Department of Pharmaceutical Technology[1], Faculty of Pharmacy, Hacettepe University, Department of Neurology[2], Faculty of Medicine, Gazi University, Department of Periodontology[3], Faculty of Dentistry, Ankara, Turkey

In vitro/in vivo studies on a buccal bioadhesive tablet formulation of carbamazepine

G. İKINCI[1], Y. ÇAPAN[1], S. ŞENEL[1], E. ALAADDINOĞLU[3], T. DALKARA[2], and A. A. HINCAL[1]

A buccoadhesive controlled-release system for delivery of carbamazepine (CBZ) was prepared by compression of hydroxypropyl methylcellulose (HPMC) and carbomer, incorporating a penetration enhancer, sodium glycodeoxycholate (GDC). The release behaviour of systems containing CBZ and various amounts of the two polymers with and without GDC was found to be non-Fickian. Formation of an interpolymer complex between HPMC and carbomer was confirmed in acidic medium by turbidity, viscosity and FT-IR measurements. Addition of the drug to the buccoadhesive formulation reduced the adhesion force significantly ($p < 0.1$). GDC did not have any effect on bioadhesion. Permeability of bovine buccal mucosa to CBZ was determined using Ussing diffusion chambers [1]. *In vivo* interaction between the tablet and tissue was examined histologically as well as by scoring mucosal irritation. Histological changes observed in the buccal epithelium after 4 h contact with the tablets containing GDC recovered completely within 24 h after removal. No measurable plasma level of CBZ was obtained either in the absence or presence of GDC.

1. Introduction

The buccal mucosa is a potential site for delivery of drugs to the systemic circulation. A drug administered across the buccal mucosa enters the systemic circulation directly, thereby minimising first-pass liver and gastro-intestinal (GI) metabolism and increasing the speed of delivery compared with oral and intramuscular routes. However, the oral mucosal permeability of some compounds is too low to allow plasma concentrations to reach therapeutic levels. Various permeation enhancers have been used to facilitate the transmucosal delivery of these compounds [2]. Among the penetration enhancers used in transmucosal formulations of drugs bile salts are most common [1, 3].

Carbamazepine (CBZ) is a widely prescribed anticonvulsant drug used in the treatment of epilepsy and trigeminal neuralgia [4]. The drug is characterised by a slow and irregular gastro-intestinal absorption because of its low water solubility [5]. Following oral administration, the amount of CBZ reaching the systemic circulation is reduced by first-pass metabolism. On repeated administration, the elimination half-life is markedly decreased due to auto-induction of the microsomal enzyme system. The limitations of conventional oral administration of CBZ can be overcome by administration via the buccal mucosa in a bioadhesive formulation which can attach the delivery system to the mucosa and allow the drug to reach the systemic circulation, bypassing the liver and escaping first-pass metabolism. HPMC and carbomer have been used in this study as principal excipients to achieve such a formulation. One of the objectives of the present study was to elucidate factors affecting the bioadhesion property of compressed tablets consisting of HPMC and carbomer. Release characteristics of CBZ from tablets with different matrices were studied *in vitro*. The permeation of CBZ across bovine buccal mucosa was investigated *ex vivo*. Histological investigations of the effect of bioadhesive tablets on the buccal mucosa and absorption of CBZ from the tablet were carried out *in vivo*.

2. Investigations, results and discussion

2.1. Characterisation of interpolymer complex formation

The formation of interpolymer complexes was studied using turbidity and viscosity measurements in media of various pH values (pH 3.0, 4.5 and 6.0). The turbidity of the HPMC/carbomer mixture solution as a function of the weight ratio of HPMC to carbomer is shown in Fig. 1. Maximum turbidity was observed at a ratio of 6 : 4 at pH 3.0. This result suggested that the interpolymer complex of HPMC/carbomer is formed in an acidic medium. Fig. 2 shows the specific viscosity of the supernatant in HPMC/carbomer mixture solutions as a function of the weight

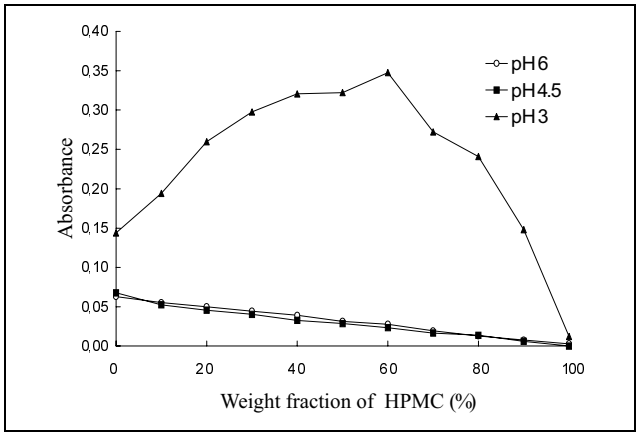

Fig. 1: Turbidity of the HPMC/CP system as a function of polymer mixing ratio in media of various pH values at 37 °C (total polymer concentration 0.05%, n = 3)

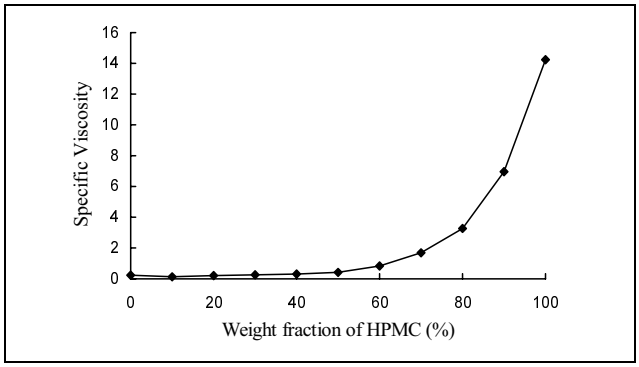

Fig. 2: Viscosity of supernatant solution in the HPMC/CP system as a function of polymer mixing ratio at pH 3 and 37 °C (total polymer concentration, 0.5%). (The viscosity was determined by the use of an Ubbelohde viscometer (Schott-Gen, Mainz, K: 0.004765))

ratio of HPMC to carbomer in the acidic medium (pH 3.0). A decrease in viscosity of the HPMC/carbomer mixture was observed indicating an interpolymer complex was formed in the acidic medium. Similar results were obtained in previous studies [6, 7].

The complexation between HPMC and carbomer was also investigated by FT-IR spectroscopy. A single peak at 1713 cm^{-1} was recorded for pure carbomer corresponding to stretching vibration of the carbonyl (C=O). When HPMC was mixed with carbomer at a 6 : 4 ratio, the peak was observed to shift to 1730 cm^{-1}, and the peak intensity decreased. These results can be considered spectroscopic evidence of interpolymer complex formation between HPMC and carbomer.

2.2. Bioadhesion properties

The adhesion forces of tablets to bovine sublingual mucosa at different HPMC/carbomer ratios are given in Table 1. No significant changes in adhesion were obtained with tablets prepared at different mixing ratios of polymers ($p > 0.1$) showing that adhesion is not affected by any interpolymer interaction. When the drug was incorporated into the formulation, a significant decrease in mean bioadhesive force was observed ($p < 0.1$). The presence of drug appears to reduce the interaction of carbomer with the tissue to form hydrogen bonds for bioadhesion. No significant difference in adhesive bond strength was observed between GDC-containing and GDC-free tablets. These results are in agreement with those given in the literature [8, 9]. Bioadhesion is important in prolonging the residence time of the delivery system at the application site in order to improve bioavailability. From the results obtained here, we can conclude that bioadhesion maintained with the formulations developed would be sufficient to provide quite a long residence time.

2.3. In vitro release of CBZ from bioadhesive tablets

The amount of CBZ released from tablets decreased with increasing polymer concentration. The percentages of CBZ released from formulation A containing 100 mg CBZ and 200 mg polymer mixture were $3.7 \pm 0.5\%$, $7.5 \pm 0.7\%$ and $13.3 \pm 1.2\%$ at 2 h, 4 h and 8 h, respectively. For formulation B (containing 200 mg CBZ and 100 mg polymer mixture) the percentages released were $4.8 \pm 1.3\%$, $9.1 \pm 1.6\%$ and $17.0 \pm 1.7\%$ and for the formulation containing GDC (C) $5.9 \pm 0.5\%$, $11.0 \pm 2.4\%$ and $19.8 \pm 1.7\%$ after 2 h, 4 h and 8 h, respectively. The other formulations showed a similar release profile, whilst the percentage of CBZ release decreased with increasing HPMC/carbomer ratio. Addition of GDC to the tablet formulation resulted in an increase in release rate. This can be explained by the increased solubility of CBZ in the presence of GDC.

Table 1: Bioadhesion force (N) for buccal adhesive tablets in contact with bovine sublingual mucus (n = 6)

Formulation*	Mean ± SD (N)
A	7.17 ± 1.33
B	5.81 ± 5.56
C	3.46 ± 2.44
D	10.46 ± 2.36
E	12.25 ± 2.93

* for composition of formulations see Table 3

The mechanism of release was investigated using the following semi-empirical equation (Eq. 1) [10]

$$Mt/M\infty = kt^n \quad (1)$$

($Mt/M\infty$; the fraction of drug released up to time t, k; a constant representing structural and geometric characteristics of the tablet, n; a diffusional exponent indicative of the mechanism of release). The calculated parameters are given in Table 2. In all cases, n was found to be close to 1, indicating apparent zero-order release.

Table 2: Kinetic constants (k), release exponent (n) and determination coefficients (r^2) following linear regression of dissolution data of buccal adhesive carbamazepine tablets (n = 6)

Formulation	N	r^2	k
A	0.90 ± 0.06	0.996	1.66
B	0.83 ± 0.10	0.995	2.03
C	0.93 ± 0.10	0.995	2.85

* for composition of formulations see Table 3.

2.4. Ex-vivo permeation of CBZ through buccal epithelium

The flux curve of CBZ across bovine buccal mucosa is shown in Fig. 3. The permeability coefficient calculated from the slope (between 1 and 8 h) of the flux curve obtained by plotting the cumulative permeated amount against time was found to be $6.62 \pm 1.34 \times 10^{-6}$ cm · s^{-1}.

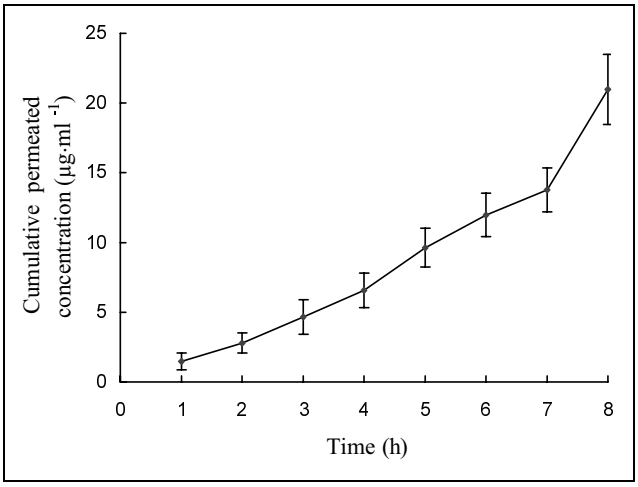

Fig. 3: Flux curve of CBZ across bovine buccal mucosa (n = 6)

2.5. Histological studies

Immediately after 4 h contact with the tablets, the buccal mucosa did not appear as smooth as the intact mucosa. The changes in appearance of the buccal mucosa were followed for 24 h. Especially with the tablets incorporating GDC, a clinically irregular and dehydrated appearance was observed. In our previous study {11] histological

Table 3: Buccal bioadhesive tablet formulation

Formulation	CBZ (mg)	HPMC (mg)	CP (mg)	GDC (mg)
A	100	100	100	–
B	200	20	80	–
C	200	15	70	15
D	–	15	70	15
E	–	15	70	–

changes such as loss of upper cell layers, formation of vacuoles and swelling in the cells were shown to occur in the buccal epithelium after 4 h contact with tablets containing GDC when compared to that with GDC free tablets. In this study, 24 h after removal of the tablet, light micrographs showed no difference in buccal epithelium between GDC free and GDC containing tablets. Thus, it can be concluded that following removal of the tablet, the mucosa recovers gradually within a 24 h period. Electron micrographs supported this observation at the ultrastructural level, with normal appearance of mitochondria and desmosomes at the end of 24 h.

2.6. Mucosal irritation

Mucosal irritation scores observed after 4 h application of GDC-free and GDC-containing tablets are shown in Fig 4. With tablets containing GDC, irritation was reported with erythema and edema formation at the site of application. After 24 h, the mucosal irritation scores were close to 0, indicating that mucosal irritation is reversible, which is also supported by light and electron micrographs.

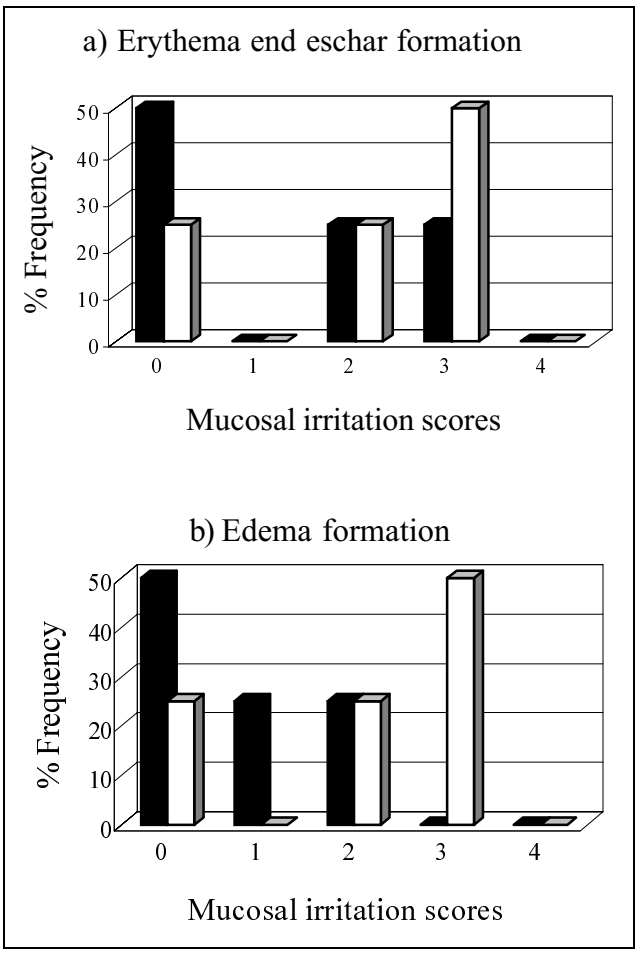

Fig. 4: Mucosal irritation scores for the GDC free (filled bars) and GDC containing (empty bars) tablets in regard to erythema and eschar formation (a) and edema formation (b) (n = 4)

2.7. Pharmacokinetic studies

Although *ex vivo* studies had shown that CBZ permeated across the buccal mucosa, following *in vivo* application of the tablet, no measurable plasma level of CBZ was obtained during 8 h either in the presence or in the absence of GDC. Thus we can conclude that the formulation developed may be proposed as a suitable formulation for buccal administrations but that the buccal mucosa seems to be not an effective route for CBZ.

Table 4: Criteria of judgement for irritation test [12]

	Buccal mucosa reaction	Score
A	Erythema and eschar formation	
	No erythema	0
	Very slight erythema	1
	Well-defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (beet root redness) to slight eschar formation (injuries in depth)	4
B	Edema formation	
	No edema	0
	Very slight edema (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

3. Experimental

3.1. Materials

Carbamazepine (Abdi İbrahim İlaç San. Tic. A.Ş., Turkey), hydroxypropyl methylcellulose (HPMC) (Methocel® K4M, Colorcon, UK), carbomer (Carbopol® 974 PNF, BF Goodrich, Belgium) (CP), magnesium stearate (E. Merck, Germany), polymethylmethacrylate (Eudragit® RSPM, Röhm Pharma, Germany) and sodium glycodeoxycholate (GDC) (Sigma, St. Louis, MO, USA) were used as received.

3.2. Formulation studies

Tablets were prepared by direct compression of CBZ with a mixture of HPMC and CP at different ratios. GDC was incorporated as a penetration enhancer and magnesium stearate as a lubricant (1% w/w). The powders were mixed and directly compressed on a single Korsch EK/O punch-tablet machine using a flat non-beveled punch of 12 mm diameter. Control tablets were prepared with the polymer mixture and magnesium stearate only. Compositions of buccal adhesive tablet formulations are given in Table 3.

3.3. Characterisation of interpolymer complex formation

Interpolymer complex formation between HPMC and carbomer was investigated by turbidity, viscosity and FT-IR measurements as described in previous studies [6, 7].

3.4. Bioadhesion studies

Tensile tests were performed on a Instron apparatus (Model 4301) using bovine sublingual mucosa [7]. Cyanoacrylate adhesive was used to fix the tablet and the bovine sublingual mucosa to the upper and lower metallic supports, respectively. 20 µl of distilled water was dropped on the tablet surface, and the tablet and mucosa were brought in contact with a force of 0.5 N and kept in this condition for 10 min. Then the tensile test was performed at a constant extension rate of 5 mm · min^{-1}.

3.5. Release studies

For in vitro release of CBZ from buccal adhesive tablets, a dissolution tester (Prolabo-Paris) (USP XXII) was used at 75 rpm paddle speed in 900 ml distilled water containing 1% sodium lauryl sulfate. Each tablet was inserted in a metal die having a central hole 12 mm in diameter, which was sealed at the lower end with paraffin wax so that the drug could be released only from the upper face of the device. Samples were withdrawn at appropriate time intervals, filtered and assayed for CBZ at 285 nm using a spectrophotometer (UV-160A Shimadzu, Japan).

3.6. Permeation of CBZ through buccal epithelium

Bovine buccal mucosa was mounted in Ussing diffusion chambers [1] with a diffusion area of 1.08 cm^2 and a compartment volume of 5 ml. After an equilibration period of 30 min with phosphate buffer solution (PBS) on both sides, the acceptor side was filled with the isotonic PBS and the do-

nor side with 0.1 mg · ml^{-1} CBZ in PBS. The diffusion studies were carried out at 34 °C and carbogen (a mixture of 95% oxygen and 5% carbon dioxide) was circulated through the cells in order to maintain the viability of the tissue and to provide stirring [1]. Samples of 0.5 ml were taken from the acceptor compartment at hourly intervals for 8 h, and replaced with the same amount of fresh buffer solution. The samples were assayed by the HPLC method described by MacKichen [13].

The cumulative amount of the drug permeated was plotted versus time and the flux was calculated from the steady-state part of the curve. The permeability coefficient (Kp) was calculated using the following equation (Eq. 2)

$$Kp = (dQ/dt)/(\Delta C \cdot A) \quad (2)$$

where dQ/dt is the average slope obtained for the 1–8 h time interval of the curve; ΔC the concentration difference across the mucosa; and A the area of diffusion.

3.7. In vivo studies

3.7.1. Histological studies

Eight non-smoking volunteers of both sexes (1 female and 7 male), aged between 21 and 24 (±1) participated in histological evaluations. Informed consent from the volunteers and the approval of the Ethics Committee of Hacettepe University was obtained. In all volunteers, the tablet was placed on the buccal mucosa in the region of the upper molar. The tablet was fixed with slight manual pressure. At the end of a 4 h period, the tablet was removed. 24 h after removal, biopsies were taken from that region and following a classical fixation, dehydration and embedding procedure [11], 1 µm sections were cut, stained with methylene blue and examined under an optical microscope (Nikon, Japan). For transmission electron microscopy, ultra thin sections (80–100 nm) were cut and collected on copper grids. Sections were counterstained with uranyl acetate and lead citrate before examination in the electron microscope (Jeol JEM 1200 EX, Japan).

3.7.2. Measurement of mucosal irritation

Mucosal irritation at the buccal site facing the buccal adhesive tablet, which was applied on the upper molar region was observed with the naked eye and photomicrographs were taken. The extent of the irritation at the buccal mucosa was evaluated by the criteria given in Table 4 [12]. Scores were given both for erythema and eschar formation, and for edema formation.

3.7.3. Pharmacokinetic studies

Eight healthy volunteers, seven females and one male, aged between 21 and 28 years (23 ± 2) and with weight between 53 and 60 kg (57 ± 3), agreed to participate in the study after explanation of the experimental protocol and written informed consent was obtained from each. The subjects received no medication for at least two weeks before the study. All subjects were judged to be healthy on the basis of medical history, physical examination, serum chemistry profile, complete blood count and urine analysis. Approval was obtained from the Ethics Committee of the Hospital of Hacettepe University.

Formulations B and C were investigated *in vivo* in a pilot study. Before placing the buccal adhesive tablet, the sides and top of the tablet, which will not be in contact with the mucosa, were coated with water impermeable polymer Eudragit® RSPM (12.5% dry Eudragit® RSPM was dissolved in a mixture of 60% (w/w) isopropyl alcohol and 40% (w/w) acetone) in order to obtain unidirectional contact and avoid an interaction with the saliva. The coated tablet was attached to the buccal mucosa for 8 h in the region of the upper molar. Samples were obtained (5 ml via an indwelling heparinized scalp-vein needle) at 1, 2, 4, 8 and 24 h after application and placed in citrated tubes. Following centrifugation, samples were stored in the deep-freeze until analysis. CBZ concentration in plasma was determined using a modification of the method proposed by MacKichan [13].

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Received January 28, 2000
Accepted February 2, 2000

Prof. Dr. Yılmaz Çapan
Hacettepe University
Faculty of Pharmacy
Department of Pharmaceutical Technology
06600 Ankara
Turkey
huetb-e@tr-net.net.tr