Photodiagnosis and Photodynamic Therapy 13 (2016) 316–322

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Photodecomposition, photomutagenicity and photocytotoxicity of retinyl palmitate under He–Ne laser photoirradiation and its effects on photodynamic therapy of cancer cells *in vitro*

Tarek Ibrahim[a,*], Mahmoud N.El Rouby[b], El-Sayed A.M. Al-Sherbini[a], Amr H.El Noury[a], Mona E. Morsy[a]

[a] National Institute of Laser Enhanced Science (NILES), Cairo University, Cairo, Egypt
[b] National Cancer Institute (NCI), Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form 6 August 2015
Accepted 8 September 2015
Available online 10 September 2015

Keywords:
Photodecomposition
Photodynamic therapy (PDT)
Photomutagenicity
Retinyl palmitate (RP)
Vitamin A

ABSTRACT

Objective: Our aim was to study photodecomposition, photomutagenicity and cytotoxicity of retinyl palmitate (RP), a principal storage form of vitamin A in humans and animals, under He–Ne laser photoirradiation. Moreover, the effect of different concentrations and timing protocol of antioxidants on photodynamic therapy (PDT) is contradictory, so the effect of RP (as antioxidant) on the PDT cytotoxicity was studied.

Methods: Photomutagenicity was tested by Ames test. Photodecomposition was studied by UV–vis spectroscopy. Cytotoxicity was measured with MTT-assay. Moreover, the effect of PDT, using hematoporphyrin derivatives (HpD) as photosensitizer under He–Ne laser irradiation (10 J/cm^2), was studied on HeLa cells either with or without RP (1–100 μM) which incubated with the cells for short or long incubation period (1 h or 24 h) prior to PDT.

Results: No photodecomposition of RP alone was obseved whereas there is a little photodecomposition of RP only in presence of HpD under irradiation with He–Ne laser. Moreover, no photomutagenicity was observed in *Salmonella typhimurium* strains under laser irradiation in presence or absence of HpD. RP alone (1–100 μM) significantly decrease the viability of HeLa cells. Laser irradiation of HeLa cells pre-incubated with RP alone for 24 h showed further significant decrease in viability of the cells. While RP incubations for 1 h before PDT had slight effect on the cells, 24 h incubation before PDT enhanced the cytotoxicity of PDT on HeLa cells.

Conclusions: RP can be used 24 h before PDT to enhance its effects. RP is not mutagenic under irradiation with He–Ne laser.

1. Introduction

Vitamin A (retinol) and its derivatives, collectively named retinoids, are required for many biological processes including cell growth, differentiation and maintenance. Retinoids are comprised of three units: a bulky hydrophobic region, a linker unit and a polar terminus which is usually a carboxylic acid. Modification of each unit has generated many more compounds [1]. Retinyl palmitate (all-trans-retinyl palmitate, RP) is a principal storage form of retinol in humans and animals that can be enzymatically hydrolyzed back to retinol *in vivo*. RP is widely used because retinol is thermally unstable while RP is relatively more stable [2,3]. Retinoids inhibit tumor formation and skin cancer development in experimental systems and in humans. Retinol is described as an inhibitor of cell growth in G1 phase. There is good evidence that the antitumor activity of retinoids is partially due to induction of cellular differentiation and/or inhibition of cell proliferation. Retinoids inhibit the proliferation of cells associated with HPV infection. They also have promising effects in inhibiting the progression of early cervical lesions to cancer [4].

The clinical evidence for a retinoid-based clinical chemopreventive approach has originated from the successful retinoids treatment of premalignant lesions such as oral leukoplakia, cervical dysplasia and xeroderma pigmentosum. Clinical trials revealed that retinoids are active in reducing some second primary cancers

* Corresponding author at: National Institute of Laser Enhanced Science (NILES), Postal Code 12613, Photochemistry Department, Cairo University, Cairo, Egypt. Fax: +20 2 35729499.
E-mail address: trkali@gmail.com (T. Ibrahim).

http://dx.doi.org/10.1016/j.pdpdt.2015.09.002
1572-1000/

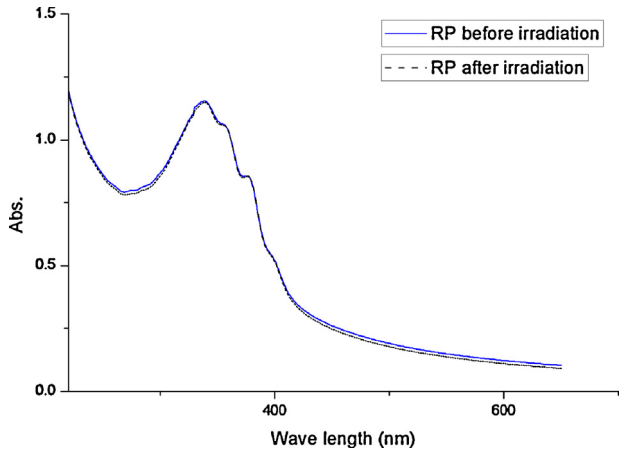

Fig. 1. UV–vis spectrum of retinyl palmitate (RP) alone before irradiation — and after irradiation ..... with red He–Ne laser (10 J/cm^2), no photodecomposition takes place under these irradiation settings.

such as aerodigestive tract tumors, second primary lung cancers and second hepatocellular carcinomas [5].

The effects of retinoids are mainly mediated by two classes of nuclear receptors, the RA receptors (RARs) and retinoid X receptors (RXRs) [6]. There are various types of retinoid-binding proteins which are located in intracellular and extracellular compartments and associated with isomeric forms of retinoids. Hence, retinoids are either associated with cellular membranes or bound to a specific retinoid-binding protein. These binding proteins solubilize and stabilize retinoids in aqueous spaces and they, along with nuclear receptors, mediate the action of retinoids [1].

Retinol and RP are considered safe as they are included in the Generally Recognized as Safe Substances Database (GRAS). Hence, they are found in cosmetic products, food and dietary supplements. However, the safety of topical retinoids was questioned in one publication and in a recent National Toxicology Program Report [7]. These reports suggested possible photomutagenicity of retinoids under ultraviolet irradiation. This suggestion contradicts a large body of data indicating that topical retinoids are chemoprotective in humans. Furthermore, these reports were immediately challenged by new reviews on the safety of RP in general and within sunscreens [8].

Photodynamic therapy (PDT) is a treatment for cancer and certain non-cancerous diseases that are generally characterized by overgrowth of unwanted or abnormal cells. PDT kills cancer cells by generation of reactive oxygen species following absorption of visible light by photosensitizers. These photosensitizers are characterized by high quantum yield to generate highly reactive cytotoxic singlet oxygen only in the presence of coherent and incoherent light [9].

Ames test is a bacterial reverse mutation assay used to test mutagenic properties of chemical compounds. This assay is carried out using several histidine dependent strains of bacteria (*Salmonella typhimurium*). Each tester strain contains a different type of mutation in the histidine operon that greatly increases their ability to detect mutagens. When the *Salmonella* tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen

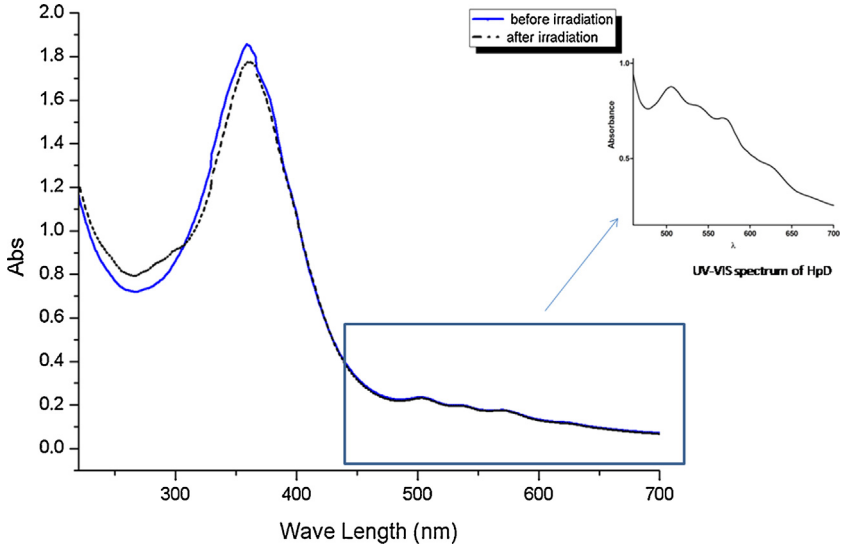

Fig. 2. UV–vis spectrum of retinyl palmitate (100 μM) mixed with HpD (25 μg/ml) in a ratio of 1:1 v/v before irradiation (—) and after irradiation (.....) with red He–Ne laser (10 J/cm^2). Inset shows a part of UV–vis spectrum of HpD to show the range of visible absorption peaks and shoulders in the red spectrum which are utilized for PDT treatment. Note that the degradation after laser irradiation takes place only in the UV range corresponding to the curve of RP which may be due to the free radical formation as a result of the photosensitization process.