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Development and in vitro/in vivo testing of mucoadhesive buccal patches releasing benzydamine and lidocaine

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Abstract

Mucoadhesive patches releasing topical drugs in the oral cavity at a slow, predetermined rate may present distinct advantages over traditional dosage forms such as mouthwashes, oral gels and lozenges. The present study was concerned with the preparation and evaluation of mucoadhesive buccal patches for controlled release of benzydamine (BNZ) and lidocaine (LDC). The drugs were used as hydrochlorides, or, to reduce their solubility and improve their release characteristics, as salts with pectin or polyacrylic acid. A LDC-tannic acid complex was also prepared and tested. After an initial screening of mucoadhesive polymers, tamarind gum (TG), a polysaccharide obtained from the seeds of *Tamarindus indica*, was selected as the adhesive component. In vitro tests, carried out on a cell line of human buccal epithelial origin, indicated a very low sensitivity for TG. The patches, prepared by compressing appropriate mixtures containing the drug salts/complexes, lactose and TG, were tested in vitro for mucoadhesion and drug release, and in vivo on human volunteers for retention and release of BNZ. The devices containing the salts of BNZ with pectin and polyacrylic acid, and the complex of LDC with tannic acid showed zero-order release kinetics in vitro. The patches adhered for over 8 h to the upper gums of the volunteers, and were perfectly tolerated. BNZ hydrochloride was released in vivo and in vitro with practically identical profiles.

Keywords: Buccal patches; Mucoadhesion; Polyacrylic acid; Polycarbophil; Xanthan gum; Tamarind gum; Pectin; Tannic acid; Benzydamine; Lidocaine; Sustained release; In vitro cellular sensitivity

1. Introduction

In recent years, the development of mucoadhesive systems as potential delivery systems for controlled release of drugs has attracted significant interest (Park and Robinson, 1984; Peppas and Buri, 1985). These dosage forms can be administered by different routes (e.g. ocular, buccal, nasal, rectal, vaginal), either for topical therapy or for systemic trans-mucosal drug delivery.

In particular, the buccal route appears to offer

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a series of advantages, such as good accessibility, robustness of the epithelium, facile removal of the dosage form in case of need, relatively low enzymatic activity, possibility of elimination of the administered dosage form from the buccal area by natural clearance mechanisms, satisfactory patient acceptance and compliance. Moreover, in the case of systemic delivery, this route offers the advantage of partly circumventing drug degradation in the GI tract and of avoiding the hepatic first pass metabolism.

Drug classes used topically in the mouth are antimicrobials, anti-inflammatories and local anaesthetics, which are commonly administered in lozenges, mouthwashes and oral gels. Drug release from these dosage forms is rapid and uncontrolled: a conventional lozenge formulation produces effective drug levels locally in the mouth for a short period (< 1 h), and repeated administration is usually limited to a maximum of 10 units per day, on account of gastric irritancy and systemic toxicity of the large quantity of drug swallowed (Collins and Deasy, 1990). Furthermore, such products are unsuitable for overnight therapy.

Aim of the present study was to develop a new mucoadhesive controlled-release device for topical buccal drug delivery. The investigation consisted essentially of the following steps: (a) preparation by compression of medicated and non-medicated buccal patches, containing different mucoadhesive polymers, (b) identification, by suitable in vitro tests, of the mucoadhesive polymer showing the best performance, (c) evaluation in vitro of the sensitivity to the polymer of a cell line of human buccal epithelial origin, (d) testing of the patches in vitro for drug release, and (e) testing on human volunteers for buccal retention and drug release. The drugs selected for the study were benzydamine (BNZ) and lidocaine (LDC). Since the drug hydrochlorides were released from the patches at fast and uncontrolled rates, salification with acidic polymers (polyacrylic acid, pectin) or (in the case of LDC) complexation with tannic acid was resorted to as an attempt to improve the release characteristics.

2. Materials and methods

2.1. Materials

The following materials were used: benzydamine hydrochloride (BNZ-HCl, A.C.R.A.F. S.p.A., Ancona, Italy); lidocaine hydrochloride (LDC HCl, Prodotti Gianni S.p.A., Milano, Italy); lidocaine base (LDC, S.I.M.S. s.r.l., Firenze, Italy); tamarind gum (TG, Glyloid® 3S, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan); polycarbophil (PCP, Noveon® AA1, Goodrich Chem. Corp., Cleveland, OH); polyacrylic acid (PAA, Carbopol® 940, Goodrich Chem. Corp., Cleveland, OH); xanthan gum (XG, Keltrol® TF, Kelco, Chicago, IL); pectin (PCT, Cesapectin® LM-32, Cesalpinia S.p.A., Bergamo, Italy); tannic acid (TA, Carlo Erba, Milan, Italy); lactose (LT, Carlo Erba, Milan, Italy); hog gastric mucin (HGM, Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan). All other reagents were of at least analytical grade.

Before use, TG and PCT were dissolved in water, filtered (0.45 μm, Sartorius 11306-47 membrane filter) and freeze-dried. The Na salt of PAA was obtained by freeze-drying a neutralised (1 N NaOH) solution of the polymer. Benzydamine base (BNZ) was obtained by extracting an alkalinised solution of BNZ-HCl with ethyl ether.

All other materials were used as received.

2.2. Analytical methods

BNZ was assayed by UV spectrophotometry (Shimadzu UV-2101 PC) at 306 nm, and LDC by HPLC (Shimadzu LC-6A liquid chromatograph equipped with SPD-M6A photodiode array detector, 20 μl-Rheodyne injector, and computer integrating system). The column (30 cm $\times$ 3.9 mm) was packed with Bondclone C18 (pore size 10 μm, Phenomenex). The mobile phase was methanol-water (60:40, v/v) containing 0.027 M Na_2SO_4 and 0.01 M $C_6H_{13}O_3SNa \cdot H_2O$ (pH adjusted at 3.5 with 10% H_2SO_4), flow rate was 0.8 ml/min. The retention time of LDC was 6.8 min; the determination was performed in the range 203–205 nm.

Table 1
Neutralisation equivalents (mEq/g) of the polymers, and quantities of drugs used for the preparation of the salts or complex

Salt forming or complexing agent	mEq/g	BNZ[a] (mg)	LDC[a] (mg)
PAA	10.00	618.80	468.66
PCT	1.40	86.63	–
TA	–	–	125.13

[a]The indicated drug amount is equivalent to 200 mg of PAA, PCT or TA.

The amount of drug in the samples was calculated using appropriate standard curves.

2.3. Preparation of BNZ and LDC salts/complexes

The neutralisation equivalents (mEq/g) of the acidic polymers, PAA and PCT, determined by potentiometric direct titration with 0.01 N NaOH, are reported in Table 1.

The BNZ salt of PCT (BNZ-PCT) was prepared by stirring in distilled water (20 ml) the amounts of drug and polymer indicated in Table 1, then by freeze-drying the mixture. The BNZ or LDC salts of PAA (BNZ-PAA and LDC-PAA) were prepared by mixing 95% ethanol solutions (20 ml), each containing the amounts of drug and polymer indicated in Table 1. The resulting precipitates were filtered off and dried in a vacuum oven.

The BNZ and LDC base content of the salts (BNZ-PCT, BNZ-PAA, and LDC-PAA) was determined by the following method. An amount of the salt (10 mg) was dissolved in 50 ml of water (BNZ-PCT) or in 50 ml of pH 6.8, 66.7 mM isotonic phosphate buffer (BNZ-PAA, and LDC-PAA); the sample was filtered (Millex AA 0.8 μm, Millipore) and analysed as indicated above.

The LDC-TA complex (LDC-TA) was prepared as indicated by Cavallito and Jewell (1958). LDC base and TA (amounts indicated in Table 1) were individually dissolved in 95% ethanol (5 ml) and the solutions were mixed. The mixture was diluted with ice water to complete precipitation of the tannate. The resulting precipitate was filtered off, washed with ice water, and dried in a vacuum oven. The amorphous solid was analysed for nitrogen content: the experimental and theoretical basic nitrogen contents (assuming 4:1, 5:1 and 6:1 LDC/TA ratios) are reported in Table 2. The 5:1 ratio appears best to represent the composition of the complex. The LDC base content of the LDC-TA complex was also assessed by HPLC analysis of a filtered (Millex AA 0.8 μm, Millipore) water solution of the material (1.0 mg in 5.0 ml). The amount of LDC found corresponded to a 5:1 complex.

2.4. Preparation of the buccal patches

Medicated patches, whose composition is indicated in Table 3, and non-medicated patches made of polymers alone (50 mg of PAA, PCP, TG or XG) were obtained by compressing for 30 s with a force of 9800 N the components (polymers alone or mixtures) with an hydraulic press (Perkin-Elmer) fitted with flat, 13.0-mm punches.

The B2 patches were dry-coated with TG by compressing on both sides of the matrices (force 9800 N) thin disks of precompressed polymer (100 mg; diameter 13.0 mm).

The amount of drug in the patches was determined by dissolving one patch in 500 ml of water. The filtered (Millex AA 0.8 μm, Millipore) solution was analysed as indicated above in Analytical methods.

Table 2
Composition of lidocaine tannate (LDC/TA)

	Ratio (base/tannic acid)		
	4:1	5:1	6:1
% N found		4.78	
% N theoretical	4.24	4.87	5.41

Table 3
Composition of the buccal patches

Patch type	Drug (mg)	TG (mg)	Lactose (mg)
B1	BNZ-HCl 10.0	200	100
B2[a]	BNZ-PCT 30.0	400	-
B3	BNZ-PAA 19.6	200	-
L1	LDC-HCl 3.0	200	100
L2	LDC-PAA 8.0	200	-
L3	LDC-TA 7.5	200	-

[a]This device was dry-coated on both sides with TG.

2.5. Mucoadhesion tests

The mucoadhesive properties of the non-medicated and medicated patches were evaluated by measuring their work of adhesion (W) on a mucous substrate (Ponchel et al., 1987). The latter consisted of a 25% w/w dispersion of HGM in water (0.125 g), spread uniformly on wet filter-paper (Saettone et al., 1989). The apparatus (Fig. 1) consisted of a testing cell connected to a custom-made tensile apparatus fitted with force and elongation transducers, whose output was fed to a computer equipped with data acquisition software (TP 5008, TiePie Engineering, NL-Leeuwarden). Before testing, all patches were hydrated by immersion (5 min, room temperature) in 66.7 mM, pH 6.8, isotonic phosphate buffer; they were then placed between the upper and lower mucous surface in the testing cell. All detachment tests were carried out at 36 ± 0.5°C in air, in the absence of external bathing fluid. The resulting force vs. elongation curves were analysed using KaleidaGraph® software (Synergy Software, Reading, PA). The W values (work of adhesion, corresponding to the area under the detachment force vs. elongation curves) reported in Fig. 2 are the average of at least six determinations.

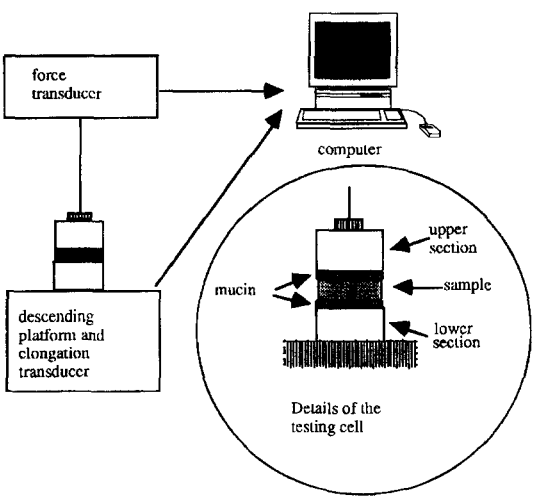

Fig. 1. Scheme of the tensile apparatus and details of the testing cell.

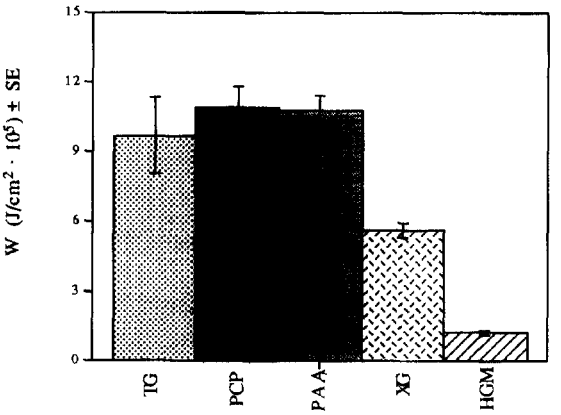

Fig. 2. Work of adhesion of the polymers under investigation (mean ± S.E., n = 6). HGM refers to the work of cohesion of mucin alone, in the absence of interposed polymers.

2.6. Cellular sensitivity tests

The cellular sensitivity of the individual excipients of the B1 patch was studied by means of the cell line TR146 of human buccal origin (Rupniak et al., 1985; Jacobsen et al., 1995), by applying an adapted colorimetric method, MTS/PMS assay. The number of viable cells after short-time and long-time (4 h and 24 h, respectively) incubation with the excipients was estimated.

2.7. In vitro release studies

The release tests were carried out at 36 ± 0.5°C using the USP XXI (US Pharmacopeia XXI, 1985) rotating basket apparatus at 75 rev./min. The dissolution medium consisted of 300 ml (B1, B2 and B3 patches), or 150 ml (L1, L2 and L3 patches) of pH 6.8, 66.7 mM isotonic phos-

phate buffer. Samples of the solution (10 ml and 5 ml for the patches containing BNZ and LDC, respectively) were withdrawn at appropriate intervals and replaced with fresh buffer. The samples were filtered (Millex AA 0.8 μm, Millipore) and analysed as indicated before.

The release data were analysed using the well known semi-empirical equation:

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ is the drug fraction released at time t, and k and n are constants incorporating structural and geometric characteristics of the drug/polymer system (Korsmeyer and Peppas, 1983; Peppas, 1985). In particular, the exponent n is related to the release mechanism: its value ranges from 0.5 (Fickian release) to 1.0 (zero-order kinetics), while n values between 0.5 and 1.0 are indicative of non-Fickian, 'anomalous' release. The n values used for analysis of the drug release mechanism from the patches were determined from log (M_t/M_∞) vs. log (t) plots; data fitting was performed on the first part of the curves ($M_t/M_\infty < 0.7$).

2.8. Release tests on human volunteers

The B1 patches were applied during the day to the upper gums of healthy volunteers ($n = 6$). The patches were removed at appropriate intervals and dissolved in water (500 ml). The filtered solutions (Millex AA 0.8 μm, Millipore) were analysed by the method indicated in Analytical methods. The amount of released BNZ was calculated by difference (initial amount − amount residual at time t).

3. Results and discussion

3.1. Mucoadhesive properties of the polymers

The results of a preliminary screening performed on four non-medicated matrices prepared with different polymers (TG, PCP, PAA and XG) are illustrated in Fig. 2. The data are reported as the work of adhesion (W) of the patches to the mucin substrate, corresponding to the area under the detachment force vs. elongation curve. The value relevant to the work of cohesion of mucin alone (HGM), in the absence of any interposed matrix, is also shown for comparison. As shown in the figure, the TG-containing matrices appeared to possess the best mucoadhesive properties, even if the differences among the tested samples were not statistically significant. The presence of drug had apparently no influence on mucoadhesion: the work of adhesion on mucin of the non-medicated matrices was not significantly different from that of corresponding medicated patches. On consideration of the results indicated above, TG was selected as the mucoadhesive component. TG is obtained from the endosperm of the seeds of the Tamarind tree (*Tamarindus indica*), which is indigenous to India and South East Asia. The seeds contain large proportions of a non-starch polysaccharide that functions as an energy reserve (Gidley et al., 1991). The exact composition of the carbohydrate is not fully known. A proposed structure consists of a main chain, β-D-(1→4) linked glucopyranosyl units, with a side chain consisting of xylopyranosyl and D-galactopyranosyl units. The molecular weight of the polysaccharide is above 50 000 (Gerard, 1980). TG has many properties similar to those of fruit pectins, and its applications in the food, cosmetic, paper, textile industry, etc. are numerous.

3.2. Cellular sensitivity tests

The investigation of short- and long-time cellular sensitivity indicated that the TR146 cells were not notably sensitive to 0.25% TG, 0.125% LT, or TG/LT (0.25:0.125%).

These results corroborated the preference for TG, already chosen on the basis of the mucoadhesive properties outlined in the previous paragraph.

3.3. In vitro release studies

The drug release profiles for the patches containing BNZ and LDC are shown in Figs. 3 and 4, respectively. For each patch, the regression coefficient, r, the n value ($\pm$95% confidence limits), the instantaneous release rate at 50% release

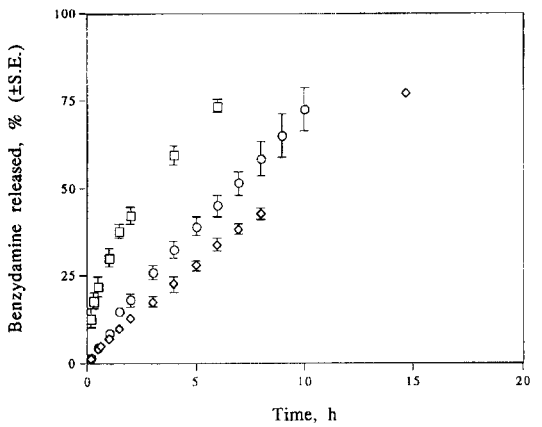

Fig. 3. Benzydamine released (%) in vitro from the B1 (□); B2 (◇) and B3 (○) patches (mean ± S.E., n = 6).

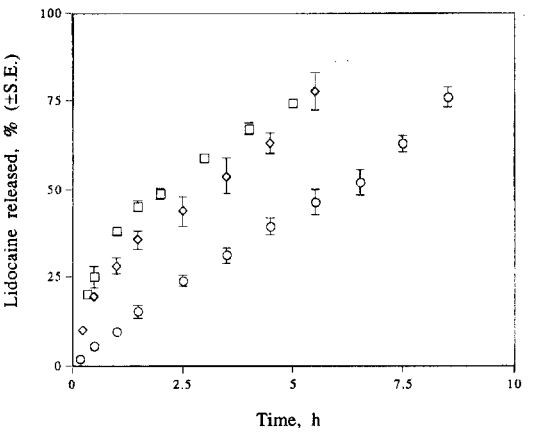

Fig. 4. Lidocaine released (%) in vitro from the L1 (□), L2 (◇), and L3 (○) patches (mean ± S.E., n = 6).

($R_{50\%}$) and the time required to reach 50% release ($t_{50\%}$) are reported in Table 4.

The data indicate that BNZ-HCl was released rapidly ($t_{50\%}$ = 2.78 h and $R_{50\%}$ = 8.83 h^{-1}) with diffusive kinetics (n = 0.492 ± 0.0073) from the B1 patch.

In the case of the B2 and B3 patches the release kinetics shifted towards zero-order (n values of 0.924 and 0.944, respectively), and the release rates (Table 4) were lower. It should be noted that the B2 patch contained the BNZ-PCT salt, and was dry coated on both sides with TG: uncoated B2 patches were found in preliminary experiments to release BNZ with 'anomalous' kinetics ($0.5 < n < 1$). Conversely, salification of BNZ with PAA, as in the B3 patch, was sufficient per se to obtain approximate zero-order release kinetics.

On the other hand, salification of LDC with PAA, as in the L2 patch, resulted in 'anomalous' release behaviour (n = 0.610 ± 0.071). Only the L3 patch, containing LDC as tannic acid complex, exhibited constant-rate release kinetics.

3.4. In vivo release studies

Fig. 5 illustrates the in vitro and in vivo release profiles of BNZ from the B1 patches: the coincidence of the two curves is evident.

The comfort, irritation, duration of adhesion and ease of removal of the patches were also evaluated during the test on human volunteers. It was noted that the patches exhibited a satisfactory adhesion to the gum tissues for over 8 h. The local tolerance was good, with only minor local reactions at the site of application (slight crum-

Table 4
In vitro release parameters of B and L patches, containing BNZ and LDC, respectively

Patch type	Regression coefficient (r)	n ± 95% C.L.	$t^{a}_{50\%}$ (h)	$R^{b}_{50\%}$ (h^{-1})
B1	0.999	0.488 ± 0.008	2.78	8.83
B2	0.998	0.924 ± 0.029	9.23	5.00
B3	0.999	0.944 ± 0.034	6.52	7.23
L1	0.996	0.464 ± 0.039	2.08	11.42
L2	0.993	0.610 ± 0.071	2.88	10.60
L3	0.999	0.925 ± 0.024	5.80	7.94

[a]Time required for release of 50% drug.
[b]Instantaneous release rate at time of 50% release.

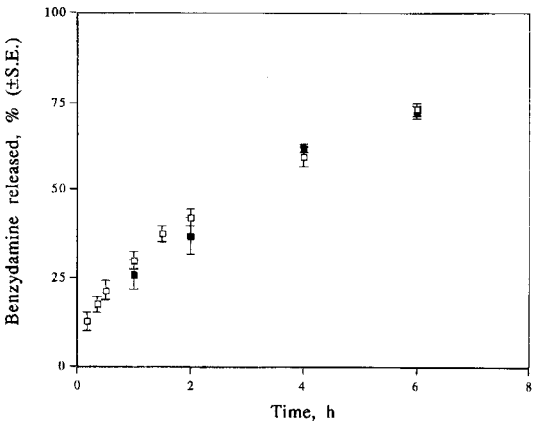

Fig. 5. Benzydamine released (%) in vitro (□) and in vivo (■) from the B1 patches (mean ± S.E., $n = 6$).

pling of the mucosa), and no reactions around the site of application.

4. Conclusions

The mucoadhesive patches tested in this study may constitute a promising system for drug administration to the oral cavity. One of their potential advantages is the preparation by a simple compression method, which could lend itself to large-scale production. The investigation also evidenced some interesting, previously unreported properties of tamarind gum (TG), which, also on account of its low in vitro cellular sensitivity, might represent a new mucoadhesive material for buccal or other mucosal delivery devices.

The model drugs, BNZ and LDC, were released at a fast rate and with diffusive kinetics when used as the hydrochlorides. In vitro tests revealed that release could be satisfactorily controlled by using the drugs in the form of less soluble salts/complexes with PCT, PAA or TA. Dry-coating with TG, tested in the case of the BNZ-PCT patches, was another effective technique for controlling release. The validity of the release tests in vitro was demonstrated by the coincidence of the in vitro and in vivo release profiles of BNZ-HCl. The preliminary test on human volunteers also demonstrated the good tolerance and long permanence of the patches.

Further studies in vivo on the present patches, and on similar devices, designed as trans-mucosal, systemic delivery systems for different drugs, are under way.

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