BYSTANDER EFFECT INDUCED BY UVA, UVB AND UVC RADIATION IN NORMAL HUMAN DERMAL FIBROBLAST

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Abstract

Bystander effect, the phenomenon which appears as biological change (mostly destructive) in cells not directly exposed to radiation but being the neighbors of irradiated cells is an important component of ionizing radiation. However, knowledge of bystander effect in the case of ultraviolet (UV) is very limited. UV spectrum is composed of three major bands, long wave (UVA), middle wave (UVB) and short wave (UVC), whose mechanisms of action are different. The aim of current studies was to compare response of skin cells to direct ultraviolet radiation and bystander effect in vitro. The endpoints used were: proliferation activity, apoptosis, necrosis and reactive oxygen species as potential mediators of bystander effect. Results indicate that bystander effect differs in dependence on UV wavelength.

1. Introduction

Radiation induced bystander effect is a phenomenon induced in cells which were not directly hit by radiation, but were exposed to signals released from irradiated cells [1]. Originally, this effect (especially damaging) has been detected in the case of ionizing radiation, particularly after low dose of alpha particles, but also after small doses of X-rays or gamma rays. The transmitted factors are free radicals, reactive oxygen species, nitric oxide, interleukin 8, tumor necrosis factor α (TNF-α) and end-products of lipid peroxidation . The signal can be transmitted through gap junctions (the cells are in close contact), or through culture medium (the cells are separated from each other) [1,2]. Bystander effect reveals in non- irradiated neighboring cells as cell death, chromosomal aberrations, reduced clonogenic survival, increased sister chromatid exchanges, formation of micronuclei, DNA strand breaks, apoptosis, changes in transcript level and gene expression [1,3].

However, recently a few publications show that bystander effect is induced by ultraviolet radiation (UV), of which the main source is the Sun. UV light emitted by the Sun is divided into three bands: UVA (320- 400nm), UVB (280- 320nm) and UVC (200-290nm). Of the solar UV radiation reaching the Earth ~95% is UVA and ~5% is UVB.[4]. UVC doesn't reach the earth's surface, because the shortest UV wavelengths are almost completely absorbed by ozone and molecular oxygen in the upper atmosphere [5], excluding the area where ozone layer is destroyed. The short wave radiation is especially dangerous for cells, because its band coincides with DNA, RNA and protein absorption spectra and induced direct damage in these molecules.

UV radiation is responsible for the induction and promotion of basal and squamous cell skin cancer [6], and is also an important etiological factor in malignant melanoma [7]. However the knowledge about participation of bystander effect in UV carcinogenesis is unknown. The aim of our study is comparison of bystander effect in normal human dermal fibroblast after exposition to different bands of UV radiation in a co-incubation system allowing permanent mutual signaling to some extent resembling situation in vivo. The cellular response (apoptosis, necrosis, proliferation) and potential molecular mediators of bystander effect (reactive oxygen species) were studied.

2. Material and methods

2.1 Cell line and experimental protocol

Normal dermal human fibroblast (NHDF) was obtained from the bank of the Center of Oncology-Gliwice. Cells were grown in Dulbecco's modified Eagle's DMEM/F12 HAM medium, supplemented with 12% of fetal bovine serum and 80μg/ml gentamicin, and were incubated at 37^{0}C in humidified air containing 5% CO_2. The transwell co-incubation system (culture plate with special insert) was used in the studies. 20 hours before irradiation cells were seeded (100 000 cells/well in 2 ml medium) into 6-well dishes. The same number of cells was seeded on inserts which were not designed to be irradiated. Transparent bottom of the insert allows the diffusion of the medium components through 0.4 μm pore size, but does not allow the

direct contact of both type of cells. Before irradiation medium was removed. Cells in wells were irradiated with different doses of UVA, UVB and UVC generated by UV Crosslinkers. Immediately after irradiation inserts were put into wells, and cells were co-cultured for required time before proper tests.

2.2 Proliferative activity (MTS) assay

MTS, a colorimetric method, in which viable cells reduces tetrazolium in colored formazan was used to determine the number of viable cells in wells and inserts. After 24h co-incubation, cells were harvested separately from wells and inserts and MTS test was performed according to manufacture procedure. Absorbance was measured at 490 nm using Elx800 universal plate reader.

2.3 Measurement of reactive oxygen species (ROS)

Reactive oxygen species, which are considered signaling molecules of bystander effect were estimated using 2',7'-dichlorofluorescein diacetate (DCFH-DA). This agent penetrates cell membranes and is deacetylated by intracellular esterases during incubation at 37°C. The non fluorescent DCFH is then oxidized by ROS to DCF and become fluorescent. After co-incubation of irradiated (UVA 20 KJ/m², UVB 10 KJ/m² and UVC 200J/m²) and bystander cells for 3, 6 and 12 h the ROS was determined by flow cytometry (Becton Dickinson FACS Canto) using the FITC configuration (488 nm laser line, LP mirror 503, BP filter 530/30) and expressed in arbitrary units.

This method is suitable for measurement of total cellular ROS. Independently superoxide radical anions were determined using MITOSOX (Molecular Probes).

2.4 Apoptosis and necrosis assays

Apoptosis and necrosis was assessed by flow cytometry using Annexin V - FITC Apoptosis Kit. Cells were harvested in required time, washed with PBS, suspended in annexin V-staining buffer, and propidium iodide (PI) and annexin V-FITC, incubated for 15 min and distribution of living, apoptotic and necrotic cells was measured by cytometry.

3. Results and discussion

In order to obtain comparable results for three bands of UV radiation we chose the different dose ranges; UVA: 5 - 20 kJ/m², UVB: 2 - 10 kJ/m² and UVC: 50 - 200 J/m² based on published data [8]. The proliferation activity measured by MTS assay showed decreasing survival with increasing doses of three bands (Fig.1).

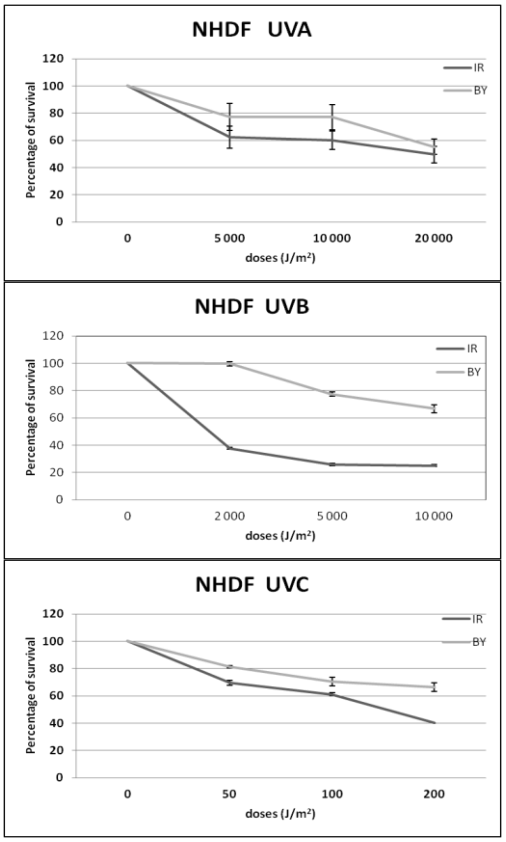

Fig.1. Proliferative activity of NHDF exposed to radiation and bystander cells estimated in MTS assay after 24 h post radiation co-incubation.

Results indicate that NHDF are the most sensitive to UVB at doses studied. However, bystander effect expressed as diminution of proliferative activity in comparison with direct effect is relatively lower after UVB. Our results are in some agreement with Whiteside and McMillian [9] who found in human keratinocytes (HaCaT) and fibroblasts (MRC5) that UVA at dose of 100 kJ/m² induces bystander effect in unirradiated cells as a reduction in clonogenic cell survival. However, UVB even in the dose 400J/m² showed no presence of bystander effect.

Reactive oxygen species which are induced by UV radiation can interact with DNA, proteins and lipid membranes leading to cellular damage. The results (Fig.2) show that ROS level appear in directly irradiated and bystander cells after irradiation with UV- A, B and C. The highest level of ROS is observed in cells directly exposed to UVA radiation, especially at 3 h. UVB is slightly less effective in ROS induction in exposed cells. Minor increase of ROS is also seen in bystander cells for UVA and UVB radiation. Results for UVC are rather unexpected (Fig. 2, low panel). We can't observe increase of ROS over control in directly irradiated cells. However, very high rise of ROS appeared in bystander cells, especially after 12 h. Observed phenomenon needs confirmation in repeated experiments.

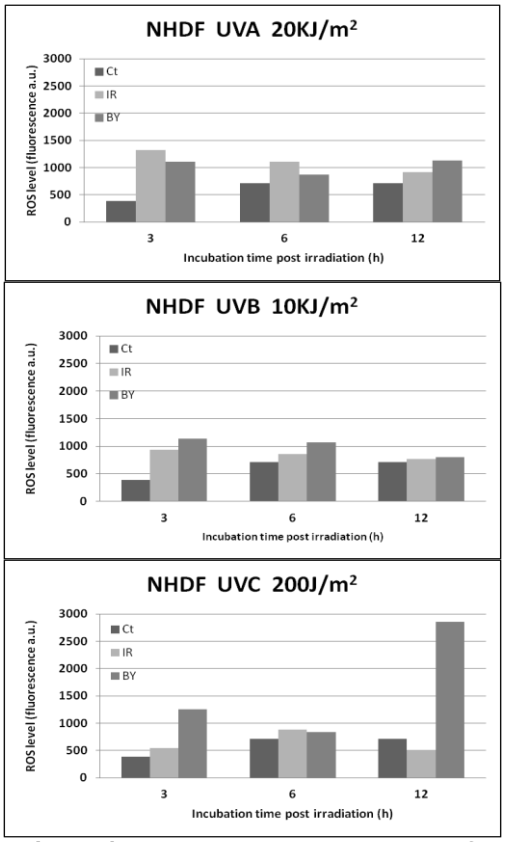

Fig.2. Flow cytometry measurement of intracellural ROS level in exposed to the highest doses of UV radiation and bystander cells.

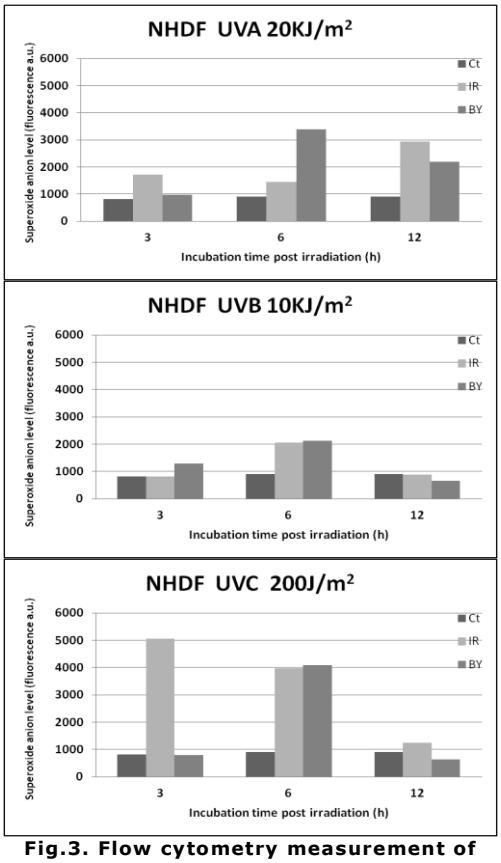

Fig.3. Flow cytometry measurement of superoxide radical anion level in exposed to radiation and bystander cells after UV radiation.

Whereas DCFH-DA measures total intracellular ROS, the MITOSOX selectively measures mitochondrial superoxide radical anion ($O_2^{\cdot-}$) which is generated as a byproduct of oxidative phosphorylation. The impairment of respiratory chain in mitochondria is thus a main source of $O_2^{\cdot-}$. The highest level of $O_2^{\cdot-}$ is observed after UVC irradiation in directly exposed cells after 3 h, and in bystander cells it is shifted in time and appeared after 6 h (Fig.3, low panel). UVA shows slight increase of $O_2^{\cdot-}$ especially after 12h and even higher increase in bystander cells after 6h (Fig.3, upper panel). UVB is the less effective in $O_2^{\cdot-}$ induction in directly hit and bystander cells (Fig. 3, middle panel).

Analysis of apoptosis indicates that UV-A and B induce comparable level of apoptosis in irradiated fibroblast (Fig.4). The highest level is observed after 12h post irradiation. UVC induces about 3 times less apoptosis. Interestingly, no apoptosis was observed in bystander cells in the case of all three bands.

Simultaneously with apoptosis we estimated necrosis in cells exposed to three bands of UV radiation and in co-incubated bystander cells. Only UVB induced necrosis in short time after exposure (3h), which is also present in bystander cells. UVC induced small amount of necrosis also after 3h. However, UVA at doses studied did not induced damage in cells leading to necrosis (Fig.5).

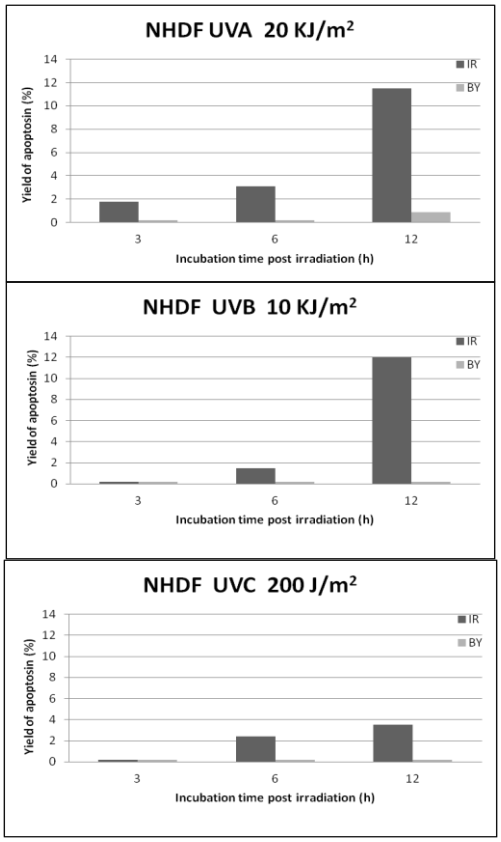

Fig.4. Yields of apoptosis in exposed to UV radiation and bystander cells after subtraction of control levels estimated in flow cytometry.

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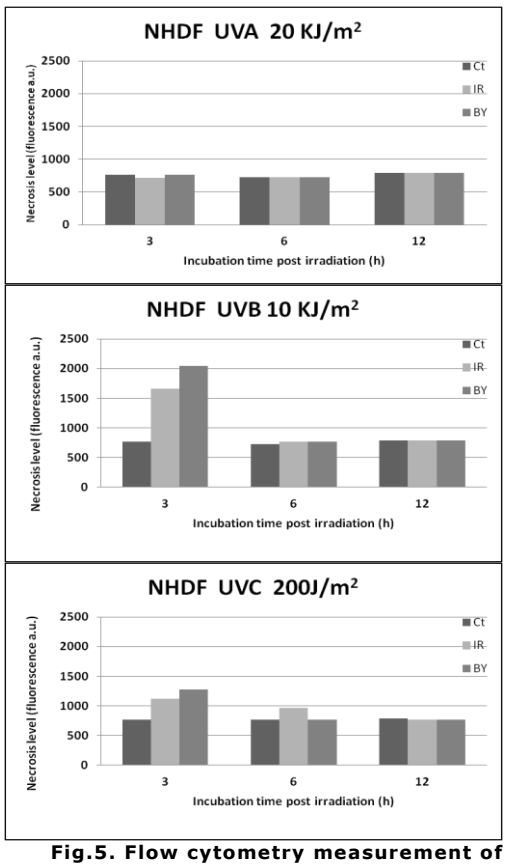

Fig.5. Flow cytometry measurement of necrosis level in exposed to radiation and bystander cells after UV radiation.

Mechanism of action of three different bands of UV radiation differ. UVA mainly acts through the generation of reactive oxygen species as singlet oxygen and hydroxyl free radicals, which can damage DNA, proteins, and lipids. UVB also can generate ROS which are mediators of bystander effect and genomic instability [10]. ROS determined in our experimental system was really generated in cells directly exposed to UVA and UVB, but not in UVC exposed cells. UVC however effectively induced ROS in bystander cells. Since UVC acts through direct damage to DNA inducing cyclobutane pirimidine dimmers (CPDs) and 6,4 photoproducts (PPs), probably bystander signals generated by UVC irradiated cells have quite different nature which needs to be elucidated. Interestingly, UVC induced high level of superoxide radical anion in comparison with UVA and UVB.

Summarizing, our results indicate that all three bands of UV radiation can induce bystander effects in non exposed cells. The nature of these effects however, can differ in dependence on UV wavelength, the dose and probably cell line. Further studies are required to gain knowledge on UV induced bystander effects and their potential hazard for human health.

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