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In vitro penetration of some β-adrenoreceptor blocking drugs through porcine buccal mucosa

P.P.H. Le Brun [1], P.L.A. Fox [2], M.E. de Vries [2] and H.E. Boddé [2]

[1] *The Hague Hospitals Pharmacy, The Hague (The Netherlands) and* [2] *Center for Bio-Pharmaceutical Sciences, Gorlaeus Laboratories, Leiden (The Netherlands)*

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Summary

Some drugs could preferably be administered by using the buccal route. To support the development of a suitable buccal dosage form we designed an in vitro penetration model to investigate drug permeability of the buccal mucosa. Porcine buccal mucosa was excised and clamped inside a two-chambered flow-through diffusion cell. The in vitro penetration of bupranolol, propranolol, oxprenolol and acebutolol was measured in triplicate (PBS-medium, pH 6.8, drug concentration 4 g/l, 37 °C). The drugs varied in lipophilicity, whereas pK_a values were comparable. From the steady-state fluxes, permeability coefficients were calculated. A correlation was found between the permeability coefficient and the drug lipophilicity, which was expressed as the octanol-buffer distribution coefficient. It was concluded that the model is useful for further permeability investigations.

Introduction

By using the buccal route, the bioavailability of a number of drugs susceptible to gastric degradation, gut metabolism or first-pass hepatic metabolism increases (Gibaldi, 1985). However, only a few drugs are indeed administered successfully via the buccal route. Buccal nitroglycerine works as rapidly as sublingual nitroglycerine (Abrams, 1984). The bioavailability of buccal morphine was 40–50% greater than after intramuscular administration although the degree of postoperative analgesia was the same (Bell et al., 1985); in terms of anxiolysis and wakefulness, the intramuscular route proved to be superior (Fisher et al., 1986). Haft and Litterer (1984) introduced the chewing of perforated nifedipine capsules as a safe and effective method to lower the blood pressure without parenteral medication. However, in a recent review the buccal absorption has been doubted after evaluation of pharmacokinetic and pharmacodynamic studies (McAllister, 1986). Buccal oxytocine was popular in the 1960s. Later this route was abandoned because of the variable absorption (Editorial, Lancet 1987).

Variable absorption seems to be a major disadvantage of the buccal route probably due to inappropriate formulations and insufficient knowledge of the absorption process. In optimizing buccal

Correspondence: P.P.H. Le Brun, The Hague Hospitals Pharmacy, P.O. Box 43100, 2504 AC The Hague, The Netherlands.

formulations muco-adhesive hydrogel patches have been developed recently (Robinson, 1985; De Vries, 1988). To support the development of a suitable buccal dosage form we designed an in vitro penetration model to investigate drug permeability of the buccal mucosa. In this paper the design of the model and its validation with a range of comparable drugs are described. Our model is derived from the one described by Squier and Hall (1985) with some modifications. It is based on the generally accepted hypothesis that the epithelium is the rate-limiting barrier in buccal absorption. The permeability of 4 β-blocking drugs was investigated: bupranolol, propranolol, oxprenolol and acebutolol, ranging from very lipophilic to hydrophilic.

Materials and Methods

Chemicals

Propranolol HCl, oxprenolol HCl, and acebutolol HCl were kindly supplied by Bufa Chemie (Castricum, The Netherlands). Bupranolol was a gift from Schwarz Pharma (Monheim, F.R.G.). Phosphate-buffered saline (PBS) was composed with p.a. chemicals (Merck, Amsterdam, The Netherlands) resp. NaCl, KCl, KH_2PO_4, and Na_2PO_4; for pH = 7.4 resp. 6.85 g, 0.20 g, 0.19 g and 1.2 g in 1 litre of distilled water; for pH = 6.8 resp. 8.00 g, 0.19 g, 0.20 g and 2.86 g in 1 litre of distilled water. Dichloromethane and EDTA-disodium were p.a. quality (Merck, Amsterdam, The Netherlands).

Tissue preparation

Buccal tissue was taken from pigs at slaughter and kept in PBS (pH = 7.4). It was used within several hours after removal. For splitting purposes the tissue was incubated during 50 min at 60 °C in 20 mmol EDTA disodium salt in PBS buffer (pH 7.4). The epithelial layer could then easily be removed from the connective tissue. The epithelium was placed between two dialysis membranes (cut-off value 5000). These membranes supported the epithelium and prevented the diffusion of macromolecular tissue and mucous components. Out of these sandwiched layers discs were punched (1 cm in diameter). Before starting a diffusion experiment, the epithelial thickness was estimated by means of a light microscope. After the experiments the epithelial layer was inspected for occasional damage.

Diffusion experiments

The punched out discs were carefully clamped inside a two-chambered flow-through diffusion cell. The characteristics of this cell have been described recently (Tiemessen et al., 1988). The effective surface of diffusion was 0.63 cm^2. Fluids could be flushed along both sides of the membranes by means of a peristaltic pump (Ismatec IPS 8). Along the mucous side of the membrane a donor solution (PBS, pH 6.8 containing a β-blocker, 2 g/l or 4 g/l) was circulated. A pH of 6.8 was chosen as a mean value of the physiological oral cavity pH (Boer et al., 1984). Along the serous side an acceptor fluid (PBS, pH 6.8) was pumped through and collected in a fraction collector (Pharmacia Frank 300) at 1 h intervals. There was no pH gradient across the buccal membrane. The flow rate on both sides was 8 ml/h. During the experiments the cells were placed in a waterbath at 37 °C. All experiments were performed in triplicate. The drug concentration of the acceptor fractions was determined (see Drug assay). The permeability for 4 β-blocking agents was determined. The drugs ranged in lipophilicity whereas the pK_a values were of the same order (Table 1) (Schoenwald and Huang, 1983).

Drug assay

All fractions collected were weighed. The pH of each sample was adjusted to 12.5 by adding 0.5 ml of 4 N NaOH. The drugs were extracted with 5.0 ml dichloromethane by shaking for 15 min using a table-shaker (Salm and Kipp). After centrifugation and separation of the layers, the UV absorption of the dichloromethane layer was determined resp. at 235, 273, 234 and 272 for propranolol, oxprenolol, acebutolol and bupranolol base (Kontron Instruments, Uvikon 860).

The concentrations were calculated using calibration curves constructed from standard solutions.

After the experiments the amount of drug remaining in the tissue was also determined. The tissue was decomposed by ultrasonication, then extracted using the same procedure as described above.

Calculation method

The permeation was determined by measuring the amount of drug in the acceptor fractions. In each case two series of experiments were performed. One with a combination of the epithelium and the supporting membranes, the other with the supporting membranes only. The cumulative amount of permeated drug, Q, per unit surface area was plotted versus time (t). In a steady-state situation the flux, J, is defined as the slope of this line: $J = dQ/Adt$ where A is the surface area.

TABLE 1

pK_a, partition coefficients, distribution coefficients, permeability coefficients and residual tissue contents of β-blocking agents

Compound	pK_a	K_p	K_d	P_e (10^{-6} cm/s)	Res. (μmol/cm^2)
Bupranolol	9.17	5012.0	21.30	15.70	59.7
Propranolol	9.23	1640.0	6.10	6.42	25.0
Oxprenolol	9.32	235.0	0.71	2.68	15.0
Acebutolol	9.20	59.0	0.23	1.27	10.4

The permeability coefficient P was calculated using the relationship $P = J/(C_d - C_a)$, where C_d and C_a are drug concentration in the donor and in the acceptor solution, respectively (Siegel, 1984).

The permeability coefficient was calculated for the combination of epithelium and supporting membranes (P_t) and for the supporting mem-

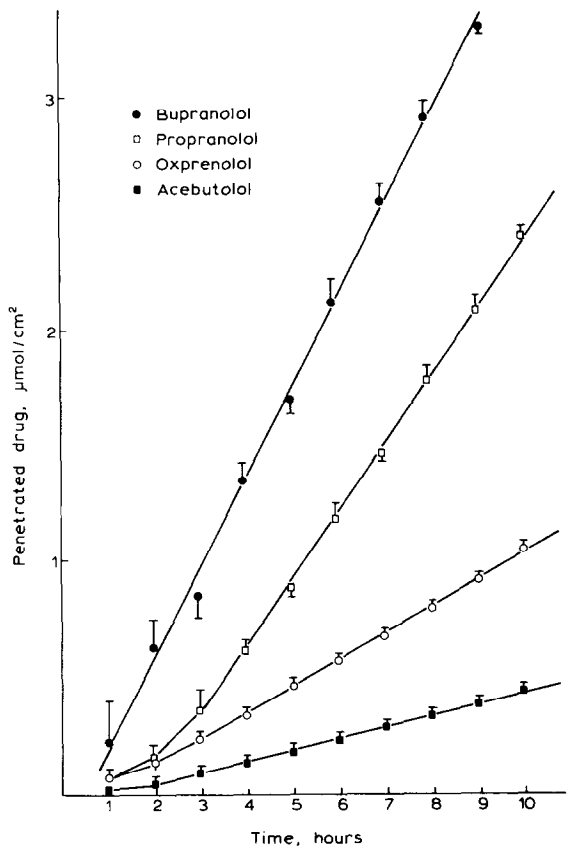

Fig. 1. Cumulative amount (±S.E.) of penetrated drug through the combination of epithelium and supporting membranes vs time.

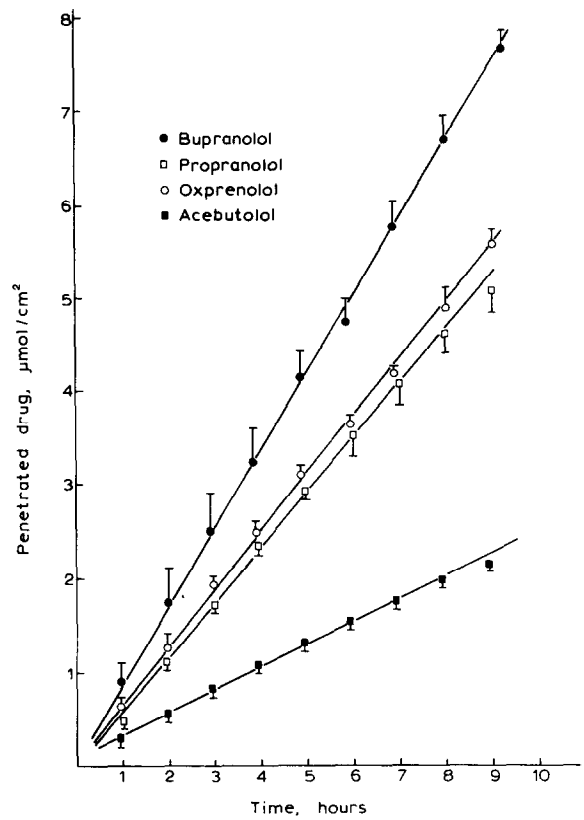

Fig. 2. Cumulative amount (±S.E.) of penetrated drug through the supporting membranes vs time.

branes alone (P_s). From these values the permeability coefficient of the epithelium (P_e) could be calculated from the relationship:
$1/P_e = 1/P_t - 1/P_s$. This relationship can easily be understood from the fact that the permeability coefficient is the reciprocal value of the resistance of the barriers: $P = 1/R$. The total resistance R_t is the sum of the partial barriers R_e (epithelium) and R_s (supporting membranes). So $1/P_e = 1/P_t - 1/P_s$. Since the thicknesses of all buccal epithelia used were in a narrow range (235 ± 35 μm), these comparative calculations can be done without making corrections for differences in epithelial thickness. The distribution coefficient, K_d, at pH 6.8 can be calculated from the partition coefficient, K_p, using the relationship:
$K_d = K_p/[1 + \text{antilog}(pK_a - pH)]$ (Schoenwald and Huang, 1983).

The dimensions of P, Q and C are respectively cm/s, μmol/cm^2 and mmol/l.

Results

The cumulative amounts of drug that had penetrated through the combination of epithelium and supporting membranes is shown in Fig. 1, and through the supporting membranes only in Fig. 2. After a short lag-time, a linear relation was found in all experiments. The calculated permeability coefficients are listed in Table 1 along with the octanol–buffer partition coefficients (Schoenwald and Huang, 1983). In each case the amount of drug that had penetrated is higher for the more lipophilic β-blocking agent. The permeability coefficient is plotted vs the distribution coefficient as shown in Fig. 3 on a double log scale. The drug residues (res) in the epithelial tissue are also listed in Table 1; the amount is higher for a more lipophilic drug.

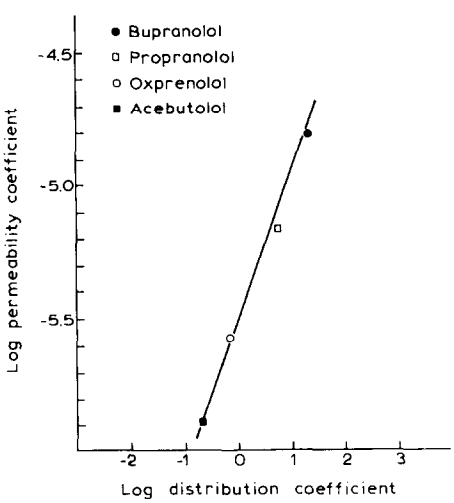

Fig. 3. Log–log plot of permeability coefficient and distribution coefficient (pH 6.8).

Discussion

Our model to simulate in vitro buccal drug penetration was found to be both useful and reliable. Firstly, the in triplicate measured steady-state fluxes were found reproducible (Fig. 1). Secondly, close examination of the tissue, after the stripping procedure and after the penetration experiments, with a light microscope did not show any major damages. Nor were structural changes found in thin sections which were studied under the electron microscope. However, more observations will have to be conducted in order to confirm these data. Furthermore, we found in a preliminary experiment a linear relationship between the propranolol donor concentration and the steady-state flux for 2, 4 and 6 g/l, respectively. For practical reasons the donor concentrations were kept at 4 g/l in the experiments with the combination of epithelium and supporting membranes; 2 g/l was used in the experiments with the supporting membranes only. It was necessary to use fresh tissue. After preservation of the tissue for several days in PBS medium (pH = 7.4) we found a decreased permeability for propranolol. Perhaps this is due to structural changes. Further examination is necessary since other studies showed an increased permeability after exposure to an aqueous environment (Squier and Hall, 1985).

The calculated permeability vs the octanol–buffer distribution coefficient (Fig. 3) showed a linear relationship on a double log scale. This observation confirms the hypothesis that in buccal drug transport the rate-limiting step is a lipophilic barrier. Whether buccal drug transport is a passive

process only or a carrier-mediated process, or a combination of both, is still uncertain (Siegel, 1984). Our data supported the predominance of a passive diffusion.

It is noteworthy that a similar correlation between permeability and distribution coefficients has been found in the corneal penetration of β-blocking drugs (Schoenwald and Huang, 1983). In these experiments a plateau has been found for very lipophilic drugs at a log distribution coefficient of ca. 2.5. In buccal penetration we have not (yet) found an optimum. It is likely that there will be a plateau for drugs with a higher lipophilicity. The important role of the lipophilicity is also shown by the amount of drug remaining in the tissue after the experiments. Since the drug donor concentrations are comparable, these data indicate a "depot function" of the mucosa. Although the theoretical water solubility has not been surpassed, binding is likely to take place in the lipophilic parts of the epithelium. One would have to take this into account when considering the in vivo situation. Binding of propranolol to oral mucosa has already been found for sublingually administered propranolol (Kates, 1977).

It is concluded that the in vitro model is useful for studying the permeability of the buccal mucosa for drugs. Currently, the model is being used for evaluating the feasibility of the buccal delivery of peptides.

References

Abrams, J., Nitrate delivery systems in perspective. *Am. J. Med.*, 75 (1984) 38–46.

Bell, M.D.D., Mishra, P., Weldon, B.D., Murray, G.R., Calvey, T.N. and Williams, N.E., Buccal morphine. A new route for analgesia? Lancet, i (1985) 71–73.

Boer, A.G. de, Leed, L.G.J. de and Breimer, D.D., Drug absorption by sublingual and rectal routes. *Br. J. Anaesthesiol.*, 56 (1984) 69–82.

Editorial, Administration of drugs by the buccal route. *Lancet*, i (1987) 66–67.

Fisher, A.P., Vine, P., Whitlock, J. and Hanna, M., Buccal morphine premedication. *Anaesthesia*, 41 (1986) 1104–1105.

Gibaldi, M., The number of drugs administered buccally is increasing. *Perspect. Clin. Pharmacol.*, 3 (1985) 49–56.

Haft, J.J. and Litterer, W.E., Chewing nifedipine to rapidly treat hypertension. *Arch. Intern. Med.*, 144 (1984) 2357–2359.

Kates, R.E., Absorption kinetics of sublingually administered propranolol. *J. Med.*, 8 (1977) 393–402.

McAllister, R.G., Kinetics and dynamics of nifedipine after oral and sublingual doses. *Am. J. Med.*, 81 (1986) 2–5.

Robinson, J.R., Bioadhesive compositions and methods of treatment therewith, *Int. Patent Class*, AOIN 25/26, 25/34 (1985).

Schoenwald, R.D. and Huang, H.S., Corneal penetration behaviour of β-blocking agents I: Physicochemical factors. *J. Pharm. Sci.*, 72 (1983) 1266–1271.

Siegel, J.A., Permeability of the oral mucosa. In: Meyer, J., Squier, C.A. and Gerson, S.J. (Eds.), *The Structure and Function of Oral Mucosa*, Pergamon, Oxford, 1984, pp. 95–108.

Squier, C.A. and Hall, B.K., In vitro permeability of porcine oral mucosa after epithelial separation, stripping and hydration. *Arch. Oral Biol.*, 30 (1985) 485–491.

Tiemessen, H.L.G.M., Boddé, H.E., Koppen, M. van, Bauer, W.C. and Junginger, H.E., A two-chambered diffusion cell with improved flow through characteristics for studying the drug permeability of biological membranes. *Acta Pharm. Technol.*, 34 (1988) 99–101.

Vries, M.E. de, Boddé, H.E., Busscher, H.J. and Junginger, H.E., Hydrogels for buccal delivery: properties relevant for muco-adhesion. *J. Biomed. Mat. Res.*, (1988) in press.