Arch Dermatol Res (2011) 303:239–246
DOI 10.1007/s00403-010-1088-6

ORIGINAL PAPER

Polyhydroxylated fatty alcohols derived from avocado suppress inflammatory response and provide non-sunscreen protection against UV-induced damage in skin cells

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Received: 3 June 2010 / Revised: 29 September 2010 / Accepted: 4 October 2010 / Published online: 27 October 2010

Abstract Exposing skin to ultraviolet (UV) radiation contributes to photoaging and to the development of skin cancer by DNA lesions and triggering inflammatory and other harmful cellular cascades. The present study tested the ability of unique lipid molecules, polyhydroxylated fatty alcohols (PFA), extracted from avocado, to reduce UVB-induced damage and inflammation in skin. Introducing PFA to keratinocytes prior to their exposure to UVB exerted a protective effect, increasing cell viability, decreasing the secretion of IL-6 and PGE_2, and enhancing DNA repair. In human skin explants, treating with PFA reduced significantly UV-induced cellular damage. These results support the idea that PFA can play an important role as a photo-protective agent in UV-induced skin damage.

Keywords UVB · Photodamage · UVB-damaged cells · Non-sunscreen protection · Avocado extract · Polyhydroxylated fatty alcohols

Electronic supplementary material The online version of this article (doi:10.1007/s00403-010-1088-6) contains supplementary material, which is available to authorized users.

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Introduction

Ultraviolet (UV) radiation from the sun was recognized long ago as the cause for many adverse effects in humans, including erythema, hyperplasia, immunosuppression, premature skin aging and the development and progression of cancer [15]. Solar UV radiation varies in energy level, ranging from short to long wavelength, and penetrates the skin according to their wavelength and interacts with different cells that are located at different depths [14]. UV radiation of shorter wavelength (UVB; 290–320 nm) is mainly absorbed in the epidermis and affects predominantly epidermal cells [9, 20], i.e., keratinocytes, which are the most abundant epidermal cells, and also other cells including melanocytes, Langerhans cells, dendritic cells, and macrophages. UV radiation of longer wavelengths (UVA; 320–400 nm) penetrates deeper and can interact with both epidermal keratinocytes and dermal fibroblasts.

Defensive mechanisms of the skin are able to protect against the harmful effect of UVB rays to only a certain extent [9]. Chronic exposure of skin to UVB triggers oxidative stress [3, 26], inflammatory cascades [3, 26], and accumulation of DNA lesions and mutations [5] especially in a tissue with impaired capacity of DNA repair and antioxidant system. Accumulation of such damage contributes to skin aging and skin cancer [5, 8, 21, 26].

Sunscreens are commonly used for preventing or ameliorating harmful effects of UV radiation on the skin [2]. However, sunscreen alone may not provide sufficient protection against skin photodamage [10, 29, 33, 36]. Non-sunscreen compounds have become more relevant to large parts of the populations in preventive skin care [19]. Using active compounds that support the skin's own defensive mechanisms or inhibit the pathological processes in photodamaged skin is highly desirable. Topical and systemic antioxidants, DNA repair enzymes, as well as some plant extracts have been reported to protect skin against various UV-induced damage models [19, 34].

In the present study, we demonstrated the anti-inflammatory and photo-protective potential of polyhydroxylated fatty alcohols (PFA) extracted from Persia gratissima (avocado).

Materials and methods

Preparation of PFA

Avocado seeds were separated from the avocado pear 'Ettinger', followed by freezing and lyophilization. Lyophilized avocado seeds were crushed and extracted with hexane for 14 h in a Soxhlet apparatus. The resulting extract was filtered and then was put into a cold room having a temperature range of 2–8°C for 12 h for the process of cool crystallization. The crystallized material, consisting of PFA, was separated from the solvent by filtration through Whatman filter paper.

In addition, PFA were obtained from the edible part of the avocado fruit. Briefly, ground avocado pears were extracted twice in ethanol at 50°C for 1 h, followed by acetone extraction at 4°C overnight. All extracts were collected and the solvents were evaporated. Dried extract was re-dissolved in 35 ml hexane. Avocado pear hexane extract was refrigerated (4°C) overnight, and precipitated PFA were separated by filtration.

Identification of the compounds was performed by using GC–MS and HPLC/MS–ECI analysis, and data were compared with previously reported information. Elucidation of the chemical structure of the compounds was based on the similarity of characteristic peaks (*m/e*) in mass spectra and the molecular peaks of sodium adducts $[M^+Na]^+$ of our PFA compared with those described earlier for PFA [27].

Cell cultures

Primary normal human epidermal keratinocytes were provided by M. Chaouat (Laboratory of Experimental Surgery, Hadassah Medical Center, Jerusalem, Israel). Cells were isolated from the skin removed during cosmetic plastic surgery. Briefly, a thin split thickness skin biopsy was taken aseptically and trypsinized overnight at 4–8°C. The epidermal layer was separated from the dermal layer and the single cells were isolated and cultured in specialized keratinocyte medium. The keratinocytes were then redistributed into flasks containing lethally irradiated 3T3 cells. The flasks were incubated at 37°C under 8–10% CO_2. Upon reaching sub-confluency the cells were redistributed to new flasks without the 3T3 feeder layer.

Organ cultures

Human scalp skin tissue samples were obtained within 2 h of surgery (with approval of the Hadassah hospital IRB committee #0273-08-HMO). Organ cultures were prepared essentially as described by Hasson and co-workers [12]. In brief, skin tissue samples were washed in DMEM and cut with a microtome (tissue sectioner, Sorvall model TC-2, Thermo Fisher Scientific, Waltham, MA) into thin slices (300 μm) and incubated in DMEM containing 10% FCS, gentamicin (15.2 μg/ml), and Ciproxin (ciprofloxacin, 19 μg/ml) at 37°C, under 5% CO_2.

UVB irradiation of tissue culture

UVB irradiation was performed with a bank of four FS40 fluorescent lamps (Philips) that emit light at wavelengths between 280 and 320 nm with a peak at 313 nm. Light intensity was determined using a Waldmann UV radiometer (Herbert Waldmann GmbH, Schwenningen, Germany). Cells were irradiated at a dose of 20–120 mJ/cm^2. Cell exposure to UV irradiation was performed in Petri dishes or in 24-well plates or on 8-well μ-slides containing pre-warmed phosphate-buffered saline (PBS), which after UV irradiation was immediately replaced by fresh growth medium supplemented with corresponding concentrations of PFA. The optimal UV dose for each kind of experiment was determined by prior titration.

UVB irradiation of human skin in organ culture

Samples of human skin ex vivo tissue were treated in growth medium with PFA at concentration 1 μg/ml for 60 min before UVB irradiation, or were UV-irradiated without PFA treatment. Immediately before UVB irradiation, the medium was removed, the samples were washed thoroughly with PBS, covered with 10 ml of PBS, and were irradiated with UVB (90 mJ/cm^2). After irradiation, PBS was changed to DMEM containing corresponding concentrations of PFA. 24 h later, skin samples were fixed for 30 min in PBS containing 4% paraformaldehyde, rinsed, and formalin-fixed paraffin-embedded (FFPE) blocks were prepared, cut (5 μm sections), deparaffinized in xylene and

dehydrated. Sections were stained with hematoxylin and eosin (H&E). Total damaged epidermal cells were recognized in the skin tissue by their pyknotic nuclei, and eosinophilic cytoplasm in histological sections stained with H&E. Using Media Cybernetics Image Pro Plus software the numbers of sunburn cells were scored under a light microscope and were determined as a percent of total epidermal cells.

Assessment of keratinocyte survival

Keratinocyte survival was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Briefly, keratinocytes at sub-confluent conditions in 24-well plates were treated with different concentrations of PFA for 24 h, followed by incubation with MTT for 4 h at 37°C. The supernatant was removed and the precipitate was dissolved in isopropanol. The absorbance of the resulting solution was measured by spectrophotometry at 540 nm.

Alternatively, in experiments with UVB irradiation, cell survival was assayed using the fluorescence-based Dead/Live kit (Molecular Probes, Eugene, OR). The kits combine fluorescent reagents to yield two-color discrimination of live cells from the dead cell. Keratinocytes were grown on 8-well μ-slides up to sub-confluent conditions. PFA-supplemented medium was added to cells for 60 min prior to UVB irradiation. After treatment, medium was removed, replaced with 100 µl PBS and the culture were exposed to UVB irradiation (20 mJ/cm^2). After UVB irradiation, PBS was immediately replaced by fresh growth medium containing corresponding concentrations of PFA, and cells were incubated for 24 h. The assay of cell survival was performed according to the manufacturer's instructions. The numbers of viable and dead cells were calculated by using confocal microscopy.

Production of IL-6 and PGE_2 by keratinocytes

IL-6 and PGE_2 were quantified in the growth medium by the ELISA method using a Human IL-6 Quantikine HS ELISA Kit and Prostaglandin E_2 Parameter Assay Kit (both R&D system, Minneapolis, MN), respectively, according to the manufacturer's instructions. Briefly, primary keratinocytes at sub-confluent conditions in 24-well plates were pretreated with PFA in growth medium for 60 min. After removing the medium, the cultures were washed thoroughly with PBS, overlayed with 300 ml of PBS, and exposed to UVB irradiation (30 mJ/cm^2). After the cell irradiation, PBS was changed to growth medium containing a corresponding concentration of PFA, and cells were incubated at 37°C, 5% CO_2 for 8 h, and medium was collected and analyzed. For investigation of the effect of TPA on IL-6 and PGE_2 production, primary human keratinocytes at sub-confluent conditions growing in 24-well plates were pretreated with PFA in the growth medium for 60 min. Then the growth medium was additionally supplemented with 1 ng/ml TPA, the cells were incubated at 37°C, 5% CO_2 for 8 h, and medium was collected and analyzed.

Real-time PCR

Primary human keratinocytes at sub-confluent conditions in 9-cm culture dishes were pretreated with PFA in growth medium for 60 min. After removing the medium, the cultures were washed thoroughly with PBS, covered with 10 ml of PBS, and irradiated with UVB (20 mJ/cm^2). After cell irradiation, PBS was changed to growth medium containing a corresponding PFA concentration. After 24 h incubation the cells were collected, total RNA was harvested, and cDNA synthesis was performed. Expression of GADD45 mRNA was determined by real-time PCR.

Real-time quantitative PCR reactions contained 10 µl SYBR Green PCR Master Mix (Applied Biosystems), 500 nmol/l of each of the specific primers, and 10 ng of cDNA in a total volume of 20 µl. We used ABI PRISM 7000 Sequence Detection System (Applied Biosystems) for sample amplification and analysis. Thermal cycling conditions were: 95°C for 10 min (enzyme activation), followed by 40 two-step cycles of 15 s at 95°C and 1 min at 60°C. All experiments were performed in triplicate and negative controls (master mix without any cDNA) were added in each run. Melting curves confirmed the presence of a single amplification product. Significant PCR fluorescent signals (*Ct*) for GADD45 of all samples were measured and normalized to a PCR fluorescent signal obtained from an endogenous reference (G3PDH). Comparative and relative quantifications of the gene products normalized to the housekeeping gene G3PDH were calculated by the $2^{-\Delta\Delta Ct}$ method. Values were expressed with reference to the value obtained from a calibrator, i.e., cDNA derived from a normal epidermis sample. The primers were (5′–3′): G3PDH forward: GAGAAGGCT GGGGCTCATTTG; G3PDH reverse: TTCACACCCATG ACGAACATG; GADD45 forward: AGGAAGTGCTCA GCAAAGCC; GADD45 reverse: GCACAACACCACGTT ATCGG.

Cyclobutane pyrimidine dimers detection

Primary human keratinocytes at sub-confluent conditions in 9-cm culture dishes were treated with PFA in growth medium for 60 min. After removing the medium, the cultures were washed thoroughly with PBS, covered with 10 ml of PBS, and irradiated with UVB (20 mJ/cm^2). After cell irradiation, PBS was changed to growth medium containing a corresponding PFA concentration, and cells were incubated at 37°C, 5% CO_2 for 24 h, followed by DNA

extraction using Wizard Genomic DNA Purification Kit (Promega, Madison, WI). In the control samples, the DNA was extracted immediately after irradiation. In brief, 5 µg/well denatured DNA was applied, in triplicate, into poly-L-lysine (Sigma) pre-coated ELISA plates, washed five times with PBS, and blocked with 2% FCS in PBS. As first antibody, an anti-thymidine dimer (ant-CPD)H3 clone 4F6 (Sigma) diluted 1:1,000 in 2% FCS in PBS was used. As second antibody, a biotin-SP-conjugated goat anti-mouse IgG diluted 1:50,000 was used, followed by peroxidase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:10,000. The peroxidase reaction was performed using 0.4 mg/ml TMB (3,3′,5,5′-tetramethylbenzidine) (Sigma, St. Louis, MO) in the presence of 0.02% H_2O_2, and color intensity was measured by spectrophotometry at 450 nm.

Data analysis

Values are shown as the mean ± standard deviation (SD). The statistical differences among groups were determined by analysis of variance (ANOVA) followed by Student–Newman–Keuls test for all pairwise comparisons. Differences were considered to be statistically significant at the level of $P < 0.05$.

Results

Chemical characterization of PFA from avocado seed

PFA consist mainly of crystalline compounds (PFA) of similar chemical structures. Elucidation of the chemical structure of the compounds (Fig. 1) was based on the similarity of characteristic peaks (*m*/*e*) in mass spectra and the molecular peaks of sodium adducts $[M^+Na]^+$ of our PFA compared with those described earlier [27]. Two main compounds of the PFA are 1-acetoxy-2,4-dihydroxy-heptadec-16-ene (compound 3, Fig. 1) and 1-acetoxy-2,4-dihydroxy-heptadec-16-yne (compound 5, Fig. 1). Their relative concentration in PFA were 32 and 53.8%, from avocado seeds, and 51 and 28.6% from avocado pears, respectively. The concentration of minor PFA constituents—compounds 1, 2, and 4 were 1.1, 4.7, and 8.4%, from avocado seeds, and 3.4, 11.1, and 5.9% from pears, respectively. GC elution profiles of PFA from avocado seeds and avocado pear are demonstrated in Online Resource 1.

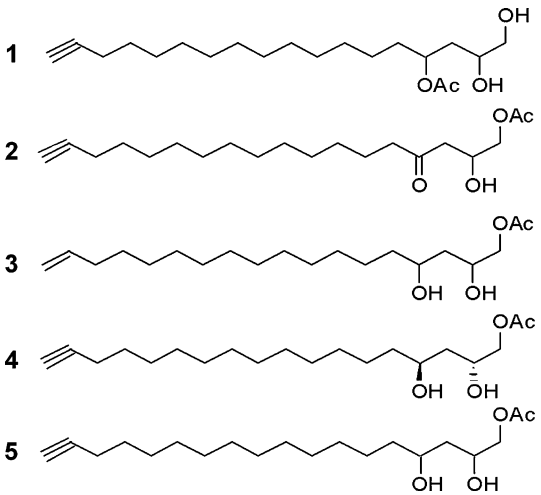

Fig. 1 Chemical structures of polyhydroxylated fatty alcohols from avocado

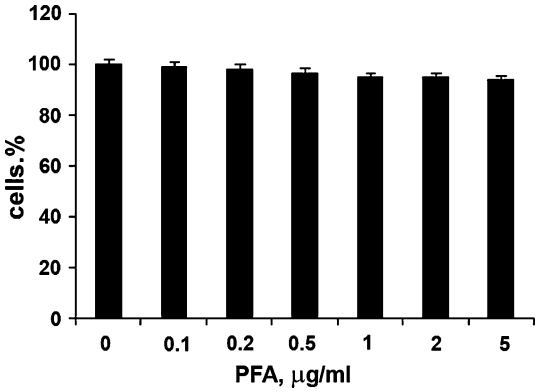

Fig. 2 Survival of keratinocytes after treatment with PFA. Survival of human primary keratinocytes in medium supplemented with different concentrations of PFA for 24 h were analyzed using MMT assay. Data are expressed as mean ± SD of four samples

Evaluation of keratinocyte viability in the presence of PFA

Treatment of primary human keratinocytes with PFA at concentrations up to 1 µg/ml did not induce cytotoxic effects (showing cell viability >95% after 24 h treatment) (Fig. 2). Using PFA at concentration 5 µg/ml resulted in 94% cell viability. Therefore, experiments were conducted at PFA concentrations up to 5 µg/ml to reduce toxicity-related issues.

Protective properties of PFA against UVB cytotoxicity in cultured keratinocytes and in human skin explants

Keratinocytes were treated with PFA before and after they were exposed to UVB irradiation at a dose of 20 mJ/cm^2. Cell viability was examined 24 h post-irradiation and compared to non-treated cells. In the PFA-treated samples (0.1 and 0.5 µg/ml) viability was 90.8 ± 7 and 91.0 ± 10%, respectively, in comparison to 61.0 ± 3% viable cells in the untreated, control samples (Fig. 3a) suggesting that PFA provided protection against UVB-induced cell death.

The ability of PFA to protect skin from photodamage induced by UVB was demonstrated using human skin

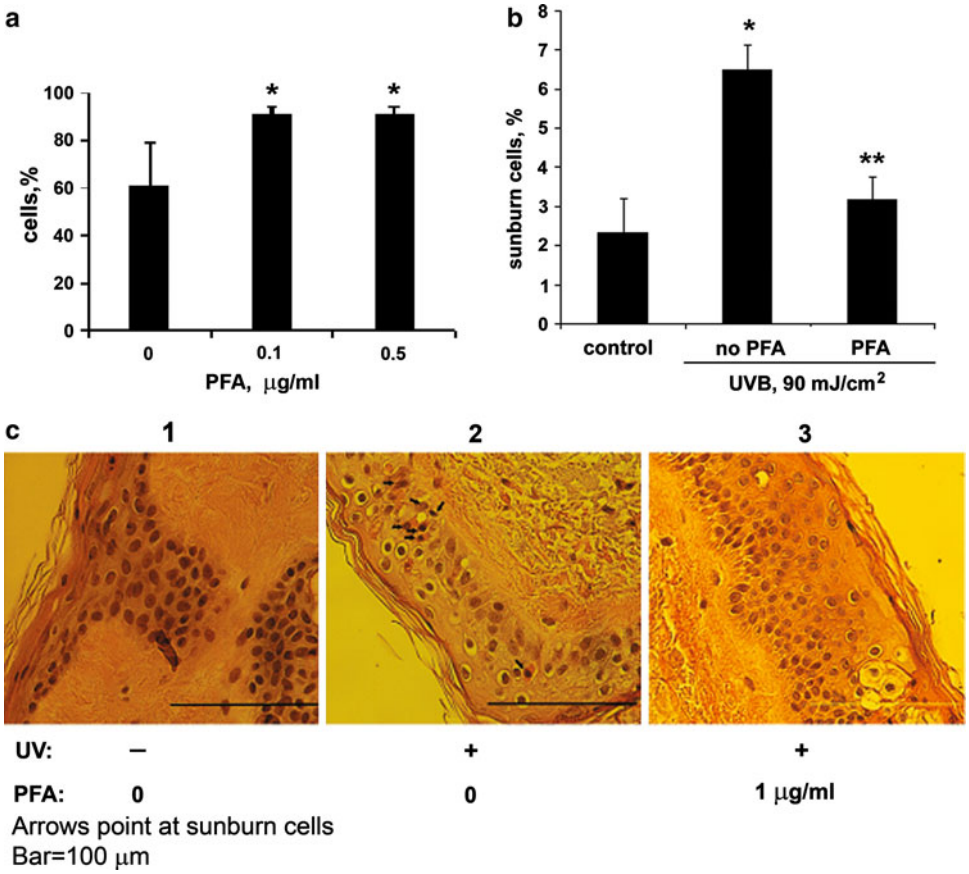

Fig. 3 Treatment with PFA before and after UVB exposure increases survival of cultured keratinocytes and protects human skin explants from sunburn cell formation. **a** Survival of primary human keratinocytes exposed to UVB (20 mJ/cm^2) with or without treatment with PFA. Cells were treated with PFA at 0.1 or 0.5 μg/ml or were without PFA treatment for 60 min prior to UVB irradiation. After UVB exposure cell were incubated for additional 24 h in the presence of corresponding PFA concentration and analysis of survival cells was performed. Data are expressed as mean ± SD of eight samples, $*P < 0.05$ compared to control. **b** Percent of sunburn cells over total cells in epidermis organ culture. Epidermal sunburn cells in UVB-irradiated skin explants treated with or without 1 μg/ml PFA for 60 min before and 24 h after UVB irradiation were visualized in histological sections stained with H&E showing typical pyknotic nuclei and eosinophilic cytoplasm. Cells were counted by using Media Cybernetics Image Pro plus software. Data are expressed as mean + SD of five to eight samples from two different donors, $*P < 0.05$, UVB-irradiated non-treated samples compared to control samples, $**P < 0.05$, UVB-irradiated samples treated with PFA compared to non-treated samples. **c** Representative image of histological sections stained with H&E: *1* non-irradiated control explants; *2* UVB-irradiated (90 mJ/cm^2) explants; *3* PFA treatment followed by UVB irradiation. *Black arrows* point at sunburn cells. Magnification ×40

explants. Exposing skin explants to UVB induced the formation of sunburn cells, recognized by pyknotic nuclei and eosinophilic cytoplasm in histological sections stained with H&E (Fig. 3c). The average percentage of sunburn cells detected in UVB-irradiated (at a dose of 90 mJ/cm^2) non-treated human skin organ culture was 6.51 ± 0.64% compared to almost half (3.19 ± 0.56%) that in samples treated with 1 μg/ml PFA (Fig. 3b). Skin explants used in the experiments were obtained from uncovered scalp skin (a chronically sun exposed site) which explain the high basal level of sunburn cells in control samples, while the number of sunburn cells in the UVB-irradiated tissue was considerably above that background.

It should be noted that PFA do not absorb light in the UVB range at wavelengths between 280 and 320 nm (see Online Resource 2), and therefore, increased viability of PFA-treated cells cannot be explained by UVB absorbance.

PFA enhance DNA repair in UVB-irradiated keratinocytes

The formation of cyclobutane pyrimidine dimers (CPD) is one of the most important characteristics of DNA damage and mutagenesis [18]. We tested the hypothesis that PFA may influence the removal of CPD from DNA in UV-exposed cells. Exposure of keratinocytes to 20 mJ/cm^2 UVB induces formation of CPD, as was measured immediately after irradiation and served as a reference for non-repaired DNA damage. To evaluate DNA repair in the irradiated cultures, the amount of CPD in cellular DNA was measured 24 h after UVB exposure and compared to the

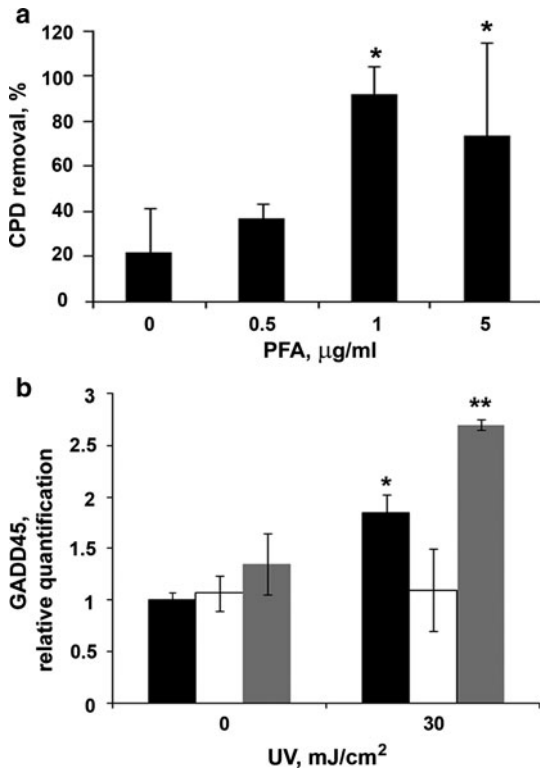

Fig. 4 Effect of PFA on removal of CPD and GADD45 expression in UVB-exposed human keratinocytes. **a** Primary human keratinocytes at sub-confluent conditions were treated with PFA at the indicated concentrations in growth medium for 60 min. The cultures were irradiated with UVB (20 mJ/cm^2) as described in "Materials and methods". DNA was extracted immediately after cell irradiation, or after additional cell incubation for 24 h in the presence of various concentration of PFA or without PFA (control). CPD concentration was determined in DNA extracts by the ELISA method using an anti-CPD antibody. Removal of CPD after 24 h is expressed as percent CPD level measured immediately after irradiation, representing the activity of repair mechanisms in the cells. Data are expressed as mean ± SD of triplicate samples.*$P < 0.05$, compared with non-treated cells. **b** Primary human keratinocytes at sub-confluent conditions were treated with PFA at concentration 1 μg/ml (*open bar*) or 5 μg/ml (*gray bar*) or were without PFA (*control-closed bar*) in growth medium for 60 min, followed by irradiation with UVB (30 mJ/cm^2) as is described in "Materials and methods". After 24 h incubation in the presence of the indicated PFA concentration, the cells were collected, total RNA was extracted, and cDNA synthesis was performed. Expression of GADD45 mRNA was determined by real-time PCR. Data are expressed as mean + SD of triplicate samples, *$P < 0.05$ compared with non-irradiated control cells, **$P < 0.05$, compared with non-treated UV-irradiated cells

non-repaired reference (Fig. 4a). CPD removal for 24 h in the cells treated with PFA at concentration 1 and 5 μg/ml was 92.3 ± 11.9 and 74.5 ± 41%, respectively, compared to about 20% in non-treated control cells. At lower PFA concentration (0.5 μg/ml) CPD removal was not significantly improved compared to control sample, achieving 37.5 ± 6.2%. Forty-eight hours post-irradiation there were no CPD detected any more, both in PFA-treated and PFA-non-treated control cells.

We further examined the possible influence of PFA in regulation of the DNA repair pathways through the expression of the stress-related gene GADD45. UV irradiation increases the transcription of several genes, including genes directly involved in nucleotide excision repair (NER) [4] and apoptosis, such as GADD45 [16, 17]. In our study, UVB irradiation (30 mJ/cm^2) of human primary keratinocytes induced an enhancement in GADD45 mRNA expression in comparison to non-irradiated cells ($P < 0.05$) (Fig. 4b). Administration of keratinocytes with PFA (1 μg/ml) prior to UVB irradiation did not significantly changed the expression of GADD45 (Fig. 4b). In contrast, in UVB-irradiated cells treated with higher PFA concentration (5 μg/ml) GADD45 expression was significantly increased ($P < 0.05$).

PFA reduce UVB-induced production of IL-6 and PGE_2 in keratinocytes

UVB-irradiation induces the production of IL-6 and PGE_2 in primary human keratinocytes (Fig. 5). Cell treatment with PFA in the range of concentrations 0.1–0.5 μg/ml significantly inhibited secretion of IL-6 by 40–50% in UVB-irradiated cells ($P < 0.05$) but not at higher PFA concentration (Fig. 5a). Treatment with PFA also inhibited PGE_2 synthesis by about 80% in UVB-exposed keratinocytes ($P < 0.05$) (Fig. 5b). Additionally, treatment of the cells with PFA also inhibited TPA-induced IL-6 and PGE_2 production in human keratinocytes by about 44 and 54% (Fig. 5c, d), respectively, showing that PFA inhibition activity is not specific for UVB as an inflammatory stimulus.

Discussion

PFA are unique crystalline lipid molecules derived from Persia gratissima (avocado) seed and the edible part of the pear. PFA have been previously found active against some cancer cell lines [24] and have been able to inhibit acetyl CoA carboxylase activity [11]. In addition, some of these compounds have demonstrated antifungal [7], antibacterial [23], and antiparasitic properties [1]. However, the photoprotective and anti-inflammatory potential of PFA on the cellular and molecular level have not been previously described.

Here we demonstrate the protective properties of PFA against UVB cytotoxicity in cultured keratinocytes and in human skin explants. That protection is shown in a number of parameters such as: increasing cell viability (Fig. 3a), decreasing the number of sunburn cells in human skin explants (Fig. 3b, c), enhancing DNA repair (Fig. 4a), and decreasing the secretion of IL-6 and PGE_2 (Fig. 5a, b). We

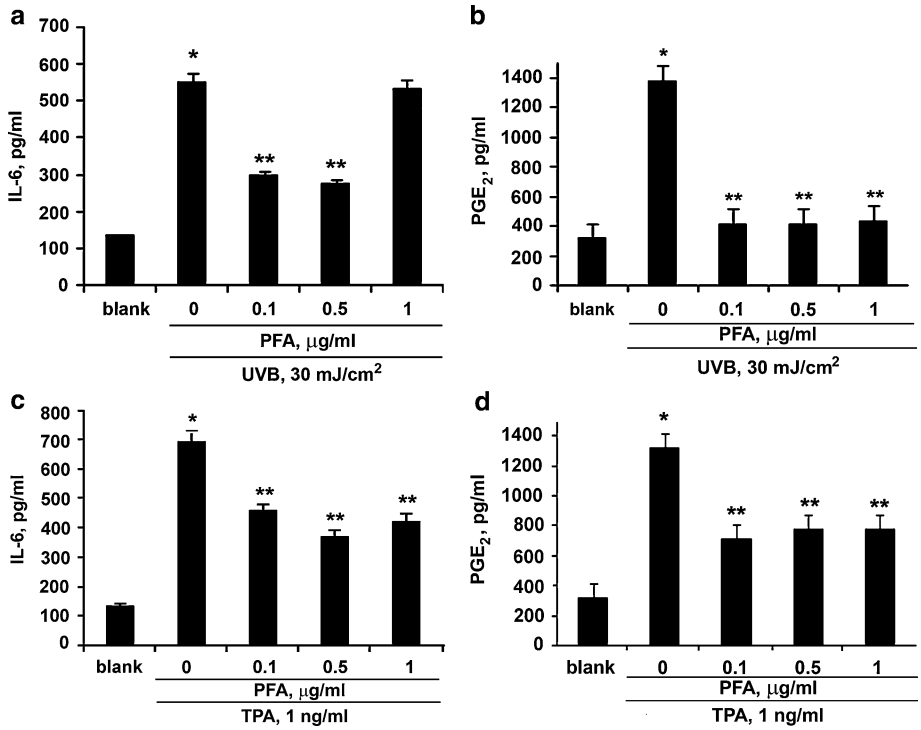

Fig. 5 PFA are capable of reducing UVB- and TPA-induced IL-6 and PGE_2 expression by keratinocytes. Primary human keratinocytes were treated with PFA at concentration 0.1, 0.5 or 1 μg/ml or without PFA (control) in growth medium for 60 min. At the end of the time, the cells were UVB-irradiated (30 mJ/cm^2) (**a**, **b**) and treated with corresponding PFA concentration as is described in "Materials and methods", or the growth medium was additionally supplemented with 1 ng/ml TPA (**c**, **d**) and the cells were incubated for 8 h more. Supernatants were analyzed for IL-6 (**a**, **c**) or for PGE_2 (**b**, **d**) concentration. Data are expressed as mean + SD of triplicate samples. *$P < 0.05$ compared with non-irradiated control cells, **$P < 0.05$, compared with non-treated UV-irradiated cells

have found that each of these parameters requires different UVB doses to demonstrate the optimal effect of PFA. For example in order to obtain sunburn cells in skin explants a minimal UVB dose 90 mJ/cm^2 is required. To detect differences in the expression of GADD45 mRNA, IL-6 and PGE_2 a UVB dose of 30 mJ/cm^2 was sufficient, while in the cells irradiated at this UVB dose complete DNA repair could not be achieved (data not shown). For demonstrating total DNA repair we had to reduce the dose still further to 20 mJ/cm^2. Under these experimental conditions PFA at concentrations of 1 and 5 μg/ml remarkably promoted removal of photoproducts (CPD) (Fig. 4a), suggesting an improvement of the DNA damage repair mechanism in keratinocytes. The formation of CPD and 6-4 pyrimidine–pyrimidone photoproducts are the most predominant DNA lesions in skin after exposure to UVB (and also UVA) [18, 25]. The main repair mechanism of UVB-induced DNA damage is NER. When skin cells are exposed to excessive UV radiation, the capacity of NER is reduced and the CPD lesions remain in the DNA and may result in cellular death, senescence, mutagenesis, and carcinogenesis of the skin [25]. Presumably, enhancement of DNA repair mechanism is one of the reasons explaining why PFA exert a protective effect on the viability of UVB-irradiated keratinocytes in culture, and on sun-damaged cell formation in UVB-irradiated human skin explants. We also analyzed possible involvement of stress-signaling protein GADD45 in photoprotective mechanism of PFA. GADD45 genes have been implicated in stress signaling in response to physiological or environmental stressors, which results in cell cycle arrest, DNA repair, cell survival, senescence, or apoptosis [16, 17]. We did not find evidence of involvement of the stress-related gene GADD45 in cell photo-protection by PFA at concentration 1 μg/ml. In contrast, cell pretreatment with PFA at concentration 5 μg/ml increases GADD45 expression in UVB-irradiated cells. This effect may be related to specific physico-chemical properties of PFA, which at high concentration acts as a detergent and causes additional stress to the cultured cells. We demonstrate that PFA is able to decrease UVB-induced secretion of the inflammatory mediator PGE_2 and of the pleiotropic cytokine IL-6 (Fig. 5a, b). Keratinocyte-derived cytokines and inflammatory mediators play an important role in the development of the inflammatory reaction in UVB-exposed skin [13, 22, 35]. Numerous studies have demonstrated that PGE_2 mediates signals that are involved in the induction of erythema, angiogenesis, vasodilatation, and vascular permeability [30, 31]. The PGE_2 signaling pathways promote photoaging and development of UVB-induced skin carcinogenesis [28]. The essential role of IL-6 in systemic inflammatory reactions has been demonstrated previously [6, 29, 32]. Taken together, our findings on inhibitory effects of PFA against UVB-induced PGE_2 and IL-6 expression in keratinocytes demonstrate anti-inflammatory properties of PFA. In addition, we demonstrate that PFA inhibit TPA-induced production of PGE_2 and IL-6 by keratinocytes (Fig. 5c, d). This observation supports the idea that PFA has anti-inflammatory capability not only against UVB-induced inflammation, but also against inflammatory reaction caused by other irritants. Since PFA do not absorb

UVB light, we suggest that they act in a non-sunscreen manner to protect against UVB-induced inflammatory induction, presumably in the same manner as they act against TPA-induced inflammation.

In conclusion, the results of the present study provide evidence that PFA protect skin cells from UVB-induced damage, enhance the DNA damage repair mechanism, and inhibit inflammation. Consequently, PFA treatment may be beneficial in the photo-protection of the skin. Further investigation should be conducted to elucidate the mechanism of PFA underlying its protective activity.

Acknowledgments This study was supported in part by the Israel Ministry of Industry and Commerce. Mr. Sigmund Geller is thanked with pleasure for editing the manuscript.

References

1. Abe F, Nagafuji S, Okawa M, Kinjo J, Akahane H, Ogura T, Martinez-Alfaro AM, Reyes-Chilpa R (2005) Trypanocidal constituents in plants 5. Evaluation of some Mexican plants for their trypanocidal activity and active constituents in the seeds of *Persea americana*. Biol Pharm Bull 28:1314–1317
2. Autier P (2009) Sunscreen abuse for intentional sun exposure. Br J Dermatol 161:40–45
3. Bennett MF, Robinson MK, Baron ED, Cooper KD (2008) Skin immune systems and inflammation: protector of the skin or promoter of aging? J Invest Dermatol Symp Proc 13:15–19
4. Bykov VJ, Sheehan JM, Hemminki K, Young AR (1999) In situ repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in human skin exposed to solar simulating radiation. J Invest Dermatol 112:326–331
5. Cadet J, Sage E, Douki T (2005) Ultraviolet radiation-mediated damage to cellular DNA. Mutat Res 571:3–17
6. Cronstein BN (2007) Interleukin-6 a key mediator of systemic and local symptoms in rheumatoid arthritis. Bull NYU Hosp Jt Dis 65:S11–S15
7. Domergue F, Helms GL, Prusky D, Browse J (2000) Antifungal compounds from idioblast cells isolated from avocado fruits. Phytochemistry 54:183–189
8. Einspahr JG, Stratton SP, Bowden GT, Alberts DS (2002) Chemoprevention of human skin cancer. Crit Rev Oncol Hematol 41:269–285
9. Halliday GM, Lyons JG (2008) Inflammatory doses of UV may not be necessary for skin carcinogenesis. Photochem Photobiol 84:272–283
10. Hanson KM, Gratton E, Bardeen CJ (2006) Sunscreen enhancement of UV-induced reactive oxygen species in the skin. Free Radic Biol Med 41:1205–1212
11. Hashimura H, Ueda C, Kawabata J, Kasai T (2001) Acetyl-CoA carboxylase inhibitors from avocado (*Persea americana* Mill.) fruits. Biosci Biotechnol Biochem 65:1656–1658
12. Hasson E, Slovatizky Y, Shimoni Y, Falk H, Panet A, Mitrani E (2005) Solid tissues can be manipulated ex vivo and used as vehicles for gene therapy. J Gene Med 7:926–935
13. Kenji K, Miyako N, Tetsuya H, Chikako N, Yoshiki M, Yoshiki T, Shuh N (2007) Prostaglandin E_2 is required for ultraviolet B-induced skin inflammation via EP2 and EP4 receptors. Lab Invest 87:49–55
14. Krutmann J (2001) The role of UVA rays in skin aging. Eur J Dermatol 11:170–171
15. Krutmann J, Gilchrest BA (2006) Photoaging of skin. In: Gilchrest BA, Krutmann J (eds) Skin aging. Springer, New York, pp 33–p44
16. Liebermann DA, Hoffman B (2008) Gadd45 in stress signaling. J Mol Signal 3:15. doi:10.1186/1750-2187-3-15
17. Maeda T, Hanna AN, Sim AB, Chua PP, Chong MT, Tron VA (2002) GADD45 regulates G2/M arrest, DNA repair, and cell death in keratinocytes following ultraviolet exposure. J Invest Dermatol 119:22–26
18. Marrot L, Meunier JR (2008) Skin DNA photodamage and its biological consequences. J Am Acad Dermatol 58:S139–S148
19. Matsui MS, Hsia A, Miller JD, Hanneman K, Scull H, Cooper KD, Baron E (2009) Non-sunscreen photoprotection: antioxidants add value to a sunscreen. J Invest Dermatol Symp Proc 14:56–59
20. Matsumura Y, Ananthaswamy HN (2004) Toxic effect of ultraviolet radiation on the skin. Toxicol Appl Pharmacol 195:298–308
21. Moriwaki S, Takahashi Y (2008) Photoaging and DNA repair. J Dermatol Sci 50:169–176
22. Müller-Decker K, Fürstenberger G (2007) The cyclooxygenase-2-mediated prostaglandin signaling is causally related to epithelial carcinogenesis. Mol Carcinog 46:705–710
23. Neeman I, Lishitz A, Kashman Y (1970) New antibacterial agent isolated from avocado pear. Appl Microbiol 19:470–473
24. Oberlies NH, Rogers LL, Martin JM, McLaughlin JL (1998) Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana*. J Nat Prod 61:781–785
25. Pagès V, Fuchs RP (2002) How DNA lesions are turned into mutations within cells? Oncogene 21:8957–8966
26. Pillai S, Oresajo C (2005) Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation—a review. Int J Cosmet Sci 27:17–34
27. Ramos-Jerz MR (2007) Phytochemical analysis of avocado seeds (*Persea americana* Mill., c.v. Hass). Cuvillier Verlag, Gottingen
28. Rundhaug JE, Miculec C, Pavone A, Fisher SM (2007) A role for cyclooxygenases-2 in ultraviolet light-induced skin carcinogenesis. Mol Carcinog 46:692–698
29. Schroeder P, Krutmann J (2010) What is need for a sunscreen to provide complete protection. Skin Therapy Lett 15:4–5
30. Toichi E, McCormick TS, Cooper KD (2002) Cell surface and cytokine phenotypes of skin immunocompetent cells involved in ultraviolet-induced immunosuppression. Methods 28:104–110
31. Trompezinski S, Pernet I, Schmitt D, Viac J (2001) UV radiation and prostaglandin E2 up-regulate vascular endothelial growth factor (VEGF) in cultured human fibroblasts. Inflamm Res 50:422–427
32. Urbanski A, Schwarz T, Neuner P, Krutmann J, Kirnbauer R, Köck A, Luger TA (1990) Ultraviolet light induces increased circulating interleukin-6 in humans. J Invest Dermatol 94:808–811
33. Vainio H, Bianchini F (2000) Cancer-preventive effects of sunscreens are uncertain. Scand J Work Environ Health 26:529–531
34. Yaar M, Gilchrest BA (2007) Photoageing: mechanism, prevention and therapy. Br J Dermatol 157:874–887
35. Yoshizumi M, Nakamura T, Kato M, Ishioka T, Kozawa K, Wakamatsu K, Kimur H (2008) Release of cytokines/chemokines and cell death in UVB-irradiated human keratinocytes, HaCaT. Cell Biol Int 32:1405–1411
36. Young AR, Walker SL (1999) Sunscreens: photoprotection of non-erythema endpoints relevant to skin cancer. Photodermatol Photoimmunol Photomed 15:221–225