Journal of Photochemistry and Photobiology B: Biology 138 (2014) 124–133

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Pyrrolidine dithiocarbamate inhibits UVB-induced skin inflammation and oxidative stress in hairless mice and exhibits antioxidant activity *in vitro*

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ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form 13 May 2014
Accepted 15 May 2014
Available online 27 May 2014

Keywords:
Antioxidant activity
Free radical
Inflammation
Oxidative stress
PDTC
UVB irradiation

ABSTRACT

Ultraviolet B (UVB) irradiation may cause oxidative stress- and inflammation-dependent skin cancer and premature aging. Pyrrolidine dithiocarbamate (PDTC) is an antioxidant and inhibits nuclear factor-κB (NF-κB) activation. In the present study, the mechanisms of PDTC were investigated in cell free oxidant/antioxidant assays, *in vivo* UVB irradiation in hairless mice and UVB-induced NFκB activation in keratinocytes. PDTC presented the ability to scavenge 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) radical (ABTS), 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) and hydroxyl radical (·OH); and also efficiently inhibited iron-dependent and -independent lipid peroxidation as well as chelated iron. *In vivo*, PDTC treatment significantly decreased UVB-induced skin edema, myeloperoxidase (MPO) activity, production of the proinflammatory cytokine interleukin-1β (IL-1β), matrix metalloproteinase-9 (MMP-9), increase of reduced glutathione (GSH) levels and antioxidant capacity of the skin tested by the ferric reducing antioxidant power (FRAP) and ABTS assays. PDTC also reduced UVB-induced IκB degradation in keratinocytes. These results demonstrate that PDTC presents antioxidant and anti-inflammatory effects *in vitro*, which line up well with the PDTC inhibition of UVB irradiation-induced skin inflammation and oxidative stress in mice. These data suggest that treatment with PDTC may be a promising approach to reduce UVB irradiation-induced skin damages and merits further pre-clinical and clinical studies.

1. Introduction

During life, the skin is exposed to exogenous environmental detrimental sources of stress. Among these sources, ultraviolet (UV) irradiation is one of the most deleterious to the skin [1].

Acute exposure to ultraviolet B (UVB) irradiation is responsible for inducing a number of disease-related changes in the skin, including erythema, edema, hyperplasia, sunburn cell formation, inflammation, while chronic UVB exposure leads to premature aging and carcinogenesis in the skin [2,3]. The reactive oxygen species (ROS) formed by exposure to UVB irradiation are presumed to play an important role in the initiation and conduction of signaling events leading to cellular response, and the skin damage may also be a result of increased oxygen radicals production during the inflammatory response to UV irradiation [4,5]. Exogenous supplementation of antioxidants can be an effective strategy to counteract the deleterious effects of the ROS generated from the excessive exposure to UV irradiation [6]. Several studies have shown the chemopreventive effects of naturally occurring as well as synthetic antioxidants agents against UV irradiation-mediated damage [7,4,8].

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http://dx.doi.org/10.1016/j.jphotobiol.2014.05.010
1011-1344/

Pyrrolidine dithiocarbamate (PDTC) is a low-molecular weight thiol compound that has been used as an antioxidant to counteract the toxic effects of free radicals. This antioxidant potential of PDTC is attributed to its thiol group which functions by neutralizing reactive oxygen intermediates [9]. It has been widely studied due to its biochemical activities, such as redox state alternation, heavy metal chelation and enzyme inhibition [10]. In fact, many studies suggest the antioxidant and therapeutic application of PDTC in diseases involving the production of free radicals [11,12]. PDTC inhibits the action of ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical in cell-based *in vitro* assays [13]. Importantly, this antioxidant activity of PDTC seems to be responsible for its inhibitory effect over nuclear factor-κB (NF-κB) activation. It is likely that PDTC prevents the ROS-induced dissociation of inhibitory factor-κB (I-κB) from NF-κB in the cell cytoplasm and as a result, active NF-κB will not translocate to the cell nucleus to exert its modulatory effect on gene expression. Additionally, PDTC interferes with κB-dependent transactivation genes [13]. As a consequence of inhibiting NF-κB activation, PDTC reduces the production of inflammatory cytokines [13].

Taking into account the above mentioned the *in vitro* antioxidant mechanisms of PDTC in cell-free systems and its therapeutic effects in UVB irradiation-induced photo-oxidative and -inflammatory damages to the skin of hairless mice and human keratinocyte cell line were investigated.

2. Materials and methods

2.1. Chemicals

Brilliant blue R, reduced glutathione (GSH), hexadecyltrimethylammonium bromide (HTAB), linoleic acid, N-ethylmaleimide, *o*-dianisidine dihydrochloride, phenylmethanesulfonyl fluoride, thiobarbituric acid (TBA), 1,10-Phenanthroline monohydrate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-(picrylhydrazyl) (DPPH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and (2,4,6-Tris(2-pyridyl)-*s*-triazine) (TPTZ) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Pyrrolidine dithiocarbamate (PDTC) was obtained from Alexis Corporation (Lausen, Lausen, Switzerland). 2-deoxy-D-ribose and bathophenanthroline (BPS) were purchased from Acros (Pittsburgh, PA, USA). Xylene cyanol was obtained from Amresco (Solon, OH, USA). ELISA kit for IL-1β determination was obtained from eBioscience (San Diego, CA, USA). Isoflurane was obtained from Abbott (Abbott Park, IL, USA).

2.2. Determination of the in vitro antioxidant activity of PDTC by different methods

2.2.1. ABTS free radical scavenging assay

The PDTC (0.08–2 μg/mL) antioxidant capacity of scavenging the free radical ABTS was determined by the decrease of absorbance at 730 nm (Evolution 60, Thermo Scientific) [14]. Samples were processed and assessed in triplicate and the ability of scavenging ABTS was calculated by the following equation:

$$\%\,\text{Of activity} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100. \quad (1)$$

2.2.2. Determination of DPPH radical scavenging activity

The PDTC (0.1–100 μg/mL) antioxidant ability to donate hydrogen and stabilize the free radical DPPH was evaluated by the reduction of DPPH radical by the change in absorbance measured at 517 nm (Evolution 60, Thermo Scientific) [15,16]. Samples were analyzed in triplicate. The results were expressed as by Eq. (1).

2.2.3. Scavenging effect on hydroxyl free radical

The hydroxyl radical (·OH) scavenging ability of PDTC was measured by the reduction of thiobarbituric acid reactive substances (TBARS) from degradation of deoxyribose by ·OH generated in Fenton reaction [17]. The scavenger ability of different concentrations of PDTC (10–500 μg/mL) was determined by the colorimetric method described [18]. The measurements were analyzed in triplicate. The scavenging of hydroxyl free radical was calculated by Eq. (1).

2.2.4. Iron-induced lipid peroxidation

Mitochondria of hairless mice were used as a source of lipid membranes to evaluate lipid peroxidation and were prepared by standard differential centrifugation techniques [19,20]. The ability of the different concentrations of PDTC (0.25–25 μg/mL) to inhibit iron-induced lipid peroxidation was evaluated by reduction of TBARS formation [21,22]. All measurements were performed in triplicate. The inhibition of iron-dependent lipoperoxidation was calculated by Eq. (1).

2.2.5. Iron-independent lipid peroxidation

The inhibitory activity of iron-independent lipid peroxidation of different concentrations of PDTC (0.5–50 μg/mL) was determined by decreasing the production of lipid hydroperoxides, a primary product of lipid peroxidation [23]. Lipid hydroperoxides were determined by previously described method [22]. All measurements were performed in triplicate. The following equation was used:

$$\%\,\text{Activity} = 1 - (\textit{absA after incubation} - \textit{absA without incubation})/(\textit{absC after incubation} - \textit{absC without incubation}) \times 100. \quad (2)$$

absA is the absorbance of sample, and *absC* is the absorbance of the control.

2.2.6. Determination of iron-chelating activity using the bathophenanthroline (BPS) assay

BPS is a strong chelator of ferrous ion that forms a colored complex when it reacts with this ion. The PDTC (0.5–500 μg/mL) chelation of iron ions was determined by colorimetric change measured at 530 and 700 nm (Evolution 60, Thermo Scientific) [19,24]. All measurements were made in triplicate. The iron chelating activity was calculated by Eq. (1).

2.3. Assessment of PDTC protective effect against UVB-induced inflammation and oxidative stress in vivo

2.3.1. Animals and experimental protocol

In vivo experiments were performed on male hairless mice (HRS/J) except by IL-1β assay that was performed on female. The animals weighing 20–30 g (2–3 months) were housed in a temperature-controlled room, 12 h light and 12 h dark cycles and with access to water and food *ad libitum*. All experiments were conducted in accordance with National Institutes of Health guidelines for the welfare of experimental animals and with the approval of the Ethics Committee of the Universidade Estadual de Londrina (Of. Circ. CEEA No. 160/2010 in December 17, 2010, registered under the number CEEA 85/10, process No. 33631.2010.82). All efforts were made to minimize the number of animals used and their suffering. The animals were divided into five groups: Group