Featured Article

Randomized, Double-Blind, Placebo-Controlled Phase IIB Trial of the Cyclooxygenase Inhibitor Ketorolac as an Oral Rinse in Oropharyngeal Leukoplakia

James L. Mulshine,[1] Jane C. Atkinson,[5] Robert O. Greer,[6] Vassiliki A. Papadimitrakopoulou,[7] Carter Van Waes,[10] Susan Rudy,[10] Jack W. Martin,[8] Seth M. Steinberg,[2] David J. Liewehr,[2] Ingalill Avis,[1] R. Ilona Linnoila,[1] Stephen Hewitt,[3] Scott M. Lippman,[7,9] Robin Frye,[4] and Paul F. Cavanaugh, Jr.[11]

[1]Intervention Section, Cell and Cancer Biology Branch, [2]Biostatistics and Data Management Section, [3]Department of Pathology and [4]Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland; [5]Clinical Research Core, National Institute of Dental and Craniofacial Research, Bethesda, MD; [6]Division of Oral Pathology and Oncology, University of Colorado School of Dentistry and University of Colorado Cancer Center, Denver, Colorado; Departments of [7]Thoracic/Head and Neck Medical Oncology, [8]Head and Neck Surgery, and [9]Clinical Cancer Prevention, University of Texas M. D. Anderson Cancer Center, Houston, Texas; [10]Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, Maryland; and [11]Over the Counter Health Care Technology Division, The Procter and Gamble Company, Mason, Ohio

Abstract

Purpose: **Nonselective cyclooxygenase (COX) inhibitors have been reported to decrease the frequency of upper aerodigestive cancers. Ketorolac tromethamine oral rinse has been shown to resolve another COX-dependent process, periodontal disease, without incurring gastrointestinal side effects. This trial evaluated if a topically delivered oral rinse containing ketorolac was as safe as and more effective than oral rinse alone in reducing the area of oral leukoplakia.**

Experimental Design: **57 patients were randomized (2:1 ratio) in a double-blind, placebo-controlled study of ketorolac (10 ml of a 0.1% ketorolac rinse solution; $n = 38$) or placebo (10 ml of rinse solution; $n = 19$) given twice daily for 30 s over 90 days. Primary end point was evaluated visually obtaining bidimensional measurement of the size of leukoplakia lesion(s) at entry and at 90 days. Secondary end point was histological assessment of the leukoplakia as sampled by serial punch biopsy and independently reviewed by three pathologists.**

Results: **The patients included 67% males, 11% non-Caucasian, and 86% used tobacco with no significant differences between the two arms. Both rinses were well tolerated with good compliance, and there was no significant difference in adverse events ($P = 0.27$). Major response rate (complete response and partial response) was 30% for ketorolac and 32% for the placebo arm. There was no significant difference in change in histology between the two arms.**

Conclusion: **Local delivery of a COX-containing oral rinse was well tolerated but produced no significant reduction in the extent of leukoplakia compared with the placebo. However, the favorable response rate to placebo arm remains unexplained and additional investigation of the tissue penetration with ketorolac is warranted.**

Introduction

Proximal aerodigestive tract cancers such as oral cavity and pharynx includes ~28,000 new cases and result in ~7,200 deaths annually in the United States alone (1). Located in a highly functional, potentially visible area of the body, these cancers can cause profound functional and cosmetic harm even when cured (2, 3). Recently there has been a growing interest in the contribution of cyclooxygenase (COX) activity in catalyzing the production of specific prostaglandin products that play a central role in carcinogenesis, including in the oropharyngeal cavity (4–7). This evidence also includes *in vivo* cancer models that nonsteroidal anti-inflammatory drugs (NSAIDs) can prevent chemically induced oral cancer and treat existing cancers (4, 8–11).

Therefore, chemoprevention agent such as NSAIDs that blocks COX activity could help in managing early oropharyngeal carcinogenesis. Potter *et al.* (12) have suggested that prostaglandins stimulate the recruitment of inflammatory cells into the tumor bed where they can bathe the transformed cells with high concentrations of proinflammatory cytokines such as interleukin (IL)-6. We have reported that this COX-dependent biological model is also relevant to oropharyngeal cancers (4). In the article, we demonstrated that oral cancer cells can make and respond to inflammatory mediators such as IL-6 and IL-8. Exposing these cells to pan COX inhibitors such as ketorolac blocked these effects especially with *in vivo* cancer models. Leukoplakia is considered a potentially premalignant lesion in the oral cavity (13, 14), which is easy to visualize and sample in a clinical trial. Surgical management of leukoplakia is standard of care. However, at present, there is no evidence of an effective treatment, including surgical interventions,

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Note: J. C. A. is currently at the University of Maryland Dental School, Baltimore, Maryland.
Requests for reprints: James L. Mulshine, Cell and Cancer Biology Branch, Upper Aerodigestive Chemoprevention Faculty, Building 10 Room 12N226, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892-1906. Phone: (301) 402-3721; Fax: (301) 402-3767; E-mail: mulshinj@mail.nih.gov.

for multifocal leukoplakia that will prevent malignant transformation to invasive oral cancer (15). In the setting of multiple or recurrent lesions, surgical management can be associated with considerable compromise of swallowing and phonation as well as other morbidities. The clinical management of leukoplakia is additionally complicated because making precise predictions in regard to malignant progression is uncertain even using histopathology (16). Our goal was to evaluate a new medical approach to the management of leukoplakia using a topical drug delivery approach with a pan COX to reduce treatment side effects.

A major concern with the use of COX inhibitors is the increased frequency of gastric ulcers, which can result in gastrointestinal bleeding severe enough to require hospitalization (17–24). We therefore chose a preparation of a pan COX inhibitor, ketorolac tromethamine that was formulated as a rinse to reduce the risk of gastrointestinal bleeding (25–27). Additionally, previous investigations with this preparation demonstrated that the rinse was more effective at delivery of the drug to the gingival sulcus around the teeth than the peroral route. The oral rinse formulation of ketorolac could inhibit prostaglandin production in the gingival crevicular fluid (GCF) for upwards of 12 h (16). We postulated that ketorolac delivered as an oral rinse may also result in more favorable drug exposure to oral cavity leukoplakia than systemically delivered drug while greatly reducing gastric cavity drug exposure. To evaluate this, we conducted a randomized placebo-controlled (double-blind) Phase II chemoprevention trial comparing an oral rinse of the pan COX inhibitor and ketorolac *versus* the vehicle rinse (without ketorolac) to determine whether a 3-month course of locally delivered rinse could cause reduction in the area of involvement with oropharyngeal leukoplakia without causing significant side effects. In this effort, we used established trial methodology (28).

Patients and Methods

This was a randomized, double-blind, placebo-controlled study of efficacy and safety of a ketorolac rinse in reducing the area of disease in the oropharynx and improving the histological appearance of the epithelium (NIH Clinical Center 98-C-018). Fifty-seven patients with oropharyngeal leukoplakia were recruited from the NIH Clinical Center, the University of Colorado Health Science Center, or M. D. Anderson Cancer Center.

Eligibility. To be eligible for the study, patients had to manifest objective evidence of oropharyngeal leukoplakia, including lesions either in the oral cavity or the oral pharynx. Individuals with leukoplakia and a previous diagnosis of head and neck or oral cancer who were free of known cancer for at least 3 months were permitted to enroll. Other eligibility criteria included the presence of bidimensionally measurable lesions that could be sampled twice as well as willingness to undergo serial photography and biopsy to document response to treatment. All eligible patients had to possess excellent performance status (performance status 0–1) and general good health (without other uncontrolled medical conditions). Patients with a hypersensitivity to aspirin, lidocaine, or NSAIDs or retinoids were excluded. Patients were also excluded if they used antibiotics, steroids, NSAIDs as well as aspirin, probenecid, or antihistamines for least $\geq$10 consecutive days, or any immunosuppressants, anticoagulants, dilantin, lithium, methotrexate, phenothiazines, investigational drugs with pharmacological activity that could compromise the test product safety during the 30 days immediately preceding the first treatment visit. Debilitating oral conditions requiring extensive dental procedures as well as a history of social or psychiatric situation interfering with study compliance were also criteria for exclusion to study enrollment. Individuals with compromised respiratory function manifested by shortness of breath with mild exertion or dependency on supplemental oxygen as well as subjects with compromised cardiovascular status, including poorly controlled angina or congestive heart failure, were excluded from participation in the study.

Clinical Trial Design. After signing an informed consent approved by the relevant Institutional Review Boards, eligible participants underwent a baseline biopsy of the most involved site of leukoplakia to exclude the presence of other conditions and confirm the absence of invasive cancer. In addition, all protocol patients underwent a head and neck screening evaluation, including nasopharyngolaryngoscopy and a detailed dental examination. Individuals found to be free of frank cancer were randomly assigned using a 2:1 ratio to 33-day cycles of either 10 ml of a 0.1% ketorolac rinse solution or 10 ml of placebo rinse solution. Both solutions were self administered twice daily by oral swish for 30 s, followed by spitting. To facilitate correct dosing for the oral rinse, a marked dosing cup and a 30-s timer were provided. As the drug is light sensitive, it was provided in a brown bottle. Clinical evaluations were made at study entry, monthly during the intervention, and 1 month after cessation of the study drugs.

As with previous ketorolac rinse studies of this duration, there were no planned treatment modifications for side effects (26, 27). If treatment-related grade 3–4 toxicity was observed, the study participant went off study and was then followed for response and toxicity. If persistent grade 2 toxicity of a 2-week duration developed from the mouth rinse, the study participant would go off study and be followed for response and toxicity. The most significant side effect from the previous Phase II periodontal trials was an increased rate of aphthous ulcers of the oral mucosa detected during the mouth exam (26, 27). In those studied, this complication did not require dose modification.

For this trial, dose compliance was monitored by diary and bottle weights. In addition to a self-reporting subject diary, study personnel questioned the patients regarding compliance with the rinse procedures on each relevant follow-up visit. On each follow-up clinic visit while on experimental therapy, subjects returned any unused drug, and the residual drug in the bottle was weighed and recorded. To additionally evaluate compliance in this study, a balanced subset of patients had serum obtained to measure the trough levels of the drug. Study participants maintained diaries regarding potential treatment side effects and were questioned by study personnel about specific complaints. In addition, hemoglobin levels were obtained at the initiation and conclusion of drug therapy to evaluate for possible occult blood loss. Smoking cessation was recommended to all smokers enrolling on the study, and referral sources for assistance in smoking cessation was provided.

Response Criteria. Criteria for disease response was as previously published at M. D. Anderson Cancer Center (28). For the leukoplakic lesions, the bidimensional surface area of the lesions were recorded by measurement of lesions on exam and confirmed with a color photograph. To provide consistency, the

oral exam and all of the oral measurements were performed by a single dentist at each site (to J. C. A., R. O. G., and J. W. M.). The objective responses are also classified as previously outlined by the M. D. Anderson group (28). Complete response was defined as disappearance of all measurable disease for at least 30 days from the inception of the treatment. Partial response was defined as a decrease in the cross-sectional areas of measurable leukoplakia by at least 50% in the product of the two longest diameters of a single lesion (or in the setting of multiple lesions, in the sum of all of these figures for all lesions) maintained for at least 30 days from the inception of treatment in the absence of any new lesions. Stable disease was defined as a decrease in surface area of $<50\%$ in the cross-sectional areas of measurable disease that lasts at least 30 days. Finally, progressive disease was defined as an increase in the cross-sectional areas or measurable disease by $>10\%$ or the appearance of a new lesion.

As a secondary study end point, we monitored histological change from baseline to study completion. There was no requirement to exhibit dysplasia to enroll on this trial. Histopathology was independently evaluated by three independent pathologists, and histological changes were graded from normal to invasive cancer in an ordinal fashion related to increasing severity of histological change. In addition, at baseline and at the one month follow-up visit, GCF levels were obtained for measuring prostaglandin E_2, IL-6, 8-isoprostane, and IL-1β as biological controls on the primary study end points. These assays were conducted by a reference lab (Assay Design, Ann Arbor, MI) using assay methodology that has been validated previously (26).

The National Cancer Institute Common Toxicity Criteria (version 2.0) was used to describe all adverse events. Ketorolac Tromethamine oral rinse is an investigational agent that has been developed by Procter & Gamble under an amendment to IND 57, 142. The placebo for this trial was the base oral rinse solution containing 20% alcohol and flavor system. The drug was manufactured by Procter & Gamble under Good Manufacturing Practices guidelines and then supplied to the National Cancer Institute for this trial via a Clinical Trials Agreement that was administered by Technology Development and Commercialization Branch, National Cancer Institute, and the Chemoprevention Branch, National Cancer Institute.

Statistical Methods. The primary objective of the study was to evaluate the response probability of ketorolac in a Phase II setting and to obtain a concurrent estimate of the response rate in a group of patients randomized to receive placebo. This study was conducted using an optimal two-stage design for a Phase II clinical trial. Patients were randomized in a double-blind fashion between ketorolac and placebo in a 2:1 ratio. Using Simon's parameter definitions (29), the undesirably low response proportion on ketorolac was 10% ($p0 = 0.10$) and the targeted response proportion was 30% ($p1 = 0.30$). The study used $\alpha = 0.05$ (the probability of incorrectly accepting the agent as if it were good) and $\beta = 0.10$ (the probability of incorrectly rejecting a good agent). The first stage of the design was to randomize 18 patients to ketorolac and 9 to placebo. Because the biostatistics monitors determined there were more than two responses to ketorolac, while maintaining the double-blind design, the accrual to the trial was completed to ensure that an overall total of 35 evaluable patients received ketorolac and 18 more received placebo. A total of 53 evaluable patients randomized to one of two treatments in a 2:1 fashion was adequate to provide 86% power to detect a difference in response rates of 10% in patients randomized to placebo and 50% in patients randomized to ketorolac using a two-tailed $\alpha = 0.05$ and 76% power to detect a difference between 10 and 45% with $\alpha = 0.05$.

The placebo arm's response estimates were derived from two studies. A previously reported IARC study of vitamin A and β-carotene for oral leukoplakia demonstrated that the response rate to placebo was 10% (30). In the first M. D. Anderson leukoplakia trial using 13-*cis*-retinoic acid, the clinically documented spontaneous reversion rate on placebo was 2 of 20 or 10% of patients (28).

Comparisons of results presented in 2×2 contingency tables were performed with a Fisher's exact or χ^2 test, as appropriate. Comparisons between dichotomous parameters (treatment arm; response/nonresponse) and ordered categorical data were performed using the Cochran-Armitage trend test (31). For 2×2 tables, which have been presented in multiple strata, the homogeneity of the resulting odds ratios

Table 1 Patient characteristics by study arm

	Treatment			
	Ketorolac	Placebo	Total	*P*
Sex				0.84
Male	25	13	38	
Female	13	6	19	
Total	38	19	57	
Race				0.65
White	33	18	51	
Non-white	5	1	6	
Total	38	19	57	
Primary site				1.00
Oral cavity	32	16	48	
Oropharynx	6	3	9	
Total	38	19	57	
Smoke				0.70
Yes	31	17	48	
No	6	2	8	
Total	37	19	56	
Cigar years				1.00
None	26	12	38	
Medium	7	7	14	
High	4	0	4	
Total	37	19	56	
Pipe years				0.35
None	28	16	44	
Medium	5	3	8	
High	4	0	4	
Total	37	19	56	
Chewing tobacco years				0.41
None	32	15	47	
Medium	2	1	3	
High	1	2	3	
Total	35	18	53	
Drinks/week[a]				0.029
None	12	4	16	
Medium	25	10	35	
High	0	5	5	
Total	37	19	56	

[a] The questionable value was classified as medium. For the analysis in which the "questionable" value was classified as high, $P = 0.018$.

Table 2 Contingency tables related to response status

	Response			
	Yes (complete response, partial response)	No (stable disease, progressive disease)	Total	*P*
Sex				0.77
Male	12	26	38	
Female	5	13	18	
Total	17	39	56	
Race				1.00
White	15	35	50	
Non-white	2	4	6	
Total	17	39	56	
Primary site				1.00
Oral cavity	14	33	47	
Oropharynx	3	6	9	
Total	17	39	56	
Smoke				0.048
Yes	17	30	47	
No	0	8	8	
Total	17	38	55	
Cigar years				1.00
None	11	26	37	
Medium	5	9	14	
High	1	3	4	
Total	17	38	55	
Pipe years				0.81
None	13	30	43	
Medium	4	4	8	
High	0	4	4	
Total	17	38	55	
Chewing tobacco years				0.79
None	14	32	46	
Medium	0	3	3	
High	1	2	3	
Total	15	37	52	
Drinks/week[a]				0.47
None	6	10	16	
Medium	10	24	34	
High	1	4	5	
Total	17	38	55	

[a] The questionable value was classified as medium. For the analysis in which the questionable value was classified as high, $P = 0.48$.

was evaluated using an exact method (32). Finally, analyses comparing age, histological change, and cigarette pack-years between response status or treatment categories were done using the Wilcoxon rank-sum test. In these analyses, smoking was simply defined as yes or no. Drinking was defined as follows: none; medium (1–14 drinks/week); and high ($\geq$ 15 drinks/week).

For the analysis of treatment toxicity, we tabulated the toxicity two ways. First, for each patient, the worst grade recorded for any toxicity was determined and then toxicity grade was categorized according to treatment. Secondly, the same tabulation as above was performed, except that the analysis was restricted to toxicities potentially ascribable to COX inhibitors, including dyspepsia, gastrointestinal bleeding, nausea, stomatitis, taste disturbance, and vomiting. For these analyses, an exact Cochran-Armitage trend test was performed.

All *P*s are two-tailed, and are not adjusted for multiple comparisons.

Results

Clinical Data. From January 1999 through May 2001, 57 patients were entered onto the study, which was conducted at three centers in the United States. All 57 patients were randomized, but 1 person on the Ketorolac arm had intolerable mouth pain with the first dose and withdrew from the study. Table 1 presents the contingency tables for treatment according to sex, race, primary site, cigarette smoking, cigar years, pipe years, chewing tobacco years, and drinks/week. Unadjusted *P*s are shown as well. There was a strong trend detected between treatment and drinks/week; there were more heavy drinkers in the placebo group than in the Ketorolac group. Otherwise the groups seem well balanced.

The contingency tables for response according to sex, race, primary site, cigarette smoking, cigar years, pipe years, chewing tobacco years, and drinks/week are presented in Table 2 with unadjusted *P*s are shown as well. There appeared to be a strong association between response and smoking. Interestingly, none of the nonsmokers responded, and slightly more smokers responded than expected by chance.

Table 3 presents the contingency tables for treatment according to response categorized four different ways. None of the unadjusted *P*s are < 0.05. We also analyzed for response rate stratified by study location. The *P* from the exact test of homogeneity of odds ratios among the three locations for response (yes/no) *versus* treatment was 0.68. Thus, the association between treatment and response was not significantly different among the three study locations. In Fig. 1, *A* and *B*, serial photographs demonstrate and example of an area of leukoplakia that completely resolved on the 90 days of oral rinse administration. However, in the bottom panels of this figure (Fig. 1, *C* and *D*), serial photomicrographs of another patient reveal no evidence of histological improvement during the course of oral rinse administration during the 90 days on this trial.

Table 3 Treatment response by study arm

	Treatment			
	Ketorolac	Placebo	Total	*P*
(Response				0.89
Yes (CR/PR)[a]	11	6	17	
No (SD/PD)	26	13	39	
Total	37	19	56	
Response				0.65
CR	4	1	5	
PR/SD/PD	33	18	51	
Total	37	19	56	
Response				1.00
CR	4	1	5	
PR	7	5	12	
No	26	13	39	
Total	37	19	56	
Response				0.88
CR	4	1	5	
PR	7	5	12	
SD	17	8	25	
PD	9	5	14	
Total	37	19	56	

[a] CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

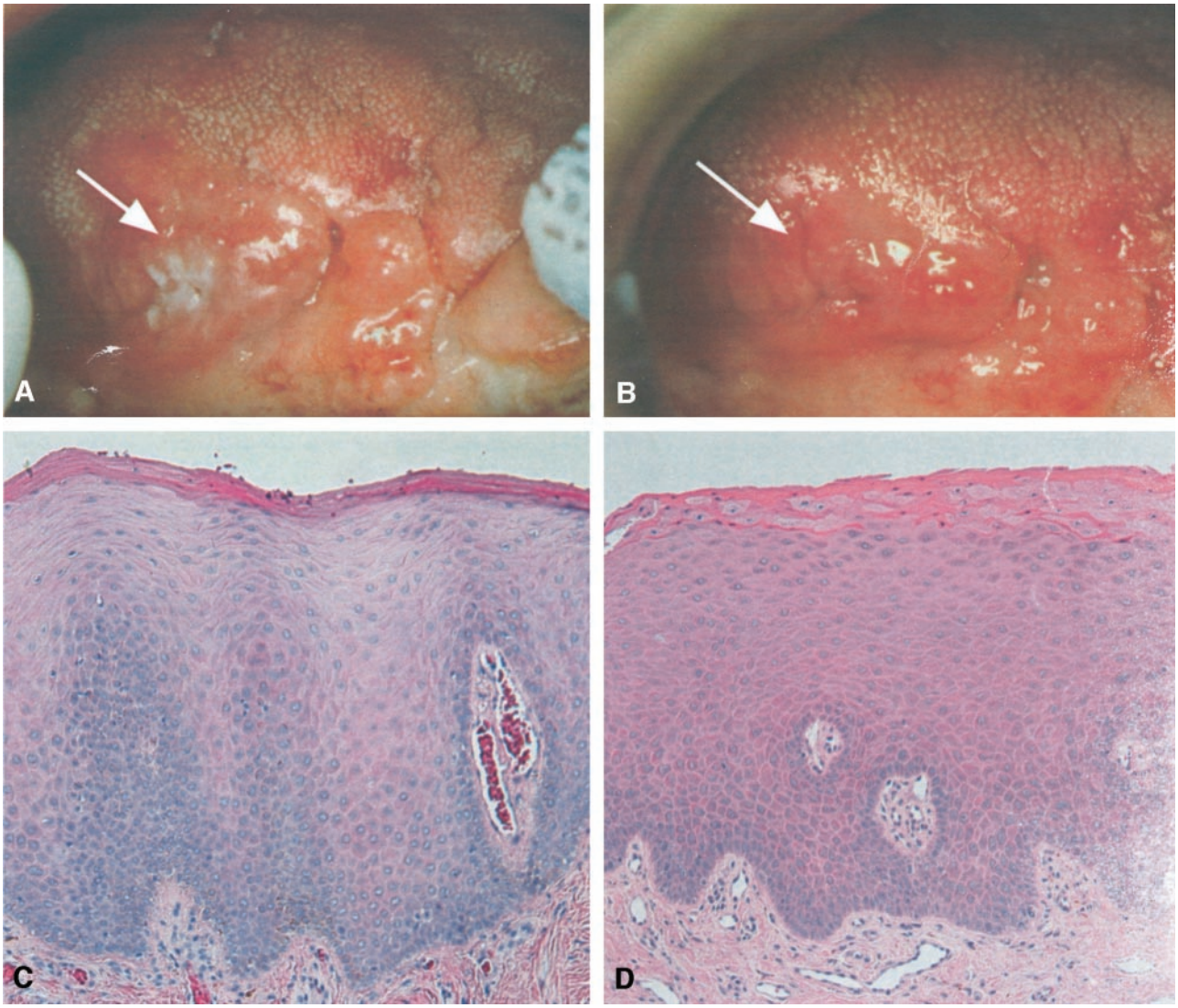

Fig. 1 *A*, picture of leukoplakia lesion from study entry evaluation in individual who had previously underwent partial glossectomy on two separate occasions for sequential primary cancers; *arrow* indicates location of the leukoplakia lesion. *B*, picture of the same individual after receiving ketorolac rinse for 90 days; *arrow* indicates area where leukoplakia was formerly located. *C*, photomicrographs of punch biopsies of another patient on the ketorolac arm of the trial showing evidence of hyperkeratosis with mild maturational atypia. *D*, photomicrograph of the same individual in *C*, but after receiving ketorolac rinse for 90 days, this biopsy did not show any evidence of histological improvement compared with the baseline biopsy.

A more detailed analysis of outcomes related to smoking or drinks/week is shown in Table 4. As shown in the contingency tables for response according to either drinks/week or any tobacco use, stratified by treatment, each of the unadjusted *P*s were ≥ 0.15. In addition, the *P*s for exact tests of homogeneity of odds ratios between the two treatments for response *versus* drinking or tobacco use were both equal to 1.0 (categorizing drinking as none *versus* any). Thus, the results for response according to either drinks/week or smoking did not vary by treatment.

In Table 5, summary statistics are presented for both age and cigarette years, grouped by either treatment or response. Each of the Wilcoxon *P*s are > 0.20, indicating that neither of the distributions of age nor cigarettes years was significantly different when classified by treatment or response.

For the secondary end point of change in histological status, the mean difference in histology from baseline to post-treatment using a graded scoring (for degree of worst dysplasia) was a decline of 1.7 categories for the ketorolac arm and a decline of 2.3 categories in the placebo arm (the *P*s for significant different from zero were 0.76 and 0.80 by a Wilcoxon signed rank test). Although there was some variability in the interpretation of individual pathologists, overall, these discrepancies did not alter the final outcome. Comparing the results between arms, using the mean differences in the worst histopathology, with scores being averages of results by the three pathologists, the *P* for the Wilcoxon rank sum test was 0.96. Thus, the two arms produced approximately equivalent, small degrees of change in histological status. It is interesting to note in Fig. 1, *C* and *D*, an example is shown of a study participant

Table 4 Response status relative to smoking and ethanol ingestion history

	Response			
	Yes	No	Total	*P*
Drinks/week,[a] Placebo				0.49
None	2	2	4	
Medium	3	7	10	
High	1	4	5	
Total	6	13	19	
Drinks/week, Ketorolac				1.00
None	4	8	12	
Medium	7	17	24	
High				
Total	11	25	36	
Any tobacco use, Placebo				1.00
Yes	6	11	17	
No	0	2	2	
Total	6	13	19	
Any tobacco use, Ketorolac				0.15
Yes	11	19	30	
No	0	6	6	
Total	11	25	36	

[a] The questionable value was classified as medium. For the analysis in which the questionable value was classified as high, $P = 0.33$.

with hyperkeratosis with mild maturational atypia that did not improve with a course of oral rinse. This case and many of the other study cases show considerable epithelial hyperplasia. As a result, the epithelium is much thicker than in the normal situation increasing the distance the drug must diffuse to get from the surface of the leukoplakia to the basement membrane.

The twice a day rinsing was tolerated well by patients in both arms of the trial. The one exception was a patient who had extreme pain with the first dose of the ketorolac rinse and left the study. An analysis of the entire worst toxicity grade reported on the trial did not show any significant difference in the adverse events between the two arms (two-tailed exact *P* for the Cochran-Armitage trend test was 0.27).

In considering the thirty eight patients on the ketorolac arm, 27 had no toxicity, 10 had grade 1 toxicity, and only 1 had grade 2 toxicity. For the 19 patients on the placebo arm, 16 had no toxicity, and the remaining 3 had only grade 1 toxicities. For this analysis, the two-tailed exact *P* for the Cochran-Armitage trend test was 0.39. There were no statistically significant differences between baseline and the completion of drug administration for any of the variables either within or between treatments. There were no significant changes in hemoglobin levels between the treatment groups during the duration of the drug administration (*P* for the exact Wilcoxon rank-sum test was 0.91). As summarized in Table 6, study subjects reported high compliance with drug schedule for both the study drug and the placebo.

Discussion

The involvement of COX activity has been implicated in a number of epithelial cancers, including oropharyngeal cancer, and has been recognized as an important chemoprevention target based on epidemiological and biological data (33–35). Efforts to inhibit COX with minimal side effects have led to the development of COX-2-selective inhibitors. COX-1 activity is thought to be essential to maintaining normal organ function, especially in the stomach, where the chronic use of pan-COX inhibitors is associated with a low frequency occurrence of serious gastric hemorrhage. Although a major step in rational drug design, the strategy of narrowing the spectrum of inhibition of COX activity still has been associated with clinically important side effects (22, 36). A growing body of evidence suggests that because COX-1 activity also produces prostaglandins as COX-2, it too can contribute to the carcinogenic process (37, 38). We explored a different strategy to enhance the therapeutic index for oropharyngeal chemoprevention using localized drug delivery of a pan-COX inhibitor only to the oral cavity.

Investigators previously reported that the ketorolac oral rinse when given twice a day was safe and effective in treating another COX-driven process of the oral cavity, periodontal disease (26, 39). In the current trial, we again found that the ketorolac rinse and placebo were well tolerated and compliance with both was high (27). The objective response rate was high for both arms, but because these rates were so close together,

Table 5 Summary statistics for age and cigarette years relative to treatment or response status

Arm	Minimum	Lower quartile	Median	Upper quartile	Maximum	Total number		*P*
						Recorded	Missing	
Age								
Ketorolac	33.00	46.00	56.50	69.00	77.00	38	0	0.25
Placebo	25.00	48.00	53.00	63.00	75.00	19	0	
Cigarette years								
Ketorolac	0.00	0.00	7.00	30.00	45.00	33	5	0.34
Placebo	0.00	10.00	17.00	30.00	41.00	17	2	

Response	Minimum	Lower quartile	Median	Upper quartile	Maximum	Total number		*P*
						Recorded	Missing	
Age								
No	25.00	46.00	56.00	66.00	77.00	39	0	0.74
Yes	30.00	48.00	55.00	69.00	77.00	17	0	
Cigarette years								
No	0.00	0.00	12.00	30.00	45.00	37	2	0.79
Yes	0.00	4.50	13.50	28.00	33.00	12	5	

Table 6 Dose compliance data during days 1–90 of drug administration

Arm	Interval (days)	Percent dose compliance					Total number		
		Minimum	Lower quartile	Median	Upper quartile	Maximum	Recorded	Missing	*P*
Ketorolac	1–30	63	100	100	100	100	35	3	0.81
Placebo	1–30	91	100	100	100	100	18	1	
Ketorolac	31–60	81	99	100	100	100	34	4	0.62
Placebo	31–60	97	100	100	100	100	18	1	
Ketorolac	61–90	55	98	100	100	100	33	5	0.28
Placebo	61–90	91	99	100	100	100	16	3	
Ketorolac	Overall	81	98	100	100	100	33	5	0.56
Placebo	Overall	94	99	100	100	100	16	3	

there is no basis to ascribe the favorable therapeutic response to the COX inhibitor. Prognostic factors in both arms were generally well balanced. Although there was a suggestion from Table 2 that there was a more favorable treatment response among the smokers, this result is not very robust. The exact *P* would have become 0.25 if only one of the 8 nonsmokers would have responded.

The frequency of significant response for both arms of this trial is 3-fold in excess of the previously reported placebo control response rate. In light of the recent discussion of placebo effect, it seems appropriate to consider whether there may be a mechanistic basis for a 20% alcohol solution contained in the base rinse to have an objective impact on the progression of leukoplakia (40). COX activity is thought to be at least in part regulated by the action of nuclear factor-κB (41, 42). Improved oral hygiene mediated by the sustained twice daily rinsing (and potentially enhanced attention to general oral hygiene related to participation in a clinical trial) could have reduced the bacterial burden in the oral cavity leading to a down-regulation of inflammatory mediators (43–47). Decreased transcriptional activity of nuclear factor-κB would reduce COX expression and potentially lead to amelioration of the leukoplakia areas (41, 48). In at least two other studies for treatment of oral inflammatory processes, higher than expected response rates were attributed to increased frequency of oral rinsing. An 18% estimated reduction in the incidence of chemotherapy-induced mucositis was found in a randomized trial testing the effectiveness of a nurse-initiated systemic oral hygiene program in which patients rinsed with 0.12% chlorhexidine or sterile water (49) Both Listerine Antiseptic (which contains 26.9% alcohol) and the hydroalcoholic control reduced the incidence of recurrent aphthous ulcerations from baseline in a 6-month, double blind clinical study (50). The contribution of improved oral hygiene to mucosal inflammation and COX status merits further study.

Because this trial did not find a benefit with the COX inhibitor, does that mean that the products of COX activity do not contribute to the process of carcinogenesis in the mouth? Such a conclusion would be at odds with growing experimental data as well as a recent epidemiological report (4, 6–11, 34, 51–54). In addition, our trial strategy is based upon the unproven assumption that regression of the area of leukoplakia or reversion of histological abnormalities will correlate with eventual reduction in the development of invasive oropharnyngeal cancers (55). This assumption is challenged by data indicating the presence of persisting genotypic alterations in normal-appearing tissue at the site of leukoplakia that completely responded clinically and histologically to chemoprevention (56). At this time, validation of the most appropriate intermediate endpoints for early oral cancer trials is a critical challenge for the field of chemoprevention. Additional research to elucidate informative biochemical, optical, or molecular surrogates of early cancer progress is a pressing need.

Another plausible explanation for the current trial result is that the duration of drug exposure, the drug dose, or the schedule of drug administration was suboptimal. Efforts to measure the prostaglandin levels in the GCF in this trial were unsuccessful because even the baseline GCF samples were found to be much lower than the observed levels of relevant lipids found with periodontal disease (26). In the prior periodontal trials, the dosing of ketorolac was based on documenting critical drug levels in the GCF (27). However, these drug levels may not be relevant to the optimal drug concentrations for leukoplakia. In reviewing the histopathology of the study cases, we found that the involved oral epithelium was routinely hyperplastic as demonstrated in Fig. 1, so drug penetration of a very polar COX inhibitor through a thick keratinized epithelial layer could conceivably be a significant obstacle to clinical efficacy.

This situation was recently reported with a different topically applied COX inhibitor in an actinic keratosis trial (57–60). When the unmodified COX inhibitor, diclofenac, was used to treat actinic keratosis lesions, it found to be ineffective. Additional evaluation revealed that the drug remained on top of the keratin at the surface of the skin when applied topically. In a systematic effort to change the depth of drug penetration with diclofenac, the partitioning of labeled diclofenac was measured with a range of formulations (60). A preparation of labeled diclofenac formulated with hyaluran was associated with the most favorable change in drug penetration. The labeled drug was now found to partition in the vicinity of basal cells at the basement membrane. Diclofenac formulated with hyaluran was found to be much more efficacious in reversing actinic keratoses as demonstrated in a subsequent randomized trial, which led to Food and Drug Administration approval for use in the treatment of actinic keratosis (57–62).

For a chemoprevention drug to be useful, it must be both safe and effective. As outlined in a recent position paper on treatment of intraepithelial neoplasia (55), this trial result underscores the need to better define the critical end point for

determining chemoprevention drug response. However, the results of this trial also suggest that epithelial-directed drug delivery has merit as a strategy for decreasing chemoprevention drug side effects (63). If local chemoprevention drug delivery is found to be safer than systemically delivered drug, this could decompress some challenges with eventual broad application of chemoprevention drugs to large populations.

After careful consideration of the results of this study along with other current research information, we conclude that ketorolac rinse as tested had no significant activity in reducing the area of involvement with leukoplakia. However, we do not think that this result excludes a possible role for COX biology in oropharyngeal carcinogenesis. To better test the strategy of direct epithelial delivery to improve therapeutic index with leukoplakia, we think that it is important to reformulate ketorolac to determine whether that enhances the penetration of that polar molecule through the keratin layer. If the partitioning of ketorolac is improved, then the use of this drug formulation should be re evaluated in a subsequent randomized trial to determine whether delivering the COX inhibitor into the oropharyngeal mucosa to the level of the basement membrane is associated with more effective COX chemoprevention. Our current hypothesis is that in leukoplakia, the clonal populations of epithelial cells at the basement membrane are the optimal target for COX drug delivery to permit effective chemoprevention.

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