Journal of Carcinogenesis

Review Article

Optimizing therapeutic efficacy of chemopreventive agents: A critical review of delivery strategies in oral cancer chemoprevention clinical trials

Andrew S. Holpuch[1], Kashappa-Goud H. Desai[2], Steven P. Schwendeman[2], Susan R. Mallery[1,3]*

[1]Division of Oral Maxillofacial Surgery, Pathology and Anesthesiology, College of Dentistry, The Ohio State University, Columbus, OH 43210, [2]Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI 48109, [3]The Ohio State University Comprehensive Cancer Center, USA

E-mail: mallery.1@osu.edu
*Corresponding author

Published: 21 September, 2011
Journal of Carcinogenesis 2011, 10:23
This article is available from: http://www.carcinogenesis.com/content/10/1/23
© 2011 Holpuch,

Received: 20 April, 2011
Accepted: 07 August 2011

Abstract

Due to its characterized progression from recognized premalignant oral epithelial changes (i.e., oral epithelial dysplasia) to invasive cancer, oral squamous cell carcinoma represents an optimal disease for chemopreventive intervention prior to malignant transformation. The primary goal of oral cancer chemoprevention is to reverse, suppress, or inhibit the progression of premalignant lesions to cancer. Over the last several decades, numerous oral cancer chemoprevention clinical trials have assessed the therapeutic efficacy of diverse chemopreventive agents. The standard of care for more advanced oral dysplastic lesions entails surgical excision and close clinical follow-up due to the potential (~33%) for local recurrence at a similar or more advanced histological stage. The purpose of this review was to identify prominent oral cancer chemoprevention clinical trials, assess their overall therapeutic efficacy, and delineate effects of local versus systemic drug administration. In addition, these compiled clinical trial data present concepts for consideration in the design and conduction of future clinical trials.

Keywords: Chemoprevention, local drug delivery, oral cancer, pharmacologic advantage

INTRODUCTION

Cancer treatment delivery strategies – Past and present

Optimal cancer therapies preferentially target cancerous cells while sparing normal, non-tumorigenic cells. These treatment outcomes, however, are rarely realized with current systemic chemotherapy regimens. Often severe dose-limiting toxicities impede the progression of treatment and/or mandate decreased therapeutic dosing.[1] These treatment alterations result in an increased risk of selecting for chemotherapy-resistant cell populations due to the low-dose induction of detoxification enzymes (i.e., phase I/II enzymes and ATP binding cassette-efflux transporters).[2] In an effort to minimize toxicity-induced treatment reductions, innovative strategies for concentrating therapeutics in tumors have been developed for systemic delivery. Notably, these novel delivery methods often exploit tumor microenvironment and protein expression profiles to preferentially target delivery to tumors (e.g., concentration of macrotherapeutics via the enhanced permeability and retention effect, antibody–therapeutic conjugates against tumor specific receptors, and hypoxia-activated prodrugs).[3-6] Another strategy introduced by

Access this article online

Quick Response Code:

Website: www.carcinogenesis.com

DOI: 10.4103/1477-3163.85185

Folkman *et al*. directed treatment toward tumor-associated angiogenesis rather than tumor cells; however, systemic delivery of angiostatic agents has not been highly successful in human applications.[7] Although tumor-targeted strategies are becoming more commonplace in clinical practice, the standard of treatment for numerous cancers remains the systemic administration of highly toxic compounds with severe side effects.[1,8,9]

Many cancers necessitate systemic delivery due to their anatomical locations, high metastatic potential, and/or advanced stage upon diagnosis. Classic examples of such malignancies include leukemia, advanced stage lymphoma, lung and hepatocellular cancers. These cancers are ideal candidates for systemic administration of chemotherapeutic compounds due to their systemic distribution and/or origin from richly vascularized tissues.[9,10] In contrast, several cancers (e.g., oral, cutaneous, and cervical carcinomas) arise at visibly accessible locations and progress through well-characterized, recognizable premalignant lesions. These cancers are, therefore, more amenable to the use of local delivery-based preventive therapies for either primary chemoprevention (suppressing progression of premalignant lesions to cancer) or secondary chemoprevention (inhibition of cancer recurrence). Despite their suitable location for local delivery strategies, numerous chemoprevention clinical trials have failed to deviate from the standard systemic delivery paradigm. Notably, in order for systemically administered agents to affect epithelial lesions, they require transport through systemic vasculature, perfusion from blood vessels in the underlying connective tissue, diffusion through interstitial spaces/epithelial basement membrane, and subsequent absorption and retention within the epithelium. This method of delivery likely requires supra-therapeutic serum concentrations (i.e., potentially toxic concentrations) to obtain therapeutically relevant drug levels in the target tissues. In contrast to these extensive delivery considerations, local delivery formulations provide targeted delivery methods that impart a pharmacologic advantage over systemic delivery schemes (i.e., optimal drug concentrations in target epithelium, reduced drug dosing and less exposure of normal tissues resulting in minimal side effects, and increased stability and apparent solubility of drugs in physiological fluids thus facilitating drug permeability across biological membranes).[11,12] Furthermore, compounds delivered locally are not subjected to first-pass hepatic metabolism, thereby limiting premature metabolic inactivation and excretion prior to the exertion of therapeutic effects in the target tissues.[12] Collectively, local formulations represent favorable treatment modalities for drug delivery to site-specific premalignant epithelial lesions.

Chemopreventive therapies to suppress the development of oral squamous cell carcinoma (OSCC), the focus of our laboratory's research, entail the use of natural and/or synthetic compounds to suppress, inhibit, or reverse the malignant transformation of oral epithelial dysplasia to OSCC.[13,14] While many promising chemopreventive agents have been evaluated in both local and systemic delivery clinical trials, only the local delivery studies have shown both chemopreventive efficacy and negligible dose-limiting side effects. Notably, the success of intraoral drug delivery strategies is governed by the effectiveness of the delivery vehicles (i.e., ability to provide effective drug localization and maximize patient compliance). Hence, numerous polymeric carriers, such as mucoadhesive gels, patches, tablets, rinses, sprays, and lozenges, have been studied for intraoral local delivery of oral medications.[13-22] Although many of these delivery strategies have been employed in oral cancer chemoprevention trials, most of these studies have failed to optimize drug delivery through the assessment of local pharmacokinetic parameters (e.g., determining oral intraepithelial drug concentrations, metabolite formation, and drug stability/release kinetics).[23-31] In addition, inconsistent enrollment criteria (e.g., the inclusion of patients with benign hyperkeratotic lesions and lack of tobacco cessation) complicate the comparative analysis of inter-trial chemopreventive efficacy. Therefore, the purpose of this review was to identify prominent oral cancer chemoprevention clinical trials, assess their overall therapeutic outcomes in those patients with histologically confirmed premalignant lesions (i.e., regression of oral epithelial dysplastic lesions), and evaluate the chemopreventive efficacy depending on the method of drug delivery (i.e., local versus systemic administration).

ORAL CANCER CHEMOPREVENTION CLINICAL TRIALS: SYSTEMIC DELIVERY

Previous oral cancer chemoprevention clinical trials that utilized systemic delivery systems have provided mixed results. The most prominent of these studies, which have evaluated the delivery of several classes of compounds, i) vitamin A derivatives, ii) cyclooxygenase 2 (COX-2) inhibitors, and iii) natural products, are reviewed [Table 1].

Vitamin A derivatives

Natural and synthetic vitamin A derivatives elicit the induction of terminal differentiation and apoptosis in epithelial cells, both promising therapeutic endpoints for oral cancer chemoprevention.[32-34] Therapeutic outcome with these derivatives is often dependent on the duration of treatment, concentration within the target site, and specific vitamin A compound under evaluation.[35-41] Notably, the vitamin A derivatives investigated in systemic delivery oral

Table 1: Systemic delivery oral cancer chemoprevention trials

Therapeutic agent	Year[Ref.]	Delivery method, schedule, and duration	Biopsies and microscopic diagnosis	Side effects	Therapeutic outcomes
Isotretinoin (13-*cis*-retinoic acid)	1986[35]	Oral capsule, 1–2 mg/kg q.d. Duration: 3 months with 6-month follow-up	Pre- and post-treatment histologically confirmed dysplastic and atypical hyperplastic lesions (n = 44)	Mild to moderate	*Isotretinoin group (n = 24):* 54% of lesions showed histological regression of dysplasia. Notably, lesion recurrence was exhibited in 9 of 16 evaluable patients *Placebo group (n = 20):* 10% of lesions showed histological regression of dysplasia (P = 0.01 relative to isotretinoin group) *Alcohol/tobacco cessation not required*
Isotretinoin and β-carotene	1993[36]	*Induction phase:* isotretinoin (oral capsule, 1.5 mg/kg q.d.) Duration: 3 months *Maintenance phase:* isotretinoin (oral capsule, 0.5 mg/kg q.d.) or β-carotene (oral capsule, 30 mg q.d.) Duration: 9 months	Pre- and post-treatment histologically confirmed dysplastic and hyperplastic NOS lesions (n = 70)	Mild to severe	*Induction phase (n = 70):* 55% of lesions exhibited complete or partial clinical regression, while 35% demonstrated stable disease. Notably, 18 of 42 patients with dysplasia had a decreased histological grade and 7 patients experienced disease progression *Maintenance phase (n = 53):* β-*carotene (n = 29)*: 45% of patients exhibited complete/partial or stable clinical response, while 55% demonstrated disease progression. *Isotretinoin (n = 24)*: 92% of patients demonstrated complete/partial or stable clinical response, while 8% experienced disease progression *Alcohol/tobacco cessation not required*
β-carotene	1999[37]	*Induction phase:* β-carotene (oral capsule, 30 mg b.i.d.) Duration: 6 months *Maintenance phase:* β-carotene (oral capsule, 30 mg b.i.d.) or placebo Duration: 12 months	Pre- and post-treatment biopsies *19 of 50 histologically confirmed lesions dysplastic	None reported	*Induction phase (n = 50):* 52% of lesions demonstrated complete or partial response, while 40% exhibited stable disease and 8% progressed. Notably, a 39% histological response rate was observed in dysplastic lesions Maintenance phase (n = 23): β-*carotene (n = 11)*: 82% of the lesions remained stable, 18% progressed. *Placebo (n = 12)*: 83% of the lesions remained stable, 17% progressed *Quantified both plasma and buccal cell β-carotene concentrations *Alcohol/tobacco cessation not required*
Isotretinoin, α-tocopherol, and interferon-α cocktail	1999[38]	Isotretinoin (oral capsule, 100 mg/m^2, q.d.) α-tocopherol (oral capsule, 1200 IU, q.d.) interferon-α (SubQ, 3 MU/m^2, twice/week) Duration: 12 months with 6-month follow-up	Pre- and post-treatment histologically confirmed oral dysplasia (n = 11)	Mild to severe	*6-month examination (n = 10):* 50% of histologically evaluated lesions demonstrated complete/partial response, while 20% exhibited stable disease and 30% progressed *12-month examination (n = 7):* 14% of histologically evaluated lesions demonstrated partial response, while 43% exhibited stable disease and 43% progressed. Notably, four patients eventually progressed to cancer *Alcohol/tobacco cessation not required*
Fenretinide	2005[39]	Oral capsule, 100 mg b.i.d. versus no treatment Duration: 12 months with 5-year follow-up	Surgically excised benign hyperkeratotic and dysplastic lesions (n = 170) *3–4% of lesions were histologically confirmed dysplasia	Mild to severe	*Fenretinide group (n = 84):* 15 patients exhibited lesional recurrences, 4 patients developed new leukoplakic lesions, and 1 patient developed oral cancer *No treatment group (n = 86):* 15 patients exhibited lesional recurrences, 10 patients developed new leukoplakic lesions, and 2 patients developed oral cancer *Alcohol/tobacco cessation not required*

Table 1 (contd...)

Table 1 (contd...)

Therapeutic agent	Year[Ref.]	Delivery method, schedule, and duration	Biopsies and microscopic diagnosis	Side effects	Therapeutic outcomes
Fenretinide	2006[40]	Oral capsule, 200 mg q.d. Duration: 3 months with 9-month follow-up	Retinoid-resistant "oral intraepithelial-neoplasia" *10 of 35 lesions histologically confirmed dysplasia	Mild to moderate	*3-month examination (n = 35):* 34% of patients exhibited clinical partial responses, while 43% of patients demonstrated stable disease and 23% progressed. Notably, 9 of 12 partial responders progressed within 9 months of trial completion *Alcohol/tobacco cessation not required*
Fenretinide	2009[41]	Oral capsule, 900 mg/m^2 b.i.d. in 3 week cycles (1 week on, 2 weeks off) Duration: 3 months with 3-month follow-up	Pre- and post-treatment histologically confirmed dysplastic lesions in 11 of 15 patients	Mild to severe	*3-month examination (n = 15):* 13% of patients demonstrated histological downgrading of dysplasia, while 47% exhibited no histological change and 40% progressed. None progressed to invasive cancer at 3-month follow-up *Trial discontinued after 3 months due to lack of efficacy *Alcohol/tobacco cessation not required*
Celecoxib	2008[49]	Oral tablet, 100, 200, or 400 mg b.i.d. or placebo Duration: 3 months	Pre- and post-treatment histologically confirmed epithelial atypia or dysplastic lesions (n = 49)	Mild to severe	*3-month examination:* No significant histological improvement rates (i.e., reversal or improvement of dysplastic grade) were observed between the placebo and Celecoxib treatment groups (P = 0.71, Fisher's exact test) *Alcohol/tobacco cessation not required (counseling offered)*
Green tea extract	2009[50]	Oral capsule, 500, 750, or 1000 mg/m^2 t.i.d. or placebo Duration: 3 months with median 27.5 month follow-up	Pre- and post-treatment histologically confirmed hyperplasia (n = 11) or dysplasia (n = 30)	Mild to moderate	*3-month examination (n = 39):* Although the histological response rates of the combined green tea extract arms were increased (21.4%) relative to placebo arms (9.1%), these findings were not statistically significant (P = 0.65) *Alcohol/tobacco cessation not required (counseling offered)*

cancer chemoprevention trials include: 13-*cis*-retinoic acid (isotretinoin), β-carotene, an isotretinoin/α-tocopherol/interferon-α cocktail, and 4-hydroxyphenylretinamide (fenretinide).[35-41]

In 1986, Hong *et al*. evaluated the effects of isotretinoin administration (oral capsule, 1–2 mg/kg q.d.) on histologically confirmed oral dysplasia.[35] Although the trial results were promising (i.e., 54% of the treated lesions showed histological reversal of dysplasia relative to 10% in the placebo group), the favorable response rate of these lesions was offset by the wide range of adverse side effects encountered throughout the study [i.e., mild (cheilitis, skin dryness, facial erythema) to moderate (conjunctivitis and hypertriglyceridemia)].[35] In an effort to expand upon the promising therapeutic outcome of Hong *et al*.'s studies, Lippman *et al*. evaluated an introductory high-dose isotretinoin treatment (oral capsule, 1.5 mg/kg q.d. for 3 months) followed by maintenance doses of either isotretinoin (oral capsule, 0.5 mg/kg q.d.) or β-carotene (oral capsule, 30 mg q.d.) for 9 months.[36] The goal of this study was to preserve responsiveness while concurrently reducing side effects. Variable side effects, however, were encountered and the inclusion of both histologically confirmed dysplasia and hyperplastic not otherwise specified (NOS) lesions complicated the interpretation of results.[36] Unlike dysplastic lesions, reactive hyperplastic lesions do not have an established malignant transformation potential, and therefore, inclusion of these lesions introduced bias toward increased therapeutic efficacy.[42,43]

Similar data evaluation challenges were encountered in a later β-carotene study (oral capsule, 30 mg b.i.d. for 6 months, followed by randomization to placebo or 30 mg b.i.d. β-carotene for 12 months), which was conducted in 50 patients (only 19 presenting with histologically confirmed dysplastic lesions) who were permitted to continue tobacco and/or alcohol use.[37] Following 6 months of treatment, 4% of the participants' lesions demonstrated complete clinical response while 48% exhibited partial clinical response (39% histological response rate in dysplastic lesions).[37] Although the indiscriminate enrollment of patients with non-dysplastic

lesions and the lack of risk factor cessation weaken the study, the quantification of β-carotene levels in both plasma and buccal cells provides a basis for target tissue concentrations in future chemoprevention studies.[37]

Furthermore, in 1999, Papadimitrakopoulou *et al*. published the results of a 12-month study evaluating a chemoprevention cocktail designed to minimize the side effects commonly associated with vitamin A treatments [i.e., isotretinoin (oral capsule, 100 mg/m^2 q.d.), α-tocopherol (oral capsule, 1200 IU q.d.), and interferon-α (subcutaneous injection, 3 MU/m^2 twice weekly)].[38,44,45] Despite the inclusion of α-tocopherol, side effects ranging up to Grade 3 toxicities were observed.[38] In addition, both histologically confirmed dysplastic laryngeal and oral lesions were included in the trial. Whereas laryngeal lesions responded favorably, oral lesions were recalcitrant to the cocktail chemopreventive approach (i.e., only 14% of histologically evaluated oral lesions demonstrated a partial response at 12 months).[38] Importantly, oral cancer chemoprevention will likely require prolonged treatment regimens. These treatment considerations combined with the adverse side effects encountered in these studies warranted the evaluation of other prospective chemopreventive compounds.

Likewise, several oral cancer chemoprevention trials have evaluated the clinical efficacy of the synthetic retinoid fenretinide based on its reduced toxicity profile and retinoid receptor-dependent and -independent effects.[39-41] In 2005, the results were published from a long-term study that evaluated the effects of low-dose treatment (oral capsules, 100 mg b.i.d.) versus no treatment for 1 year on surgically resected oral lesions, which included both histologically confirmed benign hyperkeratotic and dysplastic lesions.[39] Although this study demonstrated that fenretinide treatment protected against recurrent lesions, the inclusion of benign lesions obfuscated the determination of actual chemopreventive efficacy.[39] An additional low-dose fenretinide study (oral capsules, 200 mg q.d. for 3 months) in non-excised retinoid-resistant histologically confirmed oral dysplastic lesions demonstrated the reduction of clinical size in 34% of the patients.[40] Subsequently, a study investigating the effects of high-dose fenretinide (oral capsules, 900 mg/m^2 b.i.d. in 3 week cycles, with 1 week on, 2 weeks off) on histologically confirmed oral dysplastic lesions was discontinued after 12 weeks due to low lesional response rates.[41] Despite moderate, yet transient, treatment responses in the low-dose trials, all of these fenretinide studies encountered a range of mild to severe systemic side effects, some of which required trial discontinuation.[39-41] Notably, these studies failed to obtain the *in vitro*-established therapeutically relevant fenretinide levels of 1–10 μM in serum (i.e., high-dose treatment: $0.122 \pm 0.093\,\mu$M, low-dose continuous treatment: $0.23\,\mu$M), and were undetermined by Chiesa *et al*.[39-41,46]

Collectively, the systemic delivery of vitamin A derivatives provided modest therapeutic efficacy and were accompanied by systemic toxicities.[35-41] With the exception of the β-carotene trial by Garewal *et al.*, none of these vitamin A derivative studies evaluated oral intraepithelial compound levels, making it difficult to determine whether the negative results are due to the lack of compound efficacy or insufficient therapeutic levels within the target tissues.

COX-2 Inhibitors

COX-2 is often elevated in premalignant and malignant epithelial lesions, and is associated with suppression of apoptosis and production of reactive oxygen species, both of which are pro-tumorigenic.[47,48] Based on these well-established roles of COX-2, Papadimitrakopoulou *et al*. conducted a clinical trial evaluating the therapeutic effect of Celecoxib, a COX-2 inhibitor, in patients with histologically confirmed epithelial atypia or dysplasia.[49] Patients were assigned to placebo or Celecoxib groups (oral tablet, 100, 200, or 400 mg b.i.d. for 3 months), and pre- and post-treatment biopsies were obtained to evaluate lesional response.[49] Notably, none of the Celecoxib recipients demonstrated clinical or microscopic differences relative to placebo recipients.[49] Again, these negative results are difficult to decipher since oral intraepithelial Celecoxib levels were not determined, thus failing to demonstrate achieved therapeutic levels in the targeted epithelial lesions.

Natural products

Based on the extended duration of treatment likely required for oral cancer chemoprevention, natural products represent a promising group of compounds due to their decreased dose-limiting toxicity profiles. A recent clinical trial assessed the chemopreventive effects of green tea extract (oral capsules, 500, 750, 1000 mg/m^2 t.i.d. for 3 months) relative to placebo in patients with both histologically confirmed premalignant dysplastic lesions and benign hyperplastic lesions.[50] Although a dose-dependent trend was associated with clinical responsiveness, no significant differences were observed with regard to lesional progression to OSCC.[50] Furthermore, the inclusion of benign lesions and failure to quantify intraepithelial levels of the bioactive compounds complicate the evaluation of therapeutic efficacy.

Systemic delivery summary

These collective systemic delivery oral cancer chem-oprevention trials have yielded modest results with regard to clinical efficacy (i.e., histological and clinical lesional regression). Moreover, the failure to quantify intraepithelial chemopreventive concentrations and the frequent inclusion of benign lesions

raise concerns regarding the efficiency of drug delivery and relevancy of therapeutic outcomes. In addition, the likely need for sustained treatment regimens combined with the presence of mild to severe systemic toxicities negate the clinical utility of these chemopreventive compounds via systemic delivery methods, and accentuate the need for an alternative dosing scheme (e.g., local intraoral delivery).

ORAL CANCER CHEMOPREVENTION CLINICAL TRIALS: LOCAL DELIVERY

Previous oral cancer chemoprevention clinical trials that utilized local delivery strategies have evaluated the delivery of several classes of compounds/therapeutics [Table 2]: i) vitamin A derivatives, ii) adenoviruses, iii) cancer chemotherapy agents, iv) COX inhibitors, and v) natural products.[23-31,51,52-56]

Vitamin A derivatives

Separate studies conducted in 1983 and 1999 evaluated the topical application of isotretinoin on oral leukoplakic lesions.[23,24] Notably, oral leukoplakia is currently considered a clinical, not histopathologic, diagnostic term. Therefore, the description of clinical lesions as "oral leukoplakia" does

Table 2: Local delivery oral cancer chemoprevention trials

Therapeutic agent	Year[Ref.]	Delivery method, schedule, and duration	Biopsies and microscopic diagnosis	Side effects	Therapeutic outcomes
Isotretinoin (13-*cis*-retinoic acid)	1983[23]	1 mg oral troche (3 mg/day, 5 mg/day, 10 mg/day) Duration: 6 months with 11-month follow-up	Pre- and post-treatment histologically confirmed "oral leukoplakia" (n = 16) Note: oral leukoplakia is currently considered a clinical, not histopathologic, diagnostic term	Mild	Of 11 evaluable patients, 3 demonstrated complete clinical regression and 6 showed partial regression. Post-treatment lesion recurrence was observed in two of three patients with complete regression, and one partial responder regressed to complete *Alcohol/tobacco cessation not required*
Isotretinoin (13-*cis*-retinoic acid)	1999[24]	0.1% isotretinoin gel or placebo applied t.i.d. Duration: 4 months *Following 4 month study completion, placebo patients completed additional 4 months with 0.1% isotretinoin gel	Pre- and post-treatment histologically confirmed "oral leukoplakia" (n = 10)	None	Isotretinoin gel treatment group demonstrated clinical lesion regression. Placebo group exhibited stable disease, but when treated with isotretinoin gel the patients exhibited clinical lesion regression (one complete response and eight partial responses). Post-treatment lesion recurrence not reported *Alcohol/tobacco cessation not required*
Fenretinide	1994[25]	Topical application of capsule contents (100 mg b.i.d.) Duration: 2 months	Pre- and post-treatment biopsies of "oral leukoplakia" (n = 6) and lichen planus (n = 2) *Histology not confirmed	None	Clinical lesion regression in two of three patients at 2 months, but did not specify whether the lesions were leukoplakia or lichen planus. Topical application was well tolerated by oral mucosa. Low systemic levels of fenretinide were detected. Post-treatment lesion recurrence not reported *Alcohol/tobacco cessation not reported*
Tretinoin (all-*trans* retinoic acid)	1999[26]	0.05% tretinoin gel applied q.i.d. Duration: average of 1.5 years in biopsy-confirmed dysplasia	Pre- and post-treatment histologically confirmed oral dysplasia in 9 of 26 patients	None reported	Of patients with confirmed dysplasia, 55% exhibited clinical lesion regression or stable disease and 45% had lesions that progressed. Notably, of the 26 total trial participants (i.e., including benign lesions), 7 patients demonstrated complete remission. Three of those seven experienced post-treatment clinical lesion recurrences *Alcohol/tobacco cessation not required*
Acitretin	2000[51]	Oral mucoadhesive tablet, 10 mg b.i.d. Duration: 4 weeks	Pre- and post-treatment histologically confirmed "oral leukoplakia" (n = 21* 6 of 21 lesions dysplastic	None	10 of the 14 patients (2 of 6 dysplastic lesions) in the acitretin groups demonstrated lesional regression, while 0 of 7 in the placebo group exhibited lesional improvement. Pharmacokinetic analyses demonstrated sustained acitretin salivary levels for up to 9 hours (4.9–43 mg/ml), and low intra-lesional and systemic levels (<50 ng/ml) *Alcohol/tobacco cessation not reported*

Table 2 (contd...)

Table 2 (contd...)

Therapeutic agent	Year[Ref.]	Delivery method, schedule, and duration	Biopsies and microscopic diagnosis	Side effects	Therapeutic outcomes
ONYX-015 adenovirus	2003[27]	Oral rinse, 15 ml for 30 minutes q.d. at various intervals Duration: up to 48 weeks with 2-year follow-up	Pre- and post-treatment histologically confirmed oral dysplasia (n = 19)	Moderate	7 of the 19 dysplastic lesions demonstrated histological regression (37%), but 4 of these patients exhibited dysplastic recurrences Side effects: Circulating adenoviral antibodies/virus replication *Alcohol/tobacco cessation not reported*
Wild-type p53 adenoviral vector	2009[28,29]	Intraoral injection on days 1, 4, 7, 10, and 13 Duration: 15 days with 6-month to 2-year follow-up	Pre- and post-treatment histologically confirmed oral dysplasia (n = 18)	Moderate	4 patients exhibited complete regression without recurrence at 6 months, 12 patients demonstrated partial reduction/ stable disease, and 2 patients progressed to cancer Side effects: Injection-site tissue necrosis, fever, elevated white blood cell count, and flu-like symptoms *Alcohol/tobacco cessation not reported*
Bleomycin	1998[30]	1% (w/v) bleomycin in DMSO applied q.d. with cotton applicator for 5 minutes Duration: 2 weeks with 3.9-year mean follow-up	Pre- and post-treatment histologically confirmed oral dysplasia (n = 19)	Mild	2 patients (11%) demonstrated complete histological responses without lesional recurrence, 15 patients (79%) demonstrated partial regression, 1 patient (5%) exhibited stable disease, and 1 was lost to follow-up. Notably, five patients progressed after trial completion (three to oral cancer) Side effects: Mucosal reactions and oral sensitivity *Alcohol/tobacco cessation not required*
Ketorolac	2004[31]	0.1% ketorolac oral rinse, 10 ml for 30 seconds b.i.d. or placebo Duration: 3 months with 1-month follow-up	Pre- and post-treatment histologically confirmed benign and oral premalignant lesions (n = 57)	Mild	Ketorolac recipients (n = 38) did not demonstrate any clinical differences relative to placebo recipients (n = 19) *Alcohol/tobacco cessation recommended, but not required (counseling offered)*
Black raspberries	2008[53,54]	10% (w/w) freeze-dried black raspberry gel (0.5 g applied to lesional site q.i.d.) Duration: 6 weeks with 3- to 23-month follow-up * Pharmacokinetic study also conducted[54]	Pre- and post-treatment histologically confirmed oral premalignant lesions (n = 20)	None	45% of patients demonstrated stable disease, 35% of patients exhibited reduction of clinical lesion size and microscopic histological grade, and 20% presented with an increased histological grade. Post-treatment lesion recurrence was observed in 8/20 patients at treatment sites and in 7/20 patients at non-treated sites. Notably, induction of favorable RNA/protein expression profiles was observed *Tobacco cessation was required 6 weeks prior to and for the duration of the clinical trial. 12/20 continued alcohol use*

not differentiate between premalignant dysplastic lesions and nonmalignant reactive hyperplastic lesions. The first study in 16 patients evaluated three treatment groups (1 mg troche formulation; 3, 5, 10 mg/day for 6 months) and obtained both pre- and post-treatment biopsies.[23] Notably, of 11 evaluable patients, 3 exhibited complete clinical lesion regression, 6 had partial clinical regression, and none of the trial participants experienced "unacceptable" levels of toxicity.[23] The second study, by Piattelli *et al.*, evaluated isotretinoin gel application (0.1% isotretinoin gel t.i.d. for 4 months) or placebo in 10 patients with "biopsy-proven oral leukoplakic lesions".[24] Patients in the treatment group exhibited unspecified clinical lesional regression, while the placebo group demonstrated stable disease.[24] Following completion of the 4-month study, patients in the placebo group completed an additional 4-month study using the 0.1% isotretinoin gel and demonstrated clinical lesional regression.[24] The final results showed one complete clinical response and eight partial clinical responses with negligible side effects.[24]

In 1994, a study by Tradati *et al*. evaluated topical fenretinide application in patients with oral leukoplakia/lichen planus (100 mg b.i.d. for 2 months).[25] Although the method of topical application lacked a controlled drug delivery system

(i.e., patients broke open and applied the contents of 100 mg capsules), this study demonstrated clinical regression of pre-malignant leukoplakic lesions and benign lichen planus, no adverse side effects, and minimal drug levels in serum.[25] Notably, due to the method of intraoral delivery and likely ingestion of the capsule contents, the presence of detectable serum levels is expected. Despite the positive outcome of this small pilot study, additional local fenretinide studies were not conducted.

In 1999, Epstein and Gorsky reported their findings from a trial assessing the effects of q.i.d. topical application of a 0.05% all-*trans* retinoic acid (tretinoin) gel on oral dysplastic lesions (histologically confirmed in 9 of 26 patients).[26] Of those patients with confirmed dysplasia, five exhibited clinical lesion regression or stable disease, while four had lesions that progressed, and toxic side effects were not observed.[26] Notably, the duration and frequency of gel application varied amongst the participants, some of whom continued to smoke over the duration of the trial.[26] Clearly, lack of smoking cessation adds a major confounding variable, which hampers the proper evaluation of the trial results.

A study conducted in 2000 by Gaeta *et al*. evaluated the chemopreventive efficacy of two acitretin mucoadhesive tablet formulations (10 mg b.i.d. for 4 weeks) relative to a placebo control in 21 patients with histologically confirmed "oral leukoplakia".[51] While 10 of 14 patients in the treatment groups exhibited clinical lesion regression relative to 0 of 7 in the placebo group, only 2 of 6 patients with confirmed epithelial dysplasia demonstrated histological regression.[51] While this study demonstrated the sustainability of acitretin in saliva for 4–9 hours (formulation-dependent), a 1000-fold decrease of intra-lesional and serum levels was observed relative to salivary levels.[51] Although the sustainability in saliva is promising, delivery of therapeutically relevant levels of hydrophobic compounds (e.g., retinoids) to premalignant epithelial cells will likely require formulations containing solubility and permeability enhancers (e.g., surfactants) to facilitate compound stabilization and penetration into the oral epithelium.[55]

Adenovirus vectors

In contrast to compound-based chemoprevention through the induction of differentiation and/or apoptosis, two separate studies evaluated the therapeutic efficacy of adenovirus vector delivery for targeted cytotoxicity or gene therapy [i.e., adenovirus (ONYX-015)-mediated cytotoxicity toward cells with mutated p53 and an adenoviral vector used to insert wild-type p53].[27-29] The ONYX-015 studies, which used a mouthwash-mediated local delivery system (oral rinse, 30 minutes, 15 ml q.d. for various intervals up to 48 weeks), demonstrated histological regression of dysplastic lesions in 7 of 19 patients. The future clinical applications, however, may be limited due to the presence of circulating adenoviral antibody titers in one of the seven patients evaluated and actively replicating viruses in the oral mucosa in two of three patients.[27]

In contrast to the ONYX-015 mouthwash studies, Li *et al*. aimed to inject an adenoviral vector (injections on days 1, 4, 7, 10, and 13) to insert wild-type p53 into pre-malignant oral epithelial cells.[28,29] Results of this study demonstrated significant tissue necrosis at the injection site in 18 of 22 patients with significant side effects (e.g., circulating adenoviral antibodies, fever, elevated white blood cell counts, and flu-like symptoms).[28,29] Notably, this study did not include a vehicle control group, which hinders the ability to determine if the physical nature of the injection or the adenovirus itself was the cause of tissue necrosis.[28,29] Positive clinical outcomes, however, were noted (i.e., 22% complete clinical regression without recurrence at 6 months and 66% with partial clinical regression or stable disease).[28,29]

Cancer chemotherapy agents

Several oral cancer chemoprevention studies have evaluated the therapeutic efficacy of bleomycin, a potent DNA-damaging antibiotic.[30,57-62] The most recent of these topical studies, which evaluated bleomycin treatment [1% w/v in dimethylsulfoxide (DMSO) q.d. for 2 weeks] in patients with histologically confirmed oral dysplastic lesions, demonstrated histological responses in 89% of the patients evaluated.[30] Notably, 5 of the 19 evaluable patients demonstrated clinical lesion progression following treatment cessation (3 progressed to OSCCs).[30] In addition, this study did not assess systemic levels of bleomycin following topical application.[30] Given the known deleterious effects of bleomycin administration (i.e., pulmonary fibrosis), long-term treatment with this compound would likely be contraindicated.[63] In contrast, short-term inductive bleomycin therapy followed by long-term treatment with a less-toxic chemopreventive compound (e.g., natural products) could provide improved chemopreventive efficacy while minimizing the risk of potential deleterious systemic effects.

Cyclooxygenase inhibitors

Similar to the outcome of systemic COX-2 inhibitor studies, local application of the nonspecific COX inhibitor Ketorolac (oral rinse, 10 ml for 30 seconds b.i.d. for 3 months) exhibited comparable reductions in lesional sizes relative to placebo without eliciting any significant toxic side effects.[31] Notably, this study did not exclude benign lesions or require smoking cessation. In addition, although the authors speculated that Ketorolac was unable to penetrate the cornified epithelial

layer to reach the proliferating basal cells, they did not determine the intraepithelial Ketorolac concentration.[31]

Natural products

Black raspberry anthocyanins are potent antioxidants capable of suppressing pro-tumorigenic activation pathways (e.g., quenching reactive oxygen species-mediated signal transduction, suppressing angiogenesis, and inhibiting oxidant-responsive proinflammatory enzymes).[64-72] Due to these established effects *in vitro* and *in vivo*, our laboratories recently conducted Phase I/II oral cancer chemoprevention studies to evaluate the safety and chemopreventive efficacy of a 10% (w/w) freeze-dried black raspberry (BRB) bioadhesive gel in normal volunteers (Phase I) and in histologically confirmed premalignant oral epithelial lesions (Phase II).[53,54] Phase I studies in 10 healthy volunteers (0.5 g applied to normal mucosa q.i.d. for 6 weeks) demonstrated gel tolerability through the absence of any deleterious side effects.[53,54] Phase II studies were conducted in 20 patients (0.5 g applied to lesional site q.i.d. for 6 weeks) with oral premalignant lesions and demonstrated a significant decrease in loss of heterozygosity indices at key tumor suppressor gene loci, decreased expression of genes associated with recycling of growth factors, apoptosis inhibition, and RNA processing, and significantly reduced levels of COX-2 protein in lesional epithelium.[53,54] Notably, 45% of trial participants exhibited stable disease and 35% demonstrated a reduction of both gross lesional size and microscopic histological grade, decreased microvascular density in underlying connective tissues, and increased expression of genes associated with epithelial terminal differentiation.[53] Due to the pre- and post-treatment biopsies of confirmed premalignant lesions and required tobacco cessation, this trial facilitates critical analysis of the findings.

Interestingly, these data revealed a cohort of high-level responders, which did not correlate with a lower grade oral dysplastic lesion at the onset of treatment (i.e., lesional regression was seen in persons with moderate to higher epithelial dysplasia).[53] The basis for this variation in lesional responsiveness was speculated to reflect inter-patient variations in local pharmacokinetics including penetration, bioactivation, and sustainability of the BRB chemopreventives. Subsequent pharmacokinetic analyses in healthy volunteers (0.5 g gel applied sublingually or to retromolar pad) assessed the distribution of four main black raspberry constituents (i.e., anthocyanin metabolites) in saliva, oral mucosal tissue, and plasma.[56] Although considerable inter-patient variations of absorption and retention in saliva and oral tissue were observed, oral chemopreventive levels were significantly greater than their corresponding plasma concentrations in all participants.[56] These findings demonstrate the pharmacologic advantage provided by local delivery formulations (i.e., successful achievement of therapeutically relevant levels within the target tissue while minimizing systemic exposure).[11,12,56]

Furthermore, ongoing studies in our lab have demonstrated highly variable anthocyanin-relevant metabolic enzyme profiles (i.e., hydrolytic and Phase I/II enzymes and ATP binding cassette transporters) in 15 normal human oral mucosal samples.[73] In addition, these studies also showed participant-specific contribution of oral microflora and salivary proteins to the local recycling of anthocyanins, which may effectively increase oral epithelial exposure to the chemopreventive compounds.[73] Ultimately, these pharmacokinetic and metabolic profiling data can be used to guide future chemoprevention studies through the use of permeability enhancers and/or bioactive metabolites that are not dependent on individual metabolic profiles for bioactivation.[73] This approach would, therefore, de-emphasize metabolism-related pharmacogenomic contributions to therapeutic efficacy.

Collectively, these data formed the foundation for an ongoing National Cancer Institute supported multicenter oral cancer chemoprevention trial (NCT01192204) evaluating the effects of the 10% BRB gel in histopathologically confirmed premalignant oral lesions. This trial, conducted under the auspices of a Food and Drug Administration (FDA)-approved Investigational New Drug, is partnered with investigators at the University of Louisville and the University of North Carolina at Chapel Hill and will extend the treatment time from 6 weeks (Phase I/II trial) to 12 weeks. Notably, Phase I/II evaluative parameters (i.e., microscopic histological grading of oral premalignant lesions pre- and post-treatment, loss of heterozygosity analysis, alteration of gene expression, COX-2 and inducible nitric oxide synthase levels, and angiogenesis) and additional parameters have been included to guide post-trial assessment of therapeutic efficacy (e.g., pre-treatment oral metabolic profiling of anthocyanin-relevant bioactivation enzymes, determination of promoter DNA methylation level, and the inclusion of an investigator- and patient-blinded placebo gel cohort).

Local delivery summary

Taken together, these local delivery oral cancer chemoprevention trials have demonstrated a pharmacologic advantage over systemic delivery trials. Although adenovirus trials resulted in significant side effects, local delivery of chemopreventive compounds has been well tolerated.[23-26,30,31,53-56] In addition, to facilitate the reliable evaluation of future oral cancer chemoprevention trials, adoption of the following study parameters is recommended:

exclusive enrollment of patients with histopathologically confirmed premalignant oral epithelial lesions, alcohol/tobacco cessation, pre- and post-treatment microscopic histological grading, minimum of 12-week study duration (allows repopulation of surface epithelium since turnover occurs approximately every 28 days, and thus negates the "biopsy effect", i.e., clearing of a dysplastic lesion through frequent biopsies), determination of intraepithelial chemopreventive compound levels, and correlation of local metabolism to therapeutic efficacy.

FUTURE DIRECTIONS AND DISCUSSION

The success of intraoral local drug delivery strategies is mainly dependent on the ability of polymeric carriers to provide: i) increased apparent solubility and stability in physiological fluids, e.g., saliva, ii) appropriate rate of drug release for an optimized effect, iii) facilitated penetration and local distribution of chemopreventive compounds, and iv) flexibility to allow controlled drug delivery to various oral mucosal sites. Furthermore, due to the risk factors associated with oral cancer (i.e., tobacco and/or alcohol use), the entire oral mucosa is hypothesized to have undergone field cancerization.[74] Therefore, optimal therapeutic efficacy for oral cancer chemoprevention would likely entail both lesion-specific (topical agent) and field coverage (rinse) components. Hence, our laboratories have developed optimal intraoral drug delivery strategies for numerous chemopreventive agents by manipulating the properties of drugs (e.g., improving the apparent solubility, stability, and tissue distribution of drugs), utilizing polymeric carriers (e.g., developing mucoadhesive gels and patches, millicylindrical implants for long-term delivery, and nanoparticles), and using various delivery approaches (e.g., short- and sustained-duration drug release formulations).[13,55,75-78]

Lesion-specific, targeted therapies are currently under evaluation in our labs, and include strategies for the delivery of black raspberry extract, fenretinide, and the matrix metalloproteinase inhibitor, *N*-acetylcysteine.[75-77] In addition to the 10% BRB gel currently under Phase III evaluation, sustained release poly(DL-lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) implant formulations have been developed for the sustained delivery of black raspberry anthocyanins (PLA), fenretinide (PLGA), and *N*-acetylcysteine (PLGA).[13,75-77] Notably, these injectable implants demonstrated sustained release for 4–5 weeks *in vitro* (black raspberry extract, *N*-acetylcysteine Ca^{2+} and Mg^{2+} salts, and fenretinide) and 4 weeks *in vivo* (black raspberry extract), thus providing a potential local chemoprevention delivery method independent of daily patient compliance.[75-77] Furthermore, preliminary *in vitro* and *in vivo* studies on the development and evaluation of a novel mucoadhesive fenretinide patch demonstrate both burst and sustained release patterns imparting therapeutically relevant levels in rabbit oral mucosa.[55] In addition, our lab has demonstrated the feasibility of patch-mediated nanoparticle delivery to the basal epithelial cells and underlying connective tissue of human oral mucosal explants.[78] This nanoparticle study provides yet another mechanism for drug stabilization and subsequent local delivery to the oral epithelium.

Recent studies in our labs have also demonstrated long-term sustainability of a black raspberry oral rinse formulation designed to provide a field coverage effect.[73] Notably, rinse administration exhibited greater sustained salivary levels of anthocyanins relative to corresponding levels in pharmacokinetic studies of the 10% BRB gel.[56,73]

Based on the collective results of the local delivery chemoprevention trials, which demonstrated a pharmacologic advantage over systemic strategies by minimizing systemic toxicities while obtaining therapeutically relevant local levels, these local intraoral delivery strategies developed in our laboratories warrant further evaluation for clinical efficacy in oral cancer chemoprevention. In addition, future oral cancer chemoprevention trials should focus on similar local delivery strategies and utilize the recommended study design parameters outlined within this review.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed to the writing and revision of this review paper.

ACKNOWLEDGMENTS

The ongoing studies in our laboratories are funded by the Fanconi Anemia Research Fund and NIH grants: (R01 CA129609, RC2 CA148099, R21 CA132138 to Susan R. Mallery) (R01 HL68345 to Steven P. Schwendeman) (F30 DE020992 and T32 DE14320 to Andrew S. Holpuch).

REFERENCES

1. Deeken JF, Slack R, Marshall JL. Irinotecan and uridine diphosphate glucuronosyltransferase 1A1 pharmacogenetics: to test or not to test, that is the question. Cancer 2008;113:1502-10.
2. Gonzalez-Angulo AM, Morales-Vasquez F, Hortobagyi GN. Overview of resistance to systemic therapy in patients with breast cancer. Adv Exp Med Biol 2007;608:1-22.

3. Baban DF, Seymour LW. Control of tumour vascular permeability. Adv Drug Deliv Rev 1998;34:109-19.
4. Maeda H, Sawa T, Konno T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release 2001;74:47-61.
5. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, *et al*. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998;16:2659-71.
6. Denny WA. The role of hypoxia-activated prodrugs in cancer therapy. Lancet Oncol 2000;1:25-9.
7. Abdollahi A, Folkman J. Evading tumor evasion: current concepts and perspectives of anti-angiogenic cancer therapy. Drug Resist Updat 2010;13:16-28.
8. Murray N, Turrisi AT 3rd. A review of first-line treatment for small-cell lung cancer. J Thorac Oncol 2006;1:270-8.
9. Scagliotti G, Govindan R. Targeting angiogenesis with multitargeted tyrosine kinase inhibitors in the treatment of non-small cell lung cancer. Oncologist 2010;15:436-46.
10. Shen YC, Hsu C, Cheng AL. Molecular targeted therapy for advanced hepatocellular carcinoma: current status and future perspectives. J Gastroenterol 2010;45:794-807.
11. Shen Z, Shen T, Wientjes MG, O'Donnell MA, Au JL. Intravesical treatments of bladder cancer: review. Pharm Res 2008;25:1500-10.
12. Sood S, Shiff SJ, Yang CS, Chen X. Selection of topically applied non-steroidal anti-inflammatory drugs for oral cancer chemoprevention. Oral Oncol 2005;41:562-7.
13. Mallery SR, Stoner GD, Larsen PE, Fields HW, Rodrigo KA, Schwartz SJ, *et al*. Formulation and *in-vitro* and *in-vivo* evaluation of a mucoadhesive gel containing freeze-dried black raspberries: implications for oral cancer chemoprevention. Pharm Res 2007;24:728-37.
14. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 1976;35:1322-28.
15. Senel S, Ikinci G, Kaş S, Yousefi-Rad A, Sargon MF, Hincal AA. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. Int J Pharm 2000;193:197-203.
16. Hersh EV, DeRossi SS, Ciarrocca KN, Secreto SA, Ghassemi A. Efficacy and tolerability of an intraoral benzocaine patch in the relief of spontaneous toothache pain. J Clin Dent 2003;14:1-6.
17. Perugini P, Genta I, Conti B, Modena T, Pavanetto F. Periodontal delivery of ipriflavone: new chitosan/PLGA film delivery system for a lipophilic drug. Int J Pharm 2003;252:1-9.
18. Miyazaki S, Nakayama A, Oda M, Takada M, Attwood D. Chitosan and sodium alginate based bioadhesive tablets for intraoral drug delivery. Biol Pharm Bull 1994;17:745-7.
19. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent 2010;38 Suppl 1:S6-10.
20. Paschos E, Huth KC, Benz C, Reeka-Bardschmidt A, Hickel R. Efficacy of intraoral topical anesthetics in children. J Dent 2006;34:398-404.
21. Gravenmade EJ, Vissink A. Mucin-containing lozenges in the treatment of intraoral problems associated with Sjogren's syndrome. A double-blind crossover study in 42 patients. Oral Surg Oral Med Oral Pathol 1993;75:466-71.
22. Sudhakar Y, Kuotsu K, Bandyopadhyay AK. Buccal bioadhesive drug delivery – a promising option for orally less efficient drugs. J Control Release 2006;114:15-40.
23. Shah JP, Strong EW, DeCosse JJ, Itri L, Sellers P. Effect of retinoids on oral leukoplakia. Am J Surg 1983;146:466-70.
24. Piattelli A, Fioroni M, Santinelli A, Rubini C. bcl-2 expression and apoptotic bodies in 13-*cis*-retinoic acid (isotretinoin)-topically treated oral leukoplakia: a pilot study. Oral Oncol 1999;35:314-20.
25. Tradati N, Chiesa F, Rossi N, Grigolato R, Formelli F, Costa A, *et al*. Successful topical treatment of oral lichen planus and leukoplakias with fenretinide (4-HPR). Cancer Lett 1994;76:109-11.
26. Epstein JB, Gorsky M. Topical application of vitamin A to oral leukoplakia: A clinical case series. Cancer 1999;86:921-7.
27. Rudin CM, Cohen EE, Papadimitrakopoulou VA, Silverman S Jr, Recant W, El-Naggar AK, *et al*. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. J Clin Oncol 2003;21:4546-52.
28. Li Y, Li LJ, Zhang ST, Wang LJ, Zhang Z, Gao N, *et al*. *In vitro* and clinical studies of gene therapy with recombinant adenovirus-p53 injection for oral leukoplakia. Clin Cancer Res 2009;15:6724-31.
29. Zhang S, Li Y, Li L, Zhang Y, Gao N, Zhang Z, *et al*. Phase I study of repeated intraepithelial delivery of adenoviral p53 in patients with dysplastic oral leukoplakia. J Oral Maxillofac Surg 2009;67:1074-82.
30. Epstein JB, Gorsky M, Wong FL, Millner A. Topical bleomycin for the treatment of dysplastic oral leukoplakia. Cancer 1998;83:629-34.
31. Mulshine JL, Atkinson JC, Greer RO, Papadimitrakopoulou VA, Van Waes C, Rudy S, *et al*. Randomized, double-blind, placebo-controlled phase IIb trial of the cyclooxygenase inhibitor ketorolac as an oral rinse in oropharyngeal leukoplakia. Clin Cancer Res 2004;10:1565-73.
32. Freemantle SJ, Spinella MJ, Dmitrovsky E. Retinoids in cancer therapy and chemoprevention: promise meets resistance. Oncogene 2003;22:7305-15.
33. Smith W, Saba N. Retinoids as chemoprevention for head and neck cancer: where do we go from here? Crit Rev Oncol Hematol 2005;55:143-52.
34. Hail Jr N, Kim HJ, Lotan R. Mechanisms of fenretinide-induced apoptosis. Apoptosis 2006;11:1677-94.
35. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, *et al*. 13-*cis*-retinoic acid in the treatment of oral leukoplakia. N Engl J Med 1986;315:1501-5.
36. Lippman SM, Batsakis JG, Toth BB, Weber RS, Lee JJ, Martin JW, *et al*. Comparison of low-dose isotretinoin with beta-carotene to prevent oral carcinogenesis. N Engl J Med 1993;328:15-20.
37. Garewal HS, Katz RV, Meyskens F, Pitcock J, Morse D, Friedman S, *et al*. Beta-carotene produces sustained remissions in patients with oral leukoplakia: results of a multicenter prospective trial. Arch Otolaryngol Head Neck Surg 1999;125:1305-10.
38. Papadimitrakopoulou VA, Clayman GL, Shin DM, Myers JN, Gillenwater AM, Goepfert H, *et al*. Biochemoprevention for dysplastic lesions of the upper aerodigestive tract. Arch Otolaryngol Head Neck Surg 1999;125:1083-9.
39. Chiesa F, Tradati N, Grigolato R, Boracchi P, Biganzoli E, Crose N, *et al*. Randomized trial of fenretinide (4-HPR) to prevent recurrences, new localizations and carcinomas in patients operated on for oral leukoplakia: long-term results. Int J Cancer 2005;115:625-9.
40. Lippman SM, Lee JJ, Martin JW, El-Naggar AK, Xu X, Shin DM, *et al*. Fenretinide activity in retinoid-resistant oral leukoplakia. Clin Cancer Res 2006;12:3109-14.
41. William WN Jr, Lee JJ, Lippman SM, Martin JW, Chakravarti N, Tran HT, *et al*. High-dose fenretinide in oral leukoplakia. Cancer Prev Res (Phila) 2009;2:22-6.
42. Silverman S Jr, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer 1984;53:563-8.
43. Scuibba JJ. Oral leukoplakia. Crit Rev Oral Biol Med 1995;6:147-60.
44. Dimery IW, Hong WK, Lee JJ, Guillory-Perez C, Pham F, Fritsche HA Jr, *et al*. Phase I trial of alpha-tocopherol effects on 13-*cis*-retinoic acid toxicity. Ann Oncol 1997;8:85-9.
45. Besa EC, Abrahm JL, Bartholomew MJ, Hyzinski M, Nowell PC. Treatment with 13-cis-retinoic acid in transfusion-dependent patients with myelodysplastic syndrome and decreased toxicity with addition of alpha-tocopherol. Am J Med 1990;89:739-47.
46. Clifford JL, Menter DG, Wang M, Lotan R, Lippman SM. Retinoid receptor-dependent and -independent effects of N-(4-hydroxyphenyl)retinamide in F9 embryonal carcinoma cells. Cancer Res 1999;59:14-8.
47. Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol Chem 2006;387:365-72.
48. Prescott SM, Fitzpatrick FA. Cyclooxygenase-2 and carcinogenesis. Biochim Biophys Acta 2000;1470:M69-78.
49. Papadimitrakopoulou VA, William WN Jr, Dannenberg AJ, Lippman SM, Lee JJ, Ondrey FG, *et al*. Pilot randomized phase II study of celecoxib in oral premalignant lesions. Clin Cancer Res 2008;14:2095-101.
50. Tsao AS, Liu D, Martin J, Tang XM, Lee JJ, El-Naggar AK, *et al*. Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer Prev Res (Phila) 2009;2:931-41.
51. Gaeta GM, Gombos F, Femiano F, Battista C, Minghetti P, Montanari L, *et al*. Acitretin and treatment of oral leucoplakias. A model to have an active molecules release. J Eur Acad Dermatol Venereol 2000;14:473-8.

52. Holpuch AS, Desai KG, Phelps MP, Han B, Schwendeman SP, Mallery SR. Evaluation of a fenretinide mucoadhesive patch for local intraoral delivery [abstract]. J Dent Res 2011;90: 146204. Available from: http//www.dentalresearch.org. [Last accessed on 2011 Apr 5].
53. Mallery SR, Zwick JC, Pei P, Tong M, Larsen PE, Shumway BS, *et al*. Topical application of bioadhesive black raspberry gel modulates gene expression and reduces cyclooxygenase 2 protein in human premalignant oral lesions. Cancer Res 2008;68:4945-57.
54. Shumway BS, Kresty LA, Larsen PE, Zwick JC, Lu B, Fields HW, *et al*. Effects of topically applied bioadhesive berry gel on loss of heterozygosity indices in premalignant oral lesions. Clin Cancer Res 2008;14:2421-30.
55. Desai KG, Mallery SR, Holpuch AS, Schwendeman SP. Development and In Vitro-In Vivo Evaluation of Fenretinide-Loaded Oral Mucoadhesive Patches for Site-Specific Chemoprevention of Oral Cancer. Pharm Res 2011.[In Press].
56. Ugalde CM, Liu Z, Ren C, Chan KK, Rodrigo KA, Ling Y, *et al*. Distribution of anthocyanins delivered from a bioadhesive black raspberry gel following topical intraoral application in normal healthy volunteers. harm Res 2009;26:977-86.
57. Hayasaki K, Kitamura T, Kaneko T, Tachibana M, Kobayashi N, Tosaka K, *et al*. Application of bleomycin-iontophoresis for tumour therapy of the head and neck area. Nihon Gan Chiryo Gakkai Shi 1977;12:522-7.
58. Hisano Y, Satoh T, Suzuki M, Kanai Y. An effective case of local injection therapy of oral leukoplakia with bleomycin. Shigaku 1978;66:125-8.
59. Hammersley N, Ferguson MM, Rennie JS. Topical bleomycin in the treatment of oral leukoplakia: a pilot study. Br J Oral Maxillofac Surg 1985;23:251-8.
60. Malmström M, Hietanen J, Sane J, Sysmäläinen M. Topical treatment of oral leukoplakia with bleomycin. Br J Oral Maxillofac Surg 1988;26:491-98.
61. Wong F, Epstein J, Millner A. Treatment of oral leukoplakia with topical bleomycin: a pilot study. Cancer 1989;64:361-5.
62. Epstein JB, Wong FL, Millner A, Le ND. Topical bleomycin treatment of oral leukoplakia: a randomized double-blind clinical trial. Head Neck 1994;16: 539-44.
63. De Lena M, Guzzon A, Monfardini S, Bonadonna G. Clinical, radiologic, and histopathologic studies on pulmonary toxicity induced by treatment with bleomycin (NSC-125066). Cancer Chemother Rep 1972;56:343-56.
64. Huang C, Huang Y, Li J, Hu W, Aziz R, Tang MS, *et al*. Inhibition of benzo(a) pyrene diol-epoxide-induced transactivation of activated protein 1 and nuclear factor κB by black raspberry extracts. Cancer Res 2002;62:6857-63.
65. Huang C, Li J, Song L, Zhang D, Tong Q, Ding M, *et al*. Black raspberry extracts inhibit benzo(a)pyrene diol-epoxide-induced activator protein 1 activation and VEGF transcription by targeting the phosphotidylinositol 3-kinase/Akt pathway. Cancer Res 2006;66:581-7.
66. Hecht SS, Huang C, Stoner GD, Li J, Kenney PM, Sturla SJ, *et al*. Identification of cyanidin glycosides as constituents of freeze-dried black raspberries which inhibit anti-benzo[a]pyrene-7,8-diol-9,10-epoxide induced NFkappaB and AP-1 activity. Carcinogenesis 2006;27:1617-26.
67. Wang LS, Hecht SS, Carmella SG, Yu N, Larue B, Henry C, *et al*. Anthocyanins in black raspberries prevent esophageal tumors in rats. Cancer Prev Res (Phila) 2009;2:84-93.
68. Rodrigo KA, Rawal Y, Renner RJ, Schwartz SJ, Tian Q, Larsen PE, *et al*. Suppression of the tumorigenic phenotype in human oral squamous cell carcinoma cells by an ethanol extract derived from freeze-dried black raspberries. Nutr Cancer 2006;54:58-68.
69. Chen T, Rose ME, Hwang H, Nines RG, Stoner GD. Black raspberries inhibit N-nitrosomethylbenzylamine (NMBA)-induced angiogenesis in rat esophagus parallel to the suppression of COX-2 and iNOS. Carcinogenesis 2006;27:2301-7.
70. Chen T, Hwang H, Rose ME, Nines RG, Stoner GD. Chemopreventive properties of black raspberries in N-nitrosomethylbenzylamine-induced rat esophageal tumorigenesis: down-regulation of cyclooxygenase 2, inducible nitric oxide synthase, and c-*Jun*. Cancer Res 2006;66:2853-9.
71. Kresty LA, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, *et al*. Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. Cancer Res 2001;61:6112-9.
72. Stoner GD, Wang LS, Casto BC. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. Carcinogenesis 2008;29:1665-74.
73. Mallery SR, Budendorf DE, Larsen MP, Pei P, Tong M, Holpuch AS, *et al*. Effects of human oral mucosal tissue, saliva and oral microflora on intraoral metabolism and bioactivation of black raspberry anthocyanins. Cancer Prev Res (Phila) 2011;4:1209-21.
74. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium. Clinical Implications of Multicentric Origin. Cancer 1953;6:963-8.
75. Desai KG, Olsen KF, Mallery SR, Stoner GD, Schwendeman SP. Formulation and *in vitro-in vivo* evaluation of black raspberry extract-loaded PLGA/PLA injectable millicylindrical implants for sustained delivery of chemopreventive anthocyanins. Pharm Res 2010;27:628-43.
76. Wischke C, Zhang Y, Mittal S, Schwendeman SP. Development of PLGA-based injectable delivery systems for hydrophobic fenretini. Pharm Res 2010;27:2063-74.
77. Desai KG, Mallery SR, Schwendeman SP. Formulation and characterization of injectable Poly(DL-lactide-*co*-glycolide) implants loaded with N-acetylcysteine, a MMP inhibitor. Pharm Res 2008;25:586-97.
78. Holpuch AS, Hummel GJ, Tong M, Seghi GA, Pei P, Ma P, *et al*. Nanoparticles for local delivery to the oral mucosa: proof of principle studies. Pharm Res 2010;27:1224-36.

AUTHOR'S PROFILE

Mr. Andrew S. Holpuch is a D.D.S./Ph.D. graduate fellow at The Ohio State University College of Dentistry

Dr. Kashappa-Goud H. Desai, Ph.D. is an Assistant Research Scientist in the Department of Pharmaceutical Sciences at the University of Michigan

Dr. Steven P. Schwendeman, Ph.D. is the Ara G. Paul Professor and Chair of Pharmaceutical Sciences at the University of Michigan

Dr. Susan R. Mallery, D.D.S., Ph.D. is a Professor in the Division of Oral and Maxillofacial Surgery, Pathology, and Anesthesiology at The Ohio State University College of Dentistry

Journal of Carcinogenesis is published for Carcinogenesis Press by Medknow Publications and Media Pvt. Ltd.
Manuscripts submitted to the journal are peer reviewed and published immediately upon acceptance, cited in PubMed and archived on PubMed Central. Your research papers will be available free of charge to the entire biomedical community. Submit your next manuscript to Journal of Carcinogenesis.
www.journalonweb.com/jcar/