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Role of type I and type II reactions in DNA damage and activation of caspase 3 via mitochondrial pathway induced by photosensitized benzophenone

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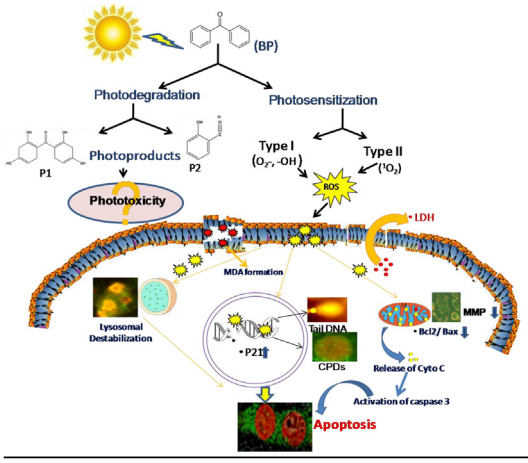
HIGHLIGHTS

- Novel photoproduct identified through LC MS/MS.
- ROS mediated DNA and membrane damage.
- Apoptotic cell death with involvement of mitochondria and lysosomes.
- P21 regulated apoptosis through decreased Bcl2/Bax ratio.
- Caspase 3 dependent apoptosis through mitochondrial pathway

GRAPHICAL ABSTRACT



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ABSTRACT

Sunscreen users have been increased, since excessive sun exposure increased the risk of skin diseases. Benzophenone (BP) and its derivatives are commonly used in sunscreens as UV blocker. Its photosafety is concern for human health. Our study showed the role of type-I and type-II radicals in activation of caspase 3 and phototoxicity of BP under sunlight/UV radiation. BP photodegraded and formed two photoproducts. BP generates reactive oxygen species (ROS) singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) through type-I and type-II photodynamic mechanisms. Photocytotoxicity significantly reduced cell viability under sunlight, UVB and UVA. DCF fluorescence confirmed intracellular ROS generation. BP showed single strand DNA breakage, further proved by cyclobutane pyrimidine dimmers (CPDs) formation. Lipid peroxidation and LDH leakage were enhanced by BP. P21 dependent cell cycle study showed sub G1 population which advocates apoptotic cell death, confirmed through AO/EB and annexin V/PI staining. BP decreased mitochondrial membrane potential,

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Sunscreens
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death protein released and activated caspase. We proposed cytochrome c regulated caspase 3 dependent apoptosis in HaCaT cell line through down regulation of Bcl2/Bax ratio. Phototoxicity potential of its photoproducts is essential to understand its total environmental fate. Hence, we conclude that BP may replace from cosmetics preparation of topical application.

1. Introduction

Ozone depletion has increased the biological damaging UV radiation on earth surface (De Fabo, 2005). Sunscreens remain an effective tool in providing protection against the known carcinogenic effects of UV radiation. However, there are a number of controversies surrounding the safety and efficacy of sunscreens as a form of photoprotection. Thus the safety of UV filters is major concern for human health consequences. Solar UVC radiation and approximately 90% of UVB radiation are absorbed by protective ozone layer and other gases present in the atmosphere. The UV radiation reaching at the earth's surface is largely composed of UVA (95%) with a small fraction of UVB (5%). Human beings are exposed to UVA in current life style, due to the popularity of tanning salons (Sage et al., 2012), which may cause hypersensitivity, photoaging, inflammation, immune-suppression and photo carcinogenesis (Meloni et al., 2010). Approximately, 5% decrease in ozone layer in next two decades may increase 10% effective UV photons on the earth surface. It has been predicted that solar photons could increase 5–8% melanomas, 10% basal cell carcinomas and 20% squamous cell carcinomas in coming decades. UV radiation may cause erythema, DNA damage, mutation, immune suppression and carcinogenesis in human beings (Tavana and Benjamin, 2010). Solar UV light damages DNA, which may result into mutation and photo carcinogenesis (Cadet et al., 2005). UVA increases prostaglandins, cytokines release and activation of NFkB transcription pathway (Muthusamy and Piva, 2010). Phototoxic reaction leads to burning sensation and pain on the exposed part of body, although it depends on the concentration of photosensitizer, immune response and sunlight exposure to human beings (Spiewak, 2009).

Sunscreens can be divided under two broad headings, based on the chemistry of its ingredients: inorganic and organic. Inorganic sunscreens, the common active ingredients are inorganic oxides like titanium dioxide and zinc oxide, which act as physical blocker by absorbing, reflecting or scattering UV radiation. Benzophenone (BP) and its derivatives are the main active ingredients of organic sunscreens product. In present scenario, BP and their twelve different derivatives are added in UV absorbing commercial products (Hayashi et al., 2006). These organic UV filters absorb UVA or UVB radiation and used in skin care products (Weisbrod et al., 2007). At present EU Cosmetics Directive has listed 28 UV filters used in cosmetic products contain 10–25% as active ingredients (Zenker et al., 2008). BP, a diarylketone and its derivatives are commonly used in sunscreens as organic UV blockers. It primarily photostabilized the odor and color in perfumes and soaps (National Toxicology Program, 2000). BP, apart from sunscreen it is used in agrochemicals, baked goods, soft candy, gelatins, puddings, pharmaceuticals, ultraviolet curing agent in sun glasses, flavor ingredient and subsequently reach into the human body (National Toxicology Program, 2000). Concentration of BP in food products ranged from 0.57 to 3.27 ppm in nonalcoholic beverages and frozen dairy products.

There are many reports which confirmed the dermal absorption of UV filters, and seen in urine and blood. Urinary concentration of BP type UV filters detected from 0.36 to 6.1 μg/L in U.S women (Kunisue et al., 2012). Previous study showed that benzophenone-3 (derivative of benzophenones) has been detected in human breast milk and urine up to 1-2% of applied amount (Gonzalez et al., 2006). In females urine approximately 60 ng/mL BP-3 was found. In the male's urine 140 ng/mL BP-3, was found. Benzophenones (BP-1, BP-2 and BP-3) had shown accumulation in blood and their molecular interaction with human serum albumin (Zhang et al., 2013). Dermal use of BP leads to hepatic, acute and subchronic systemic toxicity. BP activated pregnane X receptor regulates CYP3A and MDR1 induced CYP3A mRNA in rat liver (Mikamo et al., 2003). 14-week exposure of BP caused toxicity in liver and kidney of rats (National Toxicology Program, 2000). Increased incidences of mononuclear cell leukemia and liver lesions were observed in BP exposed male and female rats (Rhodes et al., 2007).

Previous study documented the environmental concern of sunscreen ingredients. Sunscreen ingredients enter into the environment through skin during swimming or bathing and waste water treatment plants (Schlecht et al., 2008). Glatt river of Switzerland contains BP and its derivatives up to 42 ng/L (Negreira et al., 2010). The concentration of BP-type UV filters was found 0.38 ng/g in sediment of Songhua river of China (Zhang et al., 2011). BP derivatives were detected from 78.3 to 612 ng/g of indoor dust in U.S, Korea, Japan and China (Wang et al., 2010). Recent studies have reported that topical application of sunscreens influenced reproductive hormone levels in human beings. Studies reveal that benzophenones have potential endocrine disrupting property, it mimics the endocrine hormone and subsequently alters the reproductive system, and the severe effects can be seen in the aquatic life. BP-4, frequently used absorber in cosmetics, interfere sex hormones of zebra fish embryo and adult males (Zucchi et al., 2011). BP-3 (2.4–312 μg/L) exhibits multiple hormonal activities at the transcription level in zebra fish and its embryo (Bluthgen et al., 2012). BP-2 alters sex characteristics, development and reproduction in fish (Weisbrod et al., 2007). Xenoestrogenic effects of BP stimulating the proliferation of ovarian cancer through ER signaling pathway (Park et al., 2013). Transcription profile of BP-4 revealed the expression of genes involved in hormonal pathways (Zucchi et al., 2011). Hydroxylated BP caused estrogenic and antiandrogenic activities by inhibiting endocrine action in mammals (Suzuki et al., 2005). BP-1 enhanced the number of Brd Urd positive nuclei and over expression of cyclin D1 protein. Few BPs caused genotoxicity due to their substitution (*ortho,para*-di) and increased OH substitution on benzene ring (Zhao et al., 2013).

Our study revealed the involvement of photosensitized benzophenone in mitochondrial mediated apoptosis. Mitochondria play an important role in intrinsic pathway of apoptosis, which is regulated by Bcl-2 family proteins through the pro-apoptotic protein BAX, and the anti-apoptotic protein Bcl-2. Mitochondrial confirmed its involvements in reactive oxygen species (ROS) production, down-regulation of Bcl-2 family proteins, up regulation of Bax release of death protein cytochrome c, and finally caspase-3 activation in various cell lines (Brady et al., 2011). It has been proved that ROS can enhance the mitochondrial membrane permeabilization in both models in vitro as well as in vivo (Kroemer and Reed, 2000). The balance between the levels of Bax and Bcl-2 are responsible for proper function of mitochondria, if the outer membrane of mitochondria permeabilized, causing the release of cytochrome c from mitochondria into the cytosol and