Local delivery of imiquimod in hamsters using mucoadhesive films and their residence time in human patients

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Objective. To investigate the ability of mucoadhesive films to locally deliver an immune response modifier (imiquimod) to oral mucosa.

Study Design. After determining the residence time of films in hamster cheek pouches, drug-loaded films and commercially available imiquimod cream were tested for localization of drug in mucosal tissue. The residence time of drug-free films at different locations was also tested in humans.

Results. Mucoadhesive films delivered imiquimod to the buccal mucosa with no measureable amounts in blood. In contrast, although the cream formulation resulted in higher tissue levels, it also led to significant systemic distribution of imiquimod. In humans, the films resided on tissue for up to 4 hours, increasing in the order of tongue < cheek < gingiva.

Conclusion. Preclinical findings of localized imiquimod delivery in animals and residence in humans support future investigations of the mucoadhesive system in controlled clinical trials for treating oral precancerous lesions. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;118:665-673)

Oral squamous cell carcinoma (OSCC) refers to any malignant cancer that arises from squamous epithelial cells in the oral cavity. This is the 10th most common type of cancer and was estimated to affect 41,380 new patients and cause 7890 deaths in the United States of America in 2013.[1] OSCC is commonly preceded by discolored (red or white) precancerous lesions characterized by abnormal growth (hyperplasia) and maturation (dysplasia) of epithelial cells. The likelihood of progressing to carcinoma depends on the severity of dysplasia. Early diagnosis and treatment of these oral dysplastic lesions can prevent them from progressing to OSCC and avoid further complications.[2,3]

Available treatment options for OSCC, such as radiation and chemotherapy, are used after dysplastic lesions have already progressed to OSCC, and they commonly lead to post-treatment morbidity. Although surgical resection can be performed to excise moderate to severe dysplastic lesions, the procedure results in loss of tissue and compromise of function. Hence, mucoadhesive films loaded with the immune response modifier imiquimod were designed in previous studies for preemptive, noninvasive, and localized treatment of oral dysplastic lesions.[4] Mucoadhesive drug delivery systems have been developed to localize the drug at mucosal surfaces, which avoids loss by first-pass metabolism and side effects associated with systemic delivery. In addition to increased bioavailability, adherence between the mucoadhesive polymers and absorbing tissue provides high flux and prolonged residence time of the drug at the desired site.[5] Imiquimod as Aldara cream (5% imiquimod; 3M, St. Paul, MN) was approved for treating superficial basal carcinoma. Off-label use has shown effectiveness in treating neoplasms of the vulvar epithelium, and the potential for application to melanoma of the intraepithelial oral mucosa and to oral leukoplakia has been reported based on uncontrolled single case studies.[6-9] Because retention of hydrophobic creams, such as Aldara, can be compromised in the oral cavity due to the moist tissue surfaces and continuous saliva turnover, a delivery system developed specifically for intraoral applications may improve localization of and prolong exposure to the drug.

The previously developed mucoadhesive films were able to sustain the release of imiquimod for up to 3 hours in vitro.[4,10] The ex vivo residence time, transport kinetics, and bioactivity of imiquimod-loaded films were also characterized as a function of composition. Although commonly used for initial screening of formulations, the in vitro behavior of a device will not necessarily reflect the performance in vivo because of differences in clearance rate, mechanical loading, pH,

Statement of Clinical Relevance

The treatment of oral dysplasia continues to be surveillance, resection, or ablation. Progression to cancer is difficult to predict or observe. This research could lead to treatment causing the arrest or regression of the dysplastic process in some patients.

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Received for publication May 15, 2014; returned for revision Aug 13, 2014; accepted for publication Aug 25, 2014.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2014.08.015

biochemical activities, etc. Consequently, in vivo testing is required to determine the actual residence time, release kinetics, and ability of the system to deliver the drug to tissue. Relatively few reports describe testing mucoadhesive films in animals, particularly those with applications that require prolonged residence.[11-13] The hamster cheek pouch model, which remains the most widely used for OSCC studies, provides regions of buccal mucosa with physiologic similarities to human tissue.[14,15]

The focus of the present studies was to conduct a preclinical evaluation of the performance characteristics of a mucoadhesive delivery system in vivo. After evaluating the residence time of films at different application sites in the hamster cheek pouch, the ability of the films to deliver and retain imiquimod in the oral mucosa with minimal systemic distribution was determined. Subsequently, the residence time of drug-free films at different intraoral sites was determined in human patients.

METHODS

Materials

Imiquimod was purchased from CalBiochem (White House Station, New Jersey). The polymers used for making films were polyvinylpyrrolidone (PVP) K-90 (Spectrum; New Brunswick, New Jersey) and carboxymethylcellulose (CMC; sodium salt, medium viscosity; Sigma, St. Louis, Missouri). Other chemicals used were: propylene glycol, USP grade ethanol (190 proof), methanol, poly(ethylene-co-vinyl acetate) [PEVA] with 18 wt% vinyl acetate (Sigma-Aldrich, St. Louis, Missouri); solid phase extraction tubes (STRATA XC; Phenomenex, Torrance, California); and a generic version of Aldara, 5% imiquimod (Perrigo, Dublin, Ireland).

Fabrication of films

Bilayered, imiquimod-loaded, mucoadhesive films were fabricated as described previously.[4] Briefly, aqueous solutions of PVP (1:1:1 ratio of 40% weight for volume [w/v] in deionized water: ethanol: propylene glycol) and CMC (2% w/v) were added to imiquimod solubilized in 3:7 acetate buffer (100 mM, pH 4):methanol, mixed thoroughly, and stored overnight at 43°C to remove bubbles. The backing layer was prepared by casting 10% w/v PEVA in toluene into Teflon dishes and drying at 30°C for 48 hours in sealed containers.[4] Bilayered films were subsequently made by casting the mucoadhesive polymer solution onto the PEVA and drying at 60°C. Films were then removed from the dishes and stored in a desiccator at −10°C. Mucoadhesive films with two compositions (1:2 and 2:1 PVP:CMC) and two different thicknesses were prepared (Table I).

Table I. Thickness of the PVP:CMC mucoadhesive films tested

Type (PVP:CMC)	*Thickness (mm)*
1:2 thin	0.36 ± 0.032
1:2 thick	0.55 ± 0.026
2:1 thin	0.36 ± 0.018
2:1 thick	0.47 ± 0.008

CMC, carboxymethylcellulose; *mm*, millimeter; *PVP*, polyvinylpyrrolidone.

Blank films were similarly prepared for human studies by mixing a drug-free solution of acetate buffer and methanol into the polymer solutions. Samples of diameter 7 and 10 mm were then punched and used for the hamster and human experiments, respectively. All films samples were terminally sterilized by exposure to ultra-violet light for 1.5 hours on each side in addition to aseptic fabrication using sterile solvents.

Residence time and distribution of imiquimod in hamsters

All animal studies were conducted at the University of Kentucky in accordance with a protocol approved by the Institutional Animal Care and Use Committee. Male golden Syrian hamsters weighing 90 to 115 g (Harlan, Indianapolis, Indiana) were used. With the animals under mild isoflurane anesthesia, mucoadhesive films were applied to the left cheek pouch by gentle pressure for 10 seconds. Hamsters were then transferred to an empty plastic cage with no bedding, food, or water for the remainder of the experiment. With the animals under deep anesthesia, blood was collected for drug measurement by cardiac puncture, and the animals were immediately euthanized by CO_2 asphyxiation. The treated and control (right side) cheek pouches were excised and assayed for drug content.

Pilot study. A small pilot study was initially conducted to identify the preferred films and application site for further analysis of drug distribution. The four types of mucoadhesive films shown in Table I were loaded with a low or high dose of imiquimod, that is, 0.675 mg/cm^2 (266 microgram per sample [μg/sample]) or 1.25 mg/cm^2 (533 μg/sample), respectively. After application of the films, cheek pouches were visually examined at intervals of 2 hours under mild anesthesia. The time point at which either the backing layer was spit out by the animal or the film was absent during observations was recorded as the residence time. Residence was investigated at three different locations: cheek, middle of the pouch, and deep within the pouch. After determining the preferred location to apply films, all four types at both the low dose and high dose were applied to only the middle of the pouch to assess drug delivery.