Effects of Native and Particulate Polyphenols on Dna Damage and Cell Viability After UV-C Exposure

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Research Article

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Abstract

Background

plant polyphenols have poor water solubility, resulting in low bioavailability. In order to overcome this limitation, the drug molecules can be coated with multiple layers of polymeric materials.

Methods

microcrystals of quercetin and resveratrol coated with a $(PAH/PSS)_4$ or $(CH/DexS)_4$ shell were prepared using the layer-by-layer assembly method; cultured human HaCaT keratinocytes were treated with UV-C and after that cells were incubated with native and particulate polyphenols. DNA damage, cell viability and integrity, were evaluated by comet-assay, using PrestoBlueTM reagent and lactate dehydrogenase (LDH) leakage test.

Results

the data obtained indicate that: both native and particulate polyphenols added immediately after UV-C exposure increased cell viability in a dose-dependent manner, however the efficiency of particulate polyphenols was more pronounced; quercetin coated with a $(PAH/PSS)_4$ or $(CH/DexS)_4$ shell more effectively than the native compound reduced the number of single-strand DNA breaks in the nuclei of keratinocytes exposed to UV-C radiation; native and particulate resveratrol were ineffective against DNA damage.

Conclusion

quercetin reduces cell death caused by UV-C radiation and increase DNA repair capacity. Coating quercetin with $(PAH/PSS)_4$ or $(CH/DexS)_4$ markedly enhanced its impact on DNA repair probably by facilitating transport into the nucleus.

Introduction

Overexposure to sunlight is directly associated with skin cancers, such as carcinomas [1, 2] and melanoma [3]. The promising future methods for treatment of these diseases include the use of topically administered sunlight protected creams containing sunscreen, antioxidant and repair enzymes [4–6]. Plants are the major source of natural ingredients used in sun protective formulations due to the high concentration of monoterpenes, flavonoids, organosulfides, and indoles [6–8]. Evidently, secondary plant metabolites and especially plant polyphenols (PPs) can prevent harmful consequences of UV-exposure by several ways, among which are: screen action; direct and indirect antioxidant action; anti-inflammatory

effect. A number of publications have demonstrated antioxidant and anti-inflammatory effect green tea polyphenols [9, 10], quercetin [8, 11–13] resveratrol [8, 12, 13]. Nevertheless, despite the numerous biological activities, the clinical use of PPs has been limited mainly due to their low water solubility, which results in low cellular uptake and poor skin permeability. In order to overcome these limitations, the drug molecules can be included into liposomes or into polymeric nanoparticles (NPs) exhibited great potential as a drug carrier [14–16]. Nanotechnology is one of the novel immense areas of science and engineering that provides progressive ways in different fields such as food, biomedical, drug delivery and cosmetics [17]. Thus, encapsulation of quercetin into chitosan NPs resulted in higher aqueous solubility and constant release of the flavonoids [18]. Quercetin-loaded lecithin-chitosan NPs were developed for topical application to enhance the permeation of the drug in the epidermal layer of the skin [19].

DNA can absorb high-energy short-wavelength radiation, mainly UV-C light, resulting in direct chromatin modifications and 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 [20–22]. Exposure to UV-A can also lead to chromosomal instability, however, that is associated with oxidative damage of DNA mediated by reactive oxygen species [20, 23]. Upon UV radiation, the cell elicits DNA damage response to repair the damage or in case of excessive damage to programmed cell death [24, 25]. Recently, we have reported that PPs can diminish the destructive effect of UV radiation on the skin cells, activating the process of repairing genetic damage [26]. In this work we compared effects of native and particulated forms of polyphenols on DNA damage and cell viability after exposure of HaCaT keratinocytes to UV-C.

Materials And Methods

Reagents. Quercetin (Qr), polystyrene sulfonate sodium, 70 kDa (PSS), polyallylamine hydrochloride, 58 kDa (PAH), chitosan of medium molecular weight, 450 kDa, degree of deacetylation 75–85% (CH), dextran sulfate, > 500 kDa (DexS), Dulbecco's modified Eagle's medium (DMEM), ethidium bromide (EB), practically all solvents, salts, and reagents were from Sigma-Aldrich. In addition, resveratrol (Res) from Biomol (Research Lab, Plymouth, MA), isotonic phosphate buffer pH 7.4 (PBS) from Lonza (Belgium), antibiotics from Gibco (USA), fetal bovine serum (FBS) from Capricorn (Poland) were used. Other reagents are mentioned herein below in the appropriate subsections.

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.

Preparation of layer-by-layer shell on polyphenol microcrystals. A multilayer polyelectrolyte shell on polyphenol microcrystals was prepared using the layer-by-layer assembly method [27, 28]. Briefly, polyphenol powder was dispersed in distilled water to obtain a 10 mg/mL suspension. An aliquot of a 5 mg/mL PAH or chitosan solution was added in the amount of 0.2 mL per 1 mL of the initial suspension and thoroughly dispersed for 5 min in an ultrasonic bath. After the adsorption of the polymer layer for 15

min, the microcrystals were washed with water twice to remove unadsorbed polyelectrolyte and redispersed in a volume of water equal to the initial one. The next PSS or DexS layer was deposited on the surface of microcrystals in a similar way. The adsorption of positive and negative polymers was repeated n times to obtain n bilayers. Finally, shell-coated microcrystals were redispersed in 0.9% NaCl solution in a concentration of 10,0 mg/mL. The concentration of quercetin or resveratrol in a suspension of coated microcrystals (polyphenol/$(CH/DexS)_4$ and polyphenol/$(PAH/PSS)_4$) was determined spectrophotometrically, after extraction with ethanol.

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. Before irradiation, the medium was replaced with PBS. Immediately after irradiation, PBS was replaced with serum-free DMEM (Sham-irradiated control and UV-C series), native and particulate polyphenols at a dose of 50 μmol/L (UV-C + polyphenols series).

Analysis of cells viability. The study of the effect of UV-C on the viability of cultured cells was carried out in 96-well plates. After 20 h the viability of cells 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%.

Cell integrity was evaluated by lactate dehydrogenase (LDH) leakage. In these experiments, cells were grown in 24-well plates. The activity of LDH was measured using the direct spectrophotometric assay in 1 ml of phosphate buffer (pH 7.4) containing 30 μmol/L pyruvate and 30 μmol/L NADH. 100 μl of culture medium were added and changes in optical density were measured at 340 nm for 2 min. The percentage of LDH release was calculated by dividing the activity of LDH in the medium by the LDH activity measured after complete cell lysis. None of the detergents and drugs affected LDH activity, when added directly to the reaction mixture at the concentrations used in whole cell experiments.

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 [29–31]. Briefly, cells were trypsinized, 50 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 min 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 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

Properties Of Lbl Coated Quercetin And Resveratrol Microcrystals

Layer-by-layer (LbL) assembly is an effective technique to form a thin polymeric shell around micro/nanoparticles based on sequential adsorption of electrostatically interacting polymers, moreover, hydrogen bonds and hydrophobic interactions also promote the adsorption of layers. The thickness of the shell is commonly controlled by the number of deposited bilayers n [32–33]. LbL coated quercetin microcrystals used in our experiments are characterized by a narrow size distribution (Fig. 1A, B). The number average hydrodynamic diameter of the microcrystals coated with a $(PAH/PSS)_4$ or $(CH/DexS)_4$ shell is in the range of 710–825 nm (up to 47% of the total number of particles) and 615–715 nm (up to 75%), respectively. Resveratrol microcrystals are characterized by a slightly wider size distribution (Fig. 1C, D). The hydrodynamic diameter of resveratrol coated with a $(PAH/PSS)_4$ or $(CH/DexS)_4$ shell is 1110–1485 nm (up to 63% of the total number of particles) and 615–825 nm (up to 65%), respectively.

Protection Of Human Hacat Keratinocytes Against Uv-c Cytotoxicity By Native And Particulate Polyphenols

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 (from 4 h to 20 h) using the PrestoBlueTM Reagent. The most significant effect was found after 20 h exposure, the number of viable keratinocytes decreases by more than 80% compared to the non-irradiated control (data not shown). In the following experiments, the cytoprotective activity of native and particulate polyphenols was studied 20 h after UV-C treatment. Preparations were added to cells at various concentrations immediately after UV irradiation. It was found that quercetin, resveratrol and their crystals with $(PAH/PSS)_4$ and $(CH/DexS)_4$ shells showed a dose-dependent protective effect up to 50 µmol/L in the case of native and particulate quercetin and up to 100 µmol/L in the case of native and particulate resveratrol (Fig. 2). It also needs to be stressed that comparison of the effects of quercetin with those of its nanoforms revealed that the latter are more effective in cell protection against UV-C-induced damage (Fig. 2A).

In addition to evaluation of the cell viability we also examined effects of native and particulate polyphenols on plasma membrane integrity. The LDH release assay provides fast and reliable quantification of this parameter. According to data given in Table 1 it is evident that native and particulate polyphenols almost completely reversed the effect UV-C on plasma membrane integrity estimated as the percentage of LDH release.

Table 1
Effect of native and particulate polyphenols (50 μmol/L) on the percentage of LDH release from cultured keratinocytes 20 h after exposure UV-C, 0.06 J/cm^2

Experimental conditions	LDH release, %	
	Quercetin	Resveratrol
Sham-irradiated control	8.0 ± 1,5	9.0 ± 2,1
UV-C (negative control)	79.6 ± 2.7^{***a}	73.4 ± 1.6^{***a}
UV-C and native -polyphenol	8.0 ± 4.2^{***b}	14.3 ± 9.4^{***b}
UV-C and polyphenol/(PAH/PSS)$_4$	15.5 ± 4.2^{***b}	8.0 ± 2.7^{***b}
UV-C and polyphenol/(CH/DexS)$_4$	7.0 ± 2.7^{***b}	18.7 ± 5.7^{**b}
^{***a} – P < 0.001 *vs* control; ^{***b} - P < 0.001 *vs* UV-C		

Effect Of Native And Particulate Polyphenols On Uv-induced Dna Lesions

In this line of research, we studied influence of native and particulate polyphenols on the amount of CPDs and (6 – 4)-photoproducts in nuclear DNA keratinocytes irradiated with UV-C. Cells were incubated with and without substances studied for 2 h at 37°C in 5% CO_2 atmosphere and then were harvested and subjected to alkaline version of the comet assay. This technique allows to reveal the DNA breaks [31]. Stained slides were observed using a fluorescence microscope and photographed using a digital camera (Fig. 3). Percentage of damaged DNA in the tail of the comets was scored after pooling the data from three independent experiments.

According to the data showing in Fig. 3, UV-C irradiation leaded to DNA damage, which resulted in the increase of the percentage of DNA in the comet tail near to 75% 2 h after exposure. However, when irradiated keratinocytes were incubated with quercetin, quercetin/(PAH/PSS)$_4$ or quercetin/(CH/DexS)$_4$ partial elimination of DNA lesions was found, that indicated by the decrease in percentage tail DNA (Fig. 3A). Comparison of the effects of quercetin with those of its nanoforms revealed that the latter are more effective in the elimination of DNA lesions. At the same time, neither resveratrol nor its nanoforms affected the percentage of DNA in the comet tails of HaCaT cells exposed to UV-C.

Discussion

In this work we have used UV-C radiation of 254 nm wavelength as a tool to test drugs that target DNA damage repair pathways. The advantage of this approach in contrast using hydrogen peroxide, and other oxidants lies in the fact that UV-C radiation can directly modify DNA that allows to exclude 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. 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, unrepaired DNA damage can give rise to genomic instability and induce signaling cascades, resulting in apoptotic cell death [34]. In this work, we irradiated keratinocytes by UV-C at a dose of 0.06 J/cm^2 and found significant cell injury and death 24 h after. The cellular injury and death were reduced by native and particulate polyphenols, added in culture media immediately after the irradiation. It is important to note that there is significant difference between the effect of investigated compounds on viability of cells assayed with PrestoBlueTM Reagent and on cell integrity evaluated by LDH leakage. Thus, the percentage of viable cells was increased only to 30% as compared to sham irradiation control whereas all substances studied completely prevented UV- induced LDH release. This may indicate that metabolically dead cells (which are not assessed by PrestoBlueTM) retained their cellular integrity. The protective effect of native and particulate polyphenols upon exposure of human keratinocytes to UV-C radiation may be due to a number of mechanisms, however, inhibition of cell death without DNA protection may be responsible for the increased incidence of skin tumors after UV irradiation [35, 36]. To clarify the DNA repair enhancing property of native and particulate polyphenols comet-assay followed by microscopy was used. The data received indicate that quercetin was able to decrease the number of radiation-induced photoproducts in the genomic DNA. LbL coating quercetin with $(PAH/PSS)_4$ or $(CH/DexS)_4$ enhanced its effect of DNA repair pathways. At the same time native and particulate resveratrol showed no effect on the number of UV-induced DNA photoproducts. It should also be noted' it is also noteworthy that quercetin with $(PAH/PSS)_4$ and $(CH/DexS)_4$ shells showed practically the same effects on DNA damage and cell viability after UV-C exposure.

Conclusion

Inhibition of cell death without DNA protection may be responsible for the increased incidence of skin tumors after UV irradiation. Oppositely the upregulation of DNA repair pathways resulting in reduced UV-associated mutation. Compounds which can enhance DNA repair facilities are promising for many biomedical applications. The present study was focused on the ability of native and particulate polyphenols to influence DNA repair capacity. It was found that quercetin can reduce cell death caused by UV-C radiation and decrease the number of radiation-induced photoproducts in the genomic DNA. Coating quercetin with $(PAH/PSS)_4$ or $(CH/DexS)_4$ markedly enhanced its impact on DNA repair probably by facilitating transport into the nucleus.

Abbreviations

Qr, quercetin, Res, resveratrol, DMEM, Dulbecco's modified Eagle's medium, EB, ethidium bromide, EDTA, ethylene diamine tetra-acetic acid, PBS, isotonic phosphate buffer, LDH, lactate dehydrogenase, PSS, polystyrene sulfonate sodium, PAH, polyallylamine hydrochloride, CH, chitosan, DexS, dextran sulfate,

PPs, plant polyphenols, CPDs, cyclobutane pyrimidine dimers, (6-4) photoproducts, pyrimidine-pyrimidone (6-4) photoproducts.

Declarations

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Research Involving Humans and Animals Statement Not applicable.

Informed Consent. Not applicable.

Consent for Publication. Not applicable.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest. The authors declare no competing interests.

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Figures

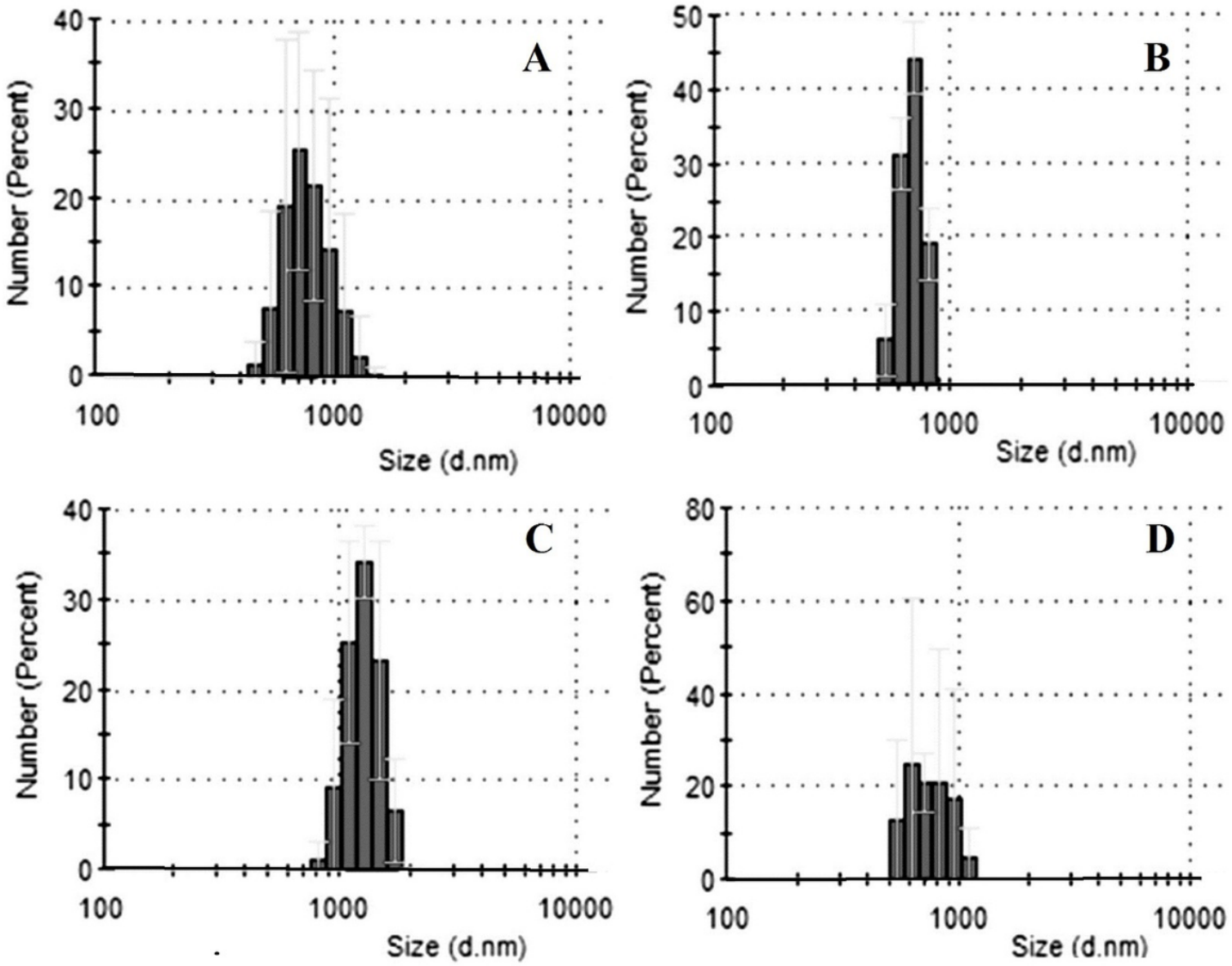

Figure 1

Size distribution diagrams of quercetin (A, B) and resveratrol (C, D) crystals with $(PAH/PSS)_4$ (A, C) and $(CH/DexS)_4$ (B, D) shells

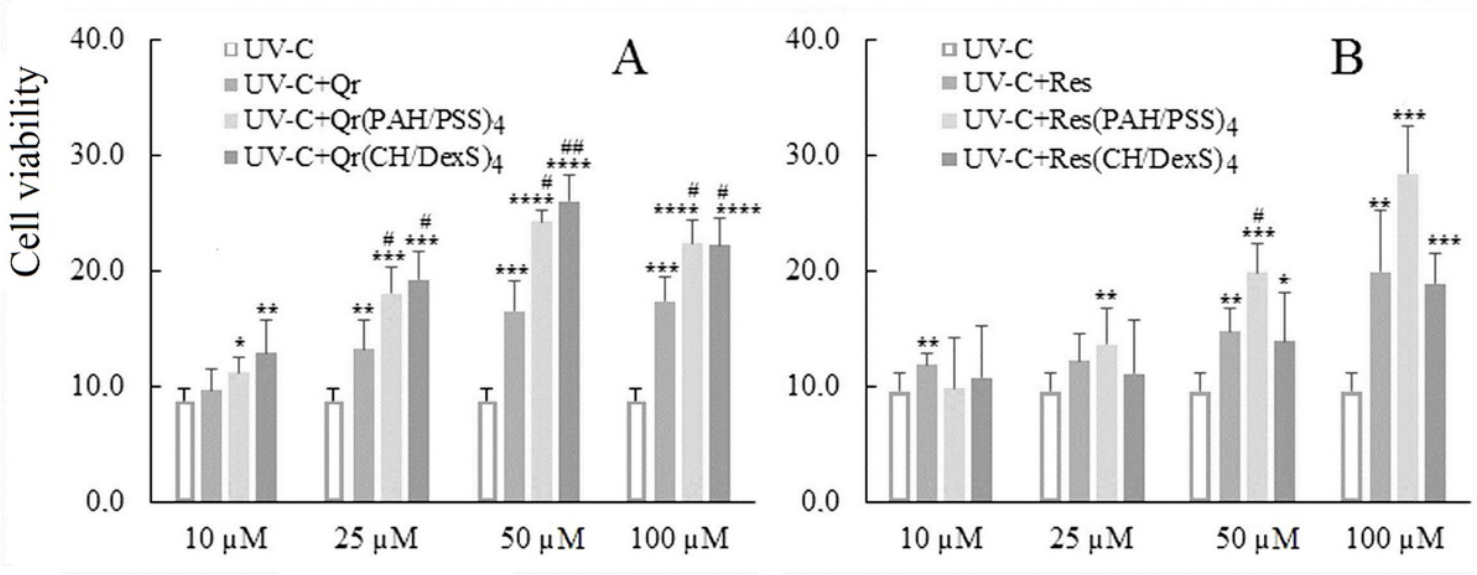

Figure 2

Effects of native and particulate polyphenols: A – quercetin; B – resveratrol, on the cell viability 20 h after exposure cultured keratinocytes to UV-C, 0.06 J/cm^2

Data are expressed as percentage to sham irradiation (mean ± SD)

* - P < 0.05; ** - P <0.01; *** - P <0.001, **** - P <0.0001 *vs* UV-C; $^{\#}$ - P <0.05; $^{\#\#}$ - P <0.01 *vs* UV-C and corresponding polyphenol

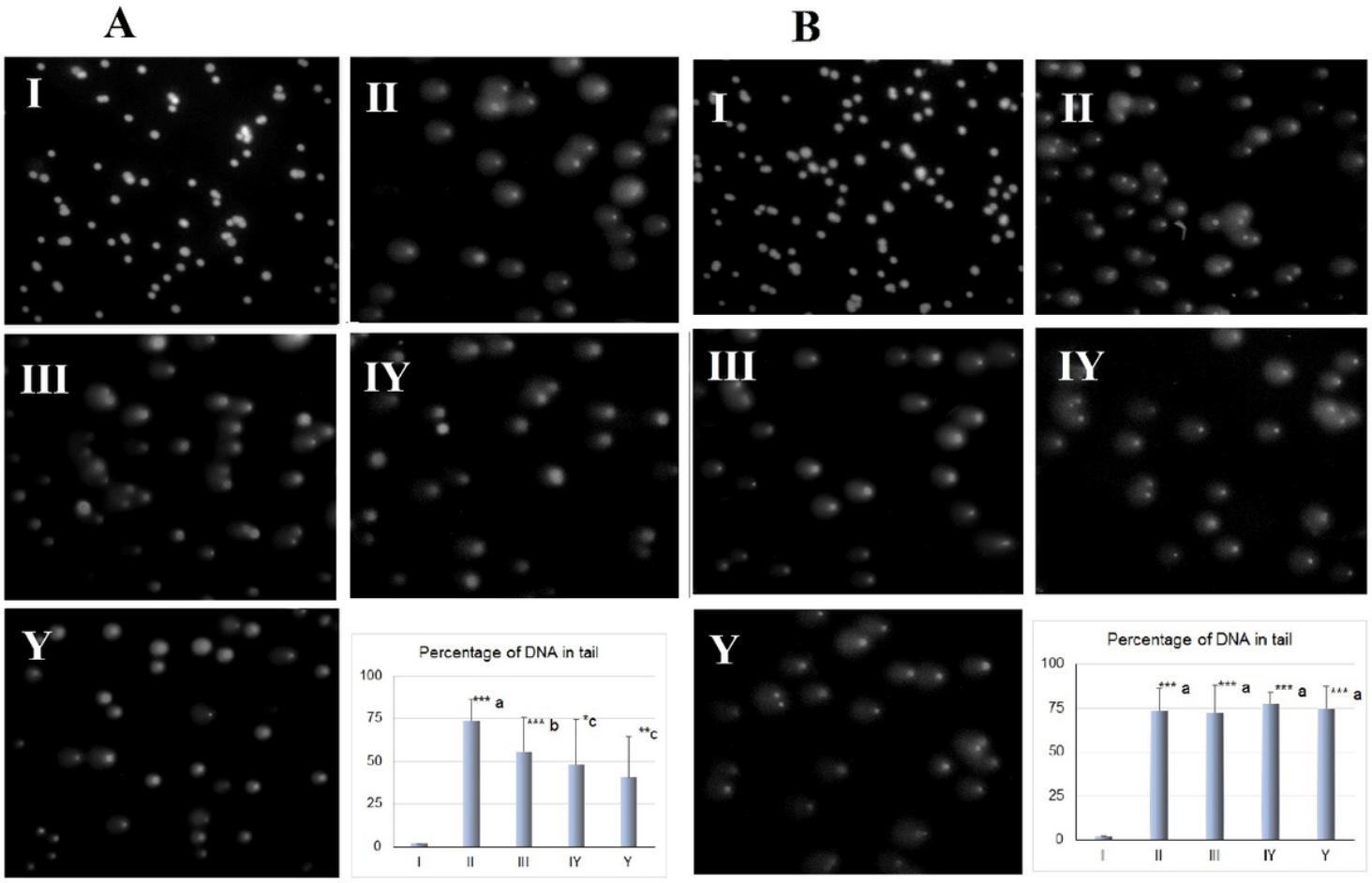

Figure 3

Effects of native and particulate polyphenols (50 μmol/L): A – quercetin; B – resveratrol, on DNA damage in cultured keratinocytes 2 h after exposure to UV-C at a dose of 0.06 J/cm^2

Data are given as representative fluorescent micrographs of DNA comets and the *corresponding diagrams* showing percentage of DNA in the "comet tail". I - sham irradiation; II - UV-C; III - UV-C and native polyphenols, IY - UV-C and polyphenol $(PAH/PSS)_4$; UV-C and polyphenol $(CH/DexS)_4$.

^{***a} - P < 0.0000001 *vs* Sham irradiation; ^{**б} - P <0.01; ^{***б} - P <0.001 *vs* UV-C; ^{*c} - P <0.05; ^{**c} - P <0.01 *vs* UV-C and corresponding polyphenol