Topical Chemoprevention of Oral Cancer With Tretinoin “Biofilm”

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Background: Oral cancer is a common malignancy. Chemoprevention is a promising treatment strategy but it produces systemic toxic effects. Topical application of chemopreventive agents is an attractive alternative that reduces toxic effects. This study is based on the hypothesis that topical application of mucosal adhesive film (MAF), as a means to deliver tretinoin, is effective and safe for oral cancer chemoprevention.

Setting: Randomized animal study conducted at the Boston University School of Medicine.

Design: This study uses the hamster cheek-pouch model to test efficacy and safety of the MAF/tretinoin patch for oral cancer prevention. The oral mucosa of 36 hamsters was painted with dimethylbenzanthracene to produce premalignant lesions. The 36 hamsters were divided into 3 groups of 12 hamsters each as follows: (1) control, no treatment; (2) systemic tretinoin (5.0 mg/kg per day, intraperitoneally); and (3) topically applied MAF/tretinoin patch (0.45 mg tretinoin/cm², once daily). Treatments continued for 40 days.

Main Outcome Measures: Tumor growth and burden were measured over time. The duration of MAF patch retention on mucosa and local tissue reaction to the treatment were also evaluated.

Results: The patch stayed on the mucosa for at least 5 hours with no evidence of inflammatory or other adverse reactions from the treated tissue. There was a significant difference in the tumor growth measurement between the control and systemic tretinoin groups (P<.001), and between the control and MAF patch groups (P<.001).

Conclusions: This is the first study, to our knowledge, to use a polymer MAF technique for oral cancer prevention. The MAF/tretinoin patch is safe and effective for such chemoprevention in the hamster model.


In carcinogenesis, genetic damage appears to be a multistep and cumulative process, often occurring over 20 to 30 years. Oral cancer is typically the product of this carcinogenic process after the entire epithelial surface at risk has been exposed to repeated insult by carcinogens, eg, tobacco. This fact strongly supports the rationale for chemoprevention, namely, to inhibit, delay, or reverse carcinogenesis before it becomes invasive disease. Numerous compounds have been extensively tested. One of the best-studied compounds to date is retinoic acid (RA), with established clinical activity against oral leukoplakia and premalignant lesions. While the effectiveness of systemic chemoprevention in oral cancer has been well demonstrated, unfortunately, its widespread use has been hindered by the toxic effects that accompany treatment, especially for individuals who must be treated for prolonged periods or who are sick from a secondary cancer. In some clinical studies, treatment-related toxic effects were so significant that many participating patients did not complete the designed intervention period. The toxic effects associated with the administration of these chemopreventive agents also prevent the use of an adequate dose and sufficient treatment period to achieve the desired results.

The use of topical chemotherapeutic approaches has been tested in clinical studies as an alternative to systemic medication. Researchers hope that the topical approach will produce fewer systemic side effects without sacrificing major advantages in chemoprevention. Furthermore, the topical medication can be applied by the patient at home with low cost and convenience. It would even offer higher efficacy than systemic medication because the drug would be delivered directly to the tar-
geted tissue. Currently, however, painting the oral mucosa with topical solution for 3 to 5 minutes is the most common topical chemoprevention protocol. Such an approach offers significantly limited clinical efficacy because it is difficult to maintain adequate agent concentration and sufficient retention time on the targeted tissue. As a transdermal delivery approach, the agent concentration and retention are critical for topical chemoprevention efficacy. For example, in a clinical study comparing local painting with concentrations of 1.0% and 0.5% bleomycin, the higher concentration was significantly superior, and resulted in better control of oral premalignant lesions. Consequently, an increase retention time of the agent applied on the local tissue can be expected to enhance efficacy. The topical painting approach cannot preserve the agent from excessive saliva flow or frequent mechanical activities in the oral cavity, which dilute and remove the medication quickly from the targeted mucosa. Furthermore, systemic toxicity increases when the medication is absorbed by a large area of “contaminated” neighboring tissue or swallowed with the saliva.

In this study, we explore the feasibility of using mucosal adhesive film (MAF), as a new vehicle and strategy of “contaminated” neighboring tissue or swallowed with the saliva.

METHODS

Male golden Syrian hamsters, 6 weeks old, were used for this study. The hamsters were routinely housed and fed standard laboratory chow and water. The study had 3 phases: (1) to determine MAF patch retention time on oral mucosa, (2) to evaluate local tissue reaction to treatment, and (3) to determine treatment efficacy for oral cancer prevention. The study was conducted in accordance with the Public Health Service policy on Human Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. The animal use protocol was approved by the Institutional Animal Care and Use Committee of the Boston University School of Medicine. Institutional guidelines regarding animal experimentation were followed.

MAF PREPARATION

The 3-layer hydroxypropylcellulose (HPC) film consists of a nonadhesive layer, an intermediate RA-containing layer, and an adhesive layer. To obtain the nonadhesive layer, a homogeneous solution of HPC (0.6 g), ethyl cellulose (0.6 g), and polyethylene glycol 400 (PEG, 0.44 g) in ethanol (20 mL) were poured into a 20-cm2 Teflon-coated cast and dried. To form the medicated layer, a solution of HPC (2.9 g), PEG (0.05 g), and tretinoin in ethanol (65 mL) was applied on the nonadhesive layer and dried. Finally, HPC (0.5 g) and PEG (0.01 g) were dissolved in ethanol (20 mL) in which pectin (1.9 g) was dispersed to form a suspension. Then the suspension was applied to the above double-layered film and dried as an adhesive layer. All of the chemicals were purchased from Sigma-Aldrich Corp, St Louis, Mo. The full thickness of the MAF is 0.2 mm.

MAF PATCH RETENTION ON MUCOSA

To ensure a sufficient MAF retention time on the targeted mucosa after topical placement, 5 hamsters were treated with “placebo” MAF (with no tretinoin). Under general anesthesia through temporary inhalation of isoflurane for 10 to 20 seconds, 2 MAF patches, 6.0 mm2 each, were placed on each side of the hamster cheek pouches. The animals usually resumed normal activities, including normal chewing and drinking, 1 to 2 minutes after the isoflurane inhalation. The placed MAF patches were examined every half hour for 6 hours after placement.

MUCOSAL REACTION TO MAF

Twelve hamsters were used in the study. They were divided into 4 groups, 3 of each: (1) placebo patch group (MAF only, with no tretinoin); (2) 0.15-mg group (using MAF patch containing 0.15-mg tretinoin/cm2); (3) 0.45-mg group; and (4) 1.35-mg group. Using the same anesthesia procedure described above, 2 MAF patches (6.0 mm2) were placed on each side of the hamster cheek pouches. The patch was changed once daily for 3 days. Any adverse responses of the patched mucosa were carefully observed when the MAF patch was changed. These responses were classified and recorded as follows: 0, normal; I, visible irritation; II, minor inflammation; III, severe inflammation; and IV, ulceration (or necrosis). Responses were documented by photography. At the end of the study, the animals were euthanized for tissue sample biopsy from the treated mucosa.

CHEMOPREVENTION OF ORAL CANCER

Earliest possible changes in the cheek pouches of the hamster model were induced with topical dimethylbenzanthracene (DMBA) painting, a common approach to create tumors in the hamster model. Briefly, under general anesthesia with carbon dioxide inhalation, the mucosa of the cheek pouches were painted with a 0.5% solution of DMBA (Sigma-Aldrich Corp) in acetone on both sides of the buccal mucosa. The painting was done with a No. 3 sable brush, 3 times per week for 3 weeks. Examination of the cheek pouches continued until there were 2 to 3 early lesions (eg, leukoplaikia-like changes) identified on each side of the pouch in most of the hamsters. Two lesions were identified from each side of cheek pouch (4 lesions from each hamster) and marked with India ink by a thorough puncture around the site with a 25-gauge needle. The lesions were also documented with a drawn “map” and photographs. Thirty-six hamsters were divided into 3 groups of 12 each: (1) control, no treatment; (2) systemic tretinoin, 3.0 mg/kg body weight per day intraperitoneally; and (3) topical MAF patch (0.45 mg tretinoin/cm2), once daily under general anesthesia. To cover an entire lesion, the patch was trimmed to 6.0 mm2. The lesion and tumor growth were examined once weekly for 40 days of the treatment. Statistical analyses were performed with Stat View statistical software (SAS Institute Inc, Cary, NC). Comparison of the tumor burden was done by using a nonparametric t test. Finally, the animals were then weighed, and euthanized to collect the treated tissue for the biopsy.

RESULTS

All of the MAF patches were retained on the targeted mucosal sites for 5 to 6 hours after placement. Afterward, the patches fell or slipped off of the initial mucosal site. There were no visible or histological changes found on the treated mucosal sites, even those mucosal...
sites treated with the 1.35-mg tretinoin patch. There was a statistically significant difference in the tumor growth measurements between the control and systemic tretinoin groups \((P<.001)\), and between the control and MAF patch groups \((P<.001)\). However, the measurement results from the systemic medication group and the MAF patch group were comparable \((P>.1)\). The results are shown in Figure 2 and the Table. There was no statistical difference found in the hamster body weight measured before and after the study, and between groups at the end of 40-day treatment.

**COMMENT**

Oral cancer is one of the most common malignancies. In 1995, it is estimated that there were approximately 30,000 new cases in the United States, resulting in 8,370 deaths. Tobacco use is known to be a key cause of oral cancer. In this country, 50 million people smoke and 12 million chew tobacco; worldwide, the corresponding figures are 1 billion and 600 million, respectively. Survival after traditional treatment for the oral cancer has improved only marginally, despite numerous advances in treatment using the most recent protocols for surgery, radiation, or chemotherapy. Despite successful primary therapy, 30% to 50% of patients have local or regional recurrence. Reversing oral carcinogenesis before its becoming invasive disease is the promise of treatment. Topical application of a chemopreventive agent can reduce the toxic effects associated with a systemic medication. Oral leukoplakia and other visible mucosal changes (such as erythroplakia), which are known to have a high risk of malignant transformation, provide a good target for topical chemoprevention.

In previous reported clinical studies, topical painting of vitamin A or other RA compounds provides some encouraging results. In addition to reduced toxic effects, topical application is easy and does not require treatment at a medical center. Therefore, the cost, compared with other interventions, is relatively low. However, up to now, the effectiveness of topical chemoprevention is controversial. In some clinical trials, topical application of medication appears effective or “marginal,” causing some regression or lower recurrence of leukoplakia. But in other studies, there is no significant efficacy in the inhibition of oral leukoplakia. Recently, topical application, again, failed to show efficacy in a clinical trial using topical vitamin A on oral leukoplakia. It is clear that, due to their inherent weaknesses, current strategies are far from being satisfactory. In the present study, the MAF patch, compared with traditional painting or other approaches, significantly prolonged retention time of the agent on the targeted mucosa. Our findings show that the MAF/tretinoin patch is very effective in preventing oral cancer, with comparable efficacy to the animals treated with systemic tretinoin \((P>.1)\). In addition to delivering tretinoin for the treatment of oral cancer, MAF preparation can be used for other chemopreventive approaches, or for treating other cancers. As well as presenting a new ideal alternative for oral cancer chemoprevention, we believe that the MAF preparation has great potential for clinical use. Furthermore, it is safe and convenient for outpatient use.

Mucosal adhesive film, made with a water-soluble polymer, has been successful for many years for the topical application of analgesics, antibiotics, or other medications to oral mucosa. It adheres very well to mucosa and is easy for patients to use at home. In a number of clinical applications, the MAF patch was shown to be safe, comfortable, and convenient. The film can be trimmed to any size or shape. It remains on the wet surface of the mucosa without displacement from chewing or tongue movement for at least 3 to 6 hours. Its effects are long-lasting because the drug is slowly absorbed by local mucosa. Patients do not complain of any significant dis-
The excellent tissue tolerance continues even when the cosa, at least in the cheek pouches of the hamster model. Our findings indicate that MAF and the tretinoin products, made with the same or similar materials as those used in human oral mucosa. Therefore, this study was undertaken. Currently, there are many MAF products available for various oral medication treatments. These products, made with the same or similar materials as those in the MAF preparation used in this study, have a very good record of the safety and local tissue tolerance when used in human oral mucosa. Therefore, this study was focused on determining the tissue tolerance to tretinoin. Our findings indicate that MAF and the tretinoin dose proposed in this study are well tolerated by oral mucosa, at least in the cheek pouches of the hamster model. The excellent tissue tolerance continues even when the tretinoin dose is increased to 1.35 mg/cm², a 3-times higher dose than actually used for the chemoprevention experiment. However, it should be noted that oral mucosa of an animal model often is more resistant to chemicals or medications than the human oral mucosa. The response of oral mucosa to tretinoin treatment may be different between hamsters and humans. Further clinical study is needed to address this issue in humans. Clinically, the systemic signs of tretinoin toxicity present primarily as dry skin, cheilitis, hypertriglyceridemia, or conjunctivitis. These symptoms may be difficult to identify in a rodent model. However, we believe that, in this study, the topical MAF/tretinoin patch should result in reduced toxic effects compared with the systemic medication. It has been known that the RA treatment toxicity is dose-dependent, and a reduced dosage will reduce toxic effects. Theoretically, even assuming total absorption of the topically applied tretinoin, each hamster will receive a maximum of only 0.11-mg tretinoin per day (proposed dose × patch size × patch number = 0.45 mg/cm² × 0.06 cm² × 4) systemically that is well below the dosage of 0.75 mg/d (dose × hamster body weight = 5.0 mg × 0.15 kg) used in hamsters treated with systemic medication.

It is possible that some of the tretinoin is systemically absorbed via the treated mucosal sites and, thus, it produces some effect on the entire mucosa and on the treatment efficacy. However, such a systemic effect cannot explain the whole treatment effectiveness. Efficacy of RA for cancer treatment is dose-dependent. In animal studies using rodent models, low-dose RA does not show any efficacy for treatment of head and neck squamous cell carcinoma until the dose is increased, at least to 1.0 mg/kg per day. As described above, there may be only 0.7 mg/kg tretinoin per day in our study, even if the drug in the MAF patch is totally and systemically absorbed (actually, 100% absorption, via such a way, is impossible). We believe that the direct effect of the topical patch on the tissue plays a key role. Otherwise, it is difficult to explain how topical tretinoin treatment could produce efficacy comparable to the systemic administration when there is at least approximately a 7-fold dosage difference between the 2 groups (0.11 mg/d vs 0.75 mg/d per hamster). Interestingly, the treatment efficacy in this study appears to be limited to the ink-marked lesions that were covered by the MAF patch. There is no evidence to show an inhibitory effect from the topical treatment on untreated lesions, even those on the same cheek pouch. This observation may imply that topical treatment can be effective only with the direct contact of patch tissue.

Clinically, the oral cavity, easily visible and accessible, is the best place to test the efficacy of any new oral cancer chemoprevention strategy. The Syrian golden hamster cheek-pouch model of carcinogenesis exhibits the closest similarity of any animal model system to the development of premalignancy in human oral cancer. Among its many advantages, the hamster model simulates many aspects of human oral cancer. For instance, there is a relative long latent period before the malignancy is produced, and cancer formation is preceded by hyperkeratotic and dysplastic lesions, very similar to the clinical situation. The model has been extensively used in chemoprevention studies in oral cancer chemoprevention, to inhibit or decrease tumor formation. This hamster cheek-pouch model has demonstrated, for the first time, the feasibility of using the MAF patch to topically deliver tretinoin for oral cancer chemoprevention. However, the choice of 0.4 mg tretinoin/cm² MAF as the treatment dose in this study is based only on our theoretical calculation. We do not know whether it is the best choice for this topical application or if it is the minimal dose required for producing the treatment efficacy. It may be possible to reduce the dose for a concomitant reduction of side effect or local tissue irritation. Further studies are warranted to find the answer and to test this topical treatment strategy in patients. We also believe that...
the same concept and strategy should be applicable to chemoprevention of skin cancer and other accessible malignancies of the tissue surface.

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REFERENCES


