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Oral mucosal adhesive ointment containing liposomal corticosteroid

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Summary

A co-polymer of methacrylic acid and methacrylic acid methyl ester (Eudispert) was used to formulate a mucoadhesive ointment. Liposomes containing triamcinolone acetonide were incorporated into (a) the Eudispert ointment, which contains 11% (w/w) of the neutralized polymer and 0.5% (w/w) gelatin, and (b) Orabase. The in vitro drug release and dissolution behaviour of these formulations were investigated. A clinical trial is currently being carried out and the initial findings indicate that the liposomal formulations are well tolerated and no local irritation has been observed.

Introduction

The treatment of ulcerative and inflammatory mucosal diseases by topical application of appropriate medication represents the most feasible and common option chosen by most dentists and physicians. Mouthwashes, oral suspensions and lozenges are the most widely used delivery systems for topical oral administration (Zegarelli, 1991; Harris and Robinson, 1992). These dosage forms give high concentration of the active ingredient but only for a short period of time. The application of ointments or creams with adhesive properties can enhance the retention time of the formulation at the site of action (Harris and Robinson, 1992). Several ointment bases have been tried in the past and in 1984, Bremecker and co-workers explored the possibilities of using a acrylic polymer based on methacrylic acid and methacrylic acid methyl ester as an adhesive ointment base for tretinoin in the treatment of lichen planus.

Aphthous stomatitis, erosive and non-erosive lichen planus are the conditions that most frequently require topical corticosteroid medication in oral medicine clinics. Triamcinolone acetonide as Kenalog in Orabase is the most widely available preparation for treatment of these conditions. Typically aphthous ulcers are up to five in number each less than 1 cm in diameter and surrounded by a halo of inflamed mucosa. Lesions are painful and last for 7–10 days. Periods of remission before the next crop of ulcers are very variable both within and between individu-

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als. Lichen planus on the oral mucosa is characterized by white striations often forming lattice-like patterns on the mucosa that are strikingly bilateral. Erythema of varying severity is often a feature and in the erosive form of the disease the mucosa becomes ulcerated. These ulcerated areas are frequently large, irregular in outline and the floor of the erosion is covered by an adherent serous exudate. Histologically lichen planus exhibits a lymphocytic infiltration below the mucosa that is sharply demarcated from the deeper submucosal tissue. Although only some patients find non-erosive lesions uncomfortable, the erosions are almost always painful and require treatment. Anti-inflammatory drugs, principally corticosteroids, have been the only reasonably successful long-term treatment for these diseases.

Topical treatment of ulcerative inflammatory diseases is associated with several general disadvantages. The most severe problem is the high permeability of the oral mucosa (Squier and Johnson, 1975). In order to localize the drugs within the epithelium, the use of liposomal encapsulation has been investigated as a possible option for oral mucosa drug delivery, during the last decade. In vivo studies have shown that liposomes have the ability to localize an encapsulated drug within the oral mucosa (Harsanyi et al., 1986) and are also fully compatible with the oral mucosa (Foong et al., 1988). These findings have also been supported by in vitro experiments (Kimura et al., 1990; Sveinsson and Mezei, 1992).

The objective of this study was to develop an adhesive oral mucosal ointment containing liposomal triamcinolone and to evaluate the product using both in vitro techniques and by conducting a small clinical trial.

Materials and Methods

Materials

Liposomes were made from soy lecithin as a phospholipid (Phospholipon 90-H, Nattermann Phospholipid GmbH, Cologne, Germany) and cholesterol (approx. 95% anhydrous, Sigma Chemical Co., St. Louis, MO). Triamcinolone acetonide (TRMA) was obtained from Apodan, Copenhagen, Denmark. Eudispert-hv was obtained as a gift from Röhm Pharma, Weiterstadt, Germany and gelatin was obtained from NMD, Oslo, Norway.

Preparation of liposomes

The liposomes were prepared by a solvent evaporation method as described earlier by Mezei and Nugent (1984). Liposomes were formulated in distiled water using soy lecithin as a phospholipid (5% w/w) and cholesterol in a 1:0.5 molar ratio. The active ingredient, triamcinolone acetonide, was encapsulated in appropriate concentrations to yield a final concentration of 0.1% (w/w) for the final product.

Microscopic examination of the liposomes

The liposomes were characterized using light microscopy and the freeze-fracture technique. Briefly, small samples of the liposome suspension were pipetted onto gold stubs and rapidly frozen by immersion in liquid nitrogen cooled Freon-22. The specimens were fractured at 1×10^{-5} Pa at a temperature of -105°C. Replicas were produced by 45° angle evaporation of platinum and supported by vertical evaporation of carbon. The replicas were cleaned and viewed under a Philips 200 Electron Microscope.

Preparation of the mucoadhesive ointments

Several materials, reported to exhibit high bioadhesive properties, (Smart et al., 1984; Smart, 1991) were tested. A co-polymer of methacrylic acid and methyl methacrylate (7:3) (Eudispert) was chosen for this study, based on its high mucoadhesive properties and compatibility with oral mucosa epithelium (Bremecker et al., 1984). The polymer was suspended in water (11% w/w) and was pre-steeped cold for 10 min under stirring by a magnetic stirrer. For each gram of Eudispert, 5.76 meq. of NaOH was added for neutralization, under rapid stirring until the preparation congealed and the ensuing reaction phase was accelerated by gently heating (45°C) for 30 min. The final product contained 11% (w/w) of the polymer, 0.5% (w/w) gelatin, which was added to improve the adhesive properties, and the liposomal drug (0.1.% w/w).

A second liposomal mucoadhesive ointment was made by incorporating the liposomal drug, at 0.1% (w/w) concentration, into Orabase. After this treatment, the liposome containing Orabase was identical to Kenalog in Orabase, with respect to appearance and sensation in the mouth. As a control a commercial product, Kenalog in Orabase, was used.

Analysis of triamcinolone acetonide

The liposome containing Eudispert ointment base was dissolved in acetonitrile/water solution (42:58 v/v) by shaking for 1 h or until the ointment dissolved. A sample of the dissolved material was diluted and filtered through a filter unit of pore size 0.45 μm (Millex-HV, Millipore Corp., Bedford, MA) and the TRMA concentration was determined using a Waters 501 HPLC system (Millipore, Waters Chromatography Division, Milford, MA). The determination was made at 254 nm using a μBondapak C_{18} column (Millipore). The mobile phase was composed of 42% acetonitrile in water and had a flow rate of 1 ml/min.

A small sample of the liposome in Orabase formulation was suspended in methanol (50°C) and sonicated for 15 min. The suspension was centrifuged and a small sample of the supernatant diluted with the mobile phase. This mixture was allowed to stand for 10 min and then centrifuged and the supernatant was assayed for the drug using HPLC as described above.

In vitro dissolution

In vitro dissolution behaviour was investigated by extruding 1 ml of the ointments into 1000 ml PBS adjusted to pH 7.2 at 37°C. The system was agitated using a magnetic stirrer and the time required to dissolve the extruded material was monitored.

In vitro drug release

In vitro drug release was investigated using a Frans diffusion cell apparatus and a synthetic membrane (Spectrum Medical Industries Inc., Terminal Annex, CA). The exposed area was 1.2 cm^{-2}. The receiving chamber was filled with PBS, pH 7.2 and sink conditions were assumed by frequently taking samples, which were replaced with a fresh buffer and by stirring with a magnetic stirrer. Samples were drawn at a predetermined intervals and drug was assayed for by using HPLC, as described above.

Clinical trial

The patient group consisted of subjects with aphthous ulceration or erosive lichen planus. To date, 35 subjects have been entered into the study, allocated alternately to one of the preparations. They were instructed to apply the ointment sparingly to affected areas of the oral mucosa three times daily. Patients have been requested to record changes in the mouth on a simple scale: worse – no change – improved, and will be reviewed at intervals of 3 months.

Results and Discussion

Microscopic studies revealed that the liposomes were multilamellar with a mean diameter of approx. 0.45 μm (Fig. 1).

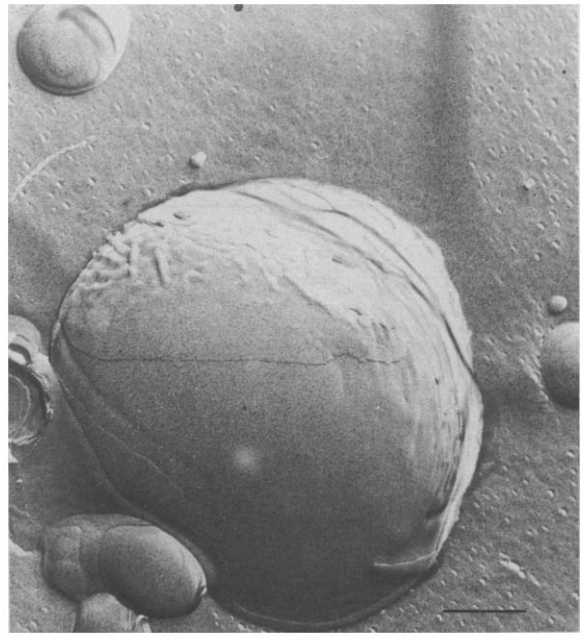

Fig. 1. Liposomes of trimacinolone acetonide visualized by TEM, (freeze-fracture). Scale bar: 0.5 μm.

In vitro dissolution test

The in vitro dissolution test was designed to determine the solubility of the different formulations. Fig. 2 represents the washing-out times for the three different formulations; Kenalog in Orabase, liposomes in Orabase and liposomes in Eudispert. Longer washing-out times were obtained for the two Orabase preparations than for the Eudispert ointment. This is not surprising as the Orabase dental paste is composed of highly hydrophobic constituents and dissolves slowly. The Eudispert ointment showed a washing-out time of approx. 10 min. Admittedly, the Eudispert ointment had a shorter washing-out time than the Orabase formulations, however, when applied on the oral mucosa the Eudispert ointment was retained on the oral mucosa for considerable period of time, even after the bulk of the ointment had eroded. Direct in vivo methods to assess the residence time of adhesive ointments in the oral cavity are not common in the literature. One reference was found where a value of 10 min for the in vivo residence time of Kenalog in Orabase in the mouth was reported (Chisholm et al., 1978).

In vitro drug release

The results from the in vitro drug release experiments indicated that the two different types of ointment bases used did not affect the rate or amount of TRMA released, although less drug

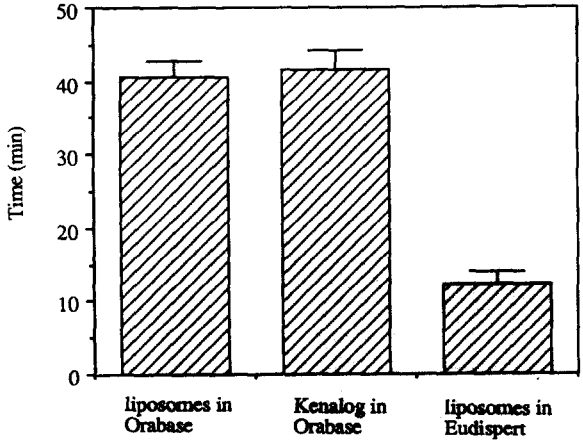

Fig. 2. Washing-out time for the three different ointments. Data are the mean of three experiments ± SD.

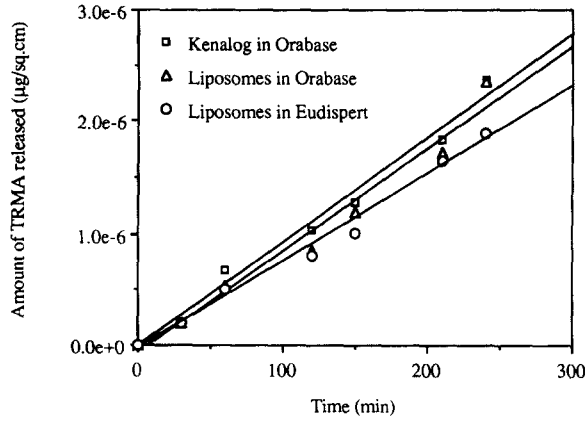

Fig. 3. Drug release expressed as a function of time for the three different ointments. Data are the mean of 3 experiments. Error bars are omitted to avoid overlapping.

was released from the formulations containing liposomal TRMA. Fig. 3 expresses the amount of drug released (μg TRMA/cm^{-2}) from the vehicles into the receiving chamber as a function of time. The corresponding apparent permeation coefficients for TRMA from Kenalog in Orabase, liposomes in Orabase and liposomes in Eudispert are $5.61e-10$, $5.48e-10$ and $4.66e-10$ cm/h, respectively. These values are approx. 4 orders of magnitude less those reported previously for the same drug when using the hamster cheek pouch model (Sveinsson and Mezei, 1992). Obviously, it can be seen from these results that the in vitro model applied can only be used for comparative purposes for different vehicles.

Clinical trial

The preparations have been well tolerated in the clinical trial, although liposomes in Orabase and Kenalog in Orabase were reported to be difficult to apply to the mucosa and gave a sticky, unpleasant sensation to several patients. There were no similar complaints about the Eudispert preparation. Clinical results are sparse at the present time and will be reported in a separate communication.

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