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Benzophenone 1 induced photogenotoxicity and apoptosis via release of cytochrome c and Smac/DIABLO at environmental UV radiation

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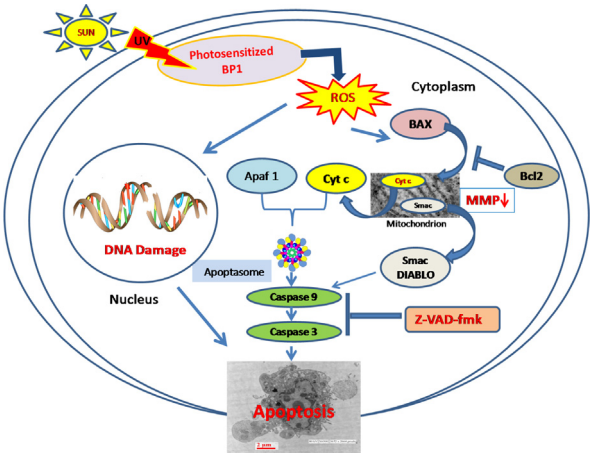
HIGHLIGHTS

- Role of Smac/DIABLO in activation of caspase 3.
- Caspase 3 dependent apoptosis via mitochondrial pathways.
- Photosensitized BP1 induced DNA damage, micronuclei and CPD_s formation.
- Role of cytochrome c and Apaf 1 in apoptosis.
- Photogenotoxic potential of BP1 in HaCaT cells at environmental UV radiation.

GRAPHICAL ABSTRACT



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ABSTRACT

Solar UV radiation is main factor of photocarcinogenesis, photoageing, and phototoxicity; thus, protection from UV radiation is major concern. Sunscreens containing UV filters are suggested as sun safe practices, but safety of UV filters remains in controversies. Benzophenone-1 (BP1) is commonly used in sunscreens as UV blocker. We assessed the photogenotoxicity and apoptotic parameters in human keratinocytes (HaCaT cells) by western blot, immunocytochemistry, flowcytometry, comet assay and TEM imaging. Our results exposed that BP1 photosensitized and generated intracellular ROS (2.02 folds) under sunlight/UVR. Decrease in cell viability was recorded as 80.06%, 60.98% and 56.24% under sunlight, UVA and UVB, respectively. Genotoxic potential of BP1 was confirmed through photomicronuclei and CPDs formation. BP1 enhanced lipid peroxidation and leakage of LDH enzyme (61.7%). Apoptotic cells were detected by AnnexinV/PI staining and sub G1 population of cell cycle. BP1 induced up regulation of apoptotic proteins Bax/Bcl2 ratio, Apaf-1, cytochrome c, Smac/DIABLO and cleaved caspase 3 was noticed. Down regulation of pro caspase 3 was inhibited by Z-VAD-fmk (inhibitor of caspase). Thus, study established the involvement of BP1 in photogenotoxicity and apoptosis via release of cytochrome c and

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Smac/DIABLO. These findings suggest sunscreen user to avoid BP1 in cosmetics preparation for its topical application.

1. Introduction

Unprotected exposure to ultraviolet radiation is the key factor to create skin cancer. Depletion of stratospheric ozone layer has increased the biological damaging ultraviolet (UV) light at earth surface. Increasing intensity of UV radiation increased the hazardous effects of UV radiation including, skin aging, skin cancer and other skin diseases. In the last 20 years, general population recognized the photocarcinogenic and photoaging properties of UV radiation (Goncalo et al., 1995). UV light can enhance the generation of free radicals which have potential to damage membranes, DNA and other cellular structures (Sobolev et al., 2000). Safety from UV radiation is a matter of concern for human health consequences. A safety measure of UV exposure includes sunscreens, sun glasses, sun cloths, and sun umbrella. Out of these the popularity of sunscreen has drastically increases due to its direct protective effect against UV radiation and growing public concern about the risk of excessive sun exposure during peak working hours of day, which may cause sunburn, photoaging and other skin disease.

Efficacy of sunscreens is measured by widely acceptable method, sun protection factor (SPF), SPF details us about the protection from ultraviolet B (UVB), but does not tell about the protection from ultraviolet A (UVA) exposure. The recent controversies regarding safety and efficacy of sunscreen use has encouraged to reassess of their use and properties. Studies have shown a direct protective effect of sunscreen use against actinic keratoses, and non-melanoma skin cancer (Young et al., 2000; Green et al., 1999). Several studies documented against the use of sunscreens and linked with increased nevus density, (predictor of melanoma) (Azizi et al., 2000). Active ingredients of sunscreen are UV blockers which prevents us either by absorbing or reflecting UV radiation. Organic sunscreens protect us by absorbing environmental UVA (320–400 nm) and UVB (280–320 nm) radiation. UVB is directly absorbed by nucleic acid resulting to photochemical damage to DNA, which may cause genetic mutations. UVA causes indirect effects on DNA via the reactive oxygen species (ROS) generation. There are evidences that UVA might have an important role in the pathogenesis of melanoma (Wang et al., 2001). Indirect evidence suggests that UVA has greater role in long-term skin damage than acute effects such as sunburn. On the basic of above evidences we have selected UVA in our experiments which support our vision that elevated intensity of UVA during peak hours of sunlight exposure would be more harmful to human being.

Nowadays, the amounts of UV filters used in sunscreens and other cosmetic products have been increasing (Kunz and Fent, 2006). BP1 a common UV filter is used in sunscreens and other cosmetics products. Benzophenones are reported in 1008 different cosmetics product in which BP1 are common in 127 products, typically at concentration <1% (Annual Review of Cosmetics Ingredient Safety Assessments, 2005). Although the use of sunscreens is considered as safe-sun practices, but earlier study has documented that UV filters may penetrate through stratum corneum and enhanced the ROS generation in presence of UV radiation (Hanson et al., 2006). The outermost layer of the skin is composed predominantly of keratinocytes that provide a barrier between host and the environment. Exposure of chemical UV absorber to skin has weakened the protective barrier which resulted in easy penetration of UV filter in blood vessels. Adverse reactions of sunscreen ingredients which include phototoxic, photoallergic, irritant contact dermatitis, and anaphylactic reactions also have been reported (Asschenfeldt et al., 2005). The Environmental Working Group (EWG) just published their 2014 guide to safe sunscreens. They reviewed approx 2000 sunscreens and more than 257 brands. They found more than 75% of the sunscreens contained toxic chemicals that can increase the risk of cancer and other health issues. A study conducted in Australian revealed that 19% of individuals have adverse reactions to sunscreens (Foley et al., 1993). Previous study showed that benzophenone-3 (derivative of benzophenones) was found in human breast milk and urine up to 1–2% of applied amount (Walters et al., 2002; Gonzalez et al., 2006). In females urine approximately 60 ng/mL BP-3 and 5 ng/mL of OMC (octyl-methoxycinnamate) and 4-MBC (3-4-methylbenzylidene) camphor, was found. In male's urine 140 ng/mL BP-3, 7 ng/mL 4-MBC and 8 ng/mL of OMC was found. Percutaneous absorption of UV filters like OMC, and 4-MBC are also reported (Janjua et al., 2004). Benzophenones such as BP-1, BP-2 and BP-3 have shown deposition in blood and their molecular interaction with serum albumin (Zhang et al., 2013). BP1 apart from accumulation in human bodies and it has potential to induce other health problems including endocrine disruption (Park et al., 2013).

Ahead of cutaneous absorption, benzophenones and its derivatives like benzophenone-2, benzophenone-4 have environmental fear too, as it contaminates environment through bathing, swimming and may mimic the endocrine hormones, the differences in reproductive hormone testosterone levels was found (Song et al., 2011 Zucchi et al., 2011). Benzophenone-2 is well known for estrogenic effects in ovariectomised rats and it may interact with estrogen reporter (Song et al., 2011).

Growth inhibition effects, reduction in cell viability and oxidative stress responses had shown by BP-3 and 4-MBC (Gao et al., 2013). Prior study revealed that aromatic ketone has photosensitizing property, ketoprofen, tiaprofenic acid, and fenofibrate, contain genotoxic response via phototoxic reaction (Placzek et al., 2013). Our study exposed the role of BP1 in release of mitochondrial death proteins and apoptosis via caspase 3 activation. Since the derivatives of benzophenone, like BP1 is most commonly used in sunscreens as photoprotective agent. Thus the safety assessment of BP1 is a matter of concern for human health consequences. But the impact of active ingredients of sunscreen (BP1) on human skin by its topical application is still unclear. Therefore, this study focuses the photosensitizing mechanism of BP1 at solar UV radiation and the exact role of mitochondrial death proteins cytochrome c and Smac/DIABLO in caspase 3 dependent apoptotic cell death.

2. Materials and methods

2.1. Chemical wares

Benzophenone1 (BP1), H_2DCFDA (2′–7′ dichloro fluorocene di acetate), NAC (N-acetyl cysteine), fetal bovine serum (FBS), DMEM F-12HAM, antibiotic and antimycotic solution, trypsin (0.25%), L-histidine, MTT, neutral red uptake (NRU), tricarboxylic acid (TCA), ascorbic acid, carbonate and phosphate buffers, RNase, and propidium iodide were procured from Sigma Chemical Co. (St. Louis, MO). Tween-20 was obtained from M.P. Biomedicals Inc. (Solon) and Hank's Balanced Salt Solution (HBSS) and PBS were purchased from In Vitrogen Corporation USA.