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Human buccal absorption. I. A method for estimating the transfer kinetics of drugs across the human buccal membrane

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Summary

A buccal perfusion cell of simple design was constructed and tested. The cell allowed 3.14 cm^2 of human buccal membrane to be perfused by 27.5 ml of an aqueous drug donor phase (pH 4.4, 300 mosM/kg) maintained at 37°C. A model drug, butyl *p*-hydroxybenzoate, was used to evaluate and validate the technique. The kinetics of drug loss from the oral cavity were estimated from knowledge of the change in drug concentration in the aqueous donor phase with time. Fifteen male and fifteen female subjects were employed in the study. Intra-subject variation ($n \geq 9$) for drug loss was shown to be small. Inter-subject variation ($n = 30$) was shown to be larger than intra-subject variation. No significant difference in the kinetics of drug loss from the buccal perfusion cell were observed between the left and right buccal membrane for any given subject (two-tailed Student's *t*-test $p > 0.05$). In addition there was no apparent difference in the rate of drug loss between male and female subjects. The buccal perfusion cell allowed drug transfer across the human buccal membrane to be easily and reliably estimated.

Introduction

A number of non-invasive methods for estimating the rate and extent of drug loss from the oral cavity have been proposed (Beckett and Triggs, 1967; Kaaber, 1974; Barsuhn et al., 1988). Beckett and Triggs (1967) introduced the buccal absorption test which involved swirling a buffered drug solution around the mouth. After a known time period the solution was expelled and the subject rinsed their mouth with buffer. Drug solution and buffer rinse were then combined, analysed for drug content and the amount of drug absorbed estimated from the difference between that entered and recovered. In order to estimate the transfer kinetics of a particular drug the buccal absorption test requires repeated swillings over different time periods up to a maximum of 15 min – a process that can take days for the mapping of a drugs kinetic profile (Tucker, 1988). In this respect Tucker (1988) reported on an improvement over the traditional buccal absorption test which enabled kinetic data to be collected in a single 15 min trial. The method involved multiple samples being withdrawn from the mouth using a positive displacement pipettor.

Neither the original buccal absorption test

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(Beckett and Triggs, 1967) or its modification (Tucker, 1988) can provide information on the rate of drug loss across different regions of the oral cavity since there is no knowing across which membrane transfer may have taken place. Also there is no control over the area across which transfer can take place.

In this respect Kaaber developed a method for investigating the transport of water and ions through known regions and fixed areas of the oral mucosa (Kaaber, 1974). He used an airtight sampling chamber comprising a disc of dry ash-free filter paper overlaid with porous membrane material. The filter paper was protected by a transparent plastic disc adhered to a piece of transparent surgical tape (Kaaber, 1974). Pimlott and Addy (1985) used a similar method to Kaaber to study steroid absorption across keritinized and non-keritinized oral mucosa sites. The vehicle for drug delivery was a three disc thickness of filter paper 7 mm in diameter. For the lip and sublingual sites drug impregnated discs were allowed to lie on the mucosa. For the palate, discs were held gently against the mucosa with tweezers while the subject was in the supine position. Schurr and Ziegler (1983) also document a disc method. They used a polytef disc with a diameter of approx. 3.5 cm corresponding to an area of about 10 cm^2 and a height of 1 cm. The disc had a central circular depression depth of 4 mm, leaving an elevated rim. A previously water-soaked filter paper disc was placed in the depression and 20 mg of crystalline protirelin spread onto the filter paper which dissolved immediately. Subsequently the device was placed in contact with the buccal mucosa.

In all of these disc methods adherence of the disc to the membrane, leakage of drug and interference from salival secretions were a problem.

Recently, Barsuhn et al. (1988) devised a closed-perfusion cell apparatus to study the transport of flurbiprofen across a 1.8 cm^2 area of human buccal membrane. The apparatus comprised a hydrophilic vinyl polysiloxane polymer cell that allowed drug solution to be continually recirculated over the buccal membrane for the duration of an experiment (42 min). Samples from a drug solution reservoir were analysed for drug content and apparent first-order disappearance rate constants calculated by linear regression analysis.

The closed-perfusion cell apparatus method successfully overcame many of the problems discussed previously. However, Barsuhn et al. (1988) document problems of perfusion circuit leakage. In addition inter-subject variation was large, disappearance rate constants varied from 2.88×10^{-5} s^{-1} to 8.07×10^{-5} s^{-1} (mean $\pm$ SD = $5.88 \pm 1.85 \times 10^{-5}$ s^{-1}, $n = 8$ subjects) for flurbiprofen at pH 5.5 (Barsuhn et al., 1988)

This study demonstrates how the transfer kinetics of a drug across the human buccal membrane can be reliably estimated using a buccal perfusion cell. The design offers fixed interfacial areas over which transfer can take place into a defined oral cavity membrane. In comparison to the closed-perfusion cell apparatus described by Barsuhn et al. (1988) the buccal perfusion cell offers larger areas over which drug transfer can take place, no leakage problems and continuous monitoring of drug loss as a function of time.

Materials and Methods

Materials

Butyl *p*-hydroxybenzoate was obtained from Sigma. Buffers comprised either 20.4 g/l potassium dihydrogen orthophosphate (pH 4.4, 37°C, 300 mosM/kg) or Sorensens glycine buffer (Diem and Lentner, 1970) adjusted to 300 mosM/kg using sodium chloride (pH 10.6, 37°C). All buffer components were of AnalaR grade, BDH Chemicals, and were used as recieved. Water was freshly distilled.

Methods

Thirty subjects (15 male, 15 female) aged 20–31 years were used in the study. Loss of butyl parahydroxybenzoate from an aqueous donor phase into the human buccal membrane was studied using a buccal perfusion cell. The buccal perfusion cell (Fig. 1) was circular with internal diameter 2 cm, internal depth 0.5 cm and allowed 3.14 cm^2 area of buccal membrane to be perfused. The cell was constructed from nylon and secured to a G-clamp using an epoxy resin compound. Small

bore Teflon circulation tubing (0.10 cm internal diameter) led from the inside of the cell through the epoxy resin and protruded to permit the cell to be connected into the perfusion circuit (Fig. 2). Perfusion circuit tubing (also Teflon) had an internal diameter of 0.2 cm. Initially the cell would be lightly clamped onto the buccal membrane, however during an experiment it maintained its position by the natural suction created by the perfusion circuit. With the buccal perfusion cell clamped onto the membrane 40–50 ml of aqueous donor phase was sucked (Model RPD Lab pump, Fluid Metering Inc., Oyster Bay, NY) via the pump head (Model PVDF pumphead, Fluid Metering Inc., Oyster Bay, NY) through the buccal perfusion cell and discarded. This period adjusted the surface pH of the membrane to that of the donor aqueous phase and allowed the investigator to eliminate air entrapped within the perfusion cell and to adjust pump flow rate. Following incorporation of the buccal perfusion cell into the perfusion circuit (Fig. 2) a second 4–5 min period elapsed during which aqueous donor phase (in the absence of drug) was recirculated around the perfusion circuit and the spectrophotometer base line set. An experiment was initiated by introduction of drug into the circulating aqueous donor phase (27.5 ml) as a 0.2 ml ethanolic bolus to produce a concentration of approx. 5×10^{-5} M. The concentration of butyl *p*-hydroxybenzoate in the donor phase (C_1) was continuously monitored by pumping the aqueous phase at 10 ± 1 ml/min

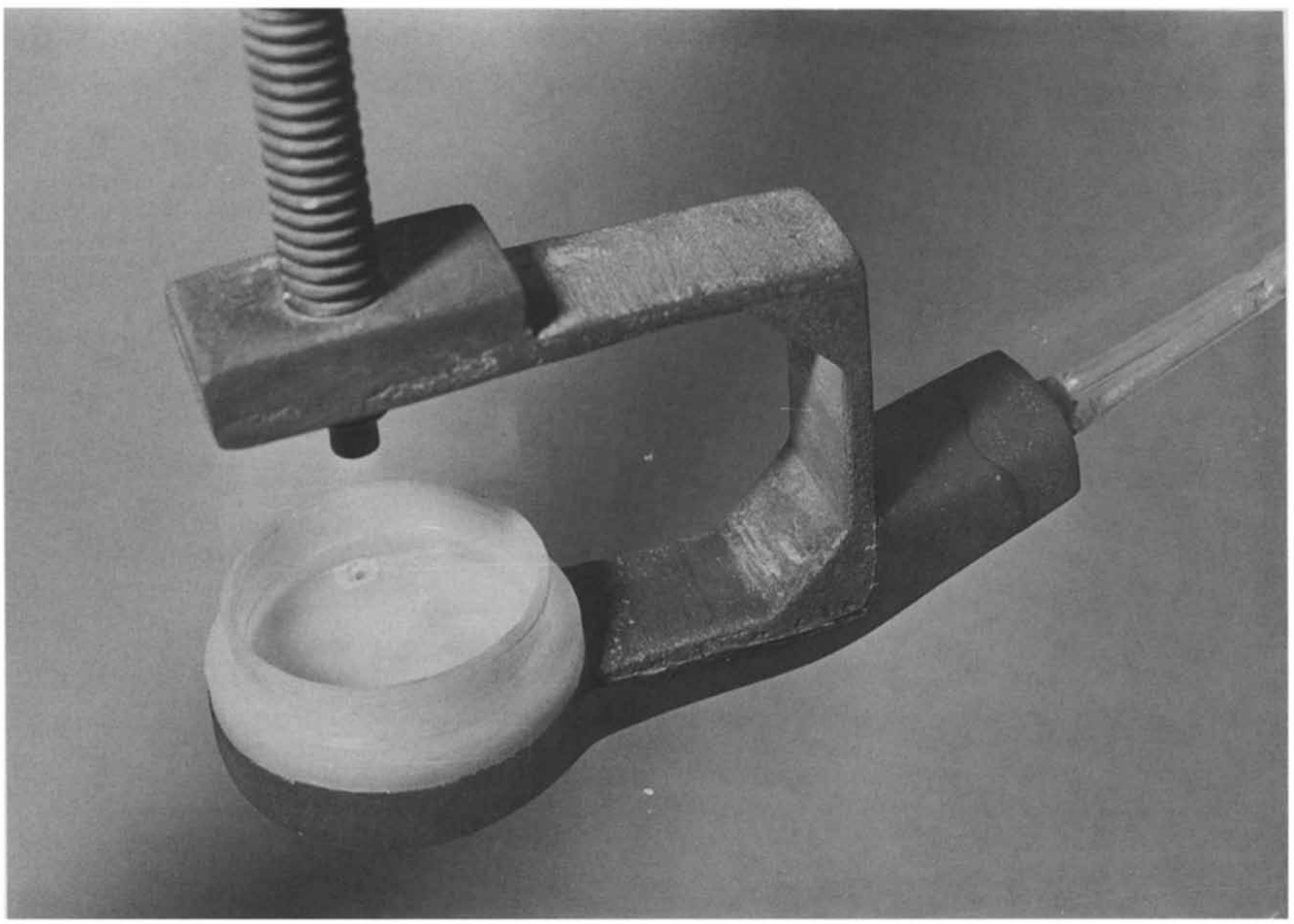

Fig. 1. Human buccal perfusion cell used in the study.

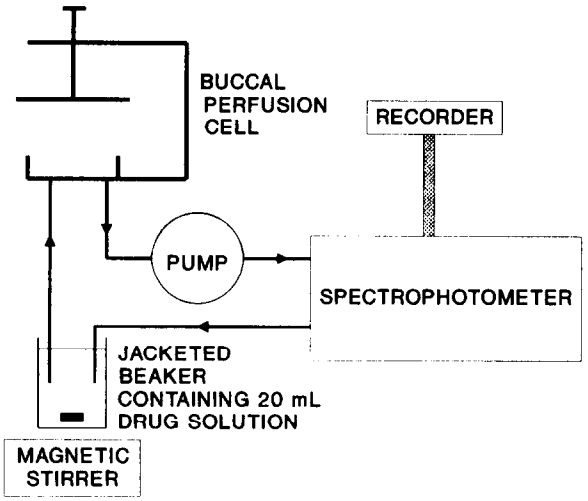

Fig. 2. Perfusion circuit showing direction of flow of aqueous donor phase.

through a flow cell in a spectrophotometer (Model 101 Spectrophotometer, Hitachi, Tokyo, Japan) and automatically recording (Model PL4 Recorder, JJ Instruments, Southampton, U.K.) the absorbance as a function of time at 267.5 nm – the isosbestic point for the compound (Byron and Rathbone, 1984). Buccal membranes underwent perfusion for at least 40 min (usually 50–60 min) and perfusion of the same membrane separated by at least 72 h. All subjects tolerated the perfusion procedure without difficulty and no irritation was apparent at the perfusion site. After removal the cell left an impression in the membrane which returned to normality within 20 min.

Aqueous donor phase volume (includes 7.5 ml perfusion circuit dead volume), pH and temperature (Model Thermomix 1419, B. Braun, Melsungen, Germany) were maintained constant throughout the duration of an experiment at 27.5 $\pm$ 0.5 ml, 4.4 $\pm$ 0.05 units and 37 $\pm$ 0.2°C, respectively. There was no detectable absorption/adsorption of drug into/onto the perfusion circuit materials or buccal perfusion cell and no transfer was observed when the solute under investigation existed predominantly (> 99%) as the ionised moeity (aqueous donor phase pH 10.6). Control experiments showed that there was no leaching of any components from the buccal membrane into the aqueous donor phase that may have interfered with the assay procedure.

Results and Discussion

Kinetics of drug loss from the buccal perfusion cell for butyl *p*-hydroxybenzoate are shown in Tables 1 and 2 for each of the subjects used in the study. Each rate constant was calculated from knowledge of the change in drug concentration in the aqueous donor phase with time based on:

$$k_{obs} = \frac{-\ln(C_1/C_1^0)}{t} \quad (1)$$

TABLE 1

Intra-subject variation for rate of butyl p-hydroxybenzoate loss into left and right human buccal membranes in four subjects and significance of difference in loss rates between a subjects left and right buccal membrane

Subject (sex)	Buccal membrane	Rate of drug loss ($k_{obs} \times 10^{-5}$ s^{-1})	Average $\pm$SD [a] ($k_{obs} \times 10^{-5}$ s^{-1})	Statistical significance of difference
MDM	right	3.81 3.61 3.92 3.39 3.99	3.74 $\pm$ 0.24	NS [b]
(male)	left	3.91 3.83 3.40 3.74 4.23	3.82 $\pm$ 0.29	
MJR	right	3.25 4.11 3.16 4.07 3.36	3.59 $\pm$ 0.46	NS
(male)	left	3.31 3.65 3.75 3.42 3.18	3.46 $\pm$ 0.24	
BRP	right	4.47 3.64 4.10 3.82 4.19	4.04 $\pm$ 0.32	NS
(male)	left	4.62 4.15 4.01 4.21 4.52	4.30 $\pm$ 0.26	
DJV	right	3.39 3.81 3.40 3.47 3.41	3.49 $\pm$ 0.18	NS
(female)	left	3.61 4.05 4.22 3.73 –	3.90 $\pm$ 0.28	

[a] Standard deviation.
[b] Not significant (two-tailed Student's *t*-test: $p > 0.05$).

TABLE 2

Inter-subject variation for rate of butyl p-hydroxybenzoate loss into the human buccal membrane in 30 apparently healthy adult subjects ($n \geq 2$)

Male subjects		Female subjects	
Subject	Rate of drug loss [a] ($k_{obs} \times 10^{-5}$ s^{-1})	Subject	Rate of drug loss [a] ($k_{obs} \times 10^{-5}$ s^{-1})
MDM	3.78 [b]	DJV [c]	3.68
BRP	4.17 [b]	SHC	3.18
MJR	3.53 [b]	RVM	3.25
TWK	3.31	BDS	4.37
KAM	3.76	MJL	3.68
RSP	4.05	EMF	3.96
SAL	3.21	FCM	3.38
KPO	3.10	SMP	3.41
JAB	3.15	NJB	3.27
DJG	3.30	SHJ	3.76
KIP	3.27	HLL	3.64
PJS	3.93	SMS	3.80
SAH	3.85	SLO	3.11
PAS	3.45	LMJ	3.12
NJG	3.29	DLW	3.42
Average (±SD)	3.54 (±0.35)	Average (±SD)	3.53 (±0.35)

[a] $n = 2$.
[b] $n = 10$.
[c] $n = 9$.

where k_{obs} is the apparent first-order rate constant for drug loss from the buccal perfusion cell (s^{-1}), C_1^0 denotes the initial drug concentration and C_1 is the drug concentration remaining at time t. Slopes ($= k_{obs}$) were determined in each case by linear regression analysis (correlation coefficient $r > 0.99$, $n \geq 15$). In general evaluation of the order and magnitude of a kinetic process usually requires the monitoring and analysis of data collected over several half-lives of the process. Measurement of drug loss over such extended periods would be inappropriate using the buccal perfusion technique described in this paper. However, the author has conducted a perfusion experiment using butyl *p*-hydroxybenzoate for a period of 180 min which provided a value for the apparent first-order rate constant for drug loss (k_{obs}) of 3.18×10^{-5} s^{-1} (linear regression analysis correlation coefficient $r = 0.999$, $n = 30$). This value compares favourably with those documented in Tables 1 and 2, thus it would appear to be valid to monitor drug loss over a limited time period (40–60 min) and to extrapolate.

Intra-subject variation for the rate of butyl parahydroxybenzoate loss into left and right buccal membranes for four subjects is shown in Table 1. Intra-subject variation appears small. Table 1 also shows that there was no significant difference in the rate of drug loss between any given subjects left and right buccal membrane (two-tailed Student's *t*-test $p > 0.05$). The rates of drug loss estimated for 30 different subjects ($n \geq 2$ for each subject) are given in Table 2 which shows that inter-subject variation in this study was greater than intra-subject variation. Intra- and inter-subject variations compare favourably with those determined using the buccal absorption test (Beckett and Triggs, 1967) and are an improvement over other cells of the type depicted in this paper (Barsuhn et al., 1988). In addition, Table 2 shows that there was no apparent difference in the rate of drug loss between male and female subjects. This is in contrast to documented differences in drug loss from the oral cavity between male and female subjects during a buccal absorption test (Odumosa and Wilson, 1971; Temple and Schesmer, 1978).

The following conclusions may be drawn from this study:

(a) The buccal perfusion cell methodology provides a simple, reliable means of estimating the kinetics of drug loss from the oral cavity into a specified oral cavity membrane under closely controlled conditions.

(b) Both intra- and inter-subject variation for the rate of drug loss from the buccal perfusion cell into the human buccal membrane were shown to be small.

(c) There appears to be no difference in the rate of drug loss between a subject's left and right buccal membrane or between male and female subjects.

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