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DNA Repair Activation and Cell Death Suppression by Plant Polyphenols in Keratinocytes Exposed to UV-irradiation

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Abstract

The work investigated effects of plant polyphenolic compounds (PPs) on responses of cultured human HaCaT keratinocytes to ultraviolet radiation in the C range (UV-C). The experimental data obtained indicate a cytoprotective effect of the PPs added immediately after UV-C exposure. The efficiency of PPs was lowered in the order: acacetin ≥ silybin > quercetin. Using the comet-assay and gamma H2AX staining followed by fluorescence microscopy it has been established that PPs accelerated H2AX phosphorylation and reduced and the number of single-strand DNA breaks in the nuclei of keratinocytes exposed to UV-C radiation. It is concluded that the PPs can diminish the destructive effect of UV radiation on the skin cells, activating the process of repairing genetic damage.

Introduction

UV radiation can affect skin cells directly damaging chromatin [1] and initiating signal processes leading to the release of pro-inflammatory cytokines that stimulate the development of inflammation. There is a substantial body of evidence supports the conclusion that chronic form of inflammation may be a causative factor in a variety of cancers [2, 3]. The results of numerous studies aimed at elucidating specific signal transduction pathways involved in UV-induced skin carcinogenesis indicate that the cellular signaling response depends on the UV wavelength. At the same time, mitogen-activated protein kinase cascades, which play an important role in the development of many UV-induced cellular responses, including the initiation of apoptosis, are activated in skin cells by all UV regions [4, 5]. If the target of UV radiation is nuclear chromatin, the DNA can directly absorb high-energy short-wavelength radiation, mainly UV-C light leading to the formation of both cyclobutane pyrimidine dimers (CPDs) between adjacent thymidine or cytosine residues as well as pyrimidine-pyrimidone (6 – 4) photoproducts ((6 – 4) photoproducts) between adjacent pyrimidine residues [1, 6, 7]. CPDs and (6 – 4) photoproducts may be also a consequence of exposure to UV-A, however, mechanism of UV-A-induced chromosomal instability is apparently different from that of short wavelength UV radiation and goes through oxidative damage of DNA mediated by reactive oxygen species [1, 8].

The cellular response to the occurrence DNA injury includes the activation of the DNA repair mechanisms [1]. One of the earliest events in DNA repair is the phosphorylation of a protein called histone H2AX. In 1998 firstly reported the phosphorylation of Ser 139 in H2AX, a variant of the core histone H2A family [9]. Upon DNA injury H2AX molecules are rapidly phosphorylated by PI3-kinases, such as ATM, ATR and DNA-PK, depending on the source of DNA damage and timing [10]. Under the action of ionizing radiation, H2AX phosphorylation started immediately after the DNA damage and reaches maximum levels after 30 min [9, 11]. However, after UV-C irradiation, H2AX phosphorylation appears within 1 h in all phases of the cell cycle. Flow cytometry of γ-H2AX content after UV exposure shows that maximum γ-H2AX content is achieved after 2 h [12].

Today, there is increasing interest in systemic and topical applications of PPs for sun protection of skin [13, 14]. Obviously, secondary plant metabolites could affect skin-UV interactions at several crucial points,

among which are: absorption of UV-light (screen action); inhibition of UV-induced free radical reactions in skin cells and extracellular matrix (direct and indirect antioxidant effects); reducing inflammatory response (anti-inflammatory effect), influence on UV-induced DNA damage and reparation. A number of publications have demonstrated antioxidant and anti-inflammatory effect of many PPs, such as green tea polyphenols [15, 16], quercetin [13, 17–19] resveratrol [13, 18–19] associated with modulation of signal transduction. However, considerably less attention has been devoted to evaluation of the influence of PPs on UV-induced DNA damage and subsequent reparation processes. Therefore, in this work we investigated effects of PPs on these responses in cultured human HaCaT keratinocytes using the comet-assay and γ-H2AX staining followed by fluorescence microscopy.

Materials And Methods

Reagents. Quercetin, Dulbecco's modified Eagle's medium (DMEM), practically all solvents, salts, and reagents were from Sigma-Aldrich (Milan, Italy). In addition, silybin and acacetin from (Extrasynthese, France), isotonic phosphate buffer pH 7.4 (PBS) from Lonza (Belgium), antibiotics from Gibco (USA), fetal bovine serum (FBS) from Capricorn (Poland). Other reagents and antibodies are mentioned herein below in the appropriate subsections. In all experiments, polyphenols were dissolved in dimethyl sulfoxide (DMSO).

Cell line. The immortalized human keratinocyte cell line HaCaT was a gift from N. E. Fusenig (Deutsches Krebsforschungszentrum, Heidelberg, Germany) and maintained in DMEM supplemented with 10% FBS, at 37°C in a humidified atmosphere containing 5% CO_2.

UV irradiation. A germicidal lamp (G 30W Sylvania), 95% of whose radiation is UV-C with a wavelength of 253.7 nm was used. The lamp was located at a distance of 10 cm from the cell plate, providing an irradiation intensity of 1.0 mW/cm^2. If not mentioned otherwise, before irradiation, the medium was replaced with PBS. Immediately after irradiation, PBS was replaced with serum-free DMEM containing DMSO (Sham-irradiated control and UV-C series) or PPs solutions in DMSO at a dose of 50 μmol/L (UV-C + PPs series).

Analysis of Cells Viability. The study of the effect of UV-C on the viability of cultured cells was carried out in 24 or 96-well plates. Cells were cultured for the specified time (18 or 24 h) after exposure under standard conditions. After 18 h the viability of cells in 24-well plates was analyzed using live/dead double fluorescent staining with acridine orange (AO) and ethidium bromide (EB) and cells were visualized and photographed using an Axiovert 25 inverted fluorescence microscope (Zeiss, Germany). After 24 h the viability of cells in 96-well plates was determined using the PrestoBlueTM Reagent (Introvigen, USA) according to the instructions. The fluorescence of resorufin was quantified on a microplate reader using an excitation of 560 nm and emission of 590 nm. The average fluorescence intensity of wells containing control cells was taken as 100%.

Immunofluorescence, Microscopy Imaging and Analysis. Cells grown on coverslips, were fixed with 10% formalin (pH 7.0) 1 h after UV irradiation, and permeabilized with 0.3% Triton X-100 for 5 min. Non-specific binding was minimized by incubating the cells in blocking buffer (PBS, 10% FBS, 1% BSA) for 1 h at 37°C, and incubated overnight at 4°C with the primary mouse monoclonal γ-H2AX (Ser-139) antibodies, 1:1,000 (Upstate Biotechnology). Secondary antibodies labeled with Alexa 488 (Molecular Probes) were added at 1:1,000, and slides were incubated at 37°C for 1 h. Cells were additionally stained with propidium iodide for nuclei visualization. Images were captured with a confocal Leica SPE microscope equipped with 63x oil-immersion objective. Immunostaining intensity of each image was quantified using the histogram tool of Photoshop software. Images used for comparison between different treatments were acquired with the same instrument settings and exposure time and were processed equally.

Analysis of DNA damage by comet assay. Cells plated in 24-well plate were cultured for 2 h after UV irradiation. Alkaline Comet-assay was performed according to Singh and Tice [20, 21]. Briefly, cells were trypsinized, 60 µl of the cell suspension of each experimental series was added to 300 µl of 0.7% low-melting agarose, and the mixture was applied to glass slides pre-coated with normal-melting agarose. The preparations were placed in a lysis buffer and kept in the dark for 20 h (4°C). Slides were then incubated with alkaline electrophoresis buffer pH 13 (0.3 M NaOH and 1 mM EDTA) for 20 min. Subsequently, electrophoresis was carried out for 20 minutes at 300 mA. The samples were washed twice in a neutralizing solution (pH 7.4, 4°C), Next, the slide was immersed in 70% ethanol for 5 min and 5 min in 96% ethanol then air dried, and stained with EB dye for 5 min. Comets were observed at 200 magnification using a fluorescence microscope Axiovert 25 (Zeiss, Germany) and documented using a digital camera. Percentage of DNA in the head (undamaged) and in the tail (damaged) was calculated for each comet using the histogram tool of Photoshop software. Data from three independent experiments were averaged for each experimental condition ($n \approx 150$ cells).

Statistical analysis. The obtained data were tabulated and analyzed by Excel program. Results are presented as means ± standard deviation (SD). Since the data were normally distributed statistical significance was evaluated using a two-tailed unpaired by Student's t-test and P values < 0.05 were considered to be significant.

Results And Discussion

PPs protection of human HaCaT keratinocytes from UV-C cytotoxicity

In preliminary experiments, the effect of UV-C radiation at doses of 0.06 J/cm^2 on the viability of human keratinocytes was studied at various time intervals after irradiation using the PrestoBlueTM Reagent. As follows from the data in Table 1, UV-C did not have a significant effect on the viability of keratinocytes 4 h after exposure, while after 24 h the number of viable keratinocytes decreases by more than 80 % compared to the non-irradiated control.

Table 1 **The effect of UV radiation on the viability of cultured keratinocytes (HaCaT) 4 and 24 h after exposure**

Experimental conditions	Percentage of viable cells	
	Time after irradiation 4 h	Time after irradiation 24 h
Sham-irradiated control	100.0 ± 14.3	100.0 ± 8.3
UV-C, 0.06 J/cm^2	89.7 ± 9.4	12.9 ± 3.9^{***a}

^{***a} - P < 0.001 *vs* sham-irradiated control

In the following experiments, we studied the cytoprotective activity of a number of PPs added to cells at a concentration of 50 μmol/L both before and after UV exposure. In the first line of experiments, cells were preincubated with PPs for 30 min, then the cells were irradiated and incubated in a medium without PPs. In the second case, PPs was added to the medium immediately after irradiation, and irradiated cells were incubated in the presence of PPs.

Table 2 **The effect of PPs (50 μmol/L) added to cells before and after UV-C radiation (0.06 J/cm^2) on the viability of cultured keratinocytes 24 h after exposure**

Experimental conditions	Percentage of viable cells	
	PPs added before UV exposure	PPs added after UV exposure
Sham-irradiated control	100.0 ± 13,5	100.0 ± 13,5
UV-C (negative control)	13.1 ± 6.1^{***a}	12.8 ± 6.1^{***a}
UV-C and acacetin	16.4 ± 7.6	39.6 ± 7.6^{***b}
UV-C and silybin	14.7 ± 2.7	38.5 ± 7.9^{***b}
UV-C and quercetin	15.8 ± 6.7	28.1 ± 6.7^{**b}

^{***a} - P < 0.0001 *vs* control; ^{**b} - P < 0,01 и ^{***b} - P < 0.001 *vs* UV-C

The data given in Table 2 indicate that PPs added after irradiation significantly increased the number of viable keratinocytes 24 h after exposure compared to UV-C irradiated cells incubated without PPs. The cytoprotective activity of PPs decreased in the following order: acacetin, silybin, resveratrol, quercetin. At the same time, PPs preincubated with cells for 30 min were ineffective.

The cytoprotective effect of acacetin and silybin against UV-C-induced cell death was confirmed by a cell viability test (Live/Dead staining) applied after 18 h. Two fluorescent dyes, AO and EB were used to distinguish between living and dead cells. The fluorescent micrographs shown in Fig. 1 demonstrate that

after exposure to UV-C at a dose of 0.06 J/cm^2 (Fig. 1b), the total number of cells significantly decreased compared to control samples (Fig. 1a), besides many UV-irradiated cells exhibited intense red fluorescence due to staining with EB, indicating injury of cell membranes. However, incubation of cells in the presence of PPs resulted in an increase in number of living cells (Fig. 1c and 1d).

Effect of acacetin added to cells after irradiation on UV-induced histone H2AX phosphorylation

The protective effect of PPs upon exposure of human keratinocytes to UV-C radiation at a dose of 0.06 J/cm2 may be due to a number of mechanisms, including an increase in the efficiency of DNA repair processes. Therefore, in following experiments, we studied the possible effect of acacetin added to cells after irradiation on UV-induced histone H2AX phosphorylation. This process is one of the earliest events in DNA repair and resulting in the appearance of phosphorylated H2AX within 1 h after UV-C exposure [12]. This time interval was chosen to assay amount of γH2AX by immunofluorescent staining using primary antibodies to γH2AX (pS139). Three separated coverslips for every experimental condition in two different experiments were used. Staining nuclei in a total of nearly 400 cells were visualized and photographed using a fluorescence microscope (Fig. 2).

Median staining intensity and mean area in pixels were measured for every nucleus. Total staining intensity of the nucleus was calculated as the result of multiplying the intensity by the area. The data shown in Table 3 indicate that UV-C irradiation caused activation of H2AX phosphorylation in keratinocytes and increase the amount of γH2AX 1 h after exposure. This results completely coincides with the data obtained earlier by T. Marty et al. [12]. It was also found that in the presence of acacetin, the process of H2AX phosphorylation was significantly accelerated and the amount of γH2AX detected in nuclei of keratinocytes 1 h after UV-C irradiation was almost twice as high as in UV-irradiated cells and four times more than in Sham irradiated control cells.

Table 3 **Effect of acacetin (50 μmol/L) added to cells after irradiation on UV-induced histone H2AX phosphorylation measured as described in "Materials and Methods"**

Experimental conditions	Median staining intensity	Median area	Median total staining intensity
Sham irradiation	1.0 ± 1.0	1.0 ± 0.5	1.0 ± 1.4
UV-C, 0.06 J/cm^2	2.6 ± 1.6 [a]	0.9 ± 0.4	2.2 ± 1.6 [a]
UV-C and acacetin	4.2 ± 2.0 [b]	1.0 ± 0.4	3.9 ± 2.9 [b]

*- The data are presented in arbitrary units (AU) normalized to sham irradiation as mean ± SD for the nucleus

[a] - P < 0.00000001 *vs* control; [b] - P <0.00000001 *vs* UV-C

Effect of PPs added to cells after irradiation on UV-induced DNA lesions

It is known that impact of UV-C irradiation on cellular DNA leads to DNA single-strand damage as a result of fast photochemical processes. The cellular response to DNA damage is the activation of repair mechanisms. The data presented in the previous section can be considered as evidence of the ability of PPs and, in particular, acacetin to activate and accelerate reparative DNA synthesis after exposure to UV-C. This phenomenon may result in a decrease in the amount of CPDs and (6-4)-photoproducts in damaged DNA. Consequently, in the following experiments we studied the amount of CPDs and (6-4)-photoproducts in nuclear DNA keratinocytes which were incubated with and without PPs after UV-C irradiation. Among the most common and accuracy methods applied to evaluate the diverse effects of genotoxic agents and to identify the gene-protective effect of potential pharmacological drugs is the comet assay. It was first developed by Ostling and Johanson in 1984 [22]. Alkaline version of the comet assay uses alkaline denaturation surrounding a DNA break to reveal the break (single or double) [23]. This technique we applied to estimate the gene-protective effect of PPs. Irradiated cells were harvested after 2 h cultivation with or without PPs and were treated as described in Materials and methods. Stained slides were observed using a fluorescence microscope and photographed using a digital camera (Fig. 3). Percentage of DNA in the head (undamaged) and the tail (damaged) was scored after pooling the data from three independent experiments (Table 4).

Table 4 **Effect of PPs (50 μmol/L) added to cells after irradiation on percentage of DNA in the "comet head" and the "comet tail" 2 h after keratinocytes exposed to UV-C**

Experimental conditions	Percentage of DNA in head	Percentage of DNA in tail
Sham irradiation	98.1 ± 0.01	1.9 ± 0.01
UV-C, 0.06 J/cm ^{2}	30.0 ± 12.4*** [a]	70.0 ± 12.4*** [a]
UV-C and quercetin	44.3 ± 19.7*** [б]	55.7 ± 19.7*** [б]
UV-C and silybin	41.8 ± 16.3*** [б]	58.2 ± 16.3*** [б]
UV-C and acacetin	39.7 ± 14.2** [б]	60.3 ± 14.2** [б]

***[a] - P < 0.0000001 *vs* Sham irradiation; **[б] - P <0.01; ***[б] - P <0.001 *vs* UV-C

According to the data showing in Fig. 3 and Table 4, UV-C irradiation resulted in DNA damage, which was manifested by the increasing the percentage of DNA in the comet tail to 70 % 2 h after exposure. All

polyphenols studied were partially able to eliminate DNA lesions as evidenced by the decrease in “comet tails” of damaged cells.

It is common knowledge that the main UV components of terrestrial radiation from sun are UV-A (95 %) and UV-B (5 %) whereas UV-C are completely absorbed by the earth's atmosphere. Therefore, for studying biological effects of solar UV radiation usually used medium and long wavelength UV light (280 to 400 nm). However, cells exposure to UV-C radiation may be useful for studying DNA reparation as a target for testing drug effects. Due to that exposure to UV-C radiation leads to direct photo-damage of DNA, this approach allows excluding the formation of a significant amount of CPDs and 6-4 photoproducts as a result of oxidative damage and therefore, to ignore antioxidant activity of tested compounds. In order to avoid screening effects, cells were irradiated with UV in the absence of PPs.

The cellular response to single-stranded DNA damage is the initiation of cell cycle arrest and DNA repair using an intact complementary strand as a template [1]. However, if complete repair does not occur, intrinsic signals resulting in apoptotic cell death [24]. In our experiments, keratinocytes were irradiated with UV-C at a dose of 0.06 J/cm^2 and significant cell death occurring 24 h after. The cells death was interfered by the addition in culture media of PPs after the irradiation. To clarify the possible mechanism underlying the anti-apoptotic effect of PPs we used comet-assay and gamma H2AX staining followed by microscopy. The data received indicate that All PPs were able to decrease the number of radiation-induced photoproducts in the genomic DNA and suggest that this occurs via the acceleration of DNA repair.

In summary, inhibition of cell death without DNA protection may be responsible for the increased incidence of skin tumors after UV irradiation [25, 26]. Oppositely the upregulation of DNA repair pathways resulting in reduced UV-associated mutation. Therefore PPs, which manifested both cell death suppression and upregulation of DNA repair may be suggested as agents for pharmaceutical applications as well as perfect ingredients for solar cosmetic products.

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Declarations

Authors’ Disclosure Statement

Conflict of Interests

The authors declare no conflict of commercial interests.

Authors’ Contribution

VK conceived and supervised the study; VK and AP defined study design, participated in planning of the experiments and wrote the manuscript; AP, TK, TS, TE performed experiments and did primary statistical

calculations.

Abbreviations

Plant polyphenolic compounds, PPs; ultraviolet radiation in the C range, UV-C; cyclobutane pyrimidine dimers, CPDs; pyrimidine-pyrimidone (6 – 4) photoproducts, (6 – 4) photoproducts; Dulbecco's modified Eagle's medium, DMEM; isotonic phosphate buffer pH 7.4, PBS; fetal bovine serum, FBS; dimethyl sulfoxide, DMSO; acridine orange, AO; ethidium bromide, EB;

References

1. Farrell AW, Halliday GM and Lyons JG (2011) Chromatin structure following UV-induced DNA damage - Repair or death? International Journal of Molecular Sciences **12**, 8063–8085.
2. Bode AM and Dong Z (2005) Signal transduction pathways in cancer development and as targets for cancer prevention. Progress in Nucleic Acid Research and Molecular Biology **79**, 237–297.
3. Adams DO and Hamilton TA (1992) Macrophages as destructive cells in host defense. *In: Inflammation: basic principles and clinical correlates.* Gallin JI, Goldstein IM, Synderman R, chief editors. New York: Raven Press, 637–662.
4. Bode AM and Dong Z (2003) Mitogen-activated protein kinase activation in UV-induced signal transduction. Science's STKE **167**, RE2.
5. Bode AM and Dong Z (2005) Signal transduction pathways in cancer development and as targets for cancer prevention. Progress in Nucleic Acid Research and Molecular Biology **79**, 237–297.
6. Setlow RB (1966) Cyclobutane-Type Pyrimidine Dimers in Polynucleotide. Science **153**, 379–386.
7. Rolfsmeier ML, Laughery MF and Haseltine A (2010) Repair of DNA Double-Strand Breaks following UV Damage in Three Sulfolobus solfataricus Strains and Cynthia. J Bacteriology **192**, 4954–4962.
8. Dahle J and Kvam E (2003) Induction of delayed mutations and chromosomal instability in fibroblasts after UVA-, UVB-, and X-radiation. Cancer Res **63**, 1464–1469.
9. Rogakou EP, Pilch DR, Orr AH, Ivanova VS and Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. Journal of Biological Chemistry **273**, 5858–5868.
10. Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S and Pommier Y (2008) GammaH2AX and cancer. Nature reviews Cancer **8**, 957–967.
11. Rogakou EP, Boon C, Redon C and Bonner WM (1999) Megabase chromatin domains involved in DNA double-strand breaks in vivo. The Journal of cell biology **146**, 905–916.
12. Marti TM, Hefner E, Feeney L, Natale V and Cleaver JE (2006) H2AX phosphorylation within the G1 phase after UV irradiation depends on nucleotide excision repair and not DNA double-strand breaks. *Proceedings of the National Academy of Sciences* **103**, 9891–9896.

13. Kostyuk VA, Potapovich AI, Albuhaydar AR, Mayer W, De Luca C and Korkina LG (2018) Natural Substances for Prevention of Skin Photo-Ageing: Screening Systems in the Development of Sunscreen and Rejuvenation Cosmetics. Rejuvenation Res **21**, 91–101.
14. Korkina L, Kostyuk V, Potapovich A, Mayer W, Talib N and De Luca C (2018) Secondary Plant Metabolites for Sun Protective Cosmetics: From Pre-Selection to Product Formulation. Cosmetics **5**, 32–52.
15. Liu ML and Yu LC (2008) Potential protection of green tea polyphenols against ultraviolet irradiation-induced injury on rat cortical neurons. Neurosci Lett **444**, 236–239.
16. Wu LY, Zheng XQ, Lu JL and Liang YR (2009) Protective effect of green tea polyphenols against ultraviolet B-induced damage to HaCaT cells. Hum Cell **22**, 18–24.
17. Vicentini FTMC, He T, Shao Y, Fonseca MJV, Verri WA, Fisher GJ and Xu Y (2011) Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF-kB pathway. J Dermatol Sci **61**, 162–168.
18. Pastore S, Potapovich A, Lulli D, Fidanza P, Kostyuk V, De Luca C, Mikhal'chik E and Korkina L (2012) Plant Polyphenols Regulate Chemokine Expression and Tissue Repair in Human Keratinocytes Through Interaction with Cytoplasmic and Nuclear Components of Epidermal Growth Factor Receptor (EGFR) System. Antioxid Redox Signal **16**, 314–328.
19. Potapovich AI, Kostyuk VA, Kostyuk TV, de Luca C and Korkina LG (2013) Effects of pre- and post-treatment with plant polyphenols on human keratinocyte responses to solar UV. Inflamm Res **6**, 773–780.
20. Singh NP, McCoy MT, Tice RR and Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res **175**, 184–191.
21. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E., Ryu JC and Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen **35**, 206–221.
22. Ostling O and Johanson KJ (1984) Microelectrophoretic study of radiationinduced DNA damages in individual mammalian cells. Biochem Biophys Res Commun **123**, 291–298.
23. Collins AR, Dobson VL, Dušinská M, Kennedy G and Štětina R (1997) The comet assay: What can it really tell us? Mutat Res **375**, 183–193.
24. Norbury CJ and Zhivotovsky B (2004) DNA damage-induced apoptosis *Oncogene* **23**, 2797–2808.
25. Dose M, Emmanuel AO, Chaumeil J, Zhang J, Sun T, Germar K, Aghajani K, Davis EM, Keerthivasan S, Bredemeyer AL, Sleckman BP, Rosen ST, Skok JA, Le Beau MM, Georgopoulos K and Gounari F (2014) β-Catenin induces T-cell transformation by promoting genomic instability. Proc Natl Acad Sci USA **111**, 391–396.
26. Gang Li, David L. Mitchell, Vincent C. Ho, John C. Reed, and Victor A (1996) Tron Decreased DNA Repair but Normal Apoptosis in Ultraviolet-Irradiated Skin of p53-Transgenic Mice. Am J Pathol **148**, 1113–1123.

Figures

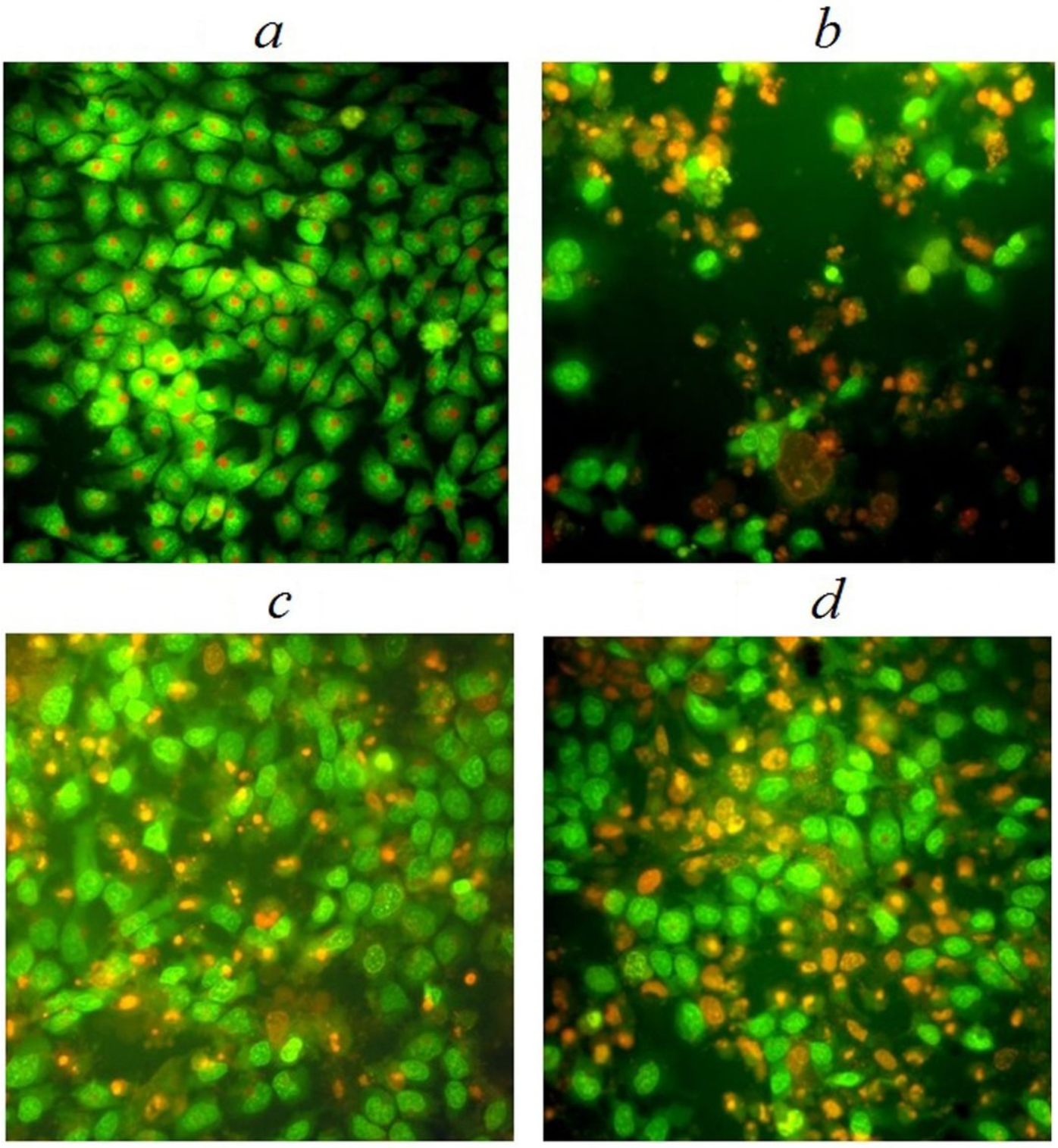

Figure 1

Representative fluorescence micrographs of the cells 18 h after exposure to UV-C at a dose of 0.06 J/cm^2. Sham-irradiated control (a), cells exposed to UV-C (negative control) (b), cells incubated after

irradiation with 50 μmol/L of acacetin (c) or of silybin (d). Cells were stained with AO and EB as described in Materials and Methods

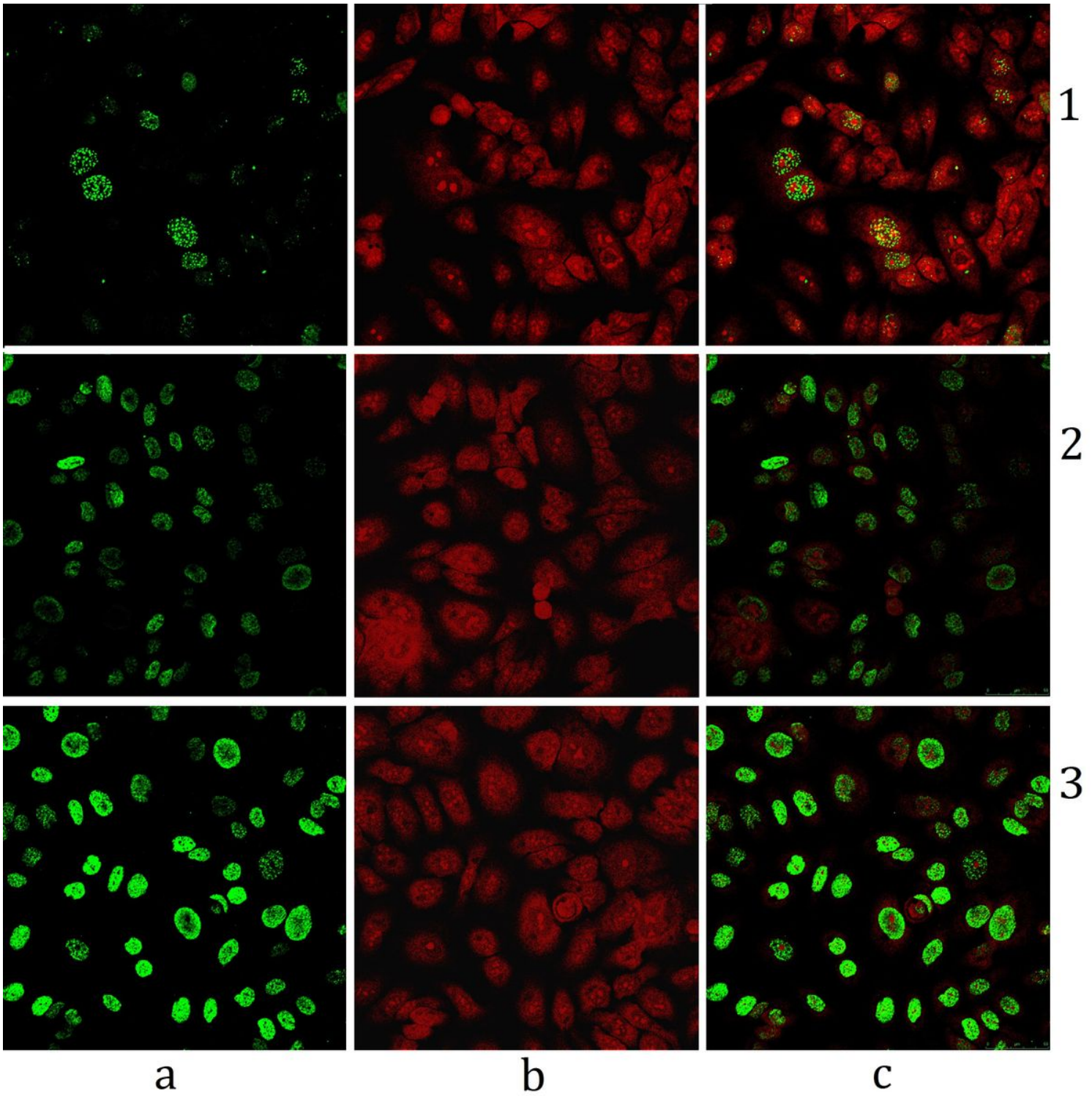

Figure 2

Representative fluorescent micrographs of HaCaT cells: 1) Sham-irradiated control, 2) 1 h after exposure to UV-C at a dose of 0.06 J/cm^2, 3) Idem as 2 but cells incubated with acacetin at a concentration of 50 μmol/L; column “a” shows staining with primary antibodies to γ H2AX and secondary antibodies conjugated with Alexa Fluor488, column “b” shows staining with propidium iodide, column “c” shows the merged images

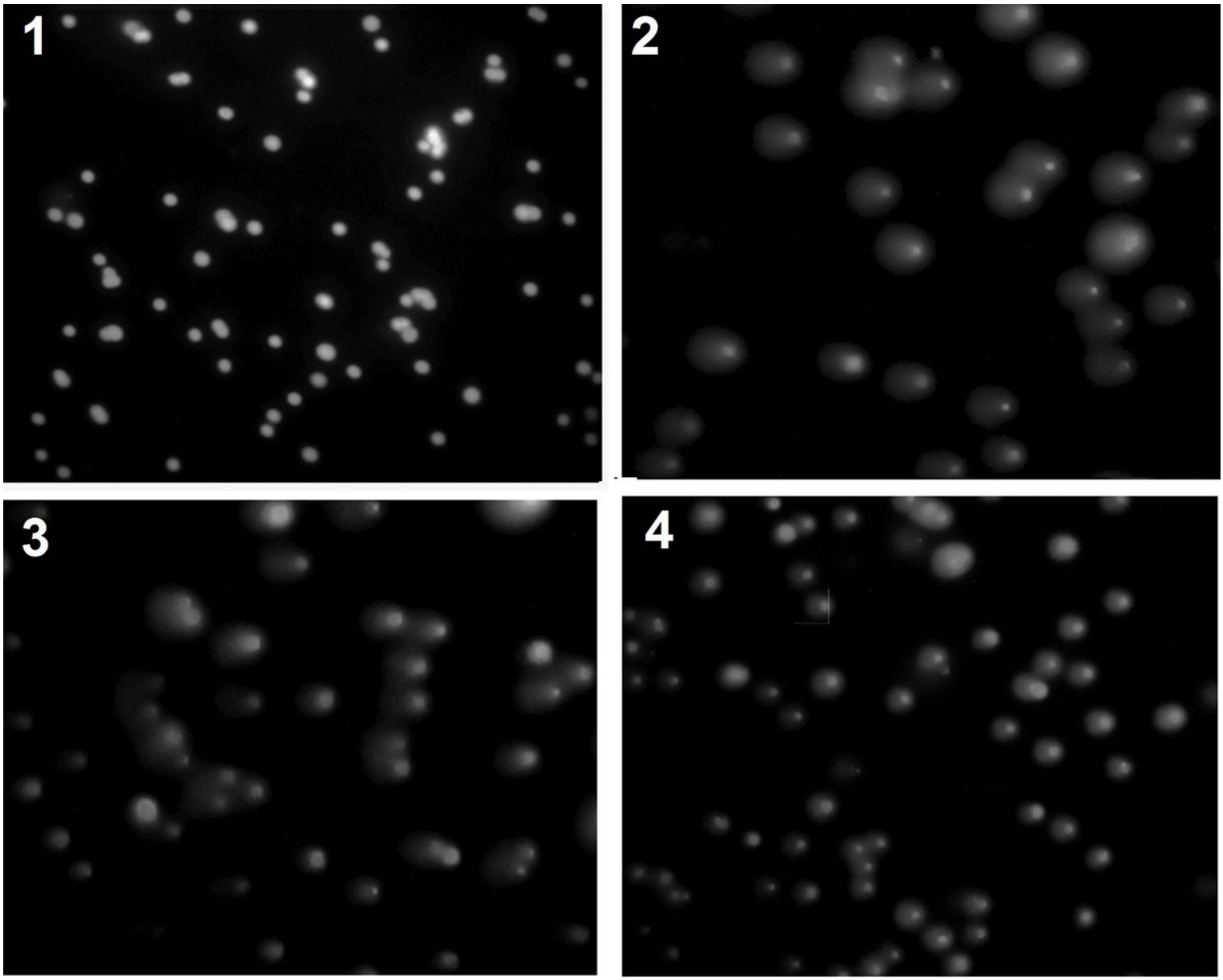

Figure 3

Representative fluorescent micrographs of DNA comets as described in Materials and Methods: 1) Sham-irradiated control, 2) 2 h after exposure to UV-C at a dose of 0.06 J/cm^2, 3) Idem as 2 but cells incubated with quercetin at a concentration of 50 μmol/L, 4) Idem as 3 but cells incubated with silybin