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Involvement of intracellular oxidative stress-sensitive pathway in phloxine B-induced photocytotoxicity in human T lymphocytic leukemia cells

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ABSTRACT

We investigated the molecular mechanisms underlying phloxine B (PhB)-induced photocytotoxicity in human T lymphocytic leukemia Jurkat cells. In addition to apoptosis-related biochemical events, photo-irradiated PhB generated intracellular reactive oxygen species (ROS), induced phosphorylation of c-Jun-*N*-terminal kinase (JNK) in an oxidative stress-dependent manner and up-regulated the gene expression of interferon (IFN)-γ, an inducer of diverse apoptosis-related molecules in activated T cells. PhB-induced apoptosis was significantly inhibited by *N*-acetyl-l-cysteine, but not by catalase, indicating that ROS generation occurred intracellularly, and by SP600125 and AG490, specific inhibitors of JNK and IFN-γ signaling, respectively, confirming their roles in the apoptotic pathway. IFN-γ up-regulation was also inhibited by SP600125, indicating that it was downstream of JNK activation. These results suggest that PhB-induced apoptosis in Jurkat cells partially involves the intracellular oxidative stress-sensitive and T cell-specific IFN-γ pathway. These data present a novel insight into the mechanisms of photocytotoxicity induced by artificial food colorants in human T lymphocytic leukemia cells.

1. Introduction

Photodynamic therapy (PDT) is a two-component treatment modality involving application of a photo-sensitizing agent (sensitizer) and photo-excitation by visible light. Photo-excited triplet state sensitizers give rise to cell-modifying reactive oxygen species (ROS), either free radicals, such as superoxide and hydroxyl radical via a Type I (electron or hydrogen transfer) mechanism or non-radical singlet molecular oxygen via a Type II (energy transfer) mechanism (Foote, 1968). Skin-related disorders, given their convenient exposure to the visible light source, are natural targets for PDT. Accordingly, PDT has been evaluated as a potential treatment for primary and secondary skin cancers, such as basal and squamous cell carcinomas and cutaneous T and B cell lymphoma, and nonmalignant skin disorders including psoriasis (Bissonnette et al., 2002). Photofrin®, the first Food and Drug Administration (FDA)-approved photosensitizer, showed promise in the treatment of psoriasis, but its disadvantages of prolonged patient photosensitivity and weak long-wavelength absorption preclude its clinical use (Josefsen and Boyle, 2008). There is therefore a need for a new type of photosensitizers.

Phloxine B (PhB; 2′,4′,5′,7′-tetrabromo-4,5,6,7-tetrachloro-fluorescein, FDA name, D&C Red No. 28; Japanese name, Red No. 104), which has an absorption maximum in the visible region at 540 nm (Inbaraj et al., 2005), is a color additive certified for use in both drugs and cosmetics in the United States, and also in foods in Japan (Fig. 1). PhB and the structurally-related fluoresceins have been evaluated as phototoxins for the potential control of fruit flies (Liquido et al., 1995; Moreno et al., 2001). In a screening of twelve kinds of artificial food colorants that have been approved by the Food Sanitation Law of Japan, we identified PhB as one of the most potent photocytotoxic agents against human promyelocytic leukemia HL-60 cell lines (Qi et al., 2011). In that study, we also found preliminary evidence that PhB-induced photocytotoxicity in human T lymphocytic leukemia Jurkat cells.

In the present study, we investigated the molecular mechanisms underlying the PhB-induced photocytotoxicity in Jurkat cells, which were employed to evaluate a PDT agent against T cell mediated skin diseases (Ke et al., 2008). Using antioxidants and signaling-selective inhibitors, we demonstrate that intracellular oxidative stress plays a pivotal role in the activation of interferon

Abbreviations: PDT, photodynamic therapy; ROS, reactive oxygen species; FDA, Food and Drug Administration; PhB, phloxine B; IFN, interferon; FBS, fetal bovine serum; NAC, *N*-acetyl-l-cysteine; H_2DCF-DA, 2′,7′-dichlorodihydrofluorescein diacetate; JNK, c-Jun-*N*-terminal kinase; Ac-DEVD-MCA, acetyl-Asp-Glu-Val-Asp-α-4-methyl-coumaryl-7-amide; PBS(−), phosphate buffer saline without calcium and magnesium; DCF, dichlorofluorescein; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; RT-PCR, reverse-transcription polymerase chain reaction; MAPK, mitogen-activated protein kinase; HIV, human immunodeficiency virus; PKR, double-stranded RNA-activated protein kinase; JAK/STAT, Janus kinase/signal transducers and activators of transcription; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB.

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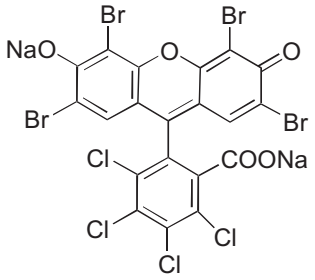

Fig. 1. Chemical structure of phloxine B (PhB).

(IFN)-γ-dependent apoptotic cell signaling. These results raise the possibility of using PhB against T cell-mediated skin diseases, such as cutaneous lymphomas and psoriasis.

2. Materials and methods

2.1. Chemicals and reagents

PhB was obtained from San-Ei Gen F.F.I., Inc. (Osaka, Japan). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco-Invitrogen (Carlsbad, CA, USA). *N*-acetyl-l-cysteine (NAC), catalase, protease and phosphatase inhibitor cocktails, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA) and SP600125 were obtained from Sigma (St., Louis, MO). Anti-phosphorylated c-Jun-*N*-terminal kinase (JNK) and anti-JNK antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA) and anti-actin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The caspsase-3 substrate, acetyl-Asp-Glu-Val-Asp-α-4-methyl-coumaryl-7-amide (Ac-DEVD-MCA), was obtained from peptide Inst. Inc. (Osaka, Japan). All other chemicals including AG490 were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Cell culture and treatment

The human T cell leukemia Jurkat cells (RIKEN Cell Bank; Tsukuba, Ibaraki, Japan) were maintained in RPMI-1640 supplemented with 10% (v/v) FBS, 50 U/ml of penicillin and 50 μg/ml of streptomycin at 37 °C under 5% CO_2 and 95% air. The Jurkat cells (4×10^5) grown in 6-well plate were treated with different concentrations of PhB and then exposed to visible light from a fluorescent lamp to exclude wavelength below 400 nm (The fluence rate; 0.4 mW/cm^2) for 1 h, followed by additional incubation for 24 h. The cells for inhibitor experiments were pretreated with catalase (1000 U/ml), NAC (10 mM), the JNK-specific inhibitor, SP600125 (10 μM), or the IFN-γ signaling inhibitor, AG490 (25 μM), for 1 h before the treatment of PhB (100 μM). The Trypan blue dye exclusion assay was carried out for the quantitative analysis of cell viability. The cells were collected by centrifugation, and resuspended in phosphate buffer saline without calcium and magnesium (PBS(−)). The cell suspensions were mixed with 0.4% Trypan blue stain. The total cells and viable cells (cell that excluded blue dye) were counted using a hemocytometer (Bürker-Türk) under a light microscope.

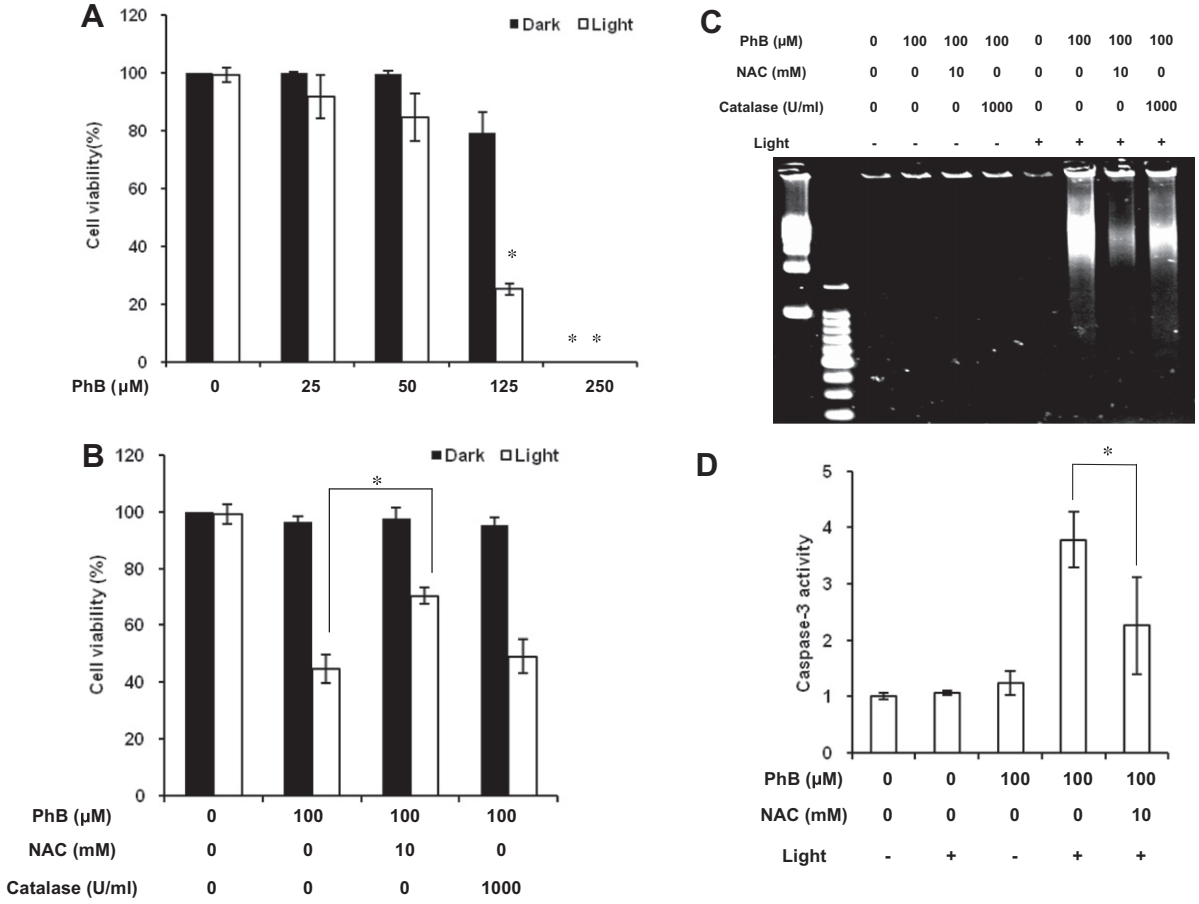

Fig. 2. Effects of NAC and catalase on PhB-induced photocytotoxicity, DNA fragmentation and caspase-3 activation in Jurkat cells. (A) Dose-dependent photocytotoxicity of PhB in Jurkat cells. Jurkat cells (4×10^5) grown in 6-well plate were incubated with the indicated concentrations of PhB for 1 h at 37 °C with or without light irradiation. After additional 24 h incubation, cell viability was measured using a trypan blue exclusion assay. The values represent means ± SD of three separate experiments (*$P < 0.05$ vs. control). (B) Jurkat cells (4×10^5 cells/ml) were pretreated with NAC (10 mM) for 1 h and treated with PhB under light irradiation for 1 h or co-treated with catalase (1000 U/ml) and PhB under light irradiation for 1 h. The cells were then incubated in the dark for 24 h. The cell viability was measured by a trypan blue exclusion assay. The values represent means ± SD of three separate experiments (*$P < 0.05$ vs. PhB with light irradiation). (C) Jurkat cells were treated in the same way as Fig. 2A. DNA fragmentation was analyzed using agarose electrophoresis. The result is representative of three independent experiments. (D) Jurkat cells (4×10^5 cells/ml) were pretreated with NAC (10 mM) for 1 h, co-treated with PhB under light irradiation for 1 h and then incubated in the dark for 6 h. The caspase-3 activity was monitored by a spectrofluorometer. The values represent means ± SD of three separate experiments (*$P < 0.05$ vs. PhB with light irradiation).