Head and Neck Chemoprevention: Recent Advances
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Significant clinical advances have been made in understanding the role of retinoids, particularly 13cRA, in the chemoprevention of head and neck squamous cell cancers.

Background: Head and neck cancers are important to human life and health in both developed and underdeveloped countries. Management of established cancers is difficult, and there is great interest in evaluating methods to prevent these tumors from developing.

Methods: The biology of carcinogenesis, including field carcinogenesis, is reviewed, together with the biology and pharmacology of the retinoids. Intervention studies of premalignant lesions have led to prospective clinical trials of the capability of various retinoids to reduce the incidence of second primary tumors in patients with “cured” squamous head and neck cancers.

Results: High-dose 13-cis-retinoic acid (13cRA) has significant activity in reversing oral leukoplakia but at a cost of substantial toxicity, and relapses occur early. An ongoing intergroup trial is underway to evaluate the capacity of low-dose (30 mg/d) 13cRA given for three years to reduce the incidence of second primary tumors in patients with “cured” squamous head and neck cancers.

Conclusions: Molecular studies of loss of heterozygosity and p53 gene mutations are advancing our understanding of field carcinogenesis and the biology, pharmacology, and effects of the retinoids used in cancer prevention. Translation of early clinical trials into large-scale intervention trials to prevent...
Strong evidence of multistep carcinogenesis came from molecular studies in premalignant tissue, particularly from studies of loss of heterozygosity (LOH). A high rate of LOH at 9p21 and 3p12-14 occurs in premalignancy (squamous dysplasia and carcinoma in situ) and in invasive carcinomas.\(^9\) Mao et al.\(^{10}\) found that LOH at 9p21 and/or 3p14 in lesion samples of oral leukoplakia patients correlated with development of squamous cell carcinoma. These LOH data suggest that clonal genetic alterations present in early stages are related to later, final stages of carcinogenesis.

### Field Carcinogenesis

The major conceptual underpinning of chemoprevention is “field” carcinogenesis. The classic example of this concept is exposure of the upper aerodigestive tract and lung to tobacco smoke. In this and other settings, the extensive, multifocal development of premalignant and malignant lesions can occur within the whole carcinogen-exposed epithelial region.\(^8\)

After being proposed by Slaughter et al.\(^{11}\) in 1953 to explain SPT development, field carcinogenesis was supported by studies of the p53 tumor suppressor gene. The association between mutations of the p53 gene and both cigarette smoking and head and neck squamous cell carcinogenesis was established by Brennan et al.\(^{12}\)

Another study\(^{13}\) involving 31 HNSCC patients found that p53 mutations in primary tumors was discordant from p53 in associated SPTs in respect to the presence or site of mutation within the p53 gene. Of the 31 patients,\(^{21}\) had p53 mutations and experienced discordance of p53 mutations between primary tumors and SPTs. This result confirmed that the developments of primary tumors and SPTs can be genetically independent events. Other studies also have found discordant p53 mutations in multiple neoplastic foci of individual patients. One recent study,\(^{14}\) however, does not support the field carcinogenesis concept. Patients with multiple primary head and neck tumors exhibited patterns of allelic loss on chromosome 9p and 3p, suggesting that at least a portion of primary tumors and associated SPTs arise from a single clone.

### Retinoid Biology, Pharmacology, and Metabolism

#### Biology and Pharmacology

The biology of retinoid activity in the body is highly complex. Controlling normal cell growth, differentiation, and loss during embryonic development is the primary physiologic function of retinoids. Retinoids function in early development to help specify embryonic axes and later control the fate of specific cell types.\(^{15,16}\)

The pharmacologic effects of retinoids in neoplastic cells -- including modulations of differentiation, proliferation, and apoptosis -- replicate the primary biologic effects of retinoids in embryonic cells.

The biology of nuclear receptors that mediate retinoid effects also is extremely complex.\(^{17,18}\) The nuclear retinoid receptors belong to a superfamily of receptors that mediates the effects of many compounds, including steroids and thyroid hormones, vitamin D, prostaglandins, and drugs that activate peroxisomal proliferation.\(^{19}\)

The two classes of retinoid receptors are retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each class has alpha, beta, and gamma subclasses, which are divided further into a large number of isoforms. Individual RARs and RXRs appear to be expressed differently in different tissues and seem to have different biologic functions.

Retinoid receptors do not function as monomers. Instead, they bind to one another to form either homodimers or heterodimers that mediate retinoid effects.\(^{17-19}\)

The pattern of associations for RARs is fairly straightforward. The three RARs (alpha, beta, and gamma) bind to the three RARs to form RAR/RXR heterodimers. The pattern of associations for RXRs is more complex.\(^{20}\)

Besides binding to RARs, RXRs can bind to many other receptor types, such as those for thyroid hormones and vitamin D, and can bind with themselves to form RXR/RXR homodimers. Therefore, a large number of receptor dimer complexes mediate the effects of retinoids that bind to and activate RXRs.\(^{21}\)

Naturally occurring retinoids tend to be pan activators of receptors, or non-receptor-specific. The natural retinoid all-trans-retinoic acid and its two isomers, 9-cis-retinoic acid (9cRA) and 13-cis-retinoic acid (13cRA), are the most widely clinically tested retinoids. These retinoic acids are readily interconverted in vivo, and so each can activate a wide spectrum of retinoid receptors, signaling pathways, and biologic effects. Current systemic therapy with these agents in many settings is limited by the substantial toxicities that accompany their beneficial effects. The side effects result from activation of multiple signaling pathways.

Synthetic retinoids have been developed that are more selectively bind with the subtly different receptor clefts of specific receptors. Synthetic retinoids can be specific for the RAR or RXR class and further for different subclasses within each class. These receptor-selective retinoids may offer new therapeutic opportunities. A synthetic retinoid selective only for RAR-alpha may be active in myeloid leukemia, or one selective only for RAR-beta may be active in squamous malignancies. Such an agent may produce less mucocutaneous and bone toxicities characteristic of retinoids, since these effects appear to be mediated by RAR-gamma.\(^{22}\)

Although individual retinoid receptors appear to have a preferred pattern of target genes, there may be considerable redundancy in the function of the individual RARs. Deletion of either RAR-alpha or RAR-gamma from cells that normally express both receptors causes a different set of changes in gene expression. Defects resulting from deletion of one set of receptors, however, can be compensated for by overexpression of the other set.\(^{23}\)

Human myeloid leukemia cells exhibit this phenomenon: deletion of endogenous RAR-alpha can be reversed in respect to receptor activity by overexpression of either RAR-beta or -gamma.\(^{24}\)

Receptor redundancy has been confirmed by studies in receptor knockout animals.\(^{25}\)

Deletion of any one receptor caused a modest alteration in developmental patterning. Deletion of two or more receptors, however, caused severe malformations.

Receptor redundancy has important implications for retinoid pharmacotherapy. One or another of the retinoid signaling pathways in tumor cells may be abrogated by mutation or functional inactivation. Ligand activation of the residual receptors and alternative signaling pathways can restore retinoid responsiveness. This occurs in acute promyelocytic leukemia cells. The apparent decrease in retinoid sensitivity in these cells is due to the promyelocytic leukemia gene-RAR translocation, which can be reversed by using pharmacologic retinoid levels to activate the residual retinoid receptors.\(^{26}\)

Neoplastic cells with disruptions of the major signaling pathways or in which retinoid effects are mediated by receptors associated also with substantial adverse effects may respond to retinoids that activate other less abundant receptors in the same neoplastic cells.

Retinoids not only regulate transcription via the activation of specific retinoid receptors (transactivation activity), but also appear to suppress the activity of other transcription factors, such as AP-1, that are critical mediators of cellular proliferative activity (transrepression activity).\(^{27-29}\)

Although researchers do not agree on mechanisms involved in transrepressive activity, recent studies suggest that AP-1 and other transcription factors compete with retinoid receptors for common “coupling factors” (proteins that control the activity of the RNA polymerase complex that transcribes genes).\(^{27-29}\)

When retinoids bind to their receptors, they can sequester these coupling factors so that they are inaccessible to other transcription factors.\(^{30}\)

Therefore, retinoids can turn down the activity of whole signaling pathways involved in either proliferative or inflammatory responses, including the pathway of AP-1, which mediates both proliferation and inflammation.
A recent surprising discovery is that the transactivating and transrepressing activities of retinoids can be dissociated. Several laboratories have developed retinoids that can inhibit AP-1-dependent processes (transrepression) without activating the transcription of retinoid-regulated genes (transactivation). Retinoid transrepression activity apparently is linked to retinoid antiproliferative activity, whereas transactivation activity is linked to induction of differentiation. For example, AP-1 selective retinoids have antiproliferative activity and do not induce cellular differentiation in a number of normal and neoplastic cell lines. Researchers are interested in the possibility that synthetic AP-1 selective retinoids may be capable of suppressing neoplastic cell growth without triggering the customary retinoid side effects of mucocutaneous and bone toxicities.

**Metabolism**

Under normal conditions, all-trans-retinoic acid and 9cRA are the physiologic retinoids that activate retinoid receptors in cells. Since these retinoids normally are not present in plasma or are present only at levels too low to be biologically significant, they must be generated intracellularly. It follows that the ability of cells both to make and breakdown retinoic acids is vitally involved in the physiologic regulation of retinoid-dependent processes. Cellular retinoic acids are generated by the enzymatic oxidation of all-trans-retinol (vitamin A), which is delivered to cells via the plasma. Four factors essentially control the levels of retinoic acids within cells: (1) the level of all-trans-retinol (vitamin A) in the plasma, (2) the ability of a cell to take up plasma retinol, (3) enzymatic activity in converting retinol to retinoic acid, and (4) enzymatic activity in deactivating intracellular retinoic acids.

Dietary vitamin A, derived either from animal tissues or from the plant pigment beta-carotene, is stored by the liver, from which it is delivered to the tissues via the plasma in a complex with two specific carrier proteins -- retinol-binding protein (RBP) and transthyretin. Although it is not known how, cells transfer retinol from RBP to the apo, or unbound, form of cytoplasmic retinol-binding protein (CRBP), which is the specific intracellular-binding protein for retinol. After transfer to CRBP, retinol can be oxidized to retinoic acid or esterified by lecithin-retinyl acyltransferase. Cells store esterified retinol for later use when temporary decreases may occur in dietary supplies of vitamin A.

The conversion of retinol to retinoic acid is controlled by the levels of CRBP and the activity of two specific enzymes (retinol and retinal dehydrogenase). The ratio of apo-CRBP to retinol-bound CRBP determines the rates of cellular uptake (from RBP) and esterification of retinol. With low ratios, less retinol is extracted from the plasma and more of that amount is esterified. With high ratios, more retinol is extracted from the plasma and more of that amount is oxidized to retinoic acid. Once inside the cell, retinol's oxidation to all-trans-retinoic acid is catalyzed by retinol and retinol dehydrogenases. Retinol dehydrogenase is an NADP-dependent enzyme and catalyzes the conversion of the retinol-CRBP complex into all-trans-retinal CRBP. Retinal dehydrogenase is an NAD-dependent enzyme and catalyzes the conversion of all-trans-retinal CRBP into all-trans-retinoic acid and apo-CRBP. Although they act is not well understood, CRBP, NADP and NAD are known to play critical roles in the regioselective regulation of the conversion of retinol to retinoic acid.

Retinoic acid formed from retinol is bound to cytoplasmic retinoic acid-binding proteins (CRABPs I and II) that are abundant in the cytoplasm and nucleoplasm of most cells. Very similar to the CRBPs, these proteins appear to facilitate the intracellular transport of fatty acids and retinoids. The key role CRABPs play in retinoid metabolism is suggested by their highly conserved structures and expression patterns among species. Since CRABP overexpression is associated with accelerated retinoid acid metabolism, CRABPs appear to facilitate the delivery of retinoic acid to the microsomal oxidases that catalyze retinoic acid degradation.

Perturbed retinoid metabolism can contribute to carcinogenesis and affect retinoid pharmacotherapeutic strategies. Deficiencies of cellular retinoic acid are tightly associated with defects in the retinoid signaling pathway and with cancer development. Low dietary vitamin A or perturbed retinoid acid metabolism can cause these deficiencies. Populations of developed countries rarely experience generalized vitamin A deficiency due to diet. Perturbations in either retinol or retinoid acid metabolism, however, can cause specific tissues to become retinoid-deficient in developed countries. For example, levels of retinoic acids in oral premalignant tissues were deficient despite normal plasma retinol and retinyl palmitate in a recent US study. Cellular vitamin A deficiency can cause perturbations in retinoid receptor expression and proliferative control that appear to help maintain a premalignant state. These abnormalities in retinoid levels may reflect alterations in the ability of premalignant cells to extract retinol from plasma or to convert it into retinoic acid, or they may reflect an accelerated rate of retinoic acid catabolism in the premalignant tissue.

Not all exogenous retinol taken up by cells is converted into biologically active retinoic acids. As discussed above, CRBP levels help to determine how much retinol is esterified and stored or oxidized to retinoic acids. One early study (conducted prior to our understanding of the importance of the ratio of apo-CRBP to bound-CRBP) indicated that high levels of CRBP are present in some human tumors and may promote retinol esterification and storage over oxidation to retinoic acids. How retinol and retinoid dehydrogenase work in tumors has not been studied. It is possible that defects in the activity of either of these enzymes, or of their cofactors NAD and NADP, may impair oxidation of retinol and reduce cellular retinoic acid levels in the presence of normal, or even elevated, cellular retinol levels.

The retinoid signaling pathway itself also may be defective. In some malignancies, for example, normal intracellular retinoic acid levels exist, but receptor signaling is defective. For instance, malignant transformation in acute promyelocytic leukemia and hepatitis B-linked hepatocellular carcinoma is associated with alterations in the structure of genes for the RARs. Defective retinoid receptors directly alter the regulation of gene expression by endogenous retinoic acid. Intact retinoid receptors also can be defective in activating gene expression. In studies of lung cancer cells, for example, retinoid and retinoid receptor levels were normal, but retinoid activation of the receptors did not always activate retinoic-acid-responsive gene transcription. These signaling breakdowns are not thoroughly understood, but they may be due to malfunctions of the factors that couple retinoid receptors to the activation of gene transcription.

One means of overcoming perturbed retinoid metabolism is with pharmacologic doses of the natural compounds retinol and retinyl esters. These compounds can be transported by serum lipoproteins as well as by RBP, thus providing alternative pathways for the delivery of the retinoids. For example, the function of RBP receptors may be impaired in human myeloid leukemia cells, but the cells are able to generate retinoic acids from retinol and retinyl esters transported by lipoproteins. Perhaps a more effective way to compensate for aberrant retinoid metabolism in transformed cells is to deliver the biologically active retinoic acid isomers --- all-trans-retinoic acid, 9cRA, or 13cRA --- directly to neoplastic cells, thus bypassing the physiologic pathways of normal retinoid metabolism altogether. Current retinoid pharmacotherapy is based on this strategy. High concentrations of retinoic acid to both normal and neoplastic cells can be achieved by systemic administration of any of these three isomers. They diffuse across plasma membranes and gain direct access to intracellular binding proteins and receptors. The ability of these retinoids to isomerize in vivo can cause fluctuations in intracellular levels of all three retinoids.

**Clinical Trials**

Retinoids have been the most studied and active chemopreventive agents in the head and neck. Retinol, retinyl palmitate, all-trans-retinoic acid, 13cRA, etretinate, and fenretinide (4-HPR) all have a record of clinical study in this region, either for the reversal of oral preinvasive lesions or for the prevention of SPTs.
Oral premalignancy lesions are of two clinical types, leukoplakia and erythroplakia. The spontaneous regression rate and transformation rate of small hyperplastic leukoplakia lesions are 30% to 40% and <5%, respectively. The risk profile is inverted, however, for erythroplakia and dysplastic leukoplakia lesions -- a 5% rate of spontaneous regression and a 30% to 40% risk of transformation. Multifocal advanced disease is rarely controlled adequately with local therapy and represents approximately 10% to 15% of all oral premalignant lesions. Squamous cancers at distant sites within the upper aerodigestive tract as well as in the oral cavity often develop in patients with oral premalignant lesions. For this reason, trials to control advanced oral premalignancy help in screening agents considered for use in preventing aerodigestive tract cancers.

In the late 1970s, supplemental beta-carotene and retinol were shown to reduce the frequency of oral micronuclei (an intermediate end-point marker of genetic damage) in studies conducted in individuals at high risk for oral cancer, such as chewers of tobacco and betel nut. Based on these data, seven subsequent trials were conducted to test the ability of supplemental beta-carotene, alone or in combination with other agents, to reverse oral leukoplakia lesions. Five were nonrandomized and were reported to have achieved response rates of from 44% to 71%. Interpreting these uncontrolled trial results is complicated by leukoplakia's 30% to 40% spontaaneous regression rate, the studies' differing response criteria, and the lack of any direct dose-response relationship in the results.

Two of the beta-carotene trials in leukoplakia were placebo controlled. Stich et al tested the three arms of combined beta-carotene plus retinol, beta-carotene alone, and placebo, which produced complete response rates of 27.5%, 14.8% and 3.0%, respectively. Partial remission rates were not reported. In Uzbekistan, a six-month trial of combined retinol plus beta-carotene plus vitamin E was placebo controlled. A significant reduction in the prevalence odds ratio (OR) of oral leukoplakia (OR=0.62; 95% confidence interval [CI]=0.39-0.98) occurred in the combination arm. The risk of progression or no change vs regression was also reduced by 40% in the combination arm, but this result was not statistically significant (OR=0.60, 95% CI=0.23-1.63).

A single-arm phase II study of alpha-tocopherol conducted by Bennet et al patients with oral leukoplakia involved 43 patients treated for 24 weeks. Twenty (46%) had clinical responses, and nine (21%) had histologic responses.

Seven randomized clinical trials have been conducted in oral premalignant lesions -- the two cited above involving beta-carotene combinations and five others involving single-agent retinoids, one of which also had a single-agent beta-carotene arm.

In 1986, Hong et al reported that high-dose 13cRA had significant activity in their prospective, randomized, double-blind clinical trial in oral leukoplakia. Clinical responses in the 13cRA vs placebo group were 67% (16 of 24) and 10% (2 of 20), respectively (P=0.002). The rate of histopathologic improvement also was significantly higher in the retinoid arm (54% vs 10%, P=0.01). Substantial toxicity and a high rate of relapse (greater than 50% within two to three months of discontinuing therapy) presented major clinical limitations within this high-dose trial.

In an effort to address the toxicity and relapse rates of the earlier Hong trial, a follow-up randomized maintenance trial with low-dose 13cRA was designed. After a three-month induction course of high-dose 13cRA, patients received a nine-month maintenance treatment with either low-dose 13cRA (0.5 mg/kg/d) or placebo (30 mg/d). As predicted in the study by Hong et al, induction of high-dose 13cRA produced a high rate of response. In the maintenance phase, only two (8%) of 24 patients in the low-dose 13cRA group had progressive leukoplakia, whereas 16 (55%) of the 29 patients on beta-carotene maintenance progressed (P<0.001). No dropouts occurred in the arm with low-dose 13cRA, which was well tolerated.

In India, Stich et al tested vitamin A (200,000 IU/wk orally for six months) compared with placebo in users of tobacco or betel nut with well-developed oral leukoplakia. Complete remission rates of the vitamin A and placebo arms were 57.1% (n=21) and 3% (n=33), respectively.

The synthetic retinamides 4-HPR and 4-HCR also have been tested in oral premalignancy. 4-HCR (40 mg/d) was significantly more active than placebo in reversing oral premalignant lesions in a trial by Han et al in 61 patients. In 1988, Chiessa et al began a randomized trial in Milan to evaluate the efficacy of systemic 4-HPR (200 mg/d for 52 weeks) as maintenance therapy vs no intervention after complete laser resection of oral premalignant lesions. Treatment included a three-day drug holiday at the end of each month to prevent the night blindness caused by 4-HPR reduction of serum retinol. In the most recent update of this study (including data from a total of 137 randomized patients), nine treatment failures (seven recurrences, two new lesions, no carcinomas) occurred in the 4-HPR group compared with 21 failures (eight recurrences, 12 new lesions, and one cancer) in the control group.

**Second Primary Tumor Trials**

Every year, patients have a 3% to 7% risk of developing SPTs following definitive treatment of early HNSCC. The spontaneous regression rate and a 30% to 40% risk of transformation. For this reason, trials to control advanced oral premalignancy help in screening agents considered for use in preventing aerodigestive tract cancers.

Oral Premalignancy Trials
overall noncompliance has been approximately 21% at two years. After completion of accrual (scheduled for June of 1998), treatment, and analysis, this comprehensive trial should define whether 13cRA is effective in preventing head and neck SPTs.

Conclusions

The fight against deadly HNSCC continues to be waged vigorously by basic scientists, epidemiologists, behavioral scientists, and clinicians. In basic research, molecular studies of LOH and p53 gene mutations are advancing our understanding of multistep and field carcinogenesis and providing direction to clinical researchers for the design of chemoprevention trials.

High incidence and mortality rates of head and neck cancers continue in developed and underdeveloped countries alike. Chemoprevention study must continue to search for novel, effective regimens to control this deadly family of cancers. Currently, one of the most promising new approaches involves translational retinoid trials.8,41,42,67-69 This study provides a paradigm of research methodology for investigating other new agents in the head and neck and in other epithelial regions.

References


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