In Vitro Oral Mucosal Absorption of Liposomal Triamcinolone Acetonide

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INTRODUCTION

The penetration of drug into and through the mucosa can be studied both *in vivo* and *in vitro*. The hamster cheek pouch has received increased attention as a model to study drug delivery through the oral mucosa (1–6). The keratinized epithelium of the hamster cheek pouch represents the major penetration barrier of the hamster cheek pouch (7,8). Keratinized mucosa is found in the human oral cavity in gingiva, dorsum of tongue, hard palate, and transitional zone of lip, but the keratin layer is absent in buccal and sublingual mucosa (9,10). However, the keratinized layer of the epithelial lining in the oral cavity is continually hydrated to a large extent, and in some places it is absent (buccal and sublingual mucosa) (10). Therefore, in treating oral inflammatory diseases, the drug applied on the oral mucosa is transported through the oral mucosa and into the systemic blood circulation. Consequently, a low concentration of the drug is observed at the site of action. Ulceration of the oral mucosa is frequently encountered by the normal population. Minor aphthous ulcers (canker sores) are the most commonly observed oral ulcers, with a prevalence of 20 to 50% of the normal population (11). In order to abort the lesions, topical steroids are frequently prescribed (11,12).

Earlier *in vivo* studies have shown that liposomes have the ability to localize an encapsulated drug within the oral mucosa (13,14). Moreover it has been demonstrated that liposomes are fully compatible with the oral mucosa (15). The objective of this study was to investigate if the hamster cheek pouch would be suitable as a model to study drug delivery from liposomes as well as from other vehicles.

MATERIALS AND METHODS

Preparation of Liposomes

The liposomes were prepared by a solvent evaporation method as described by Mezei and Nugent (16). The liposomes were formulated in 8 m*M* $CaCl_2$ solution containing (5% w/v) Phospholipon 90-H (Nattermann phospholipid GmbH, Cologne, Germany) and cholesterol, approximately 95% anhydrous (Sigma Chemical Co., St. Louis, MO), at a 1:0.5 molar ratio, containing 0.1% (w/v) triamcinolone acetonide (Lederle Cyanamid Canada, Inc., Markham, ON), along with ^{3}H-labeled triamcinolone acetonide (Amersham Corporation, Arlington Heights, IL). The liposomes were characterized using light microscopy and the freeze-fracture technique by electron microscopy. They were observed to be multilamellar, with a mean diameter of 0.45 μm.

Preparation of Control Formulations

Control preparations were prepared by dissolving the free drug (triamcinolone acetonide; 0.1%, w/v) and the radioactive tracer in Unibase o/w ointment base (Parke Davis and Co. Ltd., Brokville, ON) and Carbowax 600 (polyethylene glycol 600, Fisher Scientific Co., Fair Lawn, NJ). For the third control formulation, Kenalog in Orabase (0.1% triamcinolone acetonide dental paste USP, Princeton Pharmaceutical Products, Montreal, Quebec), only the radioactive tracer was added.

Drug Absorption and Permeation Experiments *in Vitro*

The hamsters (female Golden Syrian, 100–125 g; Canadian Hybrid Farms, Halls Harbour, NS) were sacrificed by a lethal dose of sodium pentobarbital (1.2 mg/kg intraperitonially) followed by cervical dislocation. The cheek pouches were excised, washed in Kreb's buffer, pH 7.2, and cut open into flat sheets. The open cheek pouch mucosa, with the mucosal surface facing up, was mounted between the two compartments of the diffusion cell (Skin Permeation System 1 Model LG-1083, LGA Instruments, Berkeley, CA) and held in place with an O-ring. The exposed surface area of the mucosa, where the liposome or control preparations were positioned, was 0.95 cm^2. A sample of 0.1579 g/cm^2 of liposome or control preparations was applied on the mucosal surface in the donor compartment and the receiving compartment was filled with Kreb's buffer. The diffusion cell was closed and all outlets and joints were sealed using Parafilm to prevent moisture loss. The temperature of both the receiving and the donor compartments was maintained at 37.0 ± 1°C. Sink condition was assumed by frequently taking samples which were replaced with fresh Kreb's buffer and by stirring with a magnetic stirrer.

Aliquots (0.5 ml) were removed from the receiving compartment every hour over a 6-hr period. The samples were mixed immediately with 10 ml of Ready Gel scintillation counting fluid (Beckman Instruments Inc., Fullerton, CA). At the end of the experiment the mucosa was thoroughly washed with 37°C Kreb's buffer and the exposed surface was excised, weighed, and digested using NCS Tissue Solubilizer (Amersham Corp., Arlington Heights, IL). The radioactivity of the samples from the receiving compartment and from the mucosal sample was determined in a Beckman LS 3133T liquid scintillation counter (Beckman Instruments Inc.), utilizing a quench curve prepared by external standardization.

Statistical Analysis

Data sets for each group of measurements were analyzed by ANOVA and two-sample *t* test to determine

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whether sample means were statistically different, using pooled standard deviation in Minitab, Version 7.1 (Minitab Inc, State College, PA). The difference was found significant if $P < 0.05$, except where stated otherwise.

RESULTS AND DISCUSSION

Triamcinolone acetonide (TRMA) was detected both in the mucous membrane and in the Kreb's buffer in the receiving compartment. TRMA was delivered to a significantly higher extent to the mucous tissue by the liposomal form than by the control forms [Unibase ($P < 0.003$) or Carbowax 600 ($P < 0.001$)] (Fig. 1). A possible explanation is that liposomes can act as a carrier and a slow release vehicle. The drug is carried through the keratin layer of the epithelium into the deeper layers of the tissue, where the encapsulated drug is slowly released. The liposomes, therefore, provide higher concentrations of the drug than the ointment base.

In the case of the third control, Kenalog in Orabase, difficulties were experienced in removing the remaining drug formulations from the surface of the mucosa, since this formulation contains carboxymethylcellulose, which adheres to the mucosa. Consequently, the amount of drug measured in the mucosa could be due to drug remaining on the surface of the mucosa, and not only to the drug that penetrated into the mucosa. Because of this artifact, the results are not reliable and therefore are not presented in Fig. 1.

The cumulative amount of TRMA ($\mu g/cm^2$) measured in the receiving fluid is plotted as a function of time (Fig. 2). In these plots it is assumed that a steady state in the drug delivery through the membrane is achieved (17). The time elapsed before this linear steady state is termed the lag time. A slightly higher lag time is observed for liposomes.

The cumulative amount of TRMA delivered to the receiving flask varied significantly between the vehicles after 6 hr of permeation experiment. The lag time observed in Fig. 2 is usually encountered in permeation experiments where skin or synthetic membranes are used as models and represents the time which is required for the drug to penetrate through the rate limiting stratum corneum. However, due to the high permeability of the mucous membrane, this lag time is hardly observed in these experiments, except when TRMA is incorporated into liposomes and Kenalog in Orabase. This is most likely due to the fact that the liposomes penetrate either intact or partially intact into the mucous membrane.

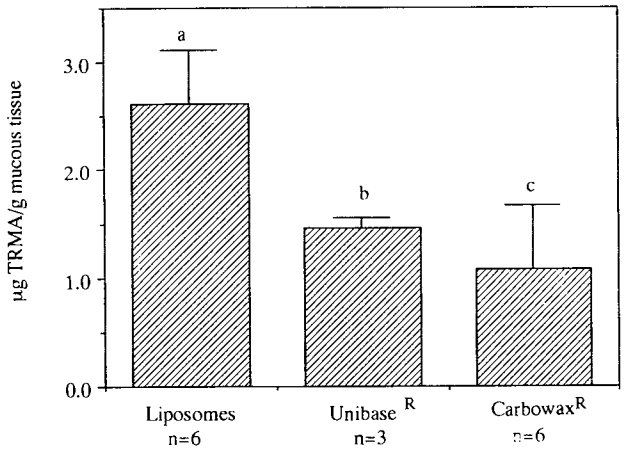

Fig. 1. Amount of triamcinolone acetonide (TRMA) accumulated in hamster cheek pouch after 6 hr. Data are the mean of three to six determinations ± SD. a is significantly different from b ($P < 0.003$) and c ($P < 0.001$).

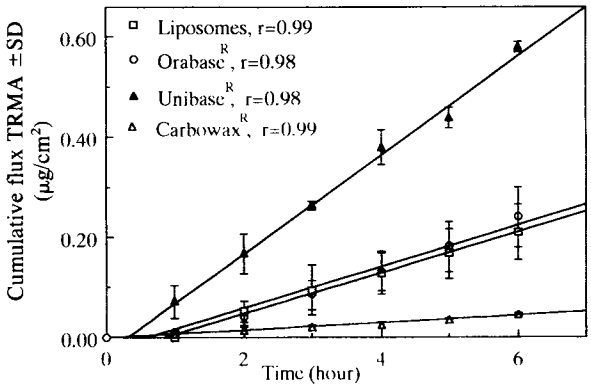

Fig. 2. Cumulative amount of triamcinolone acetonide released through the mucous membrane from the four vehicles plotted as a function of time over the period of 6 hr. Linear regression was used to fit the best line through the data points. Data are the mean of three to six determinations ± SD.

The mean values for the apparent permeability constants for TRMA, presented in Table I, were obtained from the slopes of the graphs in Fig. 2. From these results (Figs. 1 and 2), it can be seen that this particular liposome is an appropriate vehicle for local drug delivery but is not suitable as a transmucosal drug carrier. The transmucosal delivery of TRMA encapsulated in liposomes (either as the free drug or the penetration of the liposomes into the receiving fluid) lies in the same range as for the Kenalog in Orabase (Fig. 2).

CONCLUSION

Liposomal triamcinolone acetonide was delivered to a significantly greater extent to the hamster cheek pouch mucosa than the "free" drug applied in ointment form. The *in vitro* absorption technique is suitable for assessing the potentials of various vehicles in mucosal drug delivery, however, extrapolation of the results to the *in vivo* condition should be done with care.

Table I. Apparent Permeation Coefficients, K_p, for Triamcinolone Acetonide in the Four Vehicles[a]

Vehicle	K_p (cm/hr × 10^{-5})*
Liposomes	3.9 ± 1.1[a]
Unibase	10.5 ± 2.7[b]
Carbowax	0.7 ± 0.1[c]
Orabase	4.8 ± 1.4[d]

[a] Data are the means of three–six determinations ± SD.

* Superscripts a and b, $P < 0.05$; superscripts a and c, $P \leq 0.05$; superscripts a and d, $P > 0.05$.

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REFERENCES

1. K. W. Garren and A. J. Repta. Buccal drug absorption I. Comparative levels of esterase and peptidase activities in rat and hamster buccal and intestinal homogenates. *Int. J. Pharm.* **48**:189–194 (1988).
2. K. W. Garren and A. J. Repta. Buccal absorption. II. *In vitro* diffusion across the hamster cheek pouch. *J. Pharm. Sci.* **78**:160–162 (1989).
3. Y. Kurosaki, S. Hisaichi, L. Hong, T. Nakayama, and T. Kimura. Enhanced permeability of keratinised oral-mucosa to salicylic acid with 1-dodecylazacycloheptan-2-one (Azone). *In vitro* studies in hamster cheek pouch. *Int. J. Pharm.* **49**:47–55 (1989).
4. Y. Kurosaki, S. Hisaichi, C. Hamada, T. Nakayama, and T. Kimura. Effects of surfactants of the absorption of salicylic acid from hamster cheek pouch as a model of keratinized oral mucosa. *Int. J. Pharm.* **47**:13–19 (1988).
5. R. M. Tavakoli-Saberi and K. L. Audus. Cultured buccal epithelium: An *in vitro* model derived from the hamster pouch for studying drug transport and metabolism. *Pharm. Res.* **6**:160–166 (1989).
6. K. L. Audus, R. L. Bartel, I. J. Hidalgo, and R. T. Borchardt. The use of cultured epithelial and endothelial cell for drug transport and metabolism studies. *Pharm. Res.* **7**:435–451 (1990).
7. G. H. White and K. Gohari. The ultrastrucural morphology of hamster cheek pouch epithelium. *Arch. Oral Biol.* **26**:563–576 (1981).
8. C. O. Reid, J. Hardcastle, and C. J. Smith. A comparison of some of the permeability characteristics of intact and tape-stripped cheek hamster pouch *in vitro*. *J. Dent. Res.* **65**:673–676 (1986).
9. B. K. B. Berkowitz, G. R. Holland, and B. J. Moxham. Oral mucosa. In *A Colour Atlas and Textbook of Oral Anatomy*, Wolfe Medical, London, 1978, pp. 124–127.
10. C. A. Squier and N. W. Johnson. Permeability of the oral mucosa. *Br. Med. Bull.* **31**:169–175 (1975).
11. K. A. Arndt. Aphtous stomatitis. In *Manual of Dermatologic Therapeutics*, 4th ed., Little, Brown, Boston, 1989, pp. 18–21.
12. B. Rodu and C. M. Russel. Performance of a hydroxypropyl cellulose film former in normal and ulcerated oral mucosa. *Oral Surg. Oral Med. Oral. Pathol.* **65**:699–703 (1988).
13. B. B. Harsanyi, J. C. Hilchie, and M. Mezei. Liposomes as drug carriers for oral ulcers. *J. Dent. Res.* **65**:1133–1141 (1986).
14. T. Kimura, H. Nishimura, Y. Kurosaki, and T. Nakayama. Use of liposomal dosage form of flufenamic acid for treatment of oral ulcer. *Pharm. Res.* **7**:149S (1990).
15. W. C. Foong, B. B. Harsanyi, and M. Mezei. Effect of liposomes on hamster oral mucosa. *J. Biomed. Mat. Res.* **23**:1213–1229 (1989).
16. M. Mezei and F. J. Nugent. U.S. Patent 4,485,054 (1984).
17. J. Hadgraft. Mathematical models of skin absorption. In R. C. Scott, R. H. Guy, and J. Hadgraft (eds.), *Prediction of Percutaneous Penetration, Methods, Measurements, Modelling*, IBC Technical Services, London, 1989, pp. 252–263.