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In vitro permeation studies of triamcinolone acetonide mouthwashes

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Abstract

The effects of vehicle composition, contact time of mouthwash and cosolvent on permeation of triamcinolone acetonide (TA) were investigated in vitro using hamster cheek pouch mucosa and synthetic membranes. Mouthwashes containing 0.1% TA with and without the mucoadhesive carboxyvinyl polymer were formulated. Aqueous suspensions and Orabase were used as control formulations. The contact time of mouthwash was varied from 1 to 5 min. Ethanol was used as a cosolvent in various binary-water mixtures. TA was delivered to a significantly lesser extent to mucosal tissue by the mouthwash than by the aqueous suspension ($P < 0.001$), but to a higher extent than by the Orabase formulation ($P < 0.001$). No effects of contact time or the mucoadhesive polymer were observed on amount of TA accumulated in the mucosal membrane. These observations have suggested that the use of carboxyvinyl polymer and a high content of ethanol are not appropriate as vehicles for local drug delivery but are suitable for transmucosal drug carriers.

Keywords: Triamcinolone acetonide; Mouthwash; Permeation; Oral lichen planus

1. Introduction

Oral lichen planus (OLP) is a common disease, as evidenced by the large numbers of patients reported from various institutions, with an incidence of 1–2% of the general population (Eisen, 1993). OLP may be found on mucosal surfaces including the esophagus, larynx, gingiva and lips. However, most lesions occur on the posterior buccal mucosa and the tongue. These conditions frequently require topical corticosteroid medication such as 0.1% triamcinolone acetonide (TA) (Kenalog in Orabase®) or 0.5% hydrocortisone (Orabase HCA®). These formulations are appropriate for use, when the disease is not disseminated and the lesions are relatively accessible for site-specific application. Orabase is difficult to apply to the mucosa and several patients reported a sticky unpleasant sensation (Sveinsson and Holbrook, 1993). Delivery of steroids via oral rinse has the advantage of providing drug contact with distal, hard-to-reach crevices and surfaces of the

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oral cavity. In addition, disseminated drug contact will prevent new eruption (Marek, 1999). An aqueous suspension of 0.1% TA was used as an oral rinse in the treatment of patients with symptomatic OLP, and this method proved effective (Vincent, 1991; Marek, 1999).

Systems for local delivery to the oral mucosa include conventional mouthwashes, oral suspensions and lozenges. These systems provide high drug levels in the oral cavity as a whole, but only for a short time. For effective therapy with these dosage forms, therefore, it is necessary either to select drugs, that are rapidly absorbed and effective under conditions of discontinuous delivery, or to make use of frequent dosing (Harris and Robinson, 1992). As TA is a potent drug, it is difficult to provide a uniform dose using its suspension dosage form and the drug is slowly absorbed. These problems may be overcome by preparing solution dosage forms. TA is only slightly soluble in water (17.5 μg/ml at 28°C) (Behl et al., 1976), but its solubility in alcohol is 1 in 150 (Raynolds, 1993). However, high contents of alcohol in mouthwash irritate the injured mucosa and cause a burning sensation (Pader, 1994; Marek, 1999). Therefore, cosolvents such as glycerin or propylene glycol (PG) are required for formulation of TA mouthwashes (solution type). Also, use of an enhancer in formulation may improve drug absorption. Various enhancers have been used to improve the percutaneous absorption of TA, including Azone® (Chow et al., 1984; Wiechers et al., 1990), α-bisabolol (Kadir and Barry, 1991) and pyrrolidone derivatives (Sasaki et al., 1991). l-Menthol, usually used as a flavoring agent in mouthwash has been reported to enhance drug absorption through the oral mucosa (Coutel-Egros et al., 1992).

The extent of drug absorption is dependent the time of exposure of the buccal membrane to the drug (Stalker and Pollock, 1991). The contact time of mouthwash is very short as compared with the paste dosage form. However, inclusion of a mucoadhesive polymer in solution type formulations would provide longer contact time. Mucoadhesive polymers such as hydroxypropylcellulose, carbopol®934, sodium carboxymethylcellulose, gelatin and pectin have been employed in TA formulations used for the oral cavity (Nagai, 1985; Kamath and Park, 1990).

The objective of the present study was to evaluate the effects of vehicle composition, contact time of mouthwash and cosolvent on the in vitro permeation of TA.

2. Materials and methods

2.1. Materials

TA, the carboxyvinyl polymer HIVISWAKO 105 corresponding to carbomer 934, and ethanol were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Menthol was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan) and disodium EDTA from Dojindo Co. (Kumamoto, Japan). Water used throughout the experiments was membrane-filtered (Milli-Q Labo/Millipore, Tokyo, Japan). All other materials were of analytical grade, obtained commercially and used as received.

2.2. Preparation of mouthwashes and control formulations

Mouthwash was prepared by dissolving TA at 0.1% w/v in cosolvents consisting of 10% v/v ethanol and 30% v/v PG. Saccharin sodium, menthol and disodium EDTA were included as sweetener, flavoring agent and anti-oxidant, respectively. Additives were added before adding water to volume. A second mouthwash was made incorporating 0.25% HIVISWAKO 105, as a mucoadhesive polymer.

The first control preparation was made according to Marek's method (1999). 0.1% TA aqueous suspension was prepared using water as the vehicle without flavoring agent or preservatives. For the second control formulation, 0.1% TA in Orabase (Bristrol–Mayers Squibb, Thailand) was used.

2.3. In vitro permeation studies

2.3.1. Effects of vehicle composition and contact time of mouthwash

All samples for in vitro studies, as shown in Table 1, were evaluated and pH was determined

with a pH meter. Hamster cheek pouch was chosen as a model of oral mucosa. Although it is a thin, keratinized tissue more closely resembling skin than oral mucosa of human, it is reasonable to use this tissue for comparative studies because OLP can manifest on the cheek and palate (Squier and Wertz, 1996). The composition of the epithelium varies depending on the site in the oral cavity. It has a surface consisting of areas of both keratinized and non-keratinized epithelium (Rathbone and Hadgraft, 1991; Chidambaram and Srivatsava, 1995; Squier and Wertz, 1996; Shojaei, 1998). Male golden hamsters (Saitama Experiment Animal Supply Co., Japan), weighing 70–80 g were sacrificed by inhalation of an overdose of diethyl ether. Pieces of suitably sized cheek pouch buccal mucosa were excised immediately before the permeation studies. The cheek pouch thickness was measured with a micrometer. Permeation through buccal mucosa mounted on a Franz type diffusion cell apparatus, giving a diffusion area of 1.70 cm^2, was evaluated. The receiver side of the diffusion cell was filled with 16 ml of pH 7.4 isotonic phosphate buffer and stirred continuously with a magnetic stirrer. The diffusion cell was kept in a water bath at 37°C prior to the experiment. The donor side was filled with 5 ml of either preparation (sample A–D) or a sample of 0.15 g/cm^2 of Orabase was applied to the mucosal surface. For mouthwash and aqueous suspension, the contact time on the mucosa was 1 min. The contact time of mouthwash without mucoadhesive polymer was also varied from 1 to 5 min. The diffusion cell was closed and all outlets and joints were sealed using paraffin to prevent moisture loss. Samples (1 ml) were withdrawn from the receiver chamber every hour over a period of 6 h and an equal volume of buffer was immediately added to maintain a constant volume. Permeation was determined by measuring the amount of drug in the receiver chamber. At the end of the experiment, the amount of drug in the mucosa was determined. The mucosa was thoroughly washed with buffer at 37°C, and the exposed surface was excised, weighed and digested with a 2:1 mixture of chloroform and methanol (v/v). Samples of 5 ml of extracted solvents were added to the tubes with the mucosa and sonicated for 10 min. This was repeated twice. The supernatant was evaporated and then the precipitate was dissolved by 1 ml of mobile phase for HPLC. The samples were centrifuged at 13000 rpm for 3 min and filtered using a 0.45 μm disposable filter unit (Ekicrodisc® 3CR, Gelman, Tokyo, Japan). Each experiment was conducted at least in triplicate.

Synthetic membranes (Spectra/Por® CE MWCO 50000; Spectrum Medical Industries Inc. CA) were used for comparison with the hamster cheek pouch. Permeation was determined by measuring the amount of drug in the receiver chamber.

2.3.2. Effects of cosolvent

The effects of cosolvent on permeation of TA were evaluated using a Franz type diffusion cell apparatus and the synthetic membranes, as described above. TA was added to a concentration of 0.025% to cosolvent mixtures consisting of 10%, 20% or 30% (v/v) ethanol in water in the donor side. Permeation was determined by mea-

Table 1
pH and contact time on membrane of preparations with various vehicles

Sample	Preparation	pH	Contact time on membrane (min)
A	Mouthwash without mucoadhesive polymer	5.75	1
B	Mouthwash without mucoadhesive polymer	5.75	5
C	Mouthwash with mucoadhesive polymer	5.55	1
D	Aqueous suspension	5.99	1
E	Orabase	4.77	360

suring the amount of drug in the receiver chamber.

2.4. Drug assay

TA concentration was determined using an HPLC system (LC-10AS Liquid Chromatograph, SCL-10A System Controller, SIL-10A Auto Injector, SPD-10AV UV–Vis Detector, C-R6A Chromatopac, Shimadzu, Japan). The analytical conditions were as follows: column C18, HPLC Pack column, Inertsil ODS, 5 μm, 4.6 × 250 mm (GL Sciences, Inc. Japan); UV detection, 240 nm; mobile phase, 0.05 M aqueous solution of monobasic potassium phosphate: acetonitrile 60:40 (v/v); flow rate 1.5 ml/min; 50 μl injection volume.

2.5. Isopropyl myristate/vehicle partition experiments

Water, 10%, 20%, 30% v/v ethanol or 10% v/v ethanol-30% v/v PG in water and isopropyl myristate (IPM), as a model of lipophilic phase (Surber et al., 1990) were mutually saturated prior to the partitioning experiment. IPM 3 ml was added to 3 ml of the vehicle containing 0.1 mg of TA. For each determination three test tubes were placed in an incubator and shaken at 37°C for 24 h to allow equilibration. The mixture was then centrifuged at 3000 rpm for 10 min and the concentration of TA in the aqueous phase was determined by HPLC, as described above.

2.6. Data analysis

The partition coefficient (K) was calculated based on Eq. (1) where C_b and C_a are the respective overall aqueous concentrations of the drug before and after partition into IPM, respectively.

$$K = \frac{(C_b - C_a)}{C_a}. \quad (1)$$

The penetration parameters of TA (lag time (T), permeability coefficient through the membrane (P), diffusion coefficient within the membrane (D)) were calculated from penetration data. The flux through the membrane (J) was evaluated as the steady-state slope of the plot of cumulative amount of TA per unit surface area (Q) vs. time (t); $J = dQ/dt$. P was calculated from Eq. (2), where C is the drug concentration in the donor medium. The x-intercept of the extrapolated linear region of the curve gave T. D was calculated from T with known thickness of the penetration barrier (h), using Eq. (3) or from Eq. (4) with known thickness of the penetration barrier (h) and K. In case of finite dose, the penetration profiles were constructed by plotting the amount of TA penetrated vs. square root of time; D was calculated from Eq. (5).

$$J = PC, \quad (2)$$

$$T = \frac{h_2}{6D}, \quad (3)$$

$$P = \frac{(KD)}{h}, \quad (4)$$

$$\frac{M_t}{M_\alpha} = 4\left(\frac{Dt}{\pi h^2}\right)^{1/2}, \quad (5)$$

where M_α is the total amount of drug dissolved in the matrix and M_t is the amount released at time t.

The significance of differences in parameter values among samples was tested by unpaired Student's t-test. Significance was taken as $P < 0.05$.

3. Results and discussion

TA was detected both in the hamster cheek pouch mucosa and in isotonic phosphate buffer in the receiving compartment. TA was delivered to a significantly lesser extent to mucosal tissue by the mouthwash (solution form) (A) than by the aqueous suspension (D) ($P < 0.001$), but to a higher extent than by the Orabase formulation (E) ($P < 0.001$), as shown in Fig. 1. A possible explanation for these observations is that both the solution and aqueous suspension resulted in high drug levels on the mucosa but only for a short time so the absorption of TA was dependent on its K value. IPM (as model of mucosa)/vehicle-K value is an indicator of the lipophilic or hydrophobic character of a drug molecule. Passage of

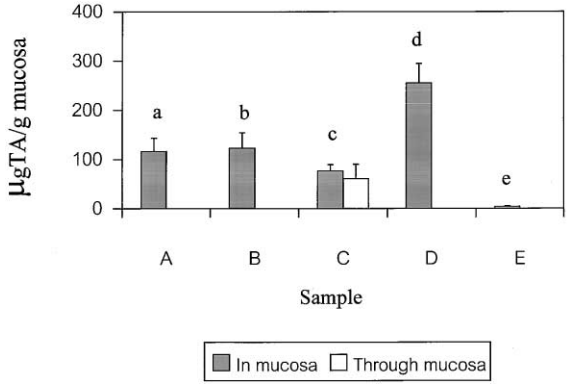

Fig. 1. Amount of TA accumulated in and permeated through the hamster cheek pouch after 6 h. Data are the means of three to five determinations ± S.D.; a is significantly different from d and e ($P < 0.001$).

drugs through lipid membranes sometimes correlates well with the K value of the drug. The K data from Table 2 supported this result. TA in water as vehicle in aqueous suspension was distributed to IPM to an extent 10-fold greater than that in 10% v/v ethanol-30% v/v PG in water as vehicle in mouthwash. In the case of Orabase, the amount of TA measured in the mucosa was similar to the value reported previously obtained using the liposomal form (Sveinsson and Mezei, 1992). TA was delivered to the mucosal tissue by the aqueous suspension to a greater higher extent than by the Orabase. This result supported the clinical observation that delivery of TA via oral rinse (0.1% or 0.2% TA aqueous suspension) is an effective method for treatment of OLP (Vincent, 1991; Eisen, 1993; Marek, 1999). There was no effect of contact time or mucoadhesive polymer on amount of TA accumulated in the mucosal membrane ($P > 0.05$). Only TA from mouthwash

Table 2
IPM/vehicle-partition coefficient (K) value of TA

Vehicles	$K \pm$ S.D.
Water	4.71 ± 0.22
10% v/v Ethanol in water	2.19 ± 0.24
20% v/v Ethanol in water	1.28 ± 0.07
30% v/v Ethanol in water	0.56 ± 0.03
10% v/v Ethanol-30% v/v PG in water	0.45 ± 0.02

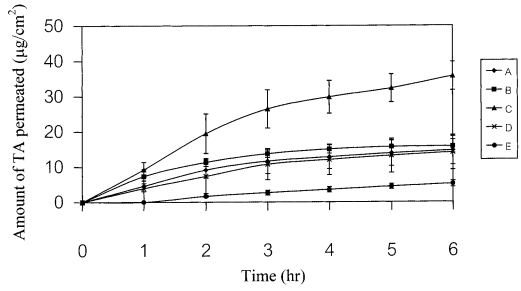

Fig. 2. Cumulative amount of TA permeated through the synthetic membrane from five samples plotted as a function of the time over the period of 6 h. Data are the means of three to four determinations ± S.D.

formulations with mucoadhesive polymer (sample C) penetrated through the hamster cheek pouch at 0.61 ± 0.24 μg/g for 6 h. The penetration profile of other samples (samples A, B, D, E) could not be observed by using hamster cheek pouch (Fig. 1), which differed from using synthetic membrane.

The cumulative amount of TA penetrated through the synthetic membrane measured in the receiving fluid was plotted as a function of time, as shown in Fig. 2. In this plots, it was assumed that a steady state of drug delivery through the membrane was achieved. The time elapsed before achieving the steady state was designed as the lag time. The lag time is usually encountered in permeation experiments where skin or synthetic membranes are used as models and represents the time required for the drug to penetrate through the rate limiting stratum corneum. However, due to either the high permeability of the membrane or dosage form, no lag time was observed in these experiments except, when TA was incorporated in Orabase. It was not surprising that Orabase acted as a slow release vehicle and can adhere to the mucosal tissue for prolonged periods. From this plot, the penetration parameters could be calculated for Orabase only. As a finite dose of TA was administered from the mouthwash and aqueous suspension under conditions of discontinuous delivery, D value could be calculated from Eq. (5) by plotting the amount of drug penetrated vs. square root of time (Fig. 3). However, it was difficult to determine M_α in these cases because

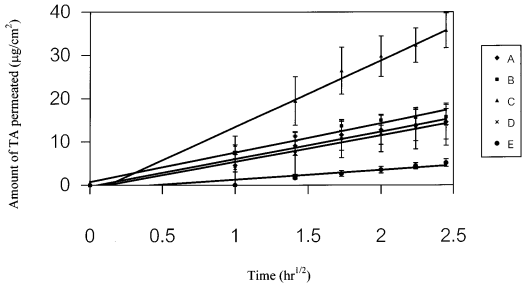

Fig. 3. Cumulative amount of TA permeated through the synthetic membrane from five samples plotted as a function of the square root of time over the period of 6 h. Data are the means of three to four determinations ± S.D.

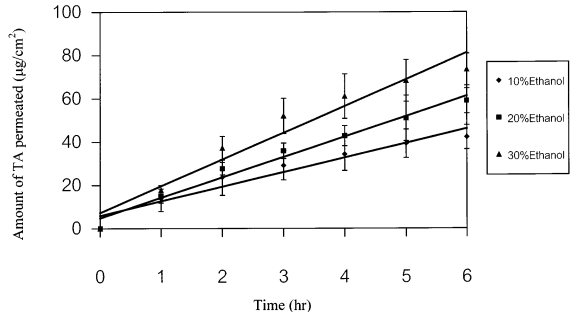

Fig. 4. Cumulative amount of TA permeated through the synthetic membrane from three cosolvents plotted as a function of the time over the period of 6 h. Data are the means of three to four determinations ± S.D.

after withdrawal of samples some of the test formulation adhered to the inside of the donor cell and was drained to the membrane under gravity during permeation studies. Due to this artifact, D value in Table 3 equal to DM_α^2 was used for comparison between samples A–D, where initial amount of TA before the samples were withdrawn from the donor cell was 6.33 mg/cm^2. The mean D values of TA in the five samples are shown in Table 3. The D value of TA from mouthwash was not significantly different from that of the aqueous suspension ($P > 0.05$). There was no effect of contact time on D value. The contact time of mouthwash is very short because it is a non-attached system, and can be readily removed from the oral cavity by swallowing. Both synthetic and natural polymers have been used as mucoadhesive polymers for buccal drug delivery (Shojaei, 1998). To formulate TA mouthwash in solution form, carboxyvinyl polymer was selected to retain the drug at the site of action. In general, dosage forms designed for buccal administration should not cause irritation and should be accepted by the patient (Shojaei, 1998). The mouthwash with 0.25% HIVISWAKO 105 was too viscous, so the use of this mouthwash may be unpleasant for patients. The high degree of permeation of drug shown in Fig. 1 and high D values shown in Table 3 indicated that this formula is not an appropriate vehicle for local drug delivery.

The cumulative amounts of TA penetrated through the synthetic membrane from three cosolvent mixtures are plotted as a function of the time in Fig. 4. The cumulative amount of TA delivered to the receiving fluid increased significantly with ethanol content. The penetration parameters of TA from various cosolvents were shown in Table 4. The observations suggested that in addition to irritating the injured mucosa (Marek, 1999), high

Table 3
Diffusion coefficient (D) value for TA in the five samples[†]

Sample	D ($\times 10^{-2}$ cm^2/h)
A	4.06 ± 2.26^a
B	4.50 ± 1.22^b
C	22.92 ± 5.12^c
D	3.89 ± 1.98^d
E	0.30 ± 0.12*

[†] D value equal to DM_α^2 was calculated using Eq. (5) and lag time (T) in Fig. 3. The thickness of synthetic membrane was 0.07 cm. Statistical differences between superscripts a and c; $P < 0.005$.

* D value was calculated using Eq. (3) and Fig. 2.

Table 4
Permeability coefficient (P) and diffusion coefficient (D) value for TA in three cosolvent mixtures[a]

Cosolvents (v/v Ethanol)	P ($\times 10^{-2}$ cm/h)	D ($\times 10^{-3}$ cm^2/h)
10%	2.69 ± 0.23	0.86
20%	3.77 ± 0.40	2.30
30%	4.94 ± 0.52	6.17

[a] P value was calculated using Eq. (2) and Fig. 4; D value was calculated using K values in Table 2 and Eq. (4).

ethanol content in mouthwash also enhanced the penetration of TA. Therefore, TA mouthwash should contain relatively low amounts of alcohol, although the recommended amount of alcohol for use in general oral rinse compositions is up to 25% (Pader, 1994).

In vitro permeation studies are suitable for assessing the usefulness of various vehicles for mucosal drug delivery. However, extrapolation of the results to in vivo conditions should be done with care because various conditions may result in alterations in permeability (Squier and Wertz, 1996). In future, clinical trials of the therapeutic effectiveness and acceptability of these preparations are required. The occurrence of local side effects such as secondary candidiasis in the oral cavity, which shows a high incidence with prolonged used of topical steroids, should be also studied.

4. Conclusions

TA in mouthwash was delivered to a significantly greater extent to hamster cheek pouch mucosa than the drug applied in Orabase. This finding suggested that use of the mouthwash is an effective method for increasing amount of drug in the oral mucosa.

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