Retinoid Chemoprevention Studies in Upper Aerodigestive Tract and Lung Carcinogenesis

Scott M. Lippman, Steven E. Benner, and Waun Ki Hong

Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

Chemoprevention is a clinical strategy to block or reverse carcinogenesis before the development of invasive cancer. Studies of chemoprevention in the lungs and upper aerodigestive tract have relied on the field carcinoma hypothesis, which predicts that diffuse epithelial injury will result from exposure of that epithelium to carcinogens. This hypothesis is supported by the frequent occurrence of multiple primary tumors within the exposed field. In addition, the understanding of carcinogenesis as a multistep process supports the use of interventions in damaged epithelium before the development of clinically invasive cancer. Current efforts are focused on applying to chemoprevention studies the increasing knowledge of the molecular events in carcinogenesis. In our program, clinical trials in lung and head and neck chemoprevention have focused on individuals with evidence of field carcinoma, i.e., a history of previous epithelial cancer or the presence of premalignant lesions. These trials include studies to develop clinically applicable intermediate markers of carcinogenesis and large Phase III trials to evaluate the efficacy of the retinoid isotretinoin in preventing second primary tumors following head and neck or lung cancers.

Introduction

Epithelial cancers of the head and neck or lung are a devastating group of diseases which account for roughly 30% of cancer deaths in the United States (1). These diseases have not been controlled with current prevention, early detection, or treatment approaches (2-5). Therefore, a great effort has been directed toward the development of effective new strategies to control these cancers. One such approach currently under intensive investigation is chemoprevention, i.e., intervention with specific agents during the premalignant process in order to suppress carcinogenesis before invasive cancer develops (6-9). This anticarcinogenic approach is supported by results from laboratory studies, in vivo experimental models, and epidemiological evidence. This paper will review the advances in aerodigestive tract cancer chemoprevention, focusing on recent retinoid studies.

Cancer chemoprevention has as its basis two fundamental concepts. The first is the theory of multistep carcinogenesis, which states that carcinogenesis in epithelial tissues evolves in sequential steps, beginning with premalignant changes and ultimately progressing to invasive cancer. The second fundamental concept, field carcinogenesis, states that carcinogen exposure diffuses the epithelium and predisposes the entire carcinogen-exposed field to the development of multiple independent cancers. Exposure of the skin to sunlight, the colon to fatty acids, and the aerodigestive tract to cigarette smoke are all examples of carcinogen exposure throughout the epithelium of an organ, resulting in diffuse injury and a higher than normal risk of multiple primary tumors (10-12).

Over the past 10 years, our group has conducted a series of randomized chemoprevention trials of the synthetic retinoid isotretinoin in the aerodigestive tract. Four trials have been completed (three in the head and neck and one in the lung), and three are still ongoing (two in the head and neck and one in the lung).

Retinoids, a class of over 3000 natural derivatives and synthetic analogues of vitamin A, are potent modulators of epithelial differentiation and carcinogenesis (13). Recent data indicate that many of the biological activities of retinoids are mediated by nuclear receptors which belong to the steroid-thyroid hormone family of receptors (14). There are two major classes of nuclear retinoid receptors, the RAR and the RXR series, each with α, β, and γ subtypes, and each subtype has multiple isoforms. In vivo carcinogenesis experiments indicate that certain retinoids can inhibit the development of invasive cancer at many epithelial sites, including the head and neck and lung. These agents act primarily in the late promotion-progression phase of carcinogenesis. Clinically, they produce a characteristic dose-related mucocutaneous toxicity syndrome which is reversible with cessation of exposure to the agent (13, 14). The retinoid isotretinoin has been shown to have significant activity in both suppressing oral carcinogenesis and preventing second primary tumors following head and neck cancer (15).

Head and Neck Carcinogenesis

Oral Premalignant Lesions. We first studied leukoplakia, the most common oral premalignant lesion, because these lesions are easy to measure and biopsy without subjecting study participants to an invasive procedure such as bronchoscopy (15). Two randomized studies have been completed. The first study tested high-dose isotretinoin in a short-term placebo-controlled study, and the second compared the efficacy of maintenance therapy with low-dose isotretinoin or β-carotene following high-dose isotretinoin induction therapy. On the basis of several encouraging uncontrolled studies of retinoid therapy in leukoplakia, the first, reported in 1986, was a Phase IIb placebo-controlled trial of high-dose isotretinoin (1-2 mg/kg/day for 3 months) in 44 patients (16). The clinical response rates were 67% in the retinoid group and 10% in the placebo group (P = 0.002). Although these initial results were encouraging, high-dose isotretinoin was associated with significant mucocutaneous toxic effects and short remission durations (median, approximately 3 months after stopping therapy).

The second, also a Phase IIb trial, was sponsored by the National Cancer Institute and was designed specifically to address the problems of severe toxicity and early relapse that were encountered in the first trial (17). A 3-month induction phase in which all patients received high-dose isotretinoin (1.5 mg/kg/day) was followed by randomization to a 9-month maintenance phase of low-dose isotretinoin (0.5 mg/kg/day) or β-carotene (30 mg/day). The primary study end point was the progression (relapse) rate during the maintenance phase. This 5-year study was recently completed with 70 registered patients. Several laboratory correlates were assessed in this trial, the early results of which are reviewed below. The clinical results were highly significant, with progression rates of 8% in the low-dose isotretinoin group and 55% in the β-carotene group (P < 0.001). These results led to the adoption of the isotretinoin dosing schedule (0.5 mg/kg/day or approximately 30 mg/day) currently under study in long-term chemoprevention trials in the lung and head and neck.
The results of several uncontrolled trials of natural agents suggest that β-carotene, vitamin E, vitamin A, and selenium have activity in oral leukoplakia (15, 18, 19). These reports require confirmation by a Phase Ib placebo-controlled trial.

Our current oral leukoplakia study, part of a National Cancer Institute-sponsored program project, compares low-dose isoretinoin to a combination of retinyl palmitate and β-carotene. The rationale for testing the natural agent combination is based on laboratory studies and limited clinical data suggesting that these agents have an enhanced combined effect and few or no toxic effects. These agents will be administered for 3 years.

Our recently completed maintenance study (17) and current ongoing long-term trial incorporate a number of laboratory correlates. These biological studies are critical for several reasons. Laboratory studies will provide invaluable data on the important biological events associated with oral carcinogenesis and the mechanisms of chemopreventive drug response and resistance. From a practical point of view, these laboratory correlates may ultimately serve as early, surrogate, or “intermediate” end points for chemoprevention trials (10, 11).

To date, these biomarkers are in an early phase of development, and no intermediate end points have been validated; i.e., expression or modulation of the markers has not been prospectively shown to correlate with the development of invasive cancer (10). Since the patients whom we are studying are chemoprevention trial participants who do not have cancer, laboratory studies must be modified to rely on minimally invasive microassay techniques which use serial sections of tiny 1-2-mm biopsy specimens. We are studying both genotypic and phenotypic markers of oral carcinogenesis (10, 20). Our genotypic studies include micronuclei frequency, a marker of clastogenic DNA damage. Micronuclei are extranuclear fragments of DNA which are formed as the result of exposure to genotoxic carcinogens. This test provides a measure of recent DNA injury and has many advantages, such as its quantitation in exfoliated cells.

Other, more specific genetic markers under study include aneuploidy in chromosomes 7 and 17 (20). We have observed polysomy (e.g., trisomy) of these chromosomes in dysplastic lesions and to a lesser degree in hyperplastic lesions. Experiments using in situ hybridization with fluorescent centromeric probes have suggested that the premalignant lesions have a clonal evolution. The finding of extra copies of chromosome 7 in these lesions is of special interest, since the epidermal growth factor receptor (EGFR) gene is located on this chromosome. Amplification of the EGFR gene has been identified in premalignant oral lesions in both animal models and human studies (10). Our data suggest that the polysomy of chromosome 7 may identify a late stage of oral carcinogenesis. Another group of genetic abnormalities, p53 mutations, are detected in a substantial percentage of both hyperplastic and dysplastic oral and bronchial lesions (21–23). Using molecular biology technologies, we are also evaluating both the RAR and RXR series of nuclear retinoid receptors. Recent data suggest that RAR-β may play an important role in the evolution of oral carcinogenesis and the responses of the lesions to retinoic acid treatment (24).

We are studying phenotypic markers of proliferation and differentiation using immunohistochemical techniques (10, 20). We have studied a number of proliferation markers and have found PCNA 3 to be the most useful and reliable of these. Assays of PCNA may be performed on paraffin-embedded tissue. Dysregulated epithelial proliferation as identified by PCNA occurs in nonmalignant tissue adjacent to head and neck cancer (25). Differentiation markers under study include cytokeratins, e.g., K1 and K19, and surface glycoproteins, such as blood group antigen A (20).

Although the sequence of genetic events associated with carcinogenesis and phenotypic changes in this region is not clear, recent molecular data, some of which are reviewed above, strongly support the concept of field carcinogenesis and suggest that there is a widespread field change in high risk subjects and cancer patients (20–28).

**Adjuvant Head and Neck Study.** We recently reported a randomized, placebo-controlled Phase III trial of high dose isoretinoin in 103 patients with stage I–IV (M0) head and neck cancer (29). The use of isoretinoin in this setting was supported by epidemiological data on nutrition, by laboratory studies, and primarily by the significant clinical activity of this agent in oral carcinogenesis (15–17). The initial report, made after a median follow-up of 32 months, indicated a highly significant reduction in the incidence of second primary tumors in the isoretinoin group (n = 49) as compared with the placebo group (n = 51); 2 patients in the isoretinoin group developed second primaries, whereas 12 in the placebo group developed second primaries (P = 0.005) (29). No significant difference was observed in primary disease recurrence or overall survival rates between the two groups. At a median follow-up of 54.5 months, the total numbers of patients with second primary tumors in the two groups remained significantly different (30). Although the observed retinoid effect on overall second primary tumor rates decreased with prolonged follow-up (P = 0.04), the strong effect has persisted for tobacco-related second primary tumors (P = 0.008). Second primary tumors develop at a constant annual rate of 4–7% in prospective studies and are the major cancer-related cause of death in patients with early-stage cancers (31–34). The results of this isoretinoin trial have provided strong support for further chemoprevention studies using retinoids to prevent second primary tumors in head and neck and lung cancers.

Our current study is a multicenter, Phase III, double-blind, placebo-controlled trial of low dose isoretinoin in approximately 1000 early-stage (T1, T2, and N0) patients after definitive local therapy. Patients in the isoretinoin treatment group will receive a dose of 30 mg/day. All patients will take the study drug for 3 years, with an additional 4 years of follow-up. This prospective study involves the Radiation Therapy Oncology Group, the M. D. Anderson Cancer Center, and its affiliated Community Clinical Oncology Program. The data evaluation stage of the trial will include a central committee for independent review and verification of all study end points.

**Lung Carcinogenesis**

The positive results of recent trials of systemic retinoids in preventing second primary tumors associated with head and neck cancers and oral carcinogenesis have sparked great interest in the potential of chemoprevention, particularly for the prevention of lung cancer, which shares a similar etiology and biology with head and neck cancers (10, 15, 20–28, 32–34).

**Premalignant Lesions.** Gouveia et al. (35) reported an uncontrolled trial of the synthetic retinoid etretinate in chronic smokers with bronchial squamous metaplasia. This group of French investigators reported that 73% (29 of 40) of the patients who received etretinate had reductions in metaplasia index; the overall mean metaplasia index decreased from 35% to 27%.

Because of the significant activity reported in this uncontrolled trial (35), we conducted a randomized Phase Ib placebo-controlled trial in a similar group of subjects; our trial used isoretinoin, which is related to etretinate. Our trial end point was the same as that used by the French group (35), i.e., squamous metaplasia identified by bronchoscopic biopsies. In this logistically complex trial, eligible chronic smokers underwent screening bronchoscopy with biopsy at six

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3 The abbreviation used is: PCNA, proliferating cell nuclear antigen.
standardized sites within the proximal lung field. Those study participants found to have significant metaplasia or any evidence of dysplasia were randomized to receive 6 months of treatment with placebo or isotretinoin (1 mg/kg/day). Subjects were reevaluated with bronchoscopy after 6 months of treatment. If the extent of metaplasia had not decreased, the treatment code was broken and subjects on placebo were crossed over to receive isotretinoin for 6 months and then undergo a third bronchoscopy. Subjects who were found to have a reduced extent of metaplasia received no further treatment but did undergo bronchoscopy with biopsy after 6 more months. Thus, participants on the study underwent a total of three fiberoptic bronchoscopies with biopsies of the same six standardized lung regions.

This study has completed accrual, and initial 6-month data for the primary end point have been analyzed (36). We observed a substantial reduction of bronchial metaplasia in both the isotretinoin (54%) and placebo (59%) groups, indicating that isotretinoin at the given dose and schedule had no impact on metaplasia and underscoring the importance of placebo-controlled trials to establish drug activity in studies of intermediate end points.

A recent placebo-controlled Phase Iib trial of the efficacy of etretinate (25 mg/day) in reversing metaplasia measured from sputum cytological samples also underscores the need for a placebo control group. In this trial, the rates of improvement in sputum cytological atypia were similar in the etretinate (32%) and placebo (30%) groups (37).

Although initial analysis of the data on the primary end point of the isotretinoin study (i.e., reversal of metaplasia) is complete, a number of biological studies on serial tissue specimens are continuing. For example, measurement of PCNA, has yielded important results. The data suggest that PCNA is a useful marker of carcinogenesis. The percentage of lesions with over 1% PCNA staining was highest in specimens with metaplasia and dysplasia. In addition, the PCNA patterns were abnormal in these higher risk lesions, with suprabasal staining suggesting dysregulated proliferation. In contrast, micronuclei frequency showed no correlation with metaplasia (10, 20). Although still ongoing, other marker studies indicate frequent p53 mutations and altered growth factor receptor expression consistent with a diffuse field injury and genomic instability.

Primary chemoprevention trials in the lung have studied individuals at increased risk of cancer because of smoking or asbestos exposure. A Finnish Phase III trial will test the efficacy of vitamin E and β-carotene in reducing lung cancer incidence in over 2,000 male smokers. A United States Phase III trial of vitamin A and β-carotene in over 15,000 heavy smokers is also under way (38).

Adjuvant Lung Study. Pastorino et al. (39) recently reported the final results of a Phase III trial of retinyl palmitate (300,000 IU/day for 12 months) in patients with resected pathological stage I non-small cell lung cancer. After a median follow-up of 46 months, the annual second primary tumor rate in the control group was 4.8%, and over 70% of second primary tumors occurred within the tobacco-related field at risk. Retinyl palmitate was associated with a 35% lower annual second primary tumor rate and a significantly longer time to development of tobacco-related second primary tumors than placebo (P = 0.045). The 5-year survival rates were not significantly different between the two study groups, due in large part to rigorous prospective follow-up, early detection of second primary tumors, and effective surgical salvage.

A United States intergroup Phase III trial of low dose isotretinoin (30 mg/day) has just been activated in patients with resected stage I non-small cell lung cancer (33, 38). The dose and schedule used in this trial were based on those used in the trial of low dose isotretinoin in preventing second primary tumors of the head and neck. The major goal of this study is to prevent second primary tumors rather than to prevent recurrence of primary lung cancer.

A European multicenter study (Euroscan) is also testing the efficacy of chemoprevention following definitive therapy of early-stage head and neck or lung cancer. This Phase III trial uses a 2 × 2 factorial design to study the efficacy of retinyl palmitate and the antioxidant N-acetylcysteine.

Conclusion

A challenge to the development of chemoprevention as an effective clinical practice is the need to develop our understanding of carcinogenesis and incorporate this into clinical trials. Chemoprevention in the lung and upper aerodigestive tract is the treatment of field carcinogenesis. The need in present studies to rely on a reduction in cancer incidence as the study end point leads to large-scale trials lasting for many years. The development of intermediate biomarkers which could be used as reliable surrogate end points for cancer incidence would greatly facilitate these trials.

A strategy being used to develop these biomarkers is to incorporate laboratory studies into clinical trials of patients with premalignant lesions such as oral leukoplakia. These patients are at greatly increased risk compared with the general population for the development of cancers. The presence of a histologically detectable lesion is associated with other carcinogenic changes within the surrounding epithelium. Clinical trials which study the reversal of these histologically apparent premalignant lesions provide an excellent opportunity to develop intermediate markers which could guide future trials (10).

Although chemoprevention is no “magic bullet,” optimal integration of effective chemoprevention into programs of local and systemic therapy for primary disease (local, regional, and distant) could have an impact on overall patient survival. However, it must be emphasized that at present, cancer chemoprevention cannot be considered standard practice (33). The recent analyses of adjuvant chemoprevention in the head and neck (29) and lung (39) are informative and suggest that retinoids may be effective in reversing the consequences of field carcinogenesis. However, both studies were relatively small, with only short-term follow-up. Furthermore, neither study was associated with prolonged survival, which in part reflects effective surgical salvage of second primary tumors in these prospectively followed patients. Therefore, the enthusiasm generated by the early results of the chemoprevention studies should be focused toward enrolling more patients in the ongoing Phase III clinical studies. In the United States, three major studies of low-dose isotretinoin are ongoing: in two, patients receive the isotretinoin following definitive therapy of early-stage head and neck cancer and in the third, isotretinoin is given after treatment for stage I non-small cell lung cancer. The current large scale United States trials are stratified by smoking status and carefully monitor patterns of smoking cessation. The multicenter Euroscan trial is also ongoing in early-stage head and neck or lung cancer patients. The results of these large-scale Phase III chemoprevention trials will have important implications for standard clinical practice.

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