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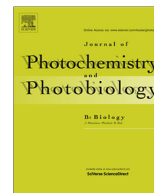

UVC inhibits collagen biosynthesis through up-regulation of NF-κB p65 signaling in cultured fibroblasts

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ABSTRACT

The effects of UVC on collagen biosynthesis, prolidase activity, expression of $\alpha_2\beta_1$ integrin, IGF-I receptor, FAK, MAP-kinases (ERK1 and ERK2) and the transcription factor NF-κB p65 were evaluated in human dermal fibroblasts. Confluent fibroblasts were treated with UVC light at a rates of 30 and 60 J/m^2. It was found that UVC-dependent decrease in collagen biosynthesis was not accompanied by parallel decrease in prolidase activity and expression. Since insulin-like growth factor receptor (IGF-IR) and $\alpha_2\beta_1$ integrin signaling are the most potent regulators of collagen biosynthesis, the effect of UVC on IGF-IR and $\alpha_2\beta_1$ integrin receptor expressions were evaluated. It was found that the exposure of the cells to UVC contributed to decrease in $\alpha_2\beta_1$ integrin receptor and FAK expression and to an increase in IGF-IR and pERK1, pERK2 expressions. It was accompanied by an increase in the expression of NF-κB p65, the known inhibitor of collagen gene expression. The data suggest that UVC-dependent decrease of collagen biosynthesis in cultured human skin fibroblasts results from decrease in $\alpha_2\beta_1$ integrin receptor signaling and activation of NF-κB p65, that is responsible for down-regulation of collagen gene expression.

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1. Introduction

The effects of UV radiation on mammalian cells have been shown to be dependent on both dose and wavelength. UVC (100–280 nm) and UVB (280–315) wavelengths are the most potent, with less biological effects observed for UVA (315–400 nm) irradiation [1,2]. The most commonly used radiation technique in studies on cells metabolism is UVB. However, there were also studies showing that UVC is a more effective wavelength or, at least, is as efficient as UVB irradiation tested for suppression of contact hypersensitivity in animal models [3,4]. In addition, a longer survival of human alloskin grafts was observed in a clinical investigation using UVC techniques [5]. UVC might be applied for clinical strategy against human colon cancers. UVC irradiation induces the removal of epidermal growth factor receptor (EGFR) from cell surface. It can protect colon cancer cells from oncogenic stimulation by EGF, resulting in cell cycle arrest [6,7]. One of major products generated in UVC-irradiated cells are reactive oxygen species (ROS), which perturbates redox balance and results in oxidative stress [8]. However, ROS are not the major cause of DNA damage in UVC-irradiated cells. It is documented that they take part in UVC-induced signal transduction pathways that modify cellular responses to UVC radiation [9–12]. Signaling is crucial for the process of collagen biosynthesis. Collagen biosynthesis in human dermal fibroblasts may depend on the activity of prolidase [13]. Prolidase [E.C.3.4.13.9] is a cytosolic enzyme which catalyses hydrolysis of imidodipeptides (mainly derived from collagen degradation), releasing proline, which is used for collagen resynthesis and cell growth [14]. Prolidase activity is stimulated through a signal mediated by collagen – β_1 integrin receptor interaction [15].

Another factor that strongly stimulates collagen biosynthesis is IGF-I, acting predominantly through the IGF-I receptor [16,17]. The effects of IGF-I include induction of collagen gene expression [18], up-regulation of prolidase activity [19], stimulation of mitotic division and prevention of apoptosis [16]. Some of these activities are regulated through NF-κB, the known inhibitor of collagen gene expression [20].

In this study, we examined the effect of UVC on cell viability, collagen biosynthesis and prolidase activity and expression, expression of $\alpha_2\beta_1$ integrin, IGF-I receptor, FAK, MAP kinases (ERK1 and ERK2) and the transcription factor – NF-κB p65 in human dermal fibroblasts.

2. Materials and methods

Alkaline phosphatase-labeled anti-mouse IgG, anti-rabbit IgG and anti-goat IgG antibodies, bacterial collagenase, Fast BCIP/NBT reagent, L-glycyl-proline, L-proline, monoclonal (rabbit) anti-FAK antibody, monoclonal (mouse) anti-IGF-IR antibody, monoclonal (mouse) anti-phosphorylated MAPK, polyclonal (rabbit) anti-IκB-α antibody, H_2O_2 and dithiothreitol were provided by Sigma Corp.,

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USA, as were most other chemicals and buffers used. Polyclonal (rabbit) ERK1/2 antibody was purchased from Cell Signaling, USA. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were products of Gibco, USA. Penicillin and streptomycin were obtained from Quality Biologicals Inc., USA. Nitrocellulose membrane (0.2 μm), sodium dodecylsulphate (SDS), polyacrylamide, molecular weight standards and Coomassie Brilliant Blue R-250 were received from Bio-Rad Laboratories, USA. L-5[^{3}H] proline (28 Ci/mmol) was purchased from Amersham, UK. Monoclonal (mouse) anti-β_1 and polyclonal (rabbit) anti-α_2-integrin antibodies, polyclonal (rabbit) NF-κB, polyclonal (goat) anti-β-actin antibody were the products of Santa Cruz Biotechnology Inc., USA. Polyclonal anti-human prolidase antibody was donated by Dr. James Phang (NCI-Frederick Cancer Research and Development Center, Frederick, MD, USA). A highly selective inhibitor of MEK1 and MEK2 (U0126) was purchased from Calbiochem, USA.

2.1. Tissue culture

All studies were performed on normal human skin fibroblasts (CRL-1474), that were purchased from American Type Culture Collection, Manassas, VA, USA. The cells were maintained in growth medium (DMEM supplemented with 10% fetal bovine serum (FBS), 2 mmol/L glutamine, 50 U/mL penicillin, 50 μg/mL streptomycin) at 37 °C in a 5% CO_2 incubator. Cells were counted in hemocytometer and cultured at 0.8×10^6 cells per 60 mm plate in 3 ml of growth medium (Costar). Cells reached confluence at day 4 and in most cases such cells were used for assays. Cells were used in the 8–14th passages.

2.2. UV exposure

Confluent cells after medium removal were washed once with PBS, covered with thin layer of fresh PBS and irradiated at 30 J/m^2 and 60 J/m^2 using Uviline CL508 crosslinker (Uvitec, United Kingdom), which deliver UVC at 254 nm. Time of irradiation was 1.5 and 3 s respectively. After that PBS was removed and new portion of growth medium was added for next 24 h. For control cells the same procedure was applied without UVC irradiation.

2.3. Cell viability assay

The assay was performed according to the method of Carmichael [21] using 3-(4,5-di-methylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT). UVC-irradiated cells were cultured 24 h in plates, washed three times with PBS and then incubated for 4 h in 1 mL of MTT solution (0.5 mg/mL of PBS) at 37 °C. The medium was removed and 1 mL of 0.1 mol/L HCl in absolute isopropanol was added to attached cells. Absorbance of converted dye in living cells was measured at a wavelength of 570 nm. UVC-irradiated cells viability was calculated as a percent of control cells.

2.4. Determination of prolidase activity

The activity of prolidase was determined according to the method of Myara et al. [22]. Protein concentration was measured by the method of Lowry et al. [23]. Enzyme activity was reported as nanomoles of proline released from synthetic substrate, during one minute per milligram of supernatant protein of cell homogenate.

2.5. Collagen production

Incorporation of radioactive precursor into proteins was measured after labeling of confluent, UVC-irradiated cells in growth medium for the last 24 h with 5[^{3}H] proline (5 μCi/mL and 28 Ci/mM) as described previously [24]. Incorporation of tracer into collagen was determined by digesting proteins with purified *Clostridium histolyticum* collagenase, according to the method of Peterkofsky [25]. Results are shown as combined values for cell plus medium fractions.

2.6. Preparation of nuclear and cytoplasmic protein extracts

Nuclear and cytoplasmic protein extracts were prepared using NE-PER Nuclear and Cytoplasmic Extraction Kit (Pierce Biotechnology, USA) according to the manufacturer's instructions.

2.7. SDS–PAGE

Slab SDS/PAGE was used, according to the method of Laemmli [26], by using 10% SDS–polyacrylamide gel.

2.8. Western immunoblot analysis

After SDS–PAGE, the gels were allowed to equilibrate for 5 min in 25 mmol/L Tris, 0.2 mol/L glycine in 20% (v/v) methanol. The protein was transferred to 0.2 μm pore-sized nitrocellulose at 100 mA for 1 h by using a LKB 2117 Multiphor II electrophoresis unit. The nitrocellulose was incubated with: polyclonal antibodies against prolidase and ERK1/2, monoclonal anti-β_1 and polyclonal anti-α_2-integrin and anti IκB-α antibodies at concentration 1:1000; polyclonal antibody against NF-κB and β-actin at concentration 1:3000; monoclonal anti-FAK antibody at concentration 1:1000; monoclonal antibodies against phosphorylated-MAPK protein at concentration 1:5000; monoclonal anti-IGF-IR antibody at concentration 1:1000 in 5% dried milk in TBS-T (20 mmol/L Tris-HCl buffer, pH 7.4, containing 150 mmol/L NaCl and 0.05% Tween 20) for 1 h. In order to analyze β_1 integrin subunit, FAK, IGF-IR and phosphorylated MAP kinases second antibody-alkaline phosphatase conjugated, anti-mouse IgG (whole molecule) was added at concentration 1:7500 in TBS-T; in order to analyze prolidase, ERK1/2 proteins, α_2-integrin subunit, IκB-α anti-rabbit IgG (whole molecule) alkaline phosphatase conjugated was added at concentration 1:5000, and in order to analyze β-actin second antibody–alkaline phosphatase conjugated, anti-goat IgG (whole molecule) was added at concentration 1:5000 in TBS-T and incubated for 30 min slowly shaking. Then nitrocellulose was washed with TBS-T (5×5 min) and submitted to Sigma-Fast BCIP/NBT reagent. The intensity of the bands was quantified by densitometric analysis using apparatus for gel documentation BioSpectrum Imaging System (UVP, USA) and presented in arbitral units normalized for β-actin.

2.9. Microscopic imaging

Phase contrast images of fibroblasts were acquired 24 h after irradiation using a Nikon Eclipse TS100-F inverted microscope equipped with DS-Fi1c cooled camera and NIS-Elements F software.

2.10. Statistical analysis

In all experiments, the mean values for three independent experiments done in duplicates ± standard deviation (S.D.) were calculated, unless otherwise indicated. The results were submitted to statistical analysis using the Student's *t*-test, accepting $P < 0.05$ as significant.

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