the highest values of k_p: 0.3231×10^2 cm/h and 0.5666×10^2 cm/h, respectively. Multiple linear regression (MLR) using least square estimation was performed on the data set with log $k_p$as dependent variable and the descriptors as predictor variables.

The potential systemic capacity after a buccal administration was predicted by estimating the plasma concentrations at steady-stated (C_{ss}). Considering the entire process of permeation ex vivo, propranolol and oxprenolol would seem to be the best candidates for administration through the buccal mucosa.

1. Introduction

In recent years, most biopharmaceutical and pharmacokinetic research have focused either on the use of new routes for drug administration or on new drug delivery systems, with the aim of obtaining improved therapeutic activity, fewer adverse effects or better patient compliance (Harris and Robinson, 1992; Squier and Wertz, 1996; Shojaei, 1998). Buccal drug delivery is a logical alternative delivery route for drugs which undergo extensive degradation in the stomach and the liver. Drugs delivered by the buccal route gain direct entry into systemic circulation (Rathbone et al., 1994; Şenel and Hincal, 2001; Hao and Heng, 2003). The permeability of buccal mucosa is between 4 to 4000 times greater than that of skin. As a result, faster onset of action for several drugs has been reported (Galey et al., 1976). Since human buccal mucosa is not widely available, animal oral mucosa is routinely used for studies in vitro. In recent studies, porcine buccal mucosa has been chosen as an animal model due to its close resemblance to human buccal mucosa in both ultra structure and enzyme activity (de Vries et al., 1990; Wertz and Squier, 1991; Xiang et al., 2002; Diaz del Consuelo et al., 2005). The permeability characteristics of porcine buccal mucosa are also similar to those of human buccal mucosa (Lesch et al., 1989).

The objective of this study was to compare the intrinsic buccal permeability characteristics of a series of beta-blockers (acebutolol, atenolol, labetalol, metoprolol, oxprenolol and propranolol) through porcine buccal mucosa. Since the same experimental conditions were maintained throughout the study, we are able to compare the possible buccal permeation of each individual drug assayed. We also attempt to establish correlations between buccal permeability and the various structural and the physicochemical descriptors of the drugs studied.

2. Materials and methods

2.1. Materials

Acebutolol chlorhydrate, atenolol base, labetalol chlorhydrate and metoprolol tartrate were supplied by Sigma–Aldrich (Madrid, Spain). Oxprenolol chlorhydrate and propranolol chlorhydrate

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were provided by Novartis and Acofarma, respectively (Barcelona, Spain). Hank's balanced salt solution (HBSS) (Composition in g/L: $CaCl_2$ = 0.14; KCl = 0.14; KH_2PO_4 = 0.06; $MgSO_4$ = 0.1; $MgCl_2$ = 0.1; NaCl = 8.0; $NaHCO_3$ = 0.35; Na_2HPO_4 = 0.09; glucose = 1) was obtained from Biological Industries (Barcelona, Spain). Phosphate buffered saline (PBS) was obtained from Dulbecco's, Life technologies (Barcelona, Spain). Acetonitrile (ACN), acetic acid, disodium hydrogen phosphate (anhydrous) and potassium phosphate (monobasic) were purchased from Panreac (Barcelona, Spain). All the chemicals were of analytical grade and used without further purification.

2.2. Analytical method

A Waters HPLC (model LC Module Plus) was used to analyse the drugs. The analyses were performed at room temperature with a C18 Atlantis reverse phase column (5 μm particle size, 4.6 × 150 mm) purchased from Waters (Barcelona, Spain). UV detection of the drug at a wavelength of 280 nm was used. The specific conditions of the mobile phase and the flow rate for each drug assayed are shown in Table 1.

Various concentrations of each drug in the buffer solution (pH 7.4) were used to construct the calibration curves. The concentration ranges were between 0.05 and 30 μg/mL (atenolol, labetalol and propranolol) and between 0.2 and 30 μg/mL (acebutolol, metoprolol and oxprenolol). The correlation coefficient that relates the theoretical and the real concentrations of the beta-blockers was, r = 0.999 in all cases. Validation of the analytical methods (n = 6) indicated that they were exact and precise. Accuracy, expressed as a relative error, ranged from −11.9% to 10.3%. Precision, expressed as a relative standard deviation, ranged from 0.5% to 14.3%. The limit of detection was between 0.102 μg/mL for atenolol and 0.532 μg/mL for acebutolol. The limit of quantification was estimated to be between 0.308 μg/mL for atenolol and 1.611 μg/mL for acebutolol. These results allow us to quantify the amount of drug in each of the samples taken at the pre-established times.

2.3. Solubility determination

Drug solubility (C_0) was determined under the same conditions as the permeation studies (pH 6.8). An excess of the drug was added to the buffer, the mixture was incubated in a shaking water bath maintained at 37 ± 1 °C for 24 h. After centrifugation at 4000 rpm, the supernatant was filtered (nylon, 0.45 μm). When the solution was appropriately diluted, the concentration of each drug was determined by HPLC. The solubility was measured in triplicate.

2.4. Determination of n-octanol-buffer solution (pH 6.8) partition coefficient (P)

The partition coefficient (P) is a characteristic physicochemical constant of drugs that indicates how lipophilic they are and it is very closely related to their capacity to penetrate lipid membranes, such as the stratum corneum of the skin and the buccal mucosa. It is defined as the relation between the concentration of non-ionized drug in an organic solvent and the concentration of the non-ionized active ingredient in an aqueous solvent.

When we consider drugs that can be ionized at physiological pH, as is the case with beta-blockers, it is better to estimate the distribution coefficient (D) (Hadgraft and Valenta, 2000; Scott and Clymer, 2002) which is a parameter that is better suited to calculating the partition coefficient of the non-ionized compound.

In the present work the logarithm of the distribution coefficient ($\log D_{\mathrm{pH\ 6.8}}$) was obtained from the literature (ChemIDplus, 2007). By applying the corresponding equation, we arrived at the logarithm of the partition coefficient ($\log P$); and then, taking the antilogarithm of $\log P$ gave us the corresponding partition coefficient (P).

Table 1
Chromatographic conditions used in each case.

Drug	Mobile phase			
	Buffer[a]	AcN	H_2O	mL/min
Acebutolol	10	25	65	2
Atenolol	10	5	85	2
Labetalol	10	25	65	2
Metoprolol	10	25	65	2
Oxprenolol	10	25	65	2
Propranolol	75	25	–	1

[a] Ammonium acetate pH 3.

2.5. Buccal mucosa permeation procedure

The porcine buccal mucosa, obtained immediately after the pigs (3–4-month-old females) had been slaughtered, was obtained from the Animal Facility at the Bellvitge Campus, University of Barcelona. The animals were slaughtered using an overdose of sodium thiopental anaesthesia. The buccal tissues were transferred from the hospital to the laboratory in containers filled with Hank's liquid. The study was approved by the Animal Experimentation Ethics Committee of the University of Barcelona and the Animal Experimentation Committee of the regional authorities (Generalitat of Catalonia).

For the permeation studies, the porcine buccal mucosa was cut to a thickness of 500 ± 50 μm (Dermatom Aesculap, Tuttlingen, Germany). This thickness corresponds to the buccal epithelial thickness, which contributes to the diffusion barrier (Sudhakar et al., 2006). The membranes were then mounted on specially designed membrane holders with a permeation orifice diameter of 1.2 cm (diffusion area: 1.1 cm^2).

Using the membrane holder, each porcine buccal membrane was mounted between the donor (1.5 mL) and the receptor (6 mL) compartments of six Franz-type diffusion cells (Franz, 1975). In the donor compartment we placed 250 μL of the solution of the drug to be studied. A pH of 6.8 was used in the donor as it represents a mean value of the physiological oral cavity pH (Le Brun et al., 1989). The receptor pH was fixed at 7.4 to simulate in vivo plasma pH.

Prior to conducting the experiments, the diffusion cells were incubated for 1 h in a water bath to equalize the temperature in all the cells at 37 ± 1 °C by means of an insulating jacket. The receptor solution was PBS, pH 7.4, which was continuously stirred at 600 rpm with a Teflon-coated bar magnet placed inside the cell. Sink conditions were ensured in all experiments after initial testing of the drug saturation concentration in the receptor medium.

Samples (300 μL) were withdrawn via syringe from the centre of the receptor compartment of the six cells at the following time intervals: 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h. The sample volume was immediately replaced with the same volume of fresh receptor medium. The cumulative amounts of the drug (μg) that had penetrated per unit surface area of the mucosa membrane (cm^2) were corrected for this sample removal and plotted versus time (h). The drug concentrations in the samples taken from the receptor compartment were assayed by HPLC.

2.6. Data analysis

The permeation profiles were analysed on the basis of a diffusion model for an infinite dose system (Okamoto et al., 1986). The permeation parameters were calculated from experimental data in steady state (straight section of the curve). The P_1 (related