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Human buccal absorption. II. A comparative study of the buccal absorption of some parahydroxybenzoic acid derivatives using the buccal absorption test and a buccal perfusion cell

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Summary

Two methods were used to study the extent and rate of drug loss from the human oral cavity of some parahydroxybenzoic acid derivatives. Extents of drug loss were measured using the buccal absorption test. Rates of drug loss were measured using a buccal perfusion cell. Both extents and rates of drug loss were shown to be dependent upon alkyl chain length. In addition both showed a similar dependence upon pH. Extents of drug loss were also shown to be independent of initial concentration and the same whether drugs were administered alone or as a multicomponent mixture. Results indicate that oral cavity membranes are essentially lipid in nature and each derivative is lost from the oral cavity by passive diffusion of the non-ionised lipid soluble form in accord with the pH-partition hypothesis. The buccal absorption test is a reliable means of estimating the extent of drug loss from the oral cavity. In contrast, the buccal perfusion cell method provides a simple and reproducible technique for estimating the rate of drug loss from the oral cavity over a fixed area of known membrane under closely controlled conditions.

Introduction

The buccal absorption test of Beckett and Triggs (1967) is a simple and reliable method for measuring the extent of drug loss from the human oral cavity for single and multicomponent mixtures of drugs. The test has been successfully used to investigate the relative importance of drug structure, contact time, initial drug concentration and pH of the solution in which the drug is held in the oral cavity (Beckett and Triggs, 1967; Beckett and Moffat, 1968, 1969, 1970; Dearden and Tomlinson, 1971a; Hicks, 1973; Manning and Evered, 1976; Temple and Schesmer, 1978; Schurmann and Turner, 1978).

The test has proved to be a useful in vivo model of passive drug transfer through lipid membranes. In this respect extents of drug loss from the oral cavity during a buccal absorption test have been successfully correlated with renal tubular reabsorption (Beckett and Triggs, 1967; Beckett and Moffat, 1968; Temple and Schesmer, 1978; Meyer et al., 1974; Kaye and Long, 1976; Beckett and Chidomere, 1977; Chan, 1979; Achari and Beckett, 1982), biological response (Dearden and Tomlinson, 1971a), drug absorption interac-

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tions (Garnham et al., 1977; McElnay et al., 1982; McElnay and Temple, 1982; McElnay and Mooney, 1983) and intestinal absorption (Manning and Evered, 1976; Evered et al., 1977, 1980; McMullan et al., 1977; Sprake and Evered, 1979; Sadoogh-Abasian and Evered, 1979; Evered and Mallett, 1983; Hunjan and Evered, 1985). However, determination of rates of drug loss from the oral cavity using this test are time consuming (Tucker, 1988) and the analysis of data complicated by the continual and erratic secretion of saliva throughout the duration of a test. Several authors have reported methods for overcoming these problems (Dearden and Tomlinson, 1971b; Schurmann and Turner, 1978; Tucker 1988) however, none have been widely used.

Recently a buccal perfusion cell was employed to determine the rate of drug loss across a known oral cavity membrane over a fixed area under defined conditions (Rathbone, 1991). The technique allowed rates of drug loss to be easily and reliably determined independent of salivary secretions.

This paper reports on a comparative study to determine the relative merits of estimating rates as opposed to extents of drug loss from the human oral cavity using the buccal absorption test and a buccal perfusion cell. Results are discussed in relation to the use of oral cavity membranes as in vivo models of passive drug transfer through biological membranes.

Materials and Methods

Materials

Methyl-, ethyl-, propyl- and butylparahydroxybenzoates were obtained from Sigma Chemical Company. Methanol HPLC grade was obtained from BDH Chemicals Ltd. Buffers comprised either 20.4 g KH_2PO_4/l (pH 4.4), isotonic citrate (pH 5.0, 5.6), isotonic phosphate (pH 6.1, 6.5), isotonic borate (pH 7.1, 7.6, 8.1, 8.6, 9.0) (Anderson, 1983) or Sorensens glycine (pH 9.5, 10.6) (Diem and Lentner, 1970). All quoted buffer pH's were measured at 37°C (Philips pH Meter, model PW9422, Pye Unicam Ltd, England). Buffer components were obtained from: sodium dihydrogen orthophosphate dihydrate, citric acid monohydrate (GPR, BDH Chemicals Ltd), disodium hydrogen orthophosphate dodecahydrate (AR, Ajax Chemicals), glycine, sodium chloride (Analar, BDH Chemicals Ltd), sodium citrate dihydrate, boric acid (Laboratory Chemicals, May and Baker Ltd).

Methods

Buccal absorption test. Three male volunteers aged 20–32 years participated in the investigations. Extents of drug loss were measured using the general method of Beckett and Triggs (1967) with the following modifications. 20 ml of drug solution containing a known amount of drug was swirled around the mouth 60 times/min. Expelled solutions were made up to volume using isotonic citrate buffer (pH 5.0). Each test was performed in triplicate.

Buccal perfusion cell. 16 subjects (11 male, 5 female), age range 19–32 years were employed in the study. Rates of drug loss were determined on at least six different occasions using the same apparatus and methodology as described previously (Rathbone, 1991). Perfusion of the membrane was continued for at least 50 min.

HPLC analysis of parahydroxybenzoic acid derivatives. HPLC analysis was performed using a Shimadzu liquid chromatograph model LC-4A, Shimadzu Corporation, Japan. For ethyl-, propyl- and butylparahydroxybenzoate the following were used: mobile phase (methanol 70%, 0.01 M buffer 30%); flow rate 1.5 ml min^{-1}; column: Brownlee Labs spheric -5 RP-18 220 × 4.6 mm 5 μm with a RP18 prefilter; detector: Shimadzu spectrophotometric detector SPD-2AS at 254 nm; 20 μl injection; Shimadzu Chromatopac C-RZAX module for integration of peak areas.

For the case of methylparahydroxybenzoate the same conditions as described above were employed except the mobile phase comprised methanol 55%, 0.01 M buffer 45%.

Results and Discussion

HPLC analysis of parahydroxybenzoic acid derivatives

The HPLC chromatograms had near-symmetrical, base-line resolved peaks with typical reten-

tion times of: methyl- 3.0, ethyl- 2.6, propyl- 3.4 and butylparahydroxybenzoate 4.4 min. Salivary secretions did not affect these retention times. The use of HPLC allowed the acids in mixtures to be separated and analysed individually.

Buccal absorption test

Extent of drug loss. The extent of drug loss was determined from knowledge of the amount of drug entered into the oral cavity and that recovered and expressed as a percentage of the original amount entered. Percentage loss in each test varied by no more than +8% from the mean value ($n = 3$) and is shown in Figs 1–3.

Contact time. The extent of drug loss for each derivative at pH 5.0, 0.01 mg/ml determined after varying contact times ranging from 30 s to 5 min shown as closed symbols in Fig. 1. Profile shapes are typical of those obtained during a buccal absorption test (Beckett and Triggs, 1967; Beckett and Moffat, 1968, 1970; Tucker, 1988). Drug loss increased rapidly with time of contact. A 3 min contact time was chosen for subsequent experiments since this represented an 'optimum contact time' that is a balance between: percentage loss from the oral cavity, subject abil-

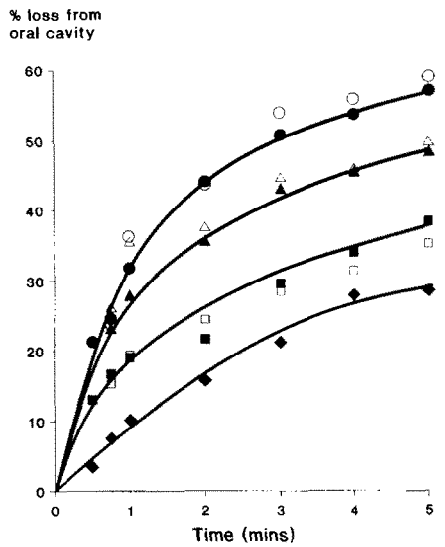

Fig. 1. Effect of contact time on the percentage loss from the oral cavity for methyl- (◆), ethyl- (■), propyl- (▲) and butylparahydroxybenzoate (●) during a buccal absorption test (0.01 mg/ml; pH 5.0). Closed symbols: compounds administered singly; open symbols: compounds administered as a multicomponent mixture. Points represent mean value of three determinations.

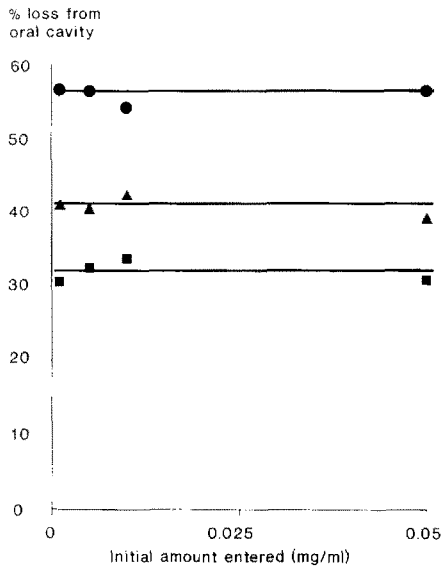

Fig. 2. Effect of initial amount entered into the oral cavity on the percentage loss from the oral cavity for ethyl- (■), propyl- (▲) and butylparahydroxybenzoate (●) during a buccal absorption test (pH 5.0; 3 min contact time). Points represent mean value of three determinations.

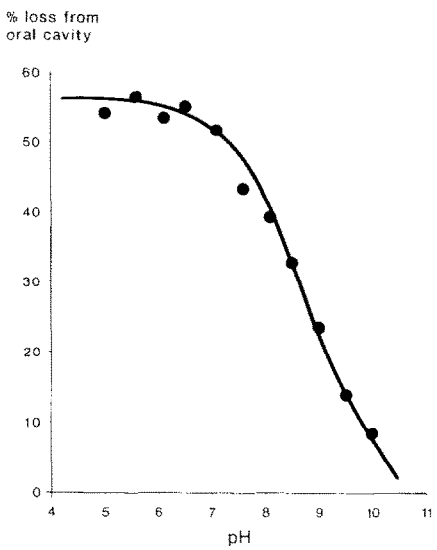

Fig. 3. Effect of pH on the percentage loss from the oral cavity for butyl parahydroxybenzoate (0.01 mg/ml; 3 min contact time). Points represent mean value of three determinations.

ity to perform the test without swallowing and saliva production.

Drug mixtures. Mixtures containing ethyl-, propyl- and butylparahydroxybenzoate were investigated at pH 5.0, 0.01 mg/ml over varying contact times and are shown as open symbols in Fig. 1. Fig. 1 shows that there is little or no difference between the profiles obtained when a drug is administered alone or as a multicomponent mixture.

Initial drug concentration. Extents of drug loss for ethyl-, propyl- and butylparahydroxybenzoate from the oral cavity were determined over the range 0.001–0.05 mg/ml (pH 5.0, 3 min contact time). Results are shown in Fig. 2 and indicate that the extent of drug loss (over a fixed contact time and given pH value) remained independent of initial drug concentration entered into the oral cavity.

pH of buffered drug solution. The effect of pH in the range 5.0–10.6 on the extent of drug loss from the oral cavity for butylparahydroxybenzoate (0.01 mg/ml, 3 min contact time) is shown in Fig. 3. Percentage loss shows a characteristic sigmoidal dependence upon pH at pH values that cause the drug to dissociate. At pH values where the parabens are essentially non-ionised, percentage loss remains independent of pH.

Buccal perfusion cell

Rate of drug loss. The apparent first-order rate constants for drug loss from the buccal perfusion cell were calculated from knowledge of the change in paraben concentration in the aqueous donor phase in the perfusion cell with time (Rathbone, 1991).

Alkyl chain length. Fig. 4 shows the effect of alkyl chain length upon the rate of drug loss for methyl-, ethyl-, propyl- and butylparahydroxybenzoates at pH 4.4. Derivatives could be ranked in order of increasing alkyl chain length showing that the rate of drug loss was dependent upon drug lipophilicity.

pH of drug donor phase. The effect of pH in the range 4.4 through 10.6 upon the rate of drug loss of butylparahydroxybenzoic acid from the buccal perfusion cell is shown in Fig. 5. At pH values where only non-ionised drug species are present the rate of loss is independent of pH. However, the characteristic sigmoidal dependence of rate of loss upon pH is observed at pH values which encourage the drug to dissociate.

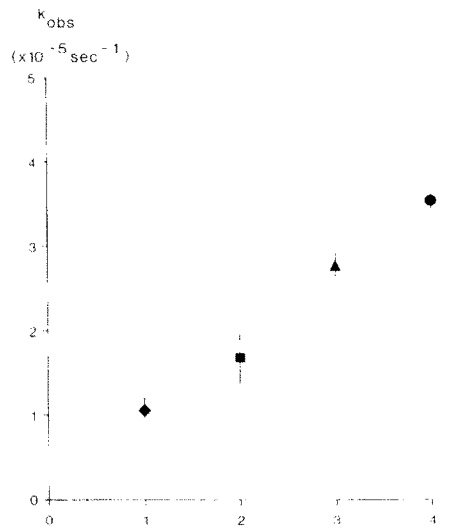

Fig. 4. Effect of drug structure on the disappearance kinetics of methyl- (◆), ethyl- (■), propyl- (▲) and butylparahydroxybenzoate (●) from the buccal perfusion cell (pH 4.4). Points represent mean ± S.D., $n \geqslant 6$.

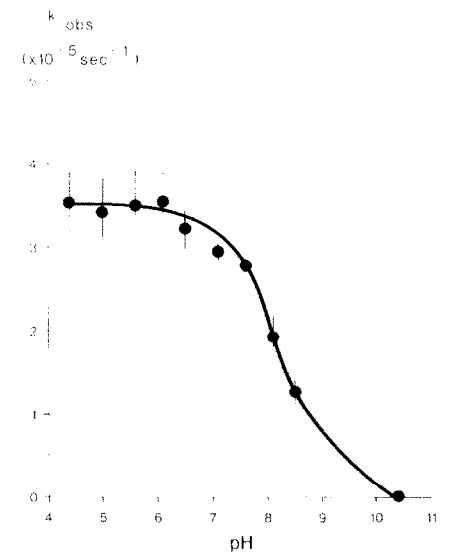

Fig. 5. Effect of pH on the disappearance kinetics of butylparahydroxybenzoate from the buccal perfusion cell. Points represent mean ± S.D., $n \geqslant 6$.

The results from both methods suggest that oral cavity membranes are essentially lipid in nature and each derivative lost from the oral cavity by the process of passive diffusion of the non-ionised lipid soluble form in accord with the pH-partition hypothesis.

The buccal absorption test represents a reliable means of estimating the extent of drug loss from the oral cavity. However, the unknown surface area and variety of membranes over which transfer can take place, the extended periods of time required to map a kinetic profile (2–3 days), interference from salivary secretions resulting in pH and volume changes throughout the duration of a test and variable (sometimes large) intra- and intersubject variations are all disadvantages of the technique when rates of drug loss from the oral cavity are required to be estimated. These disadvantages can be overcome with the buccal perfusion cell method.

The buccal perfusion cell technique provides a simple, reproducible means of determining rates of drug transfer across the human buccal membrane under closely controlled conditions. In addition the method might also allow drug disappearance from the oral cavity and appearance in the plasma to be determined simultaneously. Such a technique may provide information on the relationship between drug structure and rate constants for drug absorption and identification of those factors which govern and control drug absorption across biological membranes.

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