European Journal of Pharmaceutical Sciences 115 (2018) 233–239

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Microemulsion containing triamcinolone acetonide for buccal administration

C. Padula, I. Telò, A. Di Ianni, S. Pescina, S. Nicoli, P. Santi*

Department of Food and Drug, Parco Area delle Scienze 27/A, Università di Parma, 43124 Parma, Italy

ARTICLE INFO

Keywords:
Triamcinolone acetonide
Buccal delivery
Microemulsion
Chitosan

ABSTRACT

The aim of the present work was to investigate the potential of microemulsions for the buccal administration of triamcinolone acetonide. Microemulsions were developed by the construction of pseudoternary phase diagrams, using the aqueous titration method. Among all microemulsions prepared and tested for stability, three were selected and submitted to characterization and *in vitro* permeation/retention experiments, using pig esophageal epithelium, an accepted model of the buccal mucosa. Furthermore, one microemulsion was added of excipients (stearylamine, CTAB and chitosan) able to alter the charge of droplets.

The results obtained show that the permeation of triamcinolone acetonide across pig esophageal epithelium was not influenced by the droplet size nor by the composition, but only by the presence of chitosan, polysaccharide able to increase the transport across mono and stratified epithelia. The determination of the permeation parameters allowed us to show that chitosan acts on the diffusion parameter across the tissue and not on the partitioning parameter; for the same reason the tissue retention of triamcinolone acetonide was not modified. Triamcinolone flux (2.6 $\mu g\,cm^{-2}\,h^{-1}$) was too low to make systemic administration feasible (dose required 2.5 to 60 mg/day).

The amount of triamcinolone acetonide recovered in the mucosa after only 10 min. of microemulsion application was much higher than after overnight application of the commercial paste Omicilon® A. This suggests that triamcinolone acetonide microemulsions can be an interesting alternative to the commercial formulation to treat diseases of the buccal mucosa. Owing to the fast uptake by the tissue, the formulation can be used as a mouthwash.

1. Introduction

In the last decades microemulsions (MEs) have been studied as an interesting delivery vehicle for lipophilic drugs. These systems are easy to prepare and characterized by high thermodynamic stability; they can, in principle, solubilize both hydrophilic and lipophilic drugs and have been demonstrated to be able to enhance tissue uptake. MEs have been widely investigated for skin application, but only a few studies can be found in the literature for buccal drug delivery. Ceschel et al. tested microemulsions for the buccal delivery of *Salvia desoleana* essential oil (Ceschel et al., 2000), while Chukaewrungroj studied the use of microemulsions for the delivery of fluocinolone acetonide (Chukaewrungroj, 2016). In 2004, the efficacy of a microemulsion loaded with mometasone furoate for the treatment of oral lichen planus was demonstrated *in vivo* (Aguirre et al., 2004). Recently, promising results were obtained from the use of microemulsions, loaded into laminated sponges, for the transbuccal delivery of carvedilol (Abd-Elbary et al., 2016).

Despite the few studies, the properties of MEs can be very interesting for buccal application because the presence of a specific combination of excipients can enhance and accelerate drug uptake, issue particularly relevant given the good barrier properties of the buccal epithelium and the short residence time that characterizes buccal administration.

MEs can, in principle, be used in different forms such as mouthwashes, oral sprays or gels. Liquid dosage forms, such as mouthwashes, allow the drug to reach all the areas of the oral mucosa, thus can be used to treat diffuse diseases that affect different parts of the oral cavity (Wen and Park, 2011). The application of a liquid vehicle can be useful in the case of painful diseases, because - if formulated with non-irritant excipients - liquid formulations are better accepted compared to the application of a solid dosage form. Oral sprays share the same advantages as liquid formulations; in addition, the drug is protected from the external environment and can achieve higher concentrations at the absorption site. Finally, MEs can be thickened to obtain mucoadhesive semisolid formulations, more suitable for treating

* Corresponding author.
E-mail address: patrizia.santi@unipr.it (P. Santi).

https://doi.org/10.1016/j.ejps.2018.01.031
Received 13 November 2017; Received in revised form 15 January 2018; Accepted 16 January 2018

easily accessible and localized mucosal diseases.

Triamcinolone acetonide (TA) is a long acting corticosteroid used topically and systemically (by the parenteral and oral routes); the typical dose by injection (½ to 1/3 of the oral dose), ranges from 2.5 to 60 mg/day according to the disease, whereas topically, 0.1% formulations are used. The drug is currently employed for many inflammatory conditions of the oral cavity, such as recurrent apthous stomatitis and lichen planus (Belenguer-Guallar et al., 2014; Ramadas et al., 2016). Its permeation across and distribution into the buccal tissue have been studied *in vitro* (Shin et al., 2000) and *in vivo* (Harsanyi et al., 1986; Mumtaz and Chng, 1995; Azizi et al., 2016) from several topical formulations (tablets, mouthwashes, bioadhesive gels, *etc.*). To increase TA transport and/or retention, penetration enhancers, such as bile salts and nonionic surfactants (Shin and Kim, 2000), liposomes (Sveinsson and Holbrook, 1993), ethanol (Ungphaiboon and Maitani, 2001) and Azone® (Nicolazzo et al., 2005), were tested. In particular, Azone® doubled TA partitioning into the buccal mucosa and increased its permeation 4 times (Nicolazzo et al., 2004).

The aim of the present work was to investigate the potential of MEs for the buccal administration of TA. Microemulsion development was performed following a simple protocol based on a) drug solubility studies and excipients selection; b) construction of pseudoternary phase diagrams, using the aqueous titration method; c) accelerated stability tests. The prepared MEs were characterized for pH, structure, conductivity, droplet size distribution and zeta potential. Additives able to change the charge of the ME (stearylamine, CTAB and chitosan) were also used.

Then, selected MEs were tested across pig esophageal mucosa, an accepted model of human buccal mucosa (Diaz del Consuelo et al., 2005a). Permeation studies were performed in order to investigate TA transport across the tissue (in view of systemic administration) and mucosa retention (in view of local application) of the drug, in comparison with the commercial paste Omicilon® A.

2. Materials and Methods

2.1. Materials

Triamcinolone acetonide was purchased from Metapharmaceutical (Barcelona, Spain) while, Transcutol® HP (diethylene glycol monoethyl ether), Labrasol® (PEG-8 caprylic/capric glycerides), Peceol® (glyceryl monoleate), Labrafac lipophile® (caprylic/capric triglyceride), Labrafac® PG (propylene glycol dicaprylocaprate), Lauroglycol® 90 (propylene glycol monolaurate), Plurol oleique® (polyglyceryl-3 dioleate), Maisine® 35-1 (glyceryl monolinoleate), Capryol® 90 (Propylene glycol monocaprylate) were a gift from Gattefossé (Saint-Priest Cedex, France). Steraylamine, CTAB (cethyl-trimethyl ammonium bromide), Brij® 78 P (eicosaethylene glycol octadecyl ether) and Tween® 20 (polyoxyethylene (20) sorbitan monolaurate) were purchased from Sigma Aldrich (St. Louis, MO, USA). Chitosan (m.w. 10–1000 kDa), PEG 200, 400, 600, isopropyl myristate, myristyl myristate and propylene glycol were obtained from A.C.E.F. (Fiorenzuola, Italy). Oleic acid was obtained from Alfa Aesar (Karlsruhe, Germany) and TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) from BASF (Ludwigshafen, Germany). Sorbitan monoleate 80 (Span® 80) was a gift from Croda Ibérica SA, Spain.

For HPLC analysis, MilliQ® water was used. Acetonitrile and methanol were of HPLC grade; all other reagents were of analytical grade. Omicilon® A Orabase (Bristol-Myers Squibb Farmacêutica S.A., San Paulo, Brazil) is a dental paste composed of gelatin, pectin, and carboxymethylcellulose sodium in Plastibase® (Plasticized Hydrocarbon Gel, a polyethylene and mineral oil gel base) and contains 0.1% of TA.

2.2. Methods

2.2.1. Solubility Studies

An excess amount of TA was added to 2 ml of vehicle. Suspensions were left under magnetic stirring for 24 h at room temperature, then centrifuged for 10 min at 13000 rpm. The concentration of TA in the supernatant was determined by HPLC analysis after appropriate dilution.

2.2.2. Construction of Pseudoternary Phase Diagrams

In order to identify the region of existence of microemulsions, pseudoternary phase diagrams were built (software Origin, OriginLab, Northampton, MA, USA). Because microemulsions are made of 4 components, one axis reports “smix”, the total percentage of the mixture of surfactant/co-surfactant. The following ratios of smix were prepared: 1/1, 1/2, 2/1, v/v. Then, mixtures of oil/smix in different ratios (1/9, 1.2/8.8, 1.25/8.75, 1.5/8.5, 1.7/8.3, 2/8, 2.3/7.7, 2.5/7.5, 3/7, 3.4/6.6, 4.5/5.5, 5/5, 6/4, 7/3, 8/2, 9/1, v/v) were prepared and added of known volumes of water, in order to obtain water concentrations between 5% and 95%; after each addition, the mixtures were visually inspected for transparency, opalescence, fluidity, and phase separation.

One ME was added of stearylamine (0.5% w/v), CTAB (0.5% w/v) or chitosan (1.0% w/v); these components were dissolved in the water phase and the ME was prepared as before.

2.2.3. Thermodynamic Stability Studies

For each pseudoternary diagram, five MEs were selected from the region of existence. The selected MEs were added of TA at 0.1% w/v. Accelerated stability tests were then performed: formulations were first centrifuged for 30 min at 3500 rpm, then submitted to 6 cycles of heating (40 °C) and cooling (4 °C) of 48 h each and then to 3 cycles of freezing (−20 °C) and thawing (25 °C) of 24 h. MEs that did not pass these preliminary tests were not included in the following phase.

2.2.4. Microemulsions Characterization

Droplet size and charge were measured using the light scattering technique, at 25 °C with an incidence angle of 90°. The measures were performed on the native MEs, using a Brookhaven Instrument (PALS Zeta Potential Analyzer). Zeta potential was measured after 1:10 dilution in 1 mM KCl. The pH of o/w MEs was measured using an Orion 4 Star pH meter (Thermo Scientific, Waltham, Massachusetts, United States), at room temperature.

In order to assess isotropy, MEs were observed under a cross-polarized light microscope (Nikon, Shinjuku, Japan). Samples were deposited on a glass slide with a spatula, then covered with a covering slide in order to prevent the water evaporation.

MEs conductivity was measured at room temperature, using an AMEL 160 conductivity meter (Amel S.r.L., Milan, Italy) just after preparation and after 24 h.

MEs were analyzed for TA content by HPLC, after appropriate dilution.

2.2.5. In Vitro Permeation and Retention Studies

Permeation experiments were performed using Franz type diffusion cells, with a permeation area of 0.6 cm^2 (DISA, Milan, Italy). Pig esophageal epithelium was prepared according to Diaz Del Consuelo et al. (Diaz del Consuelo et al., 2005b). The esophageal mucosa was separated from the outer muscle layer with a scalpel and the epithelium was peeled off from the connective tissue, after immersion in distilled water at 60 °C for 60 s. The samples were frozen and used within 3 months. When needed, the tissue was thawed for 30 min in saline before mounting on the diffusion cells. The receptor compartment was filled with about 4 ml of a NaCl 0.9% solution, previously degassed in order to avoid the formation of bubbles at the tissue interface. This solution was kept at 37 °C under magnetic stirring. Experiments were performed