UVA-induced oxidative damage and cytotoxicity depend on the mode of exposure

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Abstract

The reciprocity rule (Bunsen–Roscoe law) states that a photochemical reaction is directly proportional to the total energy dose, irrespective of the dose distribution. In photomedicine the validity of this law is usually taken for granted, although the influence of radiation intensity and dose distribution are largely unknown. We have examined in a tissue culture model the effects of fractionated versus single dose exposure to UV from a metal halide source on survival, DNA synthesis, glutathione, and oxidative membrane damage. Exposure to fractionated UVA was followed by an increased rate of cell death compared to single dose exposure, when intervals between fractions where short (10–120 min). Longer intervals had the opposite effect. Corresponding results were obtained for DNA synthesis (BrdU incorporation). The increased cytotoxicity of dose fractionation with short intervals could not be abrogated by non-enzymatic antioxidants (astaxanthin, ascorbic acid, α-tocopherol). Fractionated irradiation with short intervals led to higher degree of depletion of glutathione (GSH) and to enhanced formation of thiobarbituric acid reactive substances (TBARS) in comparison to an identical single dose. Long intervals between fractions induced opposite effects. Taken together, these data indicate that immediately after UVA exposure cells are more sensitive to a further oxidative attack making repeated exposure with short intervals more cytotoxic than continuous single dose UVA. This might have implications also for responses to UVA in vivo and further studies will have to extend these findings to the situation in healthy and diseased human skin.

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Keywords: Bunsen–Roscoe law; UV; Antioxidants; Oxidative stress; Reactive oxygen species; Glutathione

1. Introduction

Solar ultraviolet radiation consists of UVC (wavelength <280 nm), UVB (280–315 nm), and UVA (315–400 nm). Only UVA and UVB are reaching the earth’s surface, because the atmosphere absorbs wavelengths <290 nm. Exposure of human skin to UV from the sun and artificial sources elicits a specific sequence of events that are dependent on dose and spectral distribution. Among the clinically apparent responses to UV are erythema, pigmentation, immunosuppression, and delayed effects such as skin aging and cancer. Although much progress has been made in the understanding of the molecular and cellular mechanisms underlying these responses, the influence of dose distribution on the photobiology of mammalian tissues has not found sufficient attention in the pertinent literature [1]. On the contrary, in most studies the observed effects are related only to wavelength and cumulative dose, irrespective of the intensity of
the source and dose distribution. In other words, the Bunsen–Roscoe rule, stating that photochemical effects depend on dose (intensity \times time) and not dose rate (intensity), is usually assumed also for complex photochemical reactions [2]. This assumption, however, is highly unlikely to hold true, taking into account the complex nature of the cellular response to UV. For UVB the main initial event is absorption by DNA resulting in the formation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts. These DNA photolesions are repaired requiring the formation of the nucleotide excision repair complex and other less important repair mechanisms [3]. Further cellular responses include the initiation of apoptosis and regulation of the expression of UV-responsive genes [4,5]. For UVA a variety of cellular chromophores have been identified, including flavins, amino acids (e.g., tryptophan, tyrosine, histidine), and formylkynurenine, the reaction product of tryptophan and singlet oxygen (\( {1}^{{\text{O}}} \)) [6–11]. However, their precise roles and relative contributions to cellular damage are not yet fully established. The main initial event after absorption of UV by these molecules is the generation of reactive oxygen species (ROS) [12]. ROS include free radicals like superoxide anion (\( {O}^{\cdot}\)) and the hydroxyl radical (OH), as well as non-radicals like hydrogen peroxide (\( {H}_{2}{O}_{2}\)) and \( {1}^{2}{O}_{2}\). ROS lead to oxidation of DNA, proteins, and to membrane damage which are considered to be important initial steps with respect to photoaging and UV-induced skin cancer [13–15]. To prevent oxidative damage, mammalian cells have developed a complex antioxidant system that includes non-enzymatic antioxidants such as \( \alpha\)-tocopherol, ascorbic acid, glutathione and \( \beta\)-carotene-oxides, and enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) [16]. Low UVA doses can lead to a substantial up-regulation of enzymatic antioxidants (e.g., manganese superoxide dismutase, MnSOD). This adaptive response correlates with protection against subsequent exposure to high UV doses and requires de novo protein synthesis [17,18].

In the present study, we investigated in a human tissue culture model the cellular response to various patterns of UV exposure to provide initial evidence on the influence of dose distribution on UV-induced tissue damage. Experimental endpoints included early cellular responses such as apoptosis, survival rate, DNA synthesis, and oxidative damage in a human keratinocyte-derived cell line. Since the output of the high pressure lamps used in this study cannot be tuned, dose fractionation with variation of interval length between the individual fractions (and in some experiments also variation of the source to target distance) was used as a surrogate for modification of intensity.

2. Methods and materials

2.1. Tissue culture

The human squamous cell carcinoma cell line A 431 (CRL 1555, ATCC, Rockville, USA) was maintained in Dulbecco medium supplemented with 10% fetal calf serum, glutamine, penicillin, and streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂.

2.2. UV irradiation

The cells were irradiated at a distance of 30 cm using a metal halide source with a filter combination that allowed for transmission between 315 and 390 nm for UVA and 290 and 320 nm for UVB (Mutzhas Supersun 5000 Solar Simulator, Munich, Germany). The output was monitored by a radiometer (IL 1700, International Light, Newburyport). Prior to UV irradiation cells were washed twice with phosphate buffered saline (PBS; with \( {Ca}^{2+}\) and \( {Mg}^{2+}\)) and left in PBS. Following irradiation PBS was replaced by fresh medium and the cells were incubated for various periods of time. For repetitive UV irradiation cells were incubated for 10–240 min between individual fractions. Mock treated cells were treated identically without UV exposure.

2.3. Antioxidants

\( \alpha\)-Tocopherol (Serva, Heidelberg, Germany) was dissolved in dimethylsulfoxide (DMSO; Sigma, Heidelberg, Germany) and used at concentrations of 5, 10, and 30 \( \mu\)M. Astaxanthin (Sigma) was dissolved in ethanol and used at concentrations of 1, 10, and 50 \( \mu\)M. Ascorbic acid (Merck, Darmstadt, Germany) was dissolved in PBS and added to the medium to reach a final concentration of 10, 50, and 100 \( \mu\)M. All antioxidants were added to subconfluent cell cultures 16 h before irradiation.

2.4. Flow cytometric analysis

UV-induced apoptosis and cell death was measured 6, 12, and 24 h after UVA and UVB irradiation using flow cytometric analysis as described elsewhere [19]. Briefly, adherent cells and cells from the supernatant were harvested and washed twice in PBS. \( 1 \times 10^{6}\) Cells were then resuspended in 100 \( \mu\)l annexin-binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂) and annexin V-FITC (Boehringer Ingelheim, Vienna, Austria) and propidium iodide (PI) were added to a final concentration of 5 \( \mu\)g/ml each. Cells were then incubated for 15 min at room temperature in the dark and subsequently diluted with 300 \( \mu\)l of annexin binding buffer. Bivariate analysis was performed using the FACSCalibur (Becton Dickinson, St. Louis, USA) equipped
with a 488 nm argon laser. Emission filters used were BP 530/30 nm (FITC) and BP 585/42 nm (PI). A minimum of 10,000 cells per sample was recorded. Cell debris were excluded from analysis based on forward and side-angle light scatter gating. Data analysis was performed using the standard CELLQuest software (Becton Dickinson, St. Louis, USA).

2.5. Viability

Viability was monitored 12 and 24 h after the first irradiation. A MTT-assay (Biomedica, Vienna, Austria) was used for the quantification of metabolically active, living cells. Dehydrogenases of active mitochondria convert the yellow colored tetrazolium compound to its red formazan derivate that is measured photometrically at 450 nm. The viability is calculated as percentage of absorbance relative to sham treated control cultures.

2.6. BrdU-assay

The replication of cellular DNA was determined by the incorporation of 5-bromo-2'-deoxyuridine (BrdU), a pyrimidine analogue, into DNA instead of thymidine. After incorporation into DNA, BrdU is detected by immunohistochemistry at an absorbance of 450/690 nm (Roche Molecular Biochemicals, Mannheim, Germany). Seventy-two hours after the first irradiation the proliferation capacity of the cells is determined photometrically.

2.7. Glutathione assay

Intracellular concentrations of the reduced form of glutathion (GSH) were assayed using a commercially available detection system (Cayman, Ann Arbor, MI, USA). This assay utilizes an enzymatic recycling method, using glutathione reductase, for the quantification of GSH. The sulfhydryl group of GSH reacts with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The rate of TNB production is directly proportional to the concentration of GSH. The absorbance is measured at 405 nm. The data were normalized according to the cell viability.

2.8. Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were assayed 30 min after UVA irradiation with a TBARS assay (Sobioda, St. Martin, France). In preliminary experiments TBARS were found to be at the highest level at this time point. Sixty minutes after exposure TBARS were back at background levels (data not shown). The supernatant was removed and 500 µl were kept frozen (−20 °C) after addition of 50 µl butylated hydroxytoluene (BHT) (2% w/w in ethanol). The mixture of the sample and working solution was heated for 60 min at 95 °C, cooled on ice and extracted with butanol. The organic phase was collected for fluorescence analysis (532/55 nm). Data are expressed in terms of TBARS equivalents normalized to the cell protein content (Pierce, Rockford, IL, USA). Values are expressed as TBARS (nmol)/total protein (µg).

2.9. Statistics

All values are indicated as mean ± SD or mean ± SEM, whatever is appropriate. For comparisons paired/unpaired t test was used as appropriate. Level of significance was p < 0.05.

3. Results

3.1. Dose fractionation increases the cytotoxic effect of UVA but not UVB

Cell death and apoptosis were determined by FACS analysis of annexin V binding and PI uptake [19]. As described earlier, annexin V-positive/PI-negative cells correspond to UV-induced early apoptotic cells that show an increase shortly after exposure. PI-positivity is an indicator of ultimate cell death. Thus, the percentage of PI-positive cells increases with time with a corresponding decrease of the annexin V-positive/PI-negative fraction. For UVA the cells were irradiated with 40, 60, and 80 J/cm² as a single dose. The same doses were separated into four equal doses with intervals of 10 min between the repetitive irradiation. For UVB we used doses of 128, 256, and 512 mJ/cm² applied according to the same irradiation regime as above. The cells were analyzed 6, 12, and 24 h after UV irradiation. Fractionated UVA irradiation at least doubled the percentage of apoptotic cells compared to the single dose at all doses and time points. This effect was observed for annexin V binding and PI uptake (Fig. 1(a)). For UVB there was no significant difference between the single dose and the fractionated irradiation (Fig. 1(b)).

3.2. Cell viability depends on the interval length between the fractions of UVA

Based on the flow cytometric observations we examined the influence of interval length on the cytotoxic effect of fractionated UVA. For these experiments we used 10, 30, 60, 120, and 240 min as an incubation period between each fraction. We delivered either 60 J/cm² as a total dose or separated into three equal fractions of 20 J/cm² each with intervals of 10, 30, 60, 120, and 240 min. Twelve hours after the first exposure cell viability was determined using the MTT-assay. Sham
irradiated cells were treated under the same conditions and their viability was compared to irradiated cells. In accordance with the results described above we found a decrease in viability after repetitive irradiation (three times 20 J/cm²) with intervals of 10 min between each fraction (viability in % of control: 47 ± 6%, mean ± SD), 30 min (33 ± 1%), and 60 min (33 ± 4%) compared to a viability of 57 ± 8% after exposure to a single dose of 60 J/cm². In contrast, fractionated exposure with longer intervals of 120 and 240 min led to a reversed effect: the viability only decreased to 77 ± 7% (Fig. 2).

3.3. Dose fractionation enhances the inhibitory effect of UVA on DNA replication

In further experiments we studied the influence of dose fractionation on DNA replication using a BrdU-assay. A 431 cells were irradiated with a single dose of 200

Fig. 1. FACS analysis of cell death after exposure to UV determined by annexin V staining and PI-uptake. (a) The cells were irradiated with UVA (40, 60, and 80 J/cm²) as single dose or divided into four equal fractions with an incubation period of 10 min between each fraction. (b) The cells were exposed to UVB (128, 256, and 512 mJ/cm²) delivered either as single dose or separated into four equal fractions with an incubation time of 10 min between every single fraction. Analysis was performed 24 h after the first irradiation. Percentages of annexin V+/PI− (“early apoptotic”) and annexin V+/PI+ (“late apoptotic”) cells are indicated.
48 J/cm² compared to three times 16 J/cm² with intervals of 60 and 240 min between the irradiation. For these experiments we used a lower total dose because at 60 J/cm² the proliferative capacity of the irradiated cells was too low to detect meaningful effects (data not shown). The proliferation activity was determined 72 h after the first irradiation by the BrdU assay. For fractionated irradiation with 60 min interval the proliferation activity was found to be 1.9-fold lower compared to the single dose of 48 J/cm² ($p = 0.007$, paired $t$ test).

By contrast, proliferative activity after repetitive irradiation with intervals of 240 min was comparable to single dose exposure (Fig. 3).

3.5. Influence of different antioxidants on the cytotoxic effect of fractionated irradiation

In the following experiments we determined the effects of $\alpha$-tocopherol (5, 10, 30 µM), astaxanthin (1, 10, 50 µM) and ascorbic acid (10, 50, 100 µM) on the fractionation related increase in UVA-induced cytotoxicity.

As expected cells supplemented with antioxidants showed a concentration dependent increase in viability after UVA exposure. $\alpha$-Tocopherol was found to be the most potent antioxidant followed by astaxanthin and ascorbic acid (Fig. 5). We used doses of 60 J/cm² compared to three times 20 J/cm² (60 min intervals) and could not overcome the fractionation effect by these substances.

3.6. GSH concentration and fractionated UVA

In order to study whether the GSH content is regulated by repetitive UVA irradiation the cell cultures were exposed to 16 J/cm² three times with intervals of either 60 or 240 min. At 6 h after UVA irradiation the GSH level was 12% lower for repetitive irradiation with 60 min intervals compared to the single dose. GSH content recovered significantly with time and reached single dose level at 24 h after exposure ($p = 0.02$).
In contrast, GSH depletion was less pronounced after repetitive irradiation with long intervals of 240 min and levels were even higher than following single dose exposure. Within the 24 h observation period no change of GSH levels was observed. A small but not significant recovery of GSH content was found after single dose exposure with time (Fig. 6).

3.7. Membrane damage is enhanced by repetitive UVA irradiation

TBARS are released into the supernatant of cell monolayers after UVA irradiation. TBARS content in the supernatant of sham-irradiated and irradiated cells was determined 30 min after each UVA exposure. After a single dose of 60 J/cm² TBARS reached a concentration of 105 ± 36 nmol/µg (mean ± SD). The first fraction of 20 J/cm² led to a TBARS level of 41 ± 22 nmol/µg, after the second fraction a significant higher amount of TBARS appeared in the supernatant (65 ± 16 nmol/µg). Finally, after the third fraction of 20 J/cm² the release of TBARS even tripled, reaching a concentration of 105 ± 13 nmol/µg. Since TBARS rapidly disappear from the supernatant after peaking at 30 min after UVA exposure the effect described above cannot be explained by accumulation of TBARS, but rather reflects increased oxidative membrane damage in cells that have been exposed to UVA before (Fig. 7(a)).

In contrast, fractionated irradiation with an incubation period of 240 min between the fractions of 20 J/cm² yields TBARS concentrations that are not significantly
different between fractions. Each fraction of 20 J/cm² induced release of about one third of the amount of TBARS that is released after exposure to 60 J/cm² (Fig. 7(b)).

4. Discussion

Exposure to UV induces molecular and biological events in human skin that may become immediately
manifest as sunburn and tanning and – with repeated exposure – are followed by late reactions including photoaging and skin cancer. A more desirable consequence of exposure to UV is its beneficial effect on various skin diseases that is widely utilized in clinical dermatology. Although in recent decades research has tremendously increased our knowledge of the photobiology of human skin, details as to how biological responses are influenced by dose- and wavelength-independent variations of exposure are largely unknown. In the present study we investigated the effect of dose fractionation and source-to-target distance in a human tissue culture model. The administered UV doses in our experiments are in a physiological range and can be easily acquired during noon. We found that fractionated exposure with short intervals (<2 h) to UVA but not to UVB leads to increased cytotoxicity and oxidative damage. Similar effects were observed without fractionation after prolonged exposure at decreased intensity (simulated by increasing the distance to the source). On the other hand UVA fractionation with long intervals (>4 h) causes less cell damage than an identical single dose.

A main result of our experiments is that not only the cumulative dose but also the timing of exposures is crucially important for the amount of oxidative damage to the cell. Preirradiation within a short interval prior to a second exposure increases the sensitivity of the cell to oxidative stress. This can be either explained by inhibition of the activity of antioxidative defense systems, by an increase of pro-oxidative molecules in the cell, or by both, resulting in a transient cellular state of increased sensitivity to further oxidative attack. This effect disappears with longer intervals between exposures, leading to the conclusion that the antioxidative defense system is able to recover within a few hours after UVA. Although the mechanisms of oxidative damage by UVA in keratinocytes have not been elucidated so far, it has been recently demonstrated that the intracellular level of “free” or “labile” iron plays an essential role in the promotion of the pro-oxidant condition after UVA [20,21]. Labile iron belonging to this intracellular pool can undergo redox cycling thereby generate toxic oxidants such as hydroxyl radical and lipid-derived alkoxyl and peroxy radicals [22]. Thus, under normal conditions cellular iron levels are tightly regulated.

![Graph](image-url)
Exposure to UVA leads to an immediate increase in labile iron that is correlated with increased rates of cell death [20,23]. It has been shown in keratinocytes and fibroblasts that the immediate increase in labile iron after UVA is dose dependent and sustained for 2 h after which it falls back to baseline at about 6 h. These kinetic results are in perfect agreement with our observation of an increase susceptibility to oxidative attack within the first 4 h after UVA exposure. Within this period the cell is in an iron-mediated pro-oxidative state that might be responsible for the observed enhancement of lipid peroxidation. The earlier described rapid photodegradation of ferritin by UVA further adds to this effect, since lack of this important iron storage protein inhibits iron sequestration [23].

Lipid peroxidation represents oxidative destruction of polyunsaturated fatty acids in an autocatalytic uncontrolled process finally resulting in the formation of lipid hydroperoxides and secondary products including a wide range of aldehyde compounds, a major one being malondialdehyde (MDA). Cholesterol and phospholipids in cellular membranes are main targets of lipid peroxidation and the produced MDA can lead to mutagenic DNA adducts [14,15,24]. OH\(^-\) is a potent inducer of lipid peroxidation [21] and from the fact that in our experiments \(^1\)O\(_2\) quenchers were not able to overcome the cytotoxic effect of dose fractionation we conclude that every single fraction might lead to an induction of a higher level of OH\(^-\). This is likely to be due to increased OH\(^-\) generation by accumulating levels of labile iron after repeated UVA exposure. In addition to this, the higher depletion of GSH after dose fractionation might further add to the increased formation of OH\(^-\). Depletion of the non-enzymatic antioxidant GSH after UVA irradiation and its protection from oxidation by antioxidants has been described earlier [25,26]. Moysan et al. suggest that on UVA irradiation GSH and oxidized glutathione (GSSG) may leak outside the cells because of membrane damage. This hypothesis is in agreement with our observation of GSH depletion and TBARS formation after repetitive UVA irradiation. Glutathione is considered as a free radical scavenger or as a cofactor for protective enzymes such as GPx [25]. GSH destroys OH\(^-\) by formation of GSSG and takes part in regeneration of \(\alpha\)-tocopherol. Our data support the view that repetitive UVA irradiation with short intervals leads to lower GSH level due to leakage and oxidation of GSH.

We observed that the cytotoxic effect of dose fractionation could not be overcome by non-enzymatic antioxidants \(\alpha\)-tocopherol, ascorbic acid, and astaxanthin, although all substances were able to significantly increase cell survival after UVA exposure. \(\alpha\)-Tocopherol is a lipid soluble non-enzymatic antioxidant acting as a direct antioxidant against \(^1\)O\(_2\) and \(O_2^-\) [27,28]. Ascorbic acid regenerates oxidized \(\alpha\)-tocopherol by donating a hydrogen ion to the tocopheryl ion. Ascorbic acid is not a very efficient ROS scavenger that is able to scavenge \(O_2^-\) and OH\(^-\) although in the presence of H\(_2\)O\(_2\) it stimulates OH\(^-\) formation. The overall effect will depend on concentration of ascorbic acid.

Astaxanthin, a \(\beta\)-carotenoid present in marine fish and shell fish is a strong and specific quencher of \(^1\)O\(_2\) [29]. Thus, our results indicate that \(^1\)O\(_2\) is unlikely to play a major role in the in the additional cytotoxicity of dose fractionation because the cytotoxic effect of dose fraction with short intervals could not be overcome by supplementation of a strong \(^1\)O\(_2\) quencher like astaxanthin. Possibly, \(\alpha\)-tocopherol, which scavenges \(^1\)O\(_2\) and \(O_2^-\) is not able to overcome the cytotoxic effect of dose fractionation because it might be consumed totally after quenching \(^1\)O\(_2\). The fact that \(^1\)O\(_2\) quenchers were not able to overcome the cytotoxic effect of dose fractionation gives additional evidence for the central role of other types of ROS, such as those generated in the \(O_2^-\rightarrow H_2O_2\rightarrow OH^-\) pathway.

Our finding of increasing rates of survival with intervals of more than 4 h between fractions point to protection through the effect of inducible antioxidative mechanisms in accordance with earlier reports. Poswig et al. [18] found that repetitive UVA irradiation with long intervals leads to an up-regulation of enzymatic antioxidants. Human fibroblasts were irradiated three times with a 24 h incubation period. MnSOD activity and mRNA level was determined after the first, second and third irradiation. Induction of enzymes activity and of mRNA levels was found after every single UVA irradiation. Moreover, it was found that preirradiation with low doses confers protection from cytotoxic effect of subsequent high-dose UVA irradiation. Correspondingly, Meeves et al. [17] found induction of GPx after repetitive UVA-irradiation with an interval of 12 h.

Whether the results demonstrated here have relevance for the in vivo response to UV has not been investigated so far. However, studies on UV carcinogenesis in mice indicate that the mode of exposure determines the total dose necessary to induce skin cancer and thus provide an in vivo example where – in addition to wavelength and total dose – the mode of exposure determines the biological response. Forbes et al. irradiated hairless mice with constant weekly doses of broadband UV (250-400 nm). The weekly doses were either delivered in one single dose or split into 3 or 5 equal doses given on 3 and 5 days per week, respectively [30]. The endpoint of this study was formation of skin tumors. Interestingly, the most fractionated pattern of irradiation was the most tumorigenic. This suggests that at equal doses fractionation with daily irradiation (with a low dose) increases the carcinogenic potential of UV compared to intermittent high doses. These results are in accordance with findings from DeGruijjl who noted that in a mouse
model higher cumulative doses were necessary to induce skin cancer if a high daily dose of UV-R (FS 40 sunlamps, 280–360 nm, peak 312 nm) was used in comparison to lower daily doses [31]. Taken together, these studies consistently demonstrate that time-dose reciprocity is absent for UV-carcinogenesis in mice as at equal cumulative doses intermittent high dose exposure has been found to be less carcinogenic.

In summary, we provide evidence that the modification of UVA radiation intensity at a constant dose has different effects on cell survival. Thus, we conclude that the rule of Bunsen and Roscoe, that was originally established for simple photochemical reactions, cannot be safely transferred to complex biological systems. Specifically, our results demonstrate for the first time that within a short period after UVA exposure cells are more vulnerable to a further oxidative attack. In our experimental system this phase of increased vulnerability lasts for about 4 h, beyond which the induction of antioxidative defense systems enhances antioxidative protection. This situation resembles the exposure of human skin during a sunny day at the beaches where UV exposure (that is often mitigated by sunscreens) is typically not continuous but interrupted by periods in the shade. Thus, extending the findings of this study to in vivo systems including healthy and diseased human skin might help to improve our current regimens in phototherapy and our understanding of photocarcinogenesis, skin aging, and photoprotection.

5. Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BHT</td>
<td>butylated hydroxytoluene</td>
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<tr>
<td>BrDU</td>
<td>5-bromo-2’-deoxyuridine</td>
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<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<tr>
<td>DTNB</td>
<td>5,5’-dithiobis(2-nitrobenzoic acid)</td>
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<td>GPx</td>
<td>glutathione peroxidase</td>
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<td>GSH</td>
<td>glutathione</td>
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References


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