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Histological and bioadhesion studies on buccal bioadhesive tablets containing a penetration enhancer sodium glycodeoxycholate

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Abstract

For many drugs, buccal route offers many advantages over conventional routes of delivery with an improved bioavailability due to the avoidance of degradation in the gastrointestinal tract and hepatic first-pass metabolism. However, the major limitation to buccal drug delivery is the permeability barrier in the buccal mucosa. Use of penetration enhancers appears to be a pertinent approach to increase the drug permeation through the buccal epithelium. Buccal bioadhesive tablet formulations enable a delivery with a plasma drug level of the desired therapeutic response for a defined period of time, and also provides a means of confining the drug and penetration enhancer to a defined region of the mucosa. In this study, a bioadhesive tablet formulation for buccal delivery was designed using a mixture of hydroxypropyl methylcellulose and carbomer, incorporated with a penetration enhancer, sodium glycodeoxycholate (GDC). In vitro bioadhesion property of the formulated tablet was examined and histological study was carried out to examine an in vivo interaction between the tablet and tissue. GDC did not affect the adhesiveness of the tablet which makes it an acceptable excipient for a buccal bioadhesive drug delivery system. Histological changes such as loss of upper cell layers and formation of vacuoles as well swelling in the cells were observed in the buccal epithelium, after 4 h contact with the tablets containing GDC. Studies on reversibility of the interaction are in progress.

Keywords: Bioadhesion; Buccal bioadhesive tablet; Histological evaluations; Human buccal mucosa; Penetration enhancer; Sodium glycodeoxycholate

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1. Introduction

The buccal mucosa offers excellent opportunities for the delivery of both locally and systemically active drugs (De Vries et al., 1991; Harris et al., 1992). It has potential advantages over other mucosal routes available: it avoids the degradation by the gastrointestinal enzymes and acids, and first-pass metabolism. Because of its excellent accessibility, self-placement of a dosage form is possible. Moreover, the drug can be removed at any time. Clinical success following oral mucosal delivery depends on the ability of a formulation to achieve and maintain plasma drug level of a defined period of time for the desired therapeutic response. At present the major limitation to buccal delivery is the low bioavailability. In order to address this problem, a first approach consists increasing the permeability of the buccal mucosa using penetration enhancers, which are able to decrease the barrier capacity of the tissue (Aungst et al., 1989; Lee et al., 1991; Hoogstraate et al., 1996; Şenel et al., 1997). Alternatively, the use of a bioadhesive system can be envisaged in order to extend the residence time and therefore the time available for drug delivery (Ishida et al., 1981; Ponchel G., 1994; Taylan et al., 1996). Obviously the issue of toxicity, both acute and chronic, will be critical to such penetration enhancing strategies.

Bioadhesive formulations have two potential applications in buccal drug delivery. Firstly, they can provide the attachment of the delivery system to the mucosa. Secondly, by means of the bioadhesive polymers, it is possible to confine drug and penetration enhancer to a defined region of the mucosa. There are many kinds of bioadhesive polymers, both synthetic and from natural sources. However when considered using as an excipient in a drug formulation, the choice of the polymer becomes more limited as the safety of the material has to be guaranteed. Being officially approved and known as safe polymers, HPMC and carbomer were chosen in this study and used at the ratio of 8:2 where a suitable bioadhesion was obtained (Taylan et al., 1996; Yazıksız et al., 1996). Bioadhesive delivery systems for buccal drug administration have been reported mainly in two parts: patch systems and tablet systems (Merkle et al., 1990; Nagai et al., 1990). Bioadhesive tablet formulation was preferred in this study.

The objective of this study was to investigate the influence of presence of penetration enhancer, sodium glycodeoxycholate (GDC), on bioadhesive properties of the buccal adhesive tablet containing carbomer and hydroxypropyl methylcellulose (HPMC) as well as in vivo effects of the bioadhesive tablets on buccal mucosa both in the presence and absence of the penetration enhancer.

2. Materials and methods

2.1. Preparation of tablets

Tablets were prepared by direct compression with a mixture of hydroxypropyl methylcellulose (HPMC) (Methocel K100, Colorcon, England) and carbomer (Carbopol® 910, BF Goodrich, Belgium) at ratio of 8:2. Sodium glycodeoxycholate (GDC) (Sigma, St. Louis,MO) was incorporated as penetration enhancer at 5% w/w concentration and 1% w/w magnesium stearate as lubricant. Control tablet contained no GDC but only the polymer mixture and lubricant. The surface area of the tablet exposed to the buccal mucosa was 1.15 cm^2.

2.2. Bioadhesion studies

Tensile experiments were done on the Instron apparutus (Model 4301) using bovine sublingual mucosa (Ponchel et al., 1987). 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 experiment was performed at a constant extension rate of 5 mm/min.

2.3. Histological studies

Six non smoker volunteers of both sexes (five female and one male), aged from 21 to 45 have participated in studies for histological evaluations

and written informed consent was obtained from each volunteer. The study was approved by the Ethical Committee of Hacettepe University.

Before placing, in order to obtain unidirectional contact and avoid an interaction with saliva, sides of the tablet which is not in contact with the mucosa was coated with polymethylmethacrylate (Eudragit®RSPM) (RöhmPharma, Germany) film. 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 press. At the end of 4 h period, the tablet was removed and from that region biopsies was taken and after a classical fixation, dehydration and embedding procedure (Şenel et al., 1994), 1μm sections were cut, stained with methylene blue and examined under a light 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. Results

3.1. Bioadhesion studies

No significant difference in adhesive bond strength was observed between the GDC-containing and GDC-free adhesive tablets ($P > 0.05$) (Table 1).

3.2. Histological studies

Tablets remained attached to the buccal mucosa during the 4 h period without any disintegration. No significant swelling was observed in the tablets (Fig. 1). Typical appearance of intact human buccal mucosa is seen in Fig. 2a. After 4 h contact with the tablet containing only polymer mixture (placebo), the surface layer of the mucosa was not smooth as the intact mucosa, showing a slight irregularity but there was no evidence of ulceration or bleeding. With the tablets containing GDC, there was a clinically irregular and dehydrated appearance accompanied with small reddish spots (Fig. 2b). Observed changes in the

Table 1
Bioadhesion forces (N) for buccal adhesive tablets in contact with bovine sublingual mucosa (n = 10)

Tablet with GDC (mean ± SD)	Tablet without GDC (mean ± SD)
9.52 ± 1.99	10.32 ± 1.87

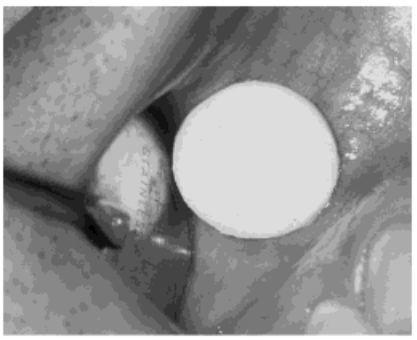

Fig. 1. Appearance of buccal adhesive tablet, after 4 h application.

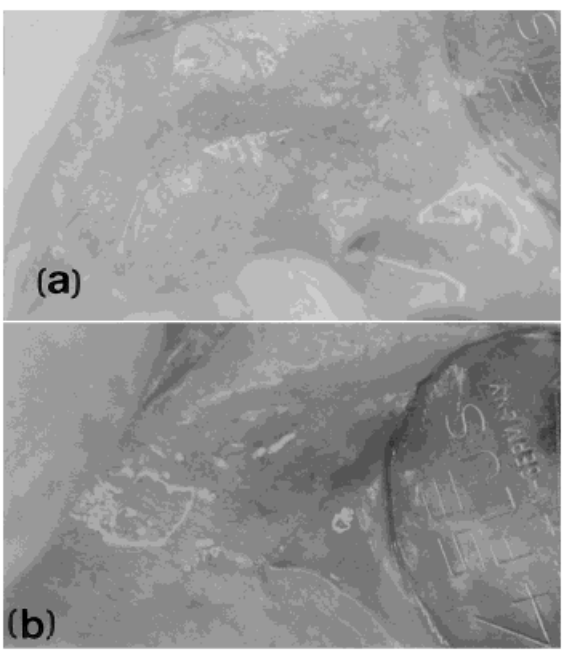

Fig. 2. Appearance of human buccal mucosa: (a) intact; (b) after removal of the adhesive tablet containing GDC at the end of 4 h.

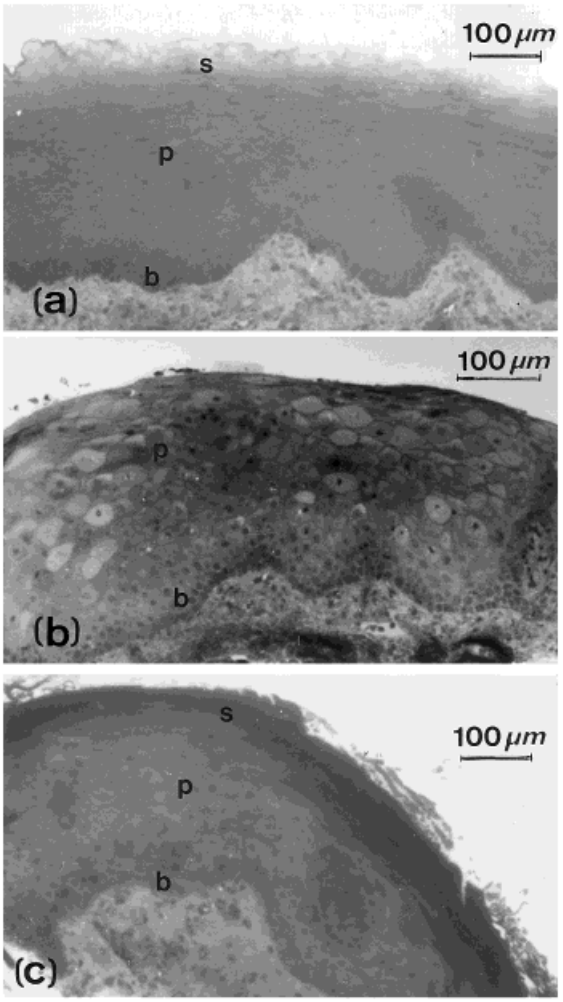

Fig. 3. Light micrographs of human buccal epithelium: (a) intact; (b) after removal of the adhesive tablet containing GDC (note the presence of vacuoles); and (c) after removal of adhesive tablet without GDC, at the end of 4 h; s, superficial cells; p, prickle cells; b, basal cells.

appearance of the mucosa after exposure to the tablets, both GDC containing and placebo, showed variation between the subjects.

Light and electron micrographs of human buccal epithelium before application of the tablets are shown in Fig. 3a and Fig. 4a. At the end of 4 h application period, with the tablets containing GDC, loss of upper cell layers and formation of vacuoles were observed as well as swelling in the cells (Fig. 3b, Fig. 4b). There was a dilation of the rough endoplasmic reticulum. In the absence of

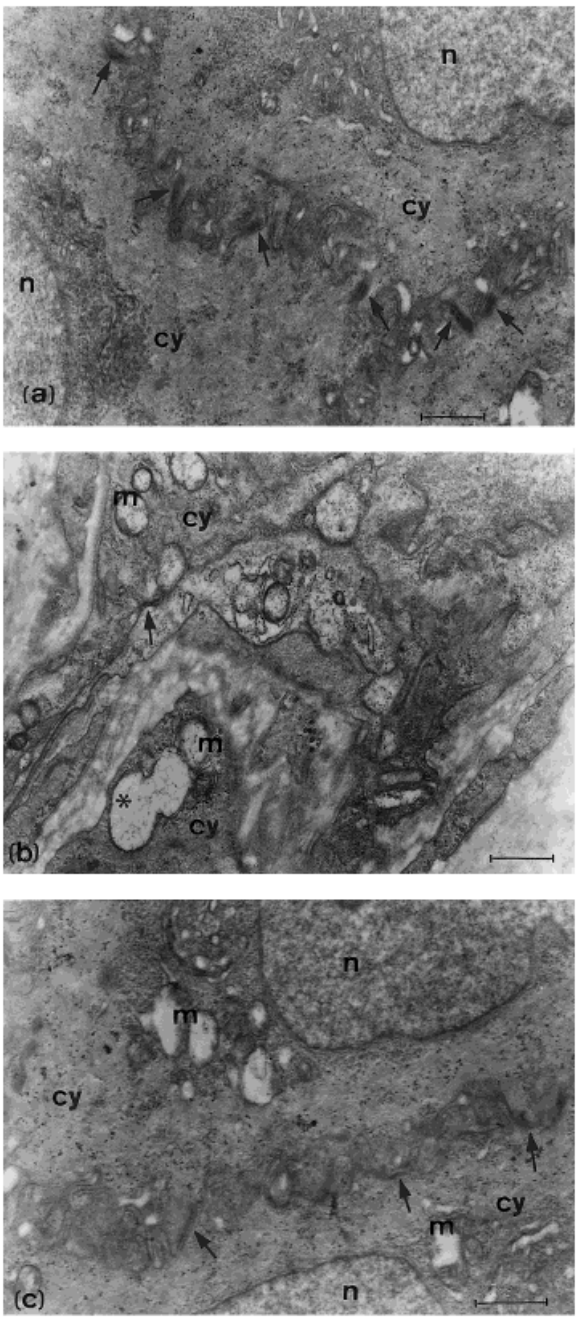

Fig. 4. Electron micrographs of human buccal epithelium: (a) intact; (n, nucleus; cy, cytoplasm); (b) after removal of the adhesive tablet containing GDC, note the swollen mitochondria, m; dilated endoplasmic reticulum(*); decrease in the number of desmosomes (illustrated by arrows); (c) after removal of the adhesive tablet without GDC, at the end of 4 h. Scale bar indicates 1 μm.

GDC, loss of upper cell layers and vacuole formation was less (Fig. 3c) yet there was an observable intracellular swelling (Fig. 4c). Swelling of the mitochondria and a decrease in the number of desmosomes was observed in both the placebo and GDC treated group, being significant in the latter group (Fig. 4a–c).

4. Discussion

Numerous studies have been done related to the bioadhesive properties of the polymers used in this study for buccal mucoadhesion however no histological studies in particular showing their effects on buccal mucosa. Histological examination of the human buccal mucosa after 4 h exposure to bioadhesive tablets containing only polymer (placebo) showed that the polymers used do not cause any serious change or damage in the buccal mucosa. As extent and frequency of contact may cause irritation following chronic applications of such systems on buccal mucosa, multiple applications are needed to be carried out to assure the long term reproducibility of these results.

GDC was chosen as the penetration enhancer and used at 5% concentration where it shows significant enhancing effect (Şenel et al., 1997). Following removal of the bioadhesive tablet containing GDC, after 4 h of application, appearance of the mucosa was not the same as the intact but it recovered gradually within 24 h period. Reversibility of the changes occur upon exposure to the tablets either with or without GDC is currently under investigation.

Histopatological examination of the human buccal mucosa revealed that significant changes occur upon 4 h of exposure to GDC containing tablets. Electron micrographs showing swelling in the cells and a significant decrease in the number of desmosomes indicate that GDC affects the mucosa causing changes in the cell membrane as well as in the cell after entering into the cells. Similar results were obtained in previous studies using porcine and bovine buccal mucosa, in vitro, after exposure to bile salts (Şenel et al., 1994, 1997).

In a previous study, the behavior of a bile salt, sodium glycocholate (GC) itself across the tissue has been studied in vitro using porcine buccal mucosa and it was shown that at 100 mM ($\cong$ 5%) concentration, there was a significant accumulation of the bile salt in the tissue which resulted in a significant enhancing effect (Şenel et al., 1998). Furthermore, the permeability of the tissue increased following the accumulation of the bile salt in the tissue, which indicates an interaction between the bile salt and the tissue. The histopatological results obtained with GDC in human confirms these previous findings. However, as mentioned above, the reversibility of this interaction is the most crucial point for the safety of this penetration enhancer.

In light micrographs, loss of upper cell layers was observed in both groups. It must be taken into consideration that removal process of the bioadhesive tablet would also cause loss of superficial cell layers. Considerable variations have been observed between the subjects. In previous studies, after exposure to GDC, a loss of superficial cell layers was observed in porcine buccal mucosa (Şenel et al., 1994) but not in bovine buccal mucosa (Şenel et al., 1997). It is obvious that changes occurring after exposure to the bile salts exhibit wide interspecies and intersubject variation. It is generally considered that oral mucosal damage caused by bile salts would be reversible and less serious than nasal mucosal damage due to the nature of oral mucosa. It has been shown by measuring their effect on mucociliary transport rate and on the ciliary beat frequency that many of the bile salts used for nasal penetration enhancement are ciliotoxic. Their membrane damaging effects have been demonstrated with the nasal epithelium of different animal models (Wheatley et. al., 1988; Ennis et al., 1990; Adriaens et al., 1997). Yet, bile-salt induced mucosal changes, or damage in buccal tissue has not been universally observed.

There are few studies of mucosal changes or damage resulting when a dosage form containing penetration enhancer/enhancers are applied to buccal mucosa. Mucosal irritation, which has led some researchers to argue against the clinical use of bile salts as penetration enhancer, was observed

to be mild and reversible in the single applications. Nakane et al. (1996) studied the mucosal irritation of a transmucosal therapeutic system (TmTs) developed for buccal delivery of luteinizing hormone- releasing hormone, containing various bile salts for permeation enhancement. The extent of irritation was observed with the naked eye and evaluated in regard to erythrema, eschar formation, and edema formation. Their results indicated that TmTs containing a bile salt was relatively safe and capable of achieving enhanced and controlled transbuccal delivery. Zhang et al. (1994) showed that it was possible to reversibly increase buccal mucosal permeability without causing any visible mucosal irritation and damaging the permeation barrier. They studied the effectiveness of the bile salts, sodium cholate and sodium taurocholate and lysophosphatidilcholine (LPC), in facilitating the transbuccal permeation of insulin. Recovery of buccal mucosa permeation barrier was investigated by measuring glucose back permeation fluxes following exposure to these enhancers. The authors utilized the fact that when a permeation enhancer is used to increase the permeability of the buccal mucosa which in its natural state is practically impermeable to glucose, the permeability to glucose will be increased. As a result glucose in the submucosal interstitial fluid permeates across the mucosa into the oral cavity. Glucose back permeation flow began to increase immediately after exposure to TC and LPC and decreased by 80% in 5–8h indicating that these enhancers transiently alter the mucosal barrier function.

Results of tensile experiments have shown that when incorporated into a bioadhesive formulation, GDC did not affect the bioadhesive properties performed by the adhesive polymers which makes it an acceptable excipient for a buccal bioadhesive drug delivery system. However, 4 h exposure to GDC containing tablets caused changes in buccal epithelium. For this reason, studies involving more frequent and prolonged applications are necessary to evaluate practicality of these penetration enhancers before any clinical application can be attempted.

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