Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology Letters 163 (2006) 30–43
Toxicology Letters
www.elsevier.com/locate/toxlet

UVA photoirradiation of retinyl palmitate—Formation of singlet oxygen and superoxide, and their role in induction of lipid peroxidation

Qingsu Xia [a], Jun Jie Yin [b], Shu-Hui Cherng [a,1], Wayne G. Wamer [b], Mary Boudreau [a], Paul C. Howard [a], Peter P. Fu [a,*]

[a] *National Center for Toxicological Research, U.S. Food and Drug Administration, Department of Biochemical Toxicology, HFT-110, 3900 NCTR Road, Jefferson, AR 72079, USA*
[b] *Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740, USA*

Received 4 August 2005; received in revised form 12 September 2005; accepted 12 September 2005
Available online 27 December 2005

Abstract

We have previously reported that photoirradiation of retinyl palmitate (RP) in ethanol with UVA light results in the formation of photodecomposition products, including 5,6-epoxy-RP and anhydroretinol (AR). Photoirradiation in the presence of a lipid, methyl linoleate, induced lipid peroxidation, suggesting that reactive oxygen species (ROS) are formed. In the present study, we employ an electron spin resonance (ESR) spin trap technique to provide direct evidence as to whether or not photoirradiation of RP by UVA light produces ROS. Photoirradiation of RP by UVA in the presence of 2,2,6,6-tetramethylpiperidine (TEMP), a specific probe for singlet oxygen, resulted in the formation of TEMPO, indicating that singlet oxygen was generated. Both 5,5-dimethyl *N*-oxide pyrroline (DMPO) and 5-*tert*-butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide (BMPO) are specific probes for superoxide. When photoirradiation of RP was conducted in the presence of the DMPO or BMPO, ESR signals for DMPO-$^{\bullet}$OOH or BMPO-$^{\bullet}$OOH were obtained. These results unambiguously confirmed the formation of superoxide radical anion. Consistent with a free radical mechanism, there was a near complete and time-dependent photodecomposition of RP and its photodecomposition products. ESR studies on the photoirradiation of 5,6-epoxy-RP and AR indicate that these compounds exhibit similar photosensitizing activities as RP under UVA light.

Keywords: Retinyl palmitate; UVA; Photoirradiation; Lipid peroxidation; Reactive oxygen species; ESR

1. Introduction

Skin is the largest organ exposed to sunlight (Mukhtar, 1995). Adverse responses to sunlight include both acute responses, such as sunburn and inflammatory disorders and chronic responses, such as cutaneous aging and skin cancer (Forbes, 1981; IARC, 1992; Mukhtar, 1995; Ahmad and Mukhtar, 2004). In addition to sunlight, the skin is exposed to a wide range of environmental chemicals and topically applied retail products. One such class of retail products is retinoid-containing dermal drugs and cosmetics. It has long been known that retinoids regulate epidermal cell growth, differentiation and maintenance. Retinoids have been used for dermatological

* Corresponding author. Tel.: +1 870 543 7207; fax: +1 870 543 7136.
E-mail address: pfu@nctr.fda.gov (P.P. Fu).
[1] Present address: Department of Food Science and Nutrition, Hung Kuang University, Sha-lu, Taichung, Taiwan, ROC.

0378-4274/$ – see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.toxlet.2005.09.010

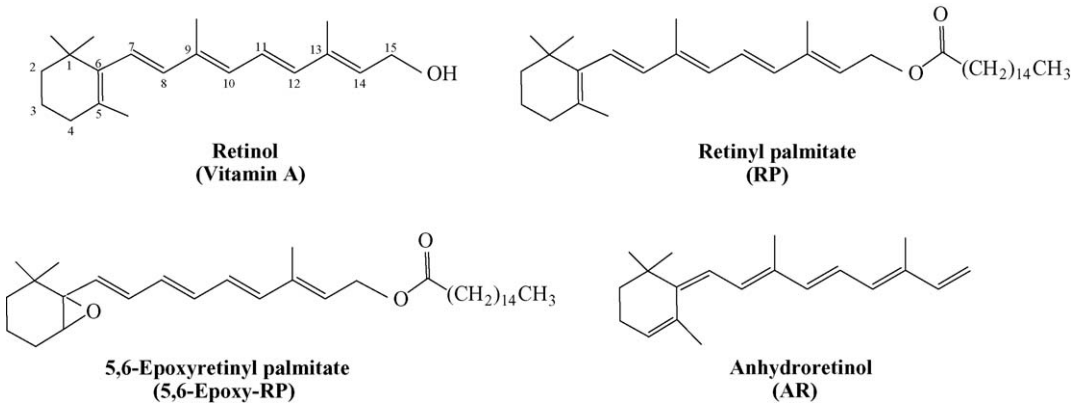

Fig. 1. Names, abbreviations and structures of retinol, retinyl palmitate, 5,6-epoxyretinyl palmitate and anhydroretinol.

applications in the management of acne and the treatment of skin diseases (Idson, 1990; Tee, 1992; IARC, 1998). Of the retinoids, retinol (Vitamin A) (Fig. 1) is thought to induce skin thickening and is incorporated into cosmetic formulations to reduce the appearance of thinning and wrinkling of skin associated with aging (Idson, 1990; IARC, 1992; Tee, 1992; Mukhtar, 1995; Ahmad and Mukhtar, 2004). A characteristic feature of Vitamin A, however, is its sensitivity to heat and light (Ji and Seo, 1999); therefore, to increase its stability, retinyl esters, such as retinyl palmitate (RP) (Fig. 1) are commonly employed in formulations for cosmetic use (Idson, 1990).

Although a number of cosmetic products contain RP, little information is available about the long-term consequences of use of these cosmetics and the effects of concomitant exposure to sunlight (Fu et al., 2002, 2003). It has been shown that topically applied RP appreciably penetrates into the human skin, resulting in significant levels of RP both inside and on top of the skin that would be available for exposure to sunlight (Boehnlein et al., 1994; Duell et al., 1997).

UV light penetrating the skin can cause both direct and indirect DNA damage (IARC, 1992; Mukhtar, 1995; Ahmad and Mukhtar, 2004). Direct DNA damage is principally caused by UVB light, and can result in cytotoxicity, mutagenicity and tumor initiation. On the other hand, DNA damage caused by UVA light is largely indirect, involving initial UVA light-dependent excitation of endogenous and exogenous photosensitizers. Photosensitizers activated by UVA light may then directly attack DNA or form reactive intermediates that subsequently attack DNA. Since RP has a maximum UV–vis absorption at 326 nm (Mukhtar, 1995), it would absorb UVA light and potentially form photodecomposition products. RP, in its excited state, may also transfer energy to molecular oxygen, leading to the formation of reactive oxygen species (ROS) which can damage DNA (Mukhtar, 1995). In addition, there is growing evidence that non-genetic damage occurs when ROS are generated, and this damage to cellular molecules can participate in disease states through altered cell signaling (Forman et al., 2002; Aslan and Ozben, 2003; Klaunig and Kamendulis, 2004; Poli et al., 2004; Trosko and Upham, 2005).

We have previously reported that photoirradiation of RP by UVA light resulted in a series of photodecomposition products (Cherng et al., 2005). The identified products include 5,6-epoxy-RP, 4-keto-RP, 11-ethoxy-12-hydroxy-RP, 13-ethoxy-14-hydroxy-RP, anhydroretinol (AR), palmitic acid, ethyl palmitate and four tentatively assigned *cis*- and *trans*-isomers of 11-ethoxy-AR (Cherng et al., 2005). The results of this study further indicated that photodecomposition of RP to yield 5,6-epoxy-RP, 4-keto-RP, 11-ethoxy-12-hydroxy-RP and 13-ethoxy-14-hydroxy-RP is mediated by a light-initiated free radical chain reaction. In contrast, AR and isomers of 11-ethoxy-AR appear to be formed through an ionic photodissociation mechanism. Photoirradiation of RP, 5,6-epoxy-RP and AR with UVA light in the presence of methyl linoleate resulted in lipid peroxide (methyl linoleate hydroperoxides) formation. This lipid peroxide formation was inhibited by dithiothreitol (DTT) (free radical scavenger), sodium azide (NaN_3) (singlet oxygen and free radical scavenger) and superoxide dismutase (SOD) (superoxide radical anion scavenger), which suggests that photoirradiation of RP, 5,6-epoxy-RP and AR by UVA light generated ROS resulting in lipid (methyl linoleate) peroxidation. Singlet oxygen has a rather short lifetime in aqueous solutions but has a longer lifetime in deuterium oxide (D_2O) (Rodgers and Snowden, 1982;