

Do Topically Applied Skin Creams Containing Retinyl Palmitate Affect the Photocarcinogenicity of Simulated Solar Light?

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ABSTRACT

Retinyl palmitate (all-*trans*-retinyl palmitate; RP) was nominated in 2001 by the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) to the National Toxicology Program (NTP) as a high priority compound for phototoxicity and photocarcinogenicity studies at the National Center for Toxicological Research (NCTR). Studies with SKH-1 hairless mice are required to test whether topical application of RP enhances the phototoxicity and photocarcinogenicity of simulated solar light and UV light. Mechanistic studies are needed to provide insight into the disposition of RP *in vitro* and on the skin of mice, and to test thoroughly whether genotoxic damage by UV-induced radicals may participate in any toxicity of topically applied RP in the presence of UV light.

Key words: Retinyl palmitate, photocarcinogenicity, SKH-1 mice

INTRODUCTION

I. Retinoids

(I) Essential Human Nutrient and Chemoprevention

Vitamin A (all-*trans*-retinol; retinol), its metabolites, analogues, and derivatives, whether natural or synthetic, are referred to as retinoids. Vitamin A, an essential human nutrient, is an important regulator in epidermal cell growth, normal cell differentiation, and cell maintenance⁽¹⁾. Its profound effects also include vision, reproduction, morphogenesis, and pattern formation. Depending on the levels of vitamin A deficiency, humans may suffer decreased night vision, irreversible blindness, xerophthalmia, skin abnormalities, anaemia, eclampsia and abruptio placentae during pregnancy, infectious disease, and vascular and heart disease^(2,3).

Retinoids have been implicated as chemoprevention agents in the prevention of various epithelial cancers and have been one of the chemoprevention agents to reach clinical trials in humans⁽⁴⁾. Epidemiological studies and intervention trials have shown vitamin A to exhibit a variety of preventive effects in humans⁽²⁾. Retinoids can also prevent

development of many types of cancers in experimental animals exposed to chemical carcinogens, including lung, mammary gland, urinary bladder, skin, oesophagus, forestomach, large intestine, liver, and thyroid^(2,5-7).

(II) Metabolic Activation Required for Biological Activities

Metabolism is required to convert retinol to biologically active metabolites, e.g., all-*trans*-retinoic acid (RA, retinoin) and its *cis*-isomers⁽²⁾. Retinyl palmitate (RP) is the principal storage form of retinol in humans and animals and can be enzymatically hydrolyzed back to retinol *in vivo*. The scheme of sequential metabolism of RP to retinol, retinal, and RA is shown in Figure 1. Both RA and its 9-*cis* isomer exhibit very high biological activities, including inducing epidermal growth and differentiation. Retinoids require binding to specific nuclear receptors, RA receptors (RARs) and retinoid X receptors (RXRs), in order to exert their biological activities. The combined ligand-receptor complex interacts with specific regulatory elements in the promoter regions of retinoid-responsive genes, thus effecting transcription regulation and playing an important role in controlling cell differentiation, growth, and reproduction (Figure 1)⁽²⁾. 9-*cis*-RA has been shown to bind with high affinity to both RAR and RXR receptors, while RA binds only to RAR^(4,5).

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Retinol, retinal, RA and RP exhibit very low solubility in water and therefore require retinoid binding proteins (RBPs) for translocation to target tissues. The mechanism of retinol uptake by target cells in the skin is not understood completely. Once in the cytoplasm, retinol and RA bind to cellular retinol-binding proteins I and II (CRBP I and II) and cellular RA binding proteins I and II (CRABP I and II), respectively. These proteins regulate intracellular concentrations of retinol and RA (and retinal).

The metabolism of RP and retinol is much more complicated than that shown in Figure 1. Although the metabolism of retinol has been extensively studied, there is still a large gap in the knowledge of how retinol is metabolized to active metabolites. The metabolism of retinol to retinal is catalyzed by alcohol dehydrogenases and short-chain dehydrogenases (reductases). Aldehyde/retinal dehydrogenases, CYP450 1A1, and CYP450 1A2 catalyze the irreversible oxidation of retinal to RA.

In summary, the effects of retinoids on cells are dependent on the bioavailability of the retinoid and the presence of retinoid receptors. Complicating this paradigm is the metabolism of retinoids to multiple products with different receptor binding and tissue distribution. Suffice it to say that the topical application of a retinoid could result in the generation and distribution of a wide variety of bioactive products.

(III) Retinoid Toxicity

The uptake of excess retinoids, e.g., hypervitaminosis A, causes toxic effects in humans. The adverse effects, both acute and chronic toxicity, can occur in the skin, circulation system, liver, nervous system, and musculoskeletal system.

In contrast to the previously described chemoprevention effect, enhancement of human cancer incidence by retinoids has also been found in a clinical study. The human study, β -Carotene and Retinol Efficacy Trial (CARET), which involved more than 18000 American adults, was halted early because an increased lung cancer incidence in the active treatment group was found⁽²⁾. Retinoids have also been reported to enhance carcinogenesis in different animal models⁽²⁾. As described below, under certain experimental conditions, retinoic acid can enhance photocarcinogenicity of mice.

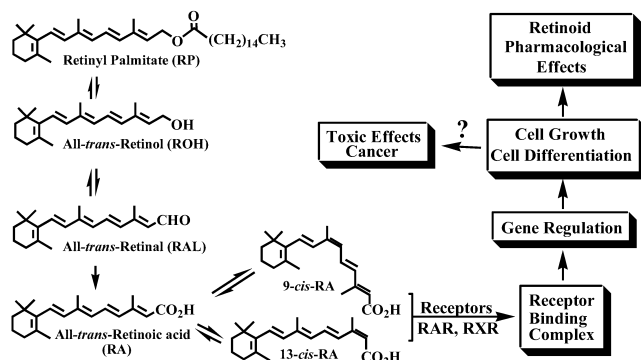


Figure 1. Proposed scheme of metabolism of RP leading to gene regulation and cell differentiation.

(1) Retinoic Acid (RA) - Effect on Photocarcinogenicity

(1-1) Sunlight Radiation Is A Human Carcinogen

Sunlight is a complete carcinogen and is responsible for the induction of squamous cell and basal cell carcinomas in humans⁽⁸⁾. The effects of photoirradiation of skin are dependent on the quantity and wavelength of the radiation. About one third of light with a wavelength of 400 nm penetrates into the epidermis to a depth 0.1 mm *in vivo*, whereas about 99% of light with a wavelength of 300 nm penetrates into only 0.03 mm of epidermis⁽⁹⁾. Light of low wavelength (higher frequency and higher photon energy) exhibits a higher capability for induction of erythema (sunburn)^(10,11). Consequently, induction of erythema in humans is wavelength dependent.

Solar light generally causes DNA damage both directly and indirectly. Direct DNA damage is principally caused by UVB (280-315 nm), resulting in cytotoxicity, mutagenicity, and initiation of tumorigenicity. Indirect DNA damage is principally caused by UVA (315-400 nm)-dependent photoactivation of endogenous and exogenous photosensitizers, which include nucleic acids, amino acids containing aromatic chromophores, proteins, porphyrins, carotenoids, retinoids, steroids, and quinones. Photoirradiation-induced skin DNA damage can be repaired by DNA repair enzymes. Residual DNA damage or mutations caused by DNA repair enzymes may give rise to activation of protooncogenes, such as the ras-oncogene, or deactivate tumor suppressor genes, such as p53^(2,9). Either of these changes could participate in the development of skin cancer.

Photoirradiation of the skin alters gene expression and protein function in cells. This results in reduction of the levels of retinoid and hormone receptors⁽²⁾, immunosuppression, altered metabolizing enzymes, modulation of membrane proteins, and altered drug-binding proteins.

(1-2) Retinoic Acid Alters Photo-induced Mouse Skin Tumors *in vivo*

RA has been used as a prescription drug for treatment of skin disorders and as an “anti-aging” agent for over 30 years. Some adverse effects reported with topically applied RA include irritation and erythema of the skin, and these effects are exacerbated in the presence of sunlight. As a result, individuals using RA are cautioned to avoid sun exposure or use SPF (skin protection factor) creams during treatment.

The effects of topically applied RA on photocarcinogenicity in mice have been investigated by several research groups⁽¹²⁻²⁰⁾. The results are quite varied, with retinoid application either increasing, decreasing, or having no effect on photocarcinogenesis. The drastically different effects on tumor incidence can probably be ascribed to differences in study design including: (i) the amount of RA applied and schedule for application, (ii) the animal model used, and (iii) the light sources and dose of light radiation. Effects of the vehicle used for RA application have also been noted⁽¹²⁾. Organic solvents, such as methanol, may cause sub-clinical irritation, resulting in enhancement of photocarcinogenesis to the skin; however, irritation induced by topical treatment

with organic solvents may not explain all the differences, since studies in enhancement of photocarcinogenesis by topically applied RA has been observed with both methanol and lotion being used as vehicles^(13,14). Vehicles formulated without antioxidants differ substantially from marketed products and allow decomposition of retinoids⁽¹²⁾. However, the studies suggest no clear association between inclusion of antioxidants in topical formulations and a study's outcome. Additionally, the importance of pigmentation in the response of the animal model to UV radiation and RA has been suggested⁽¹²⁾. A recent study indicates that topically applied RA enhances photocarcinogenesis in both lightly pigmented and albino mice, although the enhancement of photocarcinogenesis is smaller in pigmented mice⁽¹⁵⁾.

The spectral distribution and dose of incident UV radiation may also play an important role in the outcome of photocarcinogenesis studies. Topically applied RA was found to enhance photocarcinogenesis in studies in which solar simulating UV radiation was used at dose levels less than the human minimal erythema dose⁽¹³⁻¹⁷⁾. In contrast, topically applied RA was found to be equivocal or inhibit photocarcinogenesis in studies using UV radiation from unfiltered sources⁽¹⁸⁻²⁰⁾. The use of UVC (i.e., wavelengths less than 280 nm) emitting light sources not only exposes animals to wavelengths of light humans are not typically exposed, but also may induce different effects than UVB or UVA on the skin⁽²¹⁾. In addition, the doses of UV radiation in studies employing unfiltered sources exceed the human minimal erythema dose. Our current knowledge of the effects of RA on photocarcinogenesis do not allow a mechanistic explanation for the different outcomes in studies employing lower UV doses from solar simulators and higher doses from unfiltered sources. Nevertheless, the use of solar simulating UV radiation at low doses more closely resembles conditions encountered by individuals using retinoid-containing products.

Retinoic acid is currently used in a number of dermal drug products, such as Retin-A[®] (containing 0.1%, 0.05% or 0.025% tretinoin, i.e. all-*trans*-retinoic acid), which is used for acne treatment, and Renova[®] (containing 0.05% all-*trans*-retinoic acid), an adjunctive agent for mitigation of fine wrinkles, mottled hyperpigmentation, and tactile roughness of facial skin⁽²²⁾. The results of experimental photocarcinogenesis studies are mentioned in the following precautions for use of these products: "*Studies in hairless albino mice suggest that tretinoin may accelerate the tumorigenic potential of weakly carcinogenic light from a solar simulator. In other studies, when lightly pigmented hairless mice treated with tretinoin were exposed to carcinogenic doses of UVB light, the incidence and rate of development of skin tumors were reduced. Due to significantly different experimental conditions, no strict comparison of these disparate data is possible. Although the significance of these studies to man is not clear, patients should avoid or minimize exposure to sun*"⁽²²⁾. While this warning to avoid the sun when using retinoid-based products is prudent, the equivocal results

with photocarcinogenesis status in mice, leads to confusion for the physician and the user of the product.

II. Safety Concern on the Use of Retinyl Palmitate with Light Irradiation

(I) Increased Cosmetic Use of Retinyl Palmitate (RP)

The number of cosmetic retail products containing RP has increased rapidly in the last two decades. Data available from FDA's Voluntary Cosmetics Registration Program, compiled in accordance with Title 21 Section 720.4 of the Code of Federal Regulations⁽²³⁾, indicate that 102 cosmetic formulations in 1981, 355 cosmetic formulations in 1992, and 667 formulations in 2000 contained RP. Retail product categories containing RP include moisturizing preparations, skin care preparations, night skin care preparations, lipsticks, suntan gels and preparations, makeup preparations, and bath soaps and detergents⁽²⁴⁾. The Cosmetic Ingredient Review (CIR) Expert Panel, the cosmetic industry's self-governing body for evaluating the safety of cosmetic ingredients, concluded a review of safety data for retinol and RP in 1987⁽²⁵⁾. The CIR Expert Panel found that retinol and RP were safe as cosmetic ingredients in the practices of use and concentration ranges used (up to 13%) at that time.

RP (as a form of Vitamin A) is affirmed as GRAS (generally recognized as safe) as a nutrient supplement in food when used in accordance with good manufacturing practices⁽²⁶⁾. Vitamin A palmitate has also been approved as an active ingredient in OTC and prescription drugs^(27,28).

(II) Stability, Skin Absorption and Metabolism of Retinyl Palmitate

RP (all-*trans*-retinyl palmitate) is insoluble in water and glycerol, and soluble in ethanol, chloroform, and ethyl ether⁽²⁵⁾. RP is chemically unstable and its chemical stability is highly dependent on environmental conditions such as solvent, temperature, and availability of oxygen⁽²⁹⁾, yet it is more stable than retinol^(30,31). Oxidative decomposition of RP leads to a complex mixture of products⁽³²⁾. RP is also unstable under photoirradiation. It is easily thermally-isomerized to the 13-*cis* isomer. Thermal isomerization is favored in lipophilic solvents and emulsions containing high compositions of oils⁽²⁹⁾. Anhydro-retinol is one of the major decomposition products of RP⁽³³⁾.

It is important to know what products are present in cosmetic creams that were originally formulated with RP. If a significant amount of the RP is oxidized or thermally degraded, then the toxicity of these products needs to be considered.

The percutaneous absorption of RP, examined both *in vitro* and *in vivo*, has been found to be slow. Boehnlein *et al.*⁽³⁴⁾ reported that after RP was topically applied in acetone to human skin *in vitro*, about 18% of RP penetrated the skin in 30 hrs. In addition, approximately 44% of the absorbed RP was hydrolyzed to retinol by skin esterases. In a clinical

study, Duell *et al.*⁽³⁵⁾ found that topical application of a cream containing 0.6% RP resulted in elevated levels of retinol in skin measured 48 and 72 hrs after application. No increases in levels of RP were noted, indicating efficient hydrolysis of RP to retinol by cutaneous esterases.

As a result, the topical application of RP to the skin of mice or man will result in the presence of significant levels of RP and retinol 24 hrs later. In a photocarcinogenesis study, this would suggest that the RP and retinol present during the next irradiation would be subject to photodecomposition, and that the identification and toxicity of these products should be considered.

Absorbed RP is readily hydrolyzed to retinol by cutaneous esterases. In addition, skin contains the enzymes required for further metabolism of retinol to retinaldehyde (retinal) and RA. Some studies have shown that levels of RA in the skin can increase following topical application of RP or retinol. Oxidation of retinol to retinal, catalyzed by cytosolic retinol dehydrogenase, is the rate-limiting step in the production of RA⁽³⁶⁾. Alcohol dehydrogenases (class I and class IV), which actively catalyze oxidation of retinol, have been found in mouse skin⁽³⁷⁾, human keratinocytes⁽³⁸⁾, and ADH⁺ deermice but not ADH⁻ deermice⁽³⁶⁾. Retinaldehyde dehydrogenase activity, needed for conversion of retinal to RA, has also been found in skin^(3,39). In spite of the presence of cutaneous enzymes required for conversion of retinol to RA, few studies have directly demonstrated biotransformation of topically applied retinol to RA in skin^(40,41).

Duell *et al.*⁽³⁵⁾ administered 0.1, 0.3, and 0.6% RP (in ethanol with BHT) to human subjects (under occlusion) and 48-72 hrs later detected (in decreasing order) retinol, retinol linoleate, 13-*cis*-retinol, 14-hydroxy-4,14-retro-retinol, and RP, but not RA. Therefore, it would appear that the disposition and pharmacokinetics of RP in human and mouse skin is not fully understood, especially when light is introduced to the skin.

(III) Biochemical and Histological Cutaneous Changes

Topical application of RP or retinol results in biochemical changes characteristically produced by RA. Topical application of RA to human skin induces increases in RA 4-hydroxylase and cellular RA binding proteins (CRBP)⁽⁴²⁾. Duell *et al.*⁽³⁵⁾ reported that topically applied 0.6% RP produces an increase in RA 4-hydroxylase, an enzyme required for limiting levels of RA through catabolic metabolism. The elevation seen after treatment with 0.6% RP was similar to the increase elicited by 0.025% RA. Cellular RA binding protein-II (CRABPII) is essential for transport of RA from cytoplasm to the nucleus. Significant histological changes in skin are induced by topical application of RP or retinol. Topically applied RP or retinol induces epidermal hyperplasia and thickening in animals^(43,44).

Effects of topical administration of RP on human skin have been studied. Duell *et al.*⁽³⁵⁾ found that a single application of 0.6% RP in ethanol (with BHT) to human skin

resulted 4 days later in a 38% increase in epidermal thickness vs. vehicle only. In a study of 20 women⁽⁴⁵⁾, RP was administered at 0.2% for 3 months in a "water soluble" (Tween) media, and resulted in increased skin thickness and elasticity as measured with an ultrasound technique. These results were not obtained with RP in a "normal cosmetic formulation"; the author attributed this to a lack of skin penetration of RP in the latter. In a 14-day study with hairless mice (HRS/J)⁽⁴³⁾, the application of 1 mL of 0.1, 0.5, 1.5, and 5.0% RP (in oil-in-water emulsion) resulted in an inconsistent dose-response of various endpoints. Increases in protein and collagen content and epidermal thickness were observed at all RP doses, but dermal thickness increased only in the 5.0% dose group, with total skin thickness greatest in the 0.1% dose group. The instability of RP and effects of different vehicles could have contributed to the inconsistencies between studies.

(IV) Potential Enhancement of Light-induced Carcinogenicity by Retinyl Palmitate

At present, it is not known whether use of RP-containing cosmetics under concomitant exposure with light radiation is safe. Because of the slow penetration of RP into the skin, RP remaining on the skin will undergo photoreaction. There have not been any studies on the effect of topically applied RP on the carcinogenicity of UV light (simulated solar light or fluorescent lamp generated UV light); although the effects of RA on photocarcinogenesis have been studied.

When exposed to light, RP on the skin may undergo metabolism and/or photoreaction to generate reactive species exhibiting toxicological and carcinogenic properties. For example, chemicals that absorb UVA and visible light and generate reactive oxygen species (ROS) (singlet oxygen, superoxide, and hydroxy radicals) are the most widely used photosensitizers⁽⁹⁾. RP has a maximum UV-visible absorption at 326 nm⁽²⁾ and thus, may be able to absorb UVA light and act as a photosensitizer. Thus, photoactivation of RP could generate short-lived ROS that have been shown to damage DNA and proteins and lead to tumors.

III. What Is Needed to Determine the Safety of Retinyl Palmitate with Light Exposure

Regarding toxic effect to humans, currently, the long-term consequences of using cosmetics containing RP are unknown. Because clinical data indicate that topically applied RP produces many of the cutaneous changes associated with the use of drug products containing RA, and because of the association made in experimental studies between chronic application of RA and enhanced photocarcinogenesis, a determination of the effects of topically applied RP on photocarcinogenesis is essential for assessing the chronic risks posed by using cosmetic products containing RP. Thus, a study of the photocarcinogenesis of RP, under conditions relevant to the use of RP in cosmetics, is timely and important. In addition, mechanistic studies are

needed to establish the relevance of the results obtained in the selected animal model.

RP has been nominated by the U.S. FDA's Center for Food Safety and Applied Nutrition (CFSAN) and selected by the National Toxicology Program (NTP) as a high priority compound for phototoxicity and photocarcinogenicity studies at the NCTR. The nomination of RP for study is based on: (i) its increasingly widespread use of RP in cosmetic retail products for use on sun-exposed skin; (ii) the biochemical and histological cutaneous alterations elicited by RP; and (iii) the association between topical application of RA and enhancement of photocarcinogenesis.

Accordingly, a research project has been initiated at the NCTR for this study. The principal objective of this project is to study the effects of topically applied skin cream containing RP on the photocarcinogenicity of simulated solar light in SKH-1 mice. The specific aims are: (i) to determine whether or not the application of creams containing RP to the skin of SKH-1 hairless mice alters the tumor incidence induced by simulated solar light or fluorescence lamp generated UV light; and (ii) to determine the mechanisms of any alteration of tumor incidence in treated mice. These studies will provide insight into the disposition of RP *in vitro* and on the skin of mice, and test whether genotoxic damage by UV-induced radicals may participate in any toxicity of topically applied RP in the presence of UV light. As such, this goal of this study is to provide relevant information necessary for risk assessment of RP in cosmetic creams.

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曝曬日光下使用含 Retinyl Palmitate 化粧品的致癌性探討

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摘 要

以 Retinyl Palmitate 為成分之化粧品很多，然而經由日光照射後是否產生毒性及光致癌效應，目前尚未有相關報告。美國 FDA (Food and Drug Administration) 之毒理研究中心針對含有棕櫚酸視黃酯 (Retinyl Palmitate) 之化粧品，以動物模式進行有系統的致癌效應研究並對其基本藥理毒性深入探討。棕櫚酸視黃酯 (全-反-棕櫚酸視黃酯；RP) 係2001年由美國藥物食品管理局食品安全與應用營養中心 (CFSAN) 所成立國家毒理研究中心 (NCTR) 執行之國家毒性研究計畫 (NTP)，列為高優先進行光毒性及光致癌性研究之化合物。研究係以 SKH-1 裸鼠塗抹棕櫚酸視黃酯後，測試模擬太陽光與紫外線是否會增強其光毒性及光致癌性，有關致毒機制之研究提供能深入了解棕櫚酸視黃酯於體外及老鼠皮膚試驗之素質。

關鍵詞：棕櫚酸視黃酯 (Retinyl Palmitate)，光致毒性，SKH-1 裸鼠