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Enhanced bioavailability by buccal administration of triamcinolone acetonide from the bioadhesive gels in rabbits

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Abstract

The pharmacokinetics and bioavailability of triamcinolone acetonide were determined to investigate buccal absorption from the mucoadhesive gels in rabbits. The enhancing effect of sodium deoxycholate as an enhancer on the buccal absorption of triamcinolone acetonide from the mucoadhesive gels was evaluated in rabbits. Thus, 2 mg/kg of triamcinolone acetonide was administered from the mucoadhesive gels containing an enhancer (enhancer group) or not (control group) via the buccal routes and compared with intravenous routes (1 mg/kg, i.v. group). AUC of the control, enhancer and i.v group were 2374 ± 915, 3778 ± 1721 and 3945 ± 2085 h ng/ml, respectively, and the absolutive bioavailability of enhancer or i.v to control group were 159.14 or 332.35%, respectively. The average C_{max} of control and enhancer group were 263 ± 159 and 362 ± 201 ng/ml, and the mean T_{max} of the control group and enhancer group were 5.00 ± 1.67 and 4.33 ± 0.82 h, respectively, but there was no significant difference. As the triamcinolone acetonide gels containing sodium deoxycholate as an enhancer was administered to rabbits via the buccal routes, the relative bioavailability showed about 1.59-fold compared with the control group. Buccal administration of triamcinolone acetonide gels containing sodium deoxycholate as an enhancer to rabbits showed a relatively constant, sustained blood concentration with minimal fluctuation. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Triamcinolone acetonide, one of synthetic glucocorticoid of long-acting, has been used for the treatment of inflammatory disease (Falliers and Petraco, 1982; Oliver, 1982). It is absorbed rapidly from the gastrointestinal tracts, however, they are destroyed so rapidly as they pass through the liver that they are poorly effective by the oral routes. It is desirable to administer the drugs via non-oral routes such as topical, buccal, inhalation, and intramuscular routes to reduce the adverse reac-

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tions such as gastric disturbances and hepatic first effects which might occur when orally administered.

Of many drug delivery systems, there are many reports (Garren et al., 1989; Reinhold and Haus, 1989; Harris and Robinson, 1992; Vyas and Jain, 1992) on the buccal drug delivery because it has some advantages such as the abundant blood supply in buccal area, bypassing the hepatic first pass effect and excellent accessibility, etc. It is very difficult to apply ointments, solutions, creams and lotions etc. onto oral mucosa and to expect their effect for a significant period of time, because they are very easily removed by saliva-tion, temperature, tongue movement and swallow-ing. The current resurgences of interest in the buccal mucosa as a route for systemic drug deliv-ery using mocoadhesive preaparations (Mizobuchi et al., 1986; Takayanagi and Sawai, 1986; Saito et al., 1990; Park et al., 1990; Nakayama et al., 1994) through oral cavity stems from very differ-ent considerations.

Stefan and Michael (1992) Stefan et al. (1993) reported on the in vitro oral mucosal absorption of liposomal triamcinolone acetonide and in vivo correlation of the bioadhesive properties of a buccal bioadhesive miconazole slow release tablets. Reinhold and Haus (1989) reported on the evaluation of laminated mucoadhesive patches for buccal drug delivery, Vyas and Jain (1992) on the bioadhesive polymer for buccal administra-tion. Still, the major limitation to buccal delivery is the low permeation through the tissue resulting in a low bioavailability. The use of penetration enhancers is a logical approach to increase the drug permeation across the epithelium.

In my previous consecutive studies (Shin and Kim, 2000; Shin et al., 2000) to improve the permeability of triamcinolone acetonide through buccal mucosa using the various enhancers in-cluding bile salts, glycols, and non-ionic surfac-tants, sodium deoxycholate showed the best enhancing effects. The objective of this study was to evaluate the bioavailability of triamcinolone acetonide in rabbits by buccal administration of the established bioadhesive gel formulations con-taining sodium deoxycholate as an enhancer.

2. Materials and methods

2.1. Materials

Triamcinolone acetonide was obtained from Shinpoong Pharm. Co. (Seoul, Korea), poloxamer 407 was from BASF (Ludwigshafen, Germany) and carbopol 934 was from BF Goodrich (Cleve-land, USA). Methylprednisolone and sodium de-oxycholate, urethane were purchased from Sigma (USA). Heparin sodium and normal saline were from Green Cross (Seoul, Korea). Methanol was HPLC grade and all the reagents were of analyti-cal grade and used without further purification.

2.2. Preparation of triamcinolone acetonide–bioadhesive gels containing an enhancer

Poloxamer 407 (20 g) was added into water with gentle stirring at about 5°C (Shin et al., 2000) and the solution was left overnight in a refrigerator to complete polymer desolvation. Carbopol 934 (2 g) was stirred into this solution, 5 g of sodium deoxycholate and 1 g of triam-cinolone acetonide were added while stirring to the above-given polymer solution. The prepara-tion was then brought to 100 ml with the water and stored in a shaking water bath at 30°C for 2 days. The triamcinolone acetonide solution for i.v. administration was prepared by dissolving 25 mg of triamcinolone acetonide in 100 ml of 5% ethanol–saline solution.

2.3. Animal treatment

Male rabbits weighing 2.0–2.4 kg were housed individually over 2 weeks in a temperature-con-trolled environment (20–25°C). The relative hu-midity varied between 50 and 60%. They had free access to a diet and water 1 week before experi-ments unless otherwise noted. They were made to fast 24 h before the experiments.

2.4. Route of administration and withdrawal of blood samples

The rabbits were fixed on a plate and anes-thetized by subcutaneous injection of 25% ure-

thane-physiological saline (4 ml/kg). The teeth of the rabbits were fixed on the plate, while the tongue was kept tightly on the lower teeth to prevent swallowing the drugs. An infusion set equipped with a gauge (0.8 mm) hypodermic needle and winged adapter was inserted into a right femoral artery to facilitate the sampling of blood for drug analysis.

For the buccal administration, a dose of 200 mg/kg of triamcinolone acetonide gels (2 mg/kg) was administered on the buccal mucosa of the rabbits ($n = 7$). After removing 0.5 ml of arterial blood as a control sample, a single dose of 1 mg/kg of the triamcinolone, for the intravenous administration, was administered by a rapid injection via the ear vein.

Following the administration of the dose, the 2.5 ml of blood specimens were taken at specific time intervals from the cannulae inserted into the femoral artery in a heparinized-glass tubes and centrifuged at 3000 rpm for 10 min to obtain 1 ml of the plasma and frozen until analyzed. Blood samples were taken before and 10, 30 min and 1, 2, 4, 6, 8, 12, and 24 h after buccal administration and 5, 15, 30 min and 1, 2, 4, 6, 8, 12, and 24 h after intravenous administration. After taking the blood specimen, heparinized physiological saline (70 IU/ml) was inserted into the set to prevent blood coagulation. The homeostasis of the rabbits was maintained by injection of the same volume of physiological saline via the ear vein.

2.5. Determination of triamcinolone acetonide in rabbit plasma

The determination of triamcinolone acetonide in the plasma was carried out by the modified Mollmann method (Mollmann et al., 1985). A 1-ml aliquot of plasma was pipetted into a 15 ml centrifuge tube, along with 100 μl of internal standard (40 ug/ml of methylprednisolone), and 7 ml of ethylacetate and shaken for 12 min by mechanical shaking. After centrifugation for 10 min at 3000 rpm, 6 ml of the organic solvent phase was transferred to another tube and evaporated to dryness on a centrifugal evaporator under nitrogen gas at 40°C. The residue was dissolved in 0.4 ml of mobile phase by vortex mixing, then 50 μl of this solution was injected into the HPLC after Millipore filtration. Within the above mentioned concentration range, the relations were linear. The equations of the regression curves and their correlation coefficients (r) were as follows: $y = 755x - 150$ ($r = 0.997$). The intra or inter-day coefficient of variation of triamcinolone acetonide were less than 12 or 16%. The variability did not have an effect on the determination of C_{max} and AUC. The limit for the quantitative determination of triamcinolone acetonide in 1 ml plasma was 1 ng/ml.

2.6. HPLC conditions

The HPLC system consisted of a solvent delivery pump (model 910, Waters, USA), a variable UV absorbance detector, and computing intergrater. The detector wavelength was set at 254 nm and the column was used at room temperature. The stationary phase used was a Shin-pack CLC-ODS column (Shimadzu Co. column No. 425920, 4.6 × 250 mm). Mixtures of 2:1 (v/v) methanol: 10 nM citrate buffer were used as the mobile phases at a flow rate of 1.2 ml/min, The mobile phase was filtered by passing through a 0.45 um pore size membrane filter. The standard solutions were chromatographed and calibration curves were constructed on the basis of peak area measurements. The retention time of triamcinolone acetonide and internal standard from plasma chromatogram were 9.6 and 13.2 min, respectively (Fig. 1).

2.7. Pharmacokinetic data analysis

The noncompartmental pharmacokinetic analysis was performed with the LAGRAN computer program (Rocci and Jusko, 1983) which employs the Lagrange method to calculate the AUC of plasma concentration (Cp) as a function of time (t). Area under the curves was computed by LAGRAN method to reduce the errors by the trapezoidal rule. Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by area under the curve (AUC). AUMC was determined using a plot of plasma concentration multiplied by time ($C \times t$) versus

time and calculation of its area under the curve by the LAGRAN. The maximum plasma plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were determined by visual inspection of the experimental data. The elimination rate constant (K_{el}) was calculated by the regression analysis from the slope of the line and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$.

The absolute bioavailability of triamcinolone acetonide after buccal administration per the I.V. administration was calculated as given below.

$$\text{Absolute bioavailability (A.B.)} = \frac{\text{Sample AUC}}{\text{I.V. AUC}} \times \frac{\text{I.V. dose}}{\text{Sample dose}} \times 100$$

The statistical significance of the differences between the formulations was tested by the Student's paired t-test. All the values were reported as mean ± standard deviation (S.D.) of seven determinations.

2.8. Histological examination of the buccal mucosa

Histological changes on the buccal mucosa after experiment were examined. After animal experiment, the gels on the buccal mucosa were wiped off with clean tissue paper, and the excised buccal mucosa were fixed in 10% formalin by the conventional procedure, stained with hematoxylin–eosin and examined under a microscope (Aioi et al., 1993). The buccal mucosa untreated with enhancer was served as a control.

3. Results and discussion

3.1. Pharmacokinetics

3.1.1. Area under the concentration-time curve

For the purpose of studying the biopharmaceutical aspects of buccal absorption of triam-

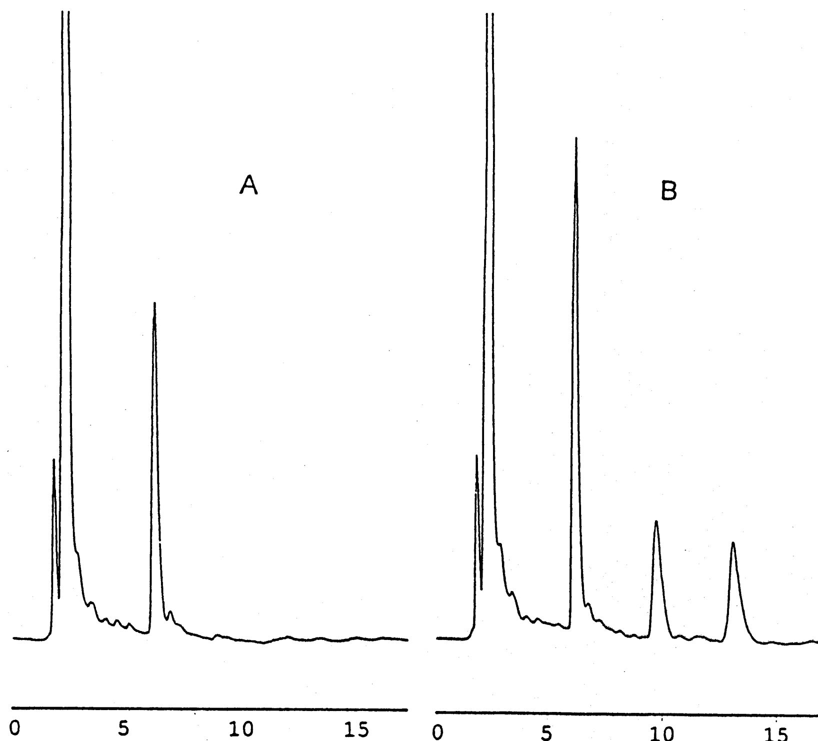

Fig. 1. Chromatograms of blank plasma (A) and plasma spiked (B) with internal standard (13.2 min) and triamcinolone acetonide (9.6 min).

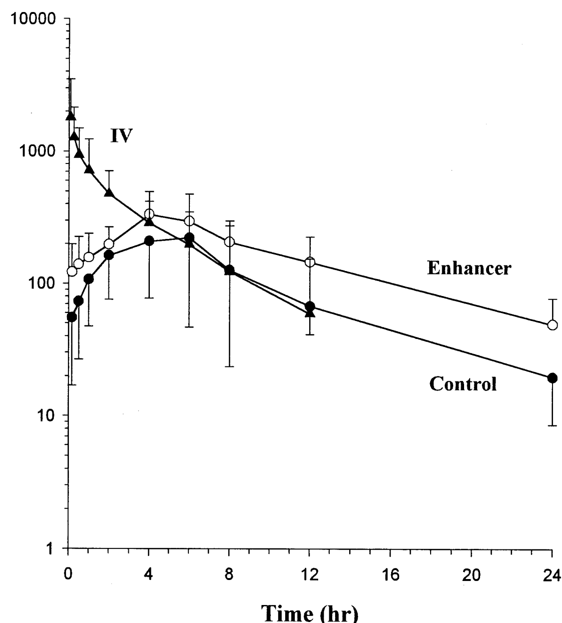

Fig. 2. Plasma concentration-time profile of triamcinolone acetonide following IV administration (1 mg/kg) and buccal administration (2 mg/kg) of the mucoadhesive gels with enhancer to rabbits ($n = 7$). The error bar represents the standard deviation of the mean. □, with enhancer (sodium deoxycholate); ●, without enhancer; ▲, I.V. administration.

Table 1
Mean plasma concentration (ng/ml) of triamcinolone acetonide from the buccal-mucoadhesive and IV administration[a]

Time (h)	Control	Enhancer	I.V.
0.083	–	–	1806 ± 1291
0.17	55.1 ± 58.2	121.7 ± 76.0	–
0.25	–	–	1275 ± 869
0.5	73.3 ± 66.6	139.5 ± 85.7	941 ± 560
1	107.5 ± 100.3	157.1 ± 81.7	717 ± 520
2	162.6 ± 146.9	197.2 ± 69.2	477 ± 233
4	208.6 ± 131.5	333.1 ± 161.0	288 ± 130
6	222.1 ± 175.3	295.6 ± 179.8	197 ± 151
8	126.3 ± 102.9	205.8 ± 90.8	123 ± 120
12	67.3 ± 26.3	145.5 ± 79.6*	59.0 ± 55
24	19.6 ± 11.0	49.0 ± 28.2*	–

[a] Each value represents the mean ± S.D. of seven determinations. *$P < 0.05$. Mucoadhesive dose of control and enhancer group = 2 mg/kg, IV dose = 1 mg/kg.

cinolone acetonide, one of the prerequisites is that the pharmacokinetic parameter after the i.v. administration should correlate with that after the buccal absorption of triamcinolone acetonide. The plasma-time concentration curve for triamcinolone acetonide after the buccal administration of 2 mg/kg of triamcinolone acetonide is shown in Fig. 2 with the i.v. administration to rabbits of a single 1 mg/kg of triamcinolone acetonide.

The average areas under the serum concentration-time curves, the value of AUC for the intravenous administration, was about 3945 ± 2085 h ng/ml. Following buccal administration of a single 2 mg/kg of triamcinolone acetonide to rabbits, the value of AUC of buccal administration with enhancer was 3778 ± 1721 h ng/ml and that without enhancer was 2374 ± 915 h ng/ml (Table 1). Within each study, no significant differences were observed among the formulations. The absolute bioavailability of AUC value of buccal administration without an enhancer showed 30.1% compared with intravenous administration. However, the absolute bioavailability of AUC value of buccal administration of triamcinolone acetonide gel containing sodium deoxycholate showed about 47.9% compared with intravenous administration. The buccal administration of triamcinolone acetonide from the gel containing sodium deoxycholate as an enhancer was higher than that from the gel without enhancer. As the triamcinolone acetonide gel containing sodium deoxycholate as an enhancer was administered via the buccal routes to rabbits, the relative bioavailability showed about 1.59-fold compared with the group without enhancer. Derendorf (Derendorf et al., 1995) reported that the absolute bioavailability of triamcinolone acetonide in oral administration showed about 23% and about 22% in inhalation. Buccal administration of triamcinolone acetonide gel containing sodium deoxycholate to rabbits showed a relatively constant, sustained blood concentration with minimal fluctuation and better bioavailability with comparing inhalation administration (Table 2 and Fig. 2).

3.1.2. Peak concentration (C_{max}) and peak time (t_{max})

Statistical analysis of the $C_{\max}$ and $t_{\max}$ values observed following the buccal administration of the triamcinolone acetonide formulations shows that enhancer group exhibited higher average

Table 2
Pharmacokinetics of triamcinolone acetonide from the buccal-mucoadhesive gels[a]

Parameters	Control	Enhancer	I.V.
AUC (h·ng/ml)	2374 ± 915	3778 ± 1721	3945 ± 2085
C_{max} (ng/ml)	263 ± 159	362 ± 201	
T_{max} (h)	5.00 ± 1.67	4.33 ± 0.82	
MRT (h)	10.25 ± 2.52	11.00 ± 2.99	
K_{el} (h)	0.144 ± 0.064	0.122 ± 0.047	0.192 ± 0.057
$T_{1/2}$ (h)	5.60 ± 2.29	6.33 ± 2.09	3.93 ± 1.31
A.B. (%)	30.1	47.9	100
R.B (%)	100	159.14	332.35

[a] Each value represents the mean ± S.D. of seven determinations. Mucoadhesive dose of control and enhancer group = 2 mg/kg, IV dose = 1 mg/kg. A.B. absolute bioavailability to IV AUC (%); R.B. relative bioavailability to control AUC (%).

C_{max} values of 362 ng/ml than those of 263 ng/ml which was achieved by the control group, but those differences were statistically insignificant. The t_{max} of the enhancer group was 4.33 h while 5.00 h from the control group (Table 2). The relative bioavailability of the enhancer group was about 159% comparing with the control group that means the enhanced absorption. The buccal administration of gels containing enhancer showed a little sustained and enhanced absorption.

3.1.3. Mean residence time (MRT), K_{el} and $T_{1/2}$

The average MRT after buccal administration was 10.25 h in control group and a little sustained 11.00 h in enhancer group. The average K_{el} was 0.122/h in the enhancer group and 0.144/h in the control group and a little decreased from the buccal administration, while 0.192/h in the intravenous administration group and statistically insignificant. The average $T_{1/2}$ was 6.33 h in enhancer group and 5.60 h in control group and a little sustained from the buccal administration, while 3.93 h in intravenous administration group and statistically insignificant.

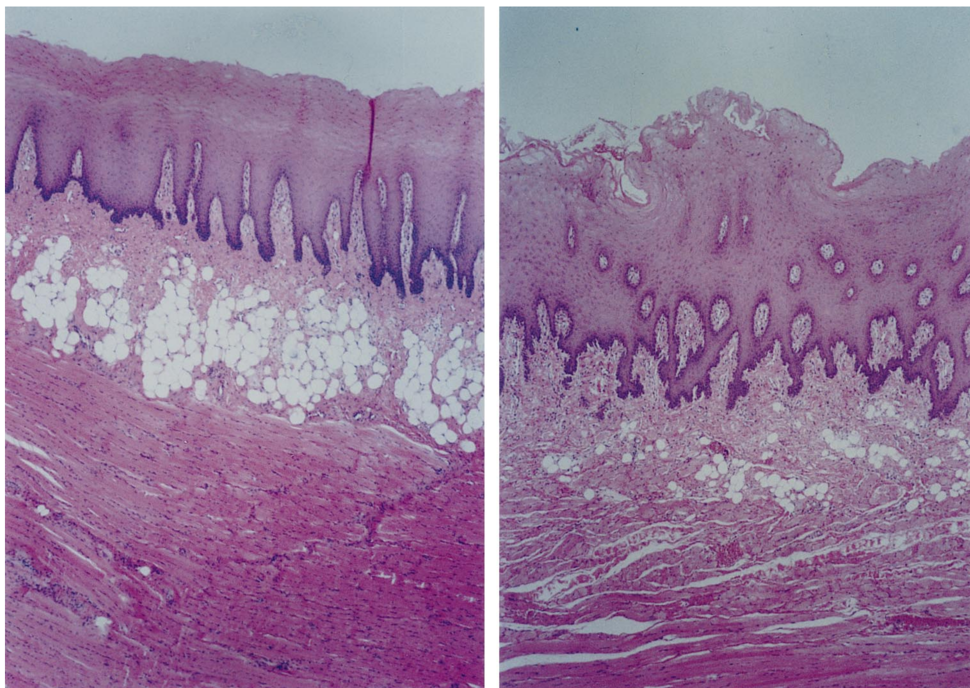

Fig. 3. Morphology of buccal mucosa of rabbits after buccal administration of triamcinolone acetonide gels containing sodium deoxycholate as an enhancer. A, without enhancer; B, with enhancer.

3.2. Histological examination of the buccal mucosa

In order to study the effects of an enhancer in triamcinolone acetonide penetration through the buccal tissues of the rabbits, the histological examination was carried out. The buccal mucosa treated with an enhancer group showed that the number of paneth cell was a little increased. But, there was no globular, cellular changes between the normal and enhancer group (Figs. 2 and 3).

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