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Pharmacology and impact of tretinoin on the treatment of premalignant squamous lesions of the oral cavity

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Abstract
Retinoids are the natural and synthetic derivatives of vitamin A. Natural and synthetic derivatives of vitamin A i.e. retinoids signal potent differentiation and growth-suppressive effects in diverse normal, premalignant, and malignant cells. Retinoids have been studied as chemoprevention for tumor, anti-angiogenic and anti-inflammatory agents. Vitamin A derivatives i.e. tretinoin modulates growth and differentiation of cells, and its deficiency enhances susceptibility to carcinogenesis. In this review chemopreventive mechanism of action (pharmacology) of vitamin A is discussed, and a review of impact of Tretinoin on the treatment of premalignant squamous lesion for oral cavity by using vitamin A derivative is included. Basically when we study its mechanism of action is unknown, but retinoids on epithelial differentiation and proliferation led to investigate the efficacy of retinoids in reversing oral leukoplakia. The objective of the current report was to review the possible role of vitamin A derivatives in the management of patients with oral lesions with a risk of transformation to carcinoma. Although administration of higher concentrations of retinoic acid results in suppression of oral leukoplakias only, its use in the treatment of patients with recurrent and persistent lesions may be justified for controlling lesions that otherwise may progress. In this regard, the vitamins derivative i.e. Tretinoin are useful pharmacological tools to reveal important pathways targeted in chemoprevention and further controlled clinical studies are needed.

Keywords: Retinoid, Tretinoin, chemoprevention, leukoplakias

Introduction
Retinoids are the natural and synthetic derivatives of vitamin A. The retinoids comprise a class of chemical compounds that are vitamers of vitamin A or are chemically related to it. Retinoids have found use in medicine where they regulate epithelial cell growth. There are three generations of retinoids:-

- First generation include retinol, retinal, tretinoin (retinoic acid), isotretinoin, and alitretinoin
- Second generation include etretinate and its metabolite acitretin
- Third generation include adapalene, bexarotene, and tazarotene

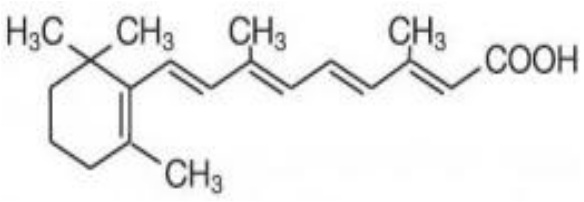
Retinoids have many important functions throughout the body including roles in vision, regulation of cell proliferation and differentiation, growth of bone tissue, immune function, and activation of tumor suppressor genes [1, 2].

Tretinoin
Retinoids, a group of naturally occurring and synthetic analogues of vitamin A, suppress carcinogenesis in various epithelial tissues [3, 4]. More importantly, retinoids also exhibited some effectiveness in clinical trials of chemoprevention of oral leukoplakia, second primary tumors in the aerodigestive tract, and skin tumor in xeroderma pigmentosum patients [5-7]. Vitamin A analogues (Retinoids) i.e. first type of generation (Tretinoin) has been used in the treatment of oral cavity premalignant mucosal lesion, which frequently develops into invasive OSCC. Various features are given below [8-10]:-

- It is highly fat-soluble, which means it easily diffuses across cell membranes.
- Tretinoin can directly and indirectly activate a wide range of retinoid receptors.
- Tretinoin is the main signalling retinoid and mediates its action through RAR–RXR heterodimers.
- Retinoic acid is bound in the cell by cellular retinoic acid binding protein (CRABP) and

inside the nucleus by two types of receptors, the retinoic acid receptor (RAR) and the retinoid X receptor (RXR).

- Tretinoin plays an important role in chemoprevention for cancers, anti-angiogenic and anti-inflammatory agents.
- Tretinoin has been shown to inhibit vascular endothelial growth factor, which is crucial for the process of angiogenesis
- Tretinoin help to transform cell types from the proliferative profile to the maturation profile, by inducing differentiation.
- Tretinoin is chemically 3,7-Dimethyl-9-(2,6,6-trimethyl-1- cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid.
- The empirical formula is C_{20} H_{28} O_2.
- Molecular weight is 300.442 g/mol.
- The structural formula is:



Retinoids in Squamous cell carcinoma

Retinoids have been shown to inhibit the growth of Squamous cell carcinoma and other malignancies. Retinoids are usually recognized for their ability to induce differentiation of various leukemic cells including SCC [11]. Premalignant Squamous lesions of the oral cavity are areas of altered epithelium that are at an increased risk for progression to Squamous cell carcinoma (SCC) [12]. Most common of these lesions is squamous dysplasia in association with leukoplakia and erythroplakia, which is the primary focus of this article. Not to be ignored, however, is the facts that up to 50% of oral SCCs cases arise from clinically normal mucosa. [13] Epidemiological studies have shown that alcohol consumption in association with tobacco is a significant risk factor for oral pre-tumor and tumor. [14, 15] The metabolized product of alcohol, acetaldehyde, is known for its carcinogenic activity. Oral Squamous cell carcinoma may occur either de novo or from the precursor lesions.

Retinoids have different features which are discussed below:-

- Retinoid metabolism is abnormal in cancer cells, compared with normal human cells.
- Retinoids plays an important role in both chemoprevention and treatment of oral SCCs.
- Retinoids are necessary for cellular growth and epithelial cell differentiation, modulating cellular gene expression.
- Retinoids can prevent cancer by inhibiting progression from premalignant to malignant stages.
- Retinoids can interfere with several events leading to tumorigenesis principally by induction of differentiation and/or apoptosis of tumour cells, and inhibition of tumour promotion in chemically induced cancers.
- Retinoids are effective for the treatment of precancerous lesions — leukoplakia, erythroplakia.
- Retinoids balances of cellular homeostasis for preventing primary and recurrent oral leukoplaktic lesions, secondary tumors, and in the regression of potentially malignant lesions.
- Retinoids treatment also found to reduce DNA synthesis, induce morphological changes, prolong cell doubling time and reduce saturation density and colony formation.

Role of RAR-expressions in oral Squamous cell carcinoma

- RARβ is involved in a diverse range of solid tumours.
- Premalignant oropharyngeal lesions have been shown to express low levels of RARβ.
- Hypermethylation of $RAR\beta_2$ is an early event in Head & Neck carcinogenesis.
- Inducibility of the RARβ *promoter by RA and/or expression of* RARβ correlates with the growth-inhibitory effect of RA in solid tumours.
- RARβ plays an important role in suppression of carcinogenesis.
- At pharmacological doses, retinoid can restores RARβ level.
- Restoration of expression could reinstate normal growth and differentiation patterns.

Basically there are two types of premalignant squamous lesion

- leukoplakia
- Erythroplakia.

Leukoplakia

Leukoplakia is defined as a predominantly white lesion or plaque affecting the oral mucosa that cannot be characterized clinically or histopathologically as any other disease and is not associated with any other physical or chemical agents except tobacco [16]. A definitive diagnosis is made when any etiological cause other than tobacco/areca nut use has been excluded and histopathology has not confirmed any other specific disorder. Leukoplakia is considered as a potentially malignant disorder with a malignancy conversion rate ranging from 0.1% to 17.5% [17]. In India, the prevalence of leukoplakia varies from 0.2% to 5.2% and malignant transformation ranges between 0.13% and 10% according to various studies [18]

Irregular, smooth to thickened leukoplakia involves the dorsal and lateral surfaces of the tongue, which demonstrated no sign of dysplasia in multiple areas of incisional biopsy. Which is shown in fig1?

Fig 1: leukoplakia in oral cavity

Erythroplakia

Erythroplakia is an uncommon but severe form of precancerous lesion defined by WHO as "any lesion of the oral mucosa that presents as bright red velvety plaques which cannot be characterized clinically or histopathologically as any other recognizable condition" [19]. There are few documented reports related to the prevalence of erythroplakia owing to its rarity and also the similarities with speckled leukoplakia. Basically Erythroplakia is characterized by a smooth, velvety clinical presentation with a homogeneous surface, without ulceration. The tissue diagnosis was

Squamous cell carcinoma in situ. Erythroplakia is shown in figure 2. Given below:-

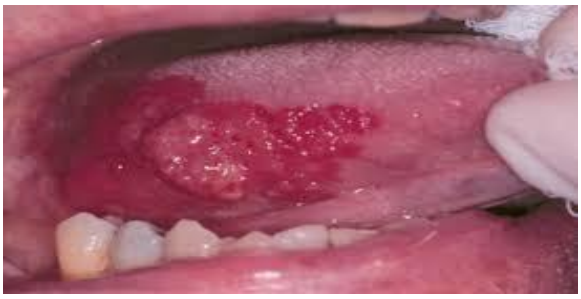


Fig 2: Erythroplakia in oral cavity

Premalignant Squamous lesions of the oral cavity most often occur on the buccal mucosa, the mandibular mucosa/sulcus, the palate, the tongue, and the floor of the mouth. [20, 21] Surgery has been considered the standard therapy for this condition, but is often not possible. The effects of retinoids on epithelial differentiation and proliferation led to investigate the efficacy of retinoids in reversing oral leukoplakia. Retinoids, including Tretinoin and retinol also showed activity in leukoplakia. All leukoplakia trials reported thus far have used reversal of the premalignant lesion as the study endpoint. With the definition of chemoprevention in mind, it can be argued that these trials actually address chemotherapy of leukoplakia rather than chemoprevention of oral cancer [22]. A follow-up of the study of Lippman *et al*. was recently published, addressing the prediction of cancer development by means of clinical parameters and molecular markers. With a median follow-up of 7 years 31.4% of the leukoplakia patients developed an upper respiratory and digestive tract tumor. Forty-one percent of the tumors had developed at sites distinct from the leukoplakia site [22].

History and Premalignant Conditions of the Oral Cavity

Tretinoin was produced in Shanghaï and first used to treat patients there [23]. Following a collaborative meeting in Shanghaï in 1987, the Chinese drug was kindly provided to subsequently treat French patients [24]. Oral cavity cancer accounts for approximately 3% of all malignancies and is a significant worldwide health problem. [25, 26] Most oral malignancies occur as Squamous cell carcinomas (SCCs); despite remarkable advances in treatment modalities, the 5-year survival rate has not significantly improved over the past several decades and still hovers at about 50-60%. [27, 28] Many oral SCCs develop from premalignant conditions of the oral cavity. A wide array of conditions have been implicated in the development of oral cancer, including leukoplakia, erythroplakia, palatal lesion of reverse cigar smoking, oral lichen planus, oral submucous fibrosis, discoid lupus erythematosus, and hereditary disorders such as dyskeratosis congenital and epidermolysis bullosa [29-31]

Despite the general accessibility of the oral cavity during physical examination, many malignancies are not diagnosed until late stages of disease. In order to prevent malignant transformation of these precursor lesions, multiple screening and detection techniques have been developed to address this problem [32, 33].

Premalignant Conditions

- **Oral submucous fibrosis**

Oral submucous fibrosis (OSF) is a chronic progressive condition found predominantly in people of Asian decent. OSF is considered to be the result of the use of the Areca nut product with resultant disruption of the extracellular matrix. The disease often manifests with diffuse involvement of the oral cavity, pharynx, and upper esophagus that appears clinically as whitish mucosa lacking elasticity. Epithelial dysplasia has been described in 7-26% of OSF tissues, and long-term studies suggest a malignant transformation rate in approximately 7% of these lesions [34]. A study by Jaitley *et al* found that mucosal smears from all 30 patients in the report with oral submucous fibrosis displayed class I cytology (i.e., characteristics indicative of benign atypical cytologic changes) [35].

- **Lichen planus, discoid lupus erythematous, and epidermolysis bullosa**

Although classified as potentially malignant conditions, the data regarding progression to malignancy for these conditions is controversial. Because of the difficulty in classifying and clinically distinguishing the varied lesions associated with these conditions, the potential for malignant transformation remains unclear [36].

Receptors and Binding Proteins

Retinoids play critical roles in growth, vision, reproduction, epithelial cell differentiation, and immune function [37]. The actions of retinoids are mediated through the nuclear retinoid receptors, which are members of the steroid/thyroid/retinoid hormone receptor family [38]. Retinoid receptors act as ligand inducible transcription factors that enhance the transcription of target genes by binding to retinoic acid response elements (RAREs) in the promoter region of retinoid-responsive genes. [39, 40] Two families of retinoid nuclear receptors have been described:-

- Retinoic acid receptors (RARs)
- Retinoid X receptors (RXRs).

The RARs (α, β, and γ) bind the naturally occurring retinoid Tretinoin with high affinity, whereas the RXRs (α, β, and γ) do not bind with Tretinoin. This multiplicity of receptors and gene pathways may in part explain the diverse effects of retinoids on a wide range of cellular processes. Under normal physiologic conditions, the concentration of Tretinoin and other naturally occurring retinoids is under tight metabolic control. The physiologic plasma concentration of Tretinoin is approximately 5 nM. Although circulating Tretinoin enters cells via passive diffusion, its contribution to intracellular Tretinoin levels is likely to be inconsequential under normal conditions because cells derive retinoic acid from intracellular oxidation of retinaldehyde, a metabolite of retinol. Intracellular Tretinoin is bound to specific binding proteins, the cellular retinoic acid binding proteins (CRABPs). CRABP I and CRABP II are highly conserved throughout evolution and appear to regulate the amount of retinoic acid capable of binding to their nuclear receptors. Binding of Tretinoin to CRABP appears to facilitate intracellular oxidative catabolism of Tretinoin to the inactive metabolite, 4-hydroxy-retinoic acid [41-45].

Mechanisms of Action

Special Features shared in mechanisms of action by Tretinoin (fig. 3) in oral premalignant lesions: [46-58]

Inhibits progression from premalignant to malignant stages and Interfere with several events leading to tumorigenesis:

- induction of differentiation;

- apoptosis of tumour cells;
- inhibition of tumour promotion

Prevents primary and recurrent oral leukoplaktic lesions, secondary tumors, and in the regression of potentially malignant lesions

- balances of cellular homeostasis;
- reduce DNA synthesis;
- induce morphological changes;
- prolong cell doubling time;
- reduce saturation density and colony formation

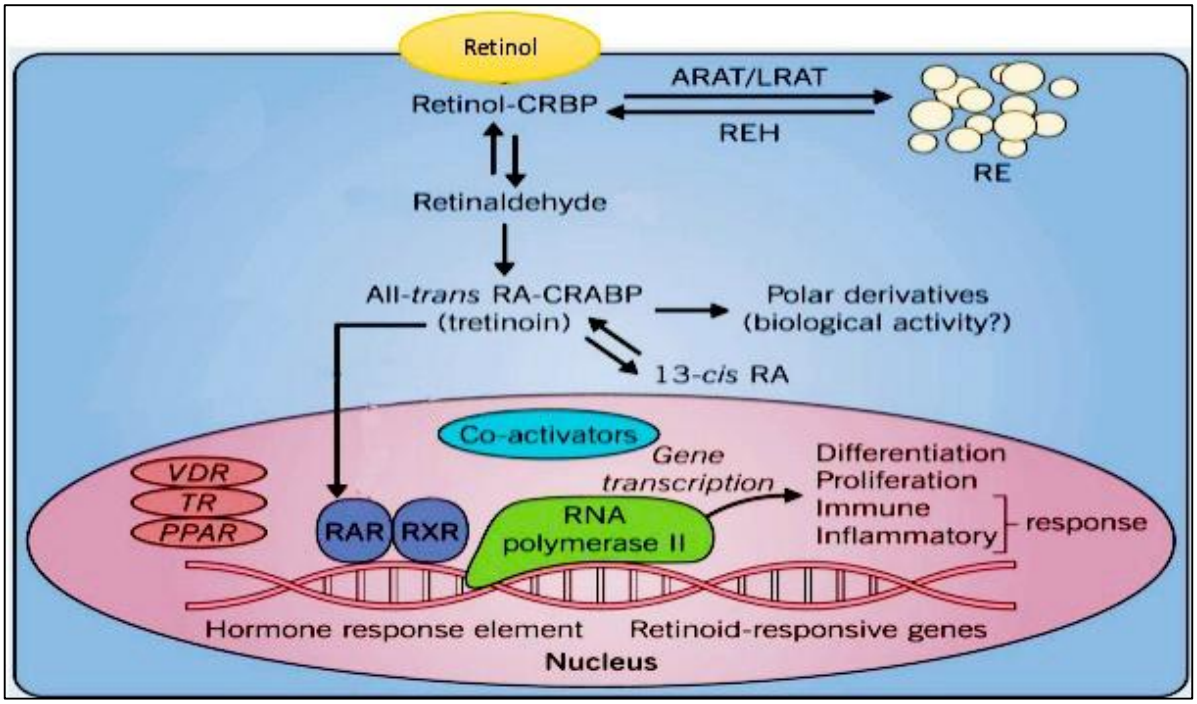


Fig 3: Mechanism of action of Tretinoin

Vitamin A (retinol) and its metabolites play important roles in vision, growth, and reproduction. Retinol, by virtue of its conversion to retinaldehyde, supports the visual requirement for vitamin A. Retinaldehyde is further converted to retinoic acid (RA), which is believed to represent the form of the vitamin that is active in cellular growth and differentiation. RA exerts its effects, at least in part, by binding to nuclear receptor proteins of the RA receptor (RAR) and retinoid X receptor (RXR) families [58]. The binding of Tretinoin to its receptor is followed by the activation or inhibition of transcription of retinoid-responsive genes. Tretinoin is a high affinity ligand for all three RAR subtypes: alpha, beta, and gamma. In addition to the nuclear receptors, cellular RA-binding proteins (CRABPs) and cellular retinol-binding proteins (CRBPs) are present in many cells [59-61]. They may play important roles in regulating retinoid metabolism and the amount of compound that gains access to the nuclear receptors [62]

Pharmacokinetics of Tretinoin

The inability to maintain adequate plasma concentrations of Tretinoin because of rapid up regulation of its catabolism is an attractive hypothesis to explain the inevitable recurrences in patients with initially responsive disease, but more recent data suggest that this mechanism alone is unlikely to be responsible for drug resistance. The low plasma concentrations of Tretinoin initially observed at the time of relapse are actually present since very early in treatment course, usually within the first week of therapy. Thus the plasma Tretinoin concentrations observed at relapse are similar to the concentrations achieved during most of the induction phase of therapy [63]. Basically Administration of Tretinoin on a continuous daily schedule results in a rapid and sustained decrease in plasma drug concentrations. This pharmacokinetic study was performed to determine if administration of Tretinoin on an intermittent schedule could overcome the rapid decrease in plasma drug concentration and provide repetitive periods of higher plasma drug exposure [64]. From the pharmacokinetic study we measured the pharmacokinetics of Tretinoin to help for treating human premalignant squamous lesions. Tretinoin permeated well into the white matter, giving peak concentration which is 6 to 7 times higher than the peak serum concentration [65] and can be calculated with pharmacokinetics analysis which is given below: [66]

Pharmacokinetic analysis

Pharmacokinetic parameters were assessed using standard noncompartmental analysis. The maximum plasma concentration (C_{max}) of Tretinoin and the corresponding time to C_{max} (t_{max}) observed were experimental values. Additionally, AUC (0, ¥) was calculated using the equation: $AUC\ (0, ¥) = AUC\ (0, t) + [C_t/l_z)$ in which AUC (0, t) is the area to the last measurable time point (t), C_t is the concentration at the last measurable time point, and l_z is the apparent first-order elimination rate constant. Accumulation of Tretinoin was not expected; therefore, AUC (0, ¥) was calculated instead of AUC (0, t) during multiple dosing. The apparent elimination half-life ($t_{1/2}$) of Tretinoin was calculated as $\ln(2)/l_z$. Clearance (CL) was calculated as dose/AUC (0,¥) and the apparent steady-state volume of distribution (V_{ss}) was calculated as [(AUCMC (0, t) /AUC (0,¥))-0.5t τ]·[dose/AUC (0,¥)], in which AUCMC (0,¥) is the area under the moment curve extrapolated to infinity and t is the duration of the

infusion. CL and the V_{ss} were calculated for Tretinoin only.

Treatment and Schedule of Administration

Treatments for Oral leukoplakia are indicated according to the extension of the lesion and its histological subtype [67, 68, 69].

Histologically, leukoplakias have been classified as:-

- Simple orthokeratosis;
- Parakeratosis with epithelial hyperplasia and minimum inflammation; and
- Hyperkeratosis with different degrees of dysplasia (16% of the cases).

Epithelial dysplasia has been categorized as mild, moderate, and severe; the latter known as in situ carcinoma due to its location within the layer of epithelial cells. [70] Surgical removal has been indicated in localized and accessible lesions. Its use has been limited in cases of extensive leukoplakias that could involve key structures or in cases of recurrent lesions after previous surgery (10 to 35% of the time). [71, 72] In lesions presenting active epithelial dysplasia, alternative therapeutic options include cryosurgery, laser surgery, and use of retinoid i.e. Tretinoin. Basically when treated lesion retinoids bind to their receptors enter the cells and, after metabolization, are transported to the nucleus by cytosolic proteins. These proteins could be structural or act in several nuclear processes such as the production of keratine, growth hormone, and apoptosis and, as a consequence, participate in cellular differentiation, embryological morphogenesis, and carcinogenesis [73, 74].

Conclusions

Although vitamin A and derivative retinoids have been successful in decreasing the clinical presence of Premalignant Squamous Lesions, the toxicity of the medications with systemic administration remains a low significant problem and has an important impact on common and long-term usage. The long-term effectiveness of chemoprevention has not been established to date, and the choice of agents requires further study. RARβ is a major mediator of retinoid growth suppression in Squamous cell carcinoma cells. RARβ and RXR are involved in growth inhibition of immortalized and transformed human bronchial epithelial cells. These and other studies provide a basis for use of retinoid nuclear receptor-selective agonists or antagonists in future chemopreventive or therapeutic trials. Perhaps combination regimens using receptor selective agonists in conjunction with other chemopreventive agents will exhibit cooperative clinical effects while reducing retinoid-associated toxicities. Advances in single or combined chemopreventive medications for the topical or systemic control of leukoplakia may decrease the potential of malignant transformation. Prospective controlled studies are needed to establish the value of chemoprevention in patients with oral leukoplakia and to assess the potential role of administration of retinoids.

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