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Photosensitized rose Bengal-induced phototoxicity on human melanoma cell line under natural sunlight exposure☆

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ABSTRACT

Rose Bengal (RB) is an anionic water-soluble xanthene dye, which used for many years to assess eye cornea and conjunctiva damage. RB showed strong absorption maxima (λ_{max}) under visible light followed by UV-B and UV-A. RB under sunlight exposure showed a time-dependent photodegradation. Our results show that photosensitized RB generates $^1O_2$ via Type-II photodynamic pathway and induced DNA damage under sunlight/UV-R exposure. 2′dGuO degradation, micronuclei formation, and single- and double-strand breakage were the outcome of photogenotoxicity caused by RB. Quenching studies with NaN_3 advocate the involvement of $^1O_2$ in RB photogenotoxicity. RB induced linoleic acid photoperoxidation, which was parallel to $^1O_2$-mediated DNA damage. Oxidative stress in A375 cell line (human melanoma cell line) was detected through DCF-DA assay. Photosensitized RB decreased maximum cellular viability under sunlight followed by UV-B and UV-A exposures. Apoptosis was detected as a pattern of cell death through the increased of caspase-3 activity, decreased mitochondrial membrane potential, and PS translocation through inner to outer plasma membrane. Increased cytosolic levels of Bax also advocate the apoptotic cell death. We propose a p53-mediated apoptosis via increased expression of Bax gene and protein. Thus, the exact mechanism behind RB phototoxicity was the involvement of $^1O_2$, which induced oxidative stress-mediated DNA and membrane damage, finally apoptotic cell death under natural sunlight exposure. The study suggests that after the use of RB, sunlight exposure may avoid to prevent from its harmful effects.

1. Introduction

Rose Bengal (RB) is a water-soluble xanthene dye used by eye care professionals to assess the cornea and conjunctiva damage [3]. It kills viruses, bacteria, and protozoa and may induce phototoxicity on retinal epithelial cells. RB causes nerve axon, corneal endothelium, heart, and pancreatic acini. RB under green light exposure kills microorganisms and cancer cells [3]. RB is an active chemotherapeutic agent for melanoma treatment [2]. Photoactive RB persists inside endosomes, Golgi apparatus, and endoplasmic reticulum (ER) except mitochondria [4,38]. RB inhibits the viability of ovarian and embryonic kidney cancer cells but less effective for normal human fibroblast [3]. Investigations have showed the cytotoxic and proapoptotic effects of RB on MCF-7 cell line, as a widely used model system for the breast cancer [2,35]) and human fetal skin fibroblasts (HFSF-PI3) as a control non-malignant cell line [36]. RB under UVR exposure with moderate intensity may avoid pain, erythema, and other side effects in the skin than photoactivated PDT agents. Such relative safety would be consistent with the long history of safe use of RB for various diagnostic applications [37].

Apoptosis is characterized by morphological changes, chromatin condensation, membrane blebbing, and DNA fragmentation [29–31]. Cell death through apoptosis is a gene-regulated phenomenon, which is induced by chemotherapeutic agents ([28]). ROS are reported to kill different kinds of cells by inducing necrosis or apoptosis [32–34]. ROS production by RB under visible green light makes its useful in dermatological diseases [4,38]. RB toxicity observed through necrosis in dark (without light) was independent of phototoxic ROS generation.

Ultraviolet (UV) light has been divided into three wave lengths such as UV-C (200–290 nm), UV-B (290–320 nm), and UV-A (320–400 nm) radiations. UV-C is absorbed by the ozone present in stratosphere and thus unable to reach at the earth's surface [5]. Biological effects of UVR are related to the generation of ROS and formation of cyclobutane

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pyrimidine dimmers [13]. Singlet oxygen is a well-known deleterious species of ROS for biological system which oxidizes DNA as well as lipids and proteins (D. [14,15]). The phototoxicity of RB persists by direct application to rabbit skin followed by sunlight exposure [17]. Non-clinical studies have shown that RB causes selective necrosis in cancer cells [2]. Photoactivated compounds generally localized within the cells for photodamage [18]. Photosensitizer generates ROS and caused apoptosis in cultured BCEN cells. The aim of this study was to explore the phototoxicity mechanism of RB at molecular level and identification of photoproducts under ambient intensities of UVR and natural sunlight by using human skin cell line.

2. Materials and methods

2.1. Radiation system

The spectral emission of UV-A has a source peak at 365 nm, whereas UV-B at 312 nm. The radiation dose was measured in mW/cm^2. The intensity of emitted light was measured by a microprocessor-controlled RMX-3 W radiometer (Vilber Lourmat) equipped with calibrated UV-A, UV-B, and UV-C detecting probes. Intensities selected for irradiation were based on dosimetry carried out between noon to 1:00 p.m. and were parallel to the ambient intensities of UV-A and UV-B reaching in sunlight at Lucknow (26°45′N latitude and 80°50′E longitude at 146 m above the mean sea level).

2.2. RB photomodification

RB was dissolved in distilled water, and a 1-μg/ml solution was prepared. RB was exposed under sunlight for (0–120 min). The photodegradation spectrum of RB was recorded between 200 and 700 nm. API 4000 QTRAP LC/MS-MS (Applied Biosystems, MDS Sciex, Toronto, Canada) was used for Q1 scan. Mass spectrometer was operated with electrospray ion source (ESI) in negative ion mode to scan the compound in the range of 500 to 1100 Da. The mass spectrometric condition for RB was optimized by continuous infusion at 10 μl/min by syringe pump (Model '11', Harvard apparatus, Holliston, USA). Exposed and control (non-irradiated) samples were diluted in acetonitrile for continuous infusion. Solvent control was also infused to compare the spectra with dark and irradiated samples.

2.3. Analysis of $^1O_2$ generation

The generation of $^1O_2$ under aerobic condition was measured in aqueous solution as per the Ray et al. [22]. The generation of $^1O_2$ was

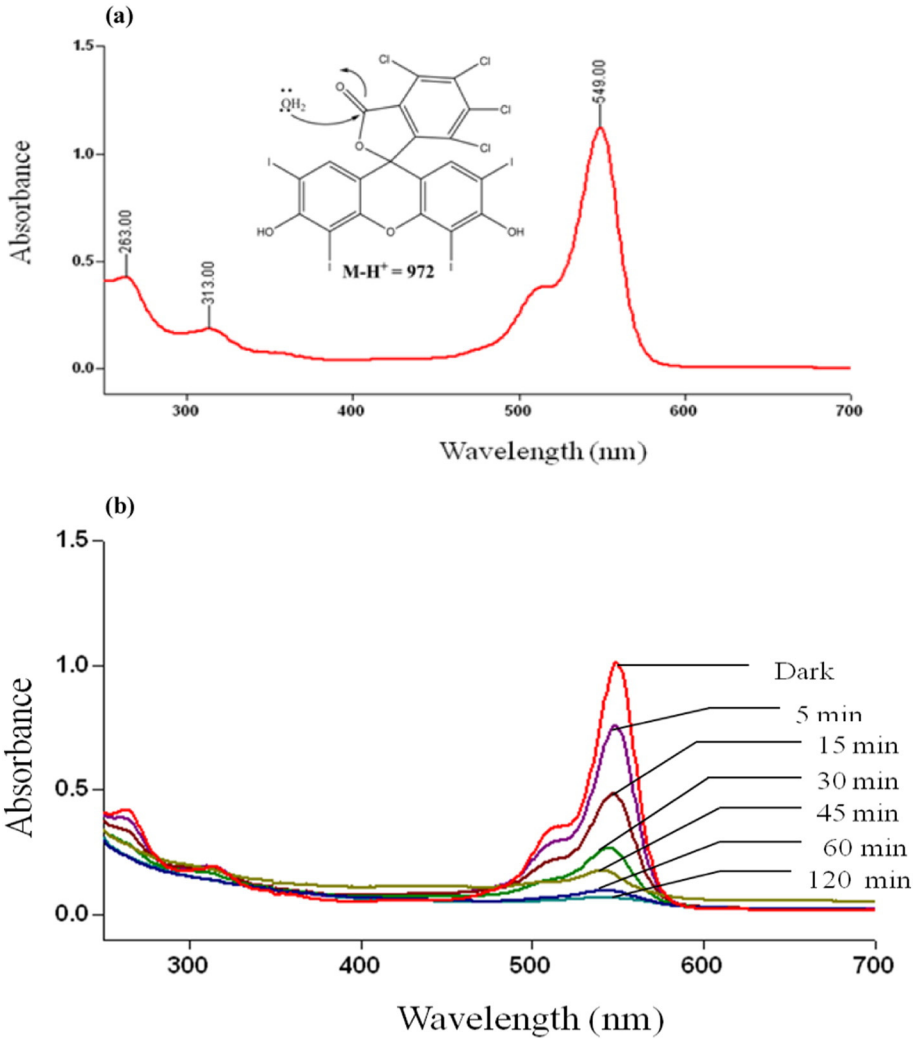

Fig. 1. Photostability study of RB under natural sunlight exposure. (a) Structure and absorption spectra of RB. (b) Photo-degradation spectra of RB (1 μg/ml) at different time intervals (0–120 min) under sunlight exposure (c and d) LC-MS spectra of RB degraded under sunlight exposure. (c) Dark control sample peak in circle, and (d) 120 min sunlight exposed sample of RB with three photoproduct having molecular wt. 638, 566, and 568. (e) Schematic representation of RB photodegradation (M-H$^+$ = 972 > 4,5,6,7-tetrachloro-3′,6′-dihydroxy-2′,4′,5′,7′-tetraiodo-3H-spiro[isobenzofuran-1,9′-xanthen]-3-one is IUPAC name of RB).