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Cherry fruit anthocyanins cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside protect against blue light-induced cytotoxicity in HaCaT cells

Hyang-Yeol Lee and Jun-Sub Kim*

Abstract

Blue light derived from multiple sources, including sunlight, generates reactive oxygen species (ROS) and negatively affects the skin in a manner similar to that of ultraviolet light. Cyanidin-3-*O*-glucoside (C3OG) and cyanidin-3-*O*-rutinoside (C3OR) are anthocyanin antioxidants that have protective effects on various tissues and cell types. However, the effects of anthocyanins on blue light-mediated changes remain unconfirmed. In this study, we determined the protective effects of C3OG and C3OR isolated and purified from waste cherry fruits (*Prunus serrulata* L. var. *tomentella* Nakai) against the blue light-induced ROS formation and inflammatory responses in HaCaT cells. It is showed that the treatment of C3OG and C3OR significantly reduced the blue light-induced cytotoxicity and ROS production in a dose dependent manner. Furthermore, we found that focal adhesion kinase (FAK) is a major upstream of blue light-induced expression of inflammatory cytokines (TNF-α, IL-6 and IL-8), and these effects were attenuated by C3OG or C3OR treatment. In the initial reaction, blue lights increased the phosphorylation of inhibitory-κB Kinase α (IKKα), c-jun N-terminal kinase (JNK), and p38. The phosphorylation of these intracellular proteins was reduced via FAK inhibitor, NAC (ROS scavenger), and anthocyanin treatments. After 24 h of blue light irradiation, C3OG or C3OR treatment markedly inhibited caspase-3-mediated apoptosis and cleaved-FAK-mediated anoikis, which is cell detachment-induced apoptosis. Therefore, our results indicate that C3OG and C3OR effectively protected human keratinocytes from harmful blue light-induced cytotoxicity and inflammation.

Keywords Blue light, Anthocyanins, Cyanidin 3-*O*-glucoside, Cyanidin 3-*O*-rutinoside, Reactive oxygen species, Focal adhesion kinase, Agri-food waste

Introduction

Similar to ultraviolet (UV) radiation, blue light can induce the generation of reactive oxygen species (ROS) and thereby cause oxidative damage to the skin [1], with exposure to blue light stimulating melanocytes and causing pigmentation problems such as spots and age spots [2, 3]. Moreover, blue light has a longer wavelength and lower energy than UV light. It can penetrate deeper into the skin [4] and promote photoaging and inflammatory changes in a wider range of skin layers than UV.

Recently accumulated evidence has indicated that red light promotes an increase in cell proliferation, whereas blue light reduces the viability of different cell types by inducing changes in mitochondrial function, including

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aberrant ROS formation [5–8]. In particular, blue light has been demonstrated to have a detrimental effect on the skin. At the molecular level, blue light (410 nm) has been established to cause a reduction in period circadian regulator 1 (a clock gene involved in the circadian rhythm) transcription and an increase in ROS production, DNA damage, and release of inflammatory cytokines (interleukin [IL]-1α, IL-6, IL-8, and tumor necrosis factor-alpha [TNF-α]) in keratinocytes [9]. At longer wavelengths, blue light (470–480 nm) has been found to promote an increase in ROS release, which is dependent on transient receptor potential vanilloid 1 [10]. Also, blue light at 460 nm was shown to induce an increase in ROS production by mediating flavin excitation, which may, in turn, cause aging of skin [1]. Furthermore, high-intensity blue light (412, 419, 426, and 453 nm) is toxic to endothelial cells and keratinocytes [11], and blue-violet light (380–495 nm) has been demonstrated to have destructive effects on the carotenoids in the skin [12]. Collectively, the findings of these studies thus indicate that ROS generation is the main factor underlying the detrimental effects attributable to blue light.

Focal adhesion kinase (FAK) is a protein tyrosine kinase activated via integrins and growth factor receptors [13]. Having been activated, FAK promotes cell migration and proliferation, including those associated with tumor metastasis and angiogenesis [14, 15], and plays a major role in inflammatory cytokine signaling [16, 17]. Notably, ROS is linked to the activation of several protein tyrosine kinases, including FAK, proline-rich tyrosine kinase 2, and Src [18–21]. Furthermore, we have previously shown that FAK is activated by ROS generated during exposure to illumination from different light-emitting diodes (LEDs) and is involved in downstream signaling pathways, including those of mitogen-activated protein kinase (MAPK) and inhibitory-κB Kinase α (IKKα) [8].

The springtime cherry blossom festival is a particularly important tourist event in countries such as Japan and Korea [22]. Consequently, cherry trees are extensively planted in many areas as a policy to enhance the added value of a region. However, the large numbers of fruits produced by these trees (*Prunus serrulata* L. var. *tomentella* Nakai) tend to have an unpleasant taste and texture and hence go to waste. In this study, we show the functional utility of this wasted commodity, which can contribute to resource recycling and creating added value.

Anthocyanins, which are produced in abundance in colored fruits and vegetables, such as berries, red grapes, purple sweet potatoes, and red cabbages, are among the most important and interesting classes of flavonoids, and accumulating evidence is indicating their beneficial effects in preventing human diseases associated with oxidative stress [23]. Among the diverse variety of anthocyanins, cyanidin-3-*O*-glucoside (C3OG) has been identified as the most common and abundant anthocyanins in fruits [24], and the findings of numerous studies have indicated that C3OG has beneficial effects on the treatment of a range of inflammatory disorders, aging, cancer, and diabetes [25]. To date, however, it has yet to be established whether anthocyanins have any beneficial effects with respect to blue light-induced cytotoxicity.

Results

Effects of cyanidin 3-*O*-glucoside (C3OG) and cyanidin 3-*O*-rutinoside (C3OR) on blue light-induced cytotoxicity in HaCaT cells

In our previous study, we demonstrated that high-intensity LED blue light has a cytotoxic effect on human skin keratinocytes (HaCaT cells) mediated via the generation of large amounts of ROS [8]. Further, C3OG and C3OR can contribute to inhibiting intracellular ROS production induced by diverse stimuli [26, 27]. Consequently, in the present study, we evaluated the effects of C3OG or C3OR isolated from cherry fruits on blue light-induced cytotoxicity.

We found that, compared with the controls, whereas blue light at lower light intensities (2,500 and 5,000 lx) gradually increased cell viability, exposure to higher intensities (10,000 and 20,000 lx) significantly reduced viability (Fig. 1A). Based on these observations, we selected blue light of 20,000 lx, which had the highest cytotoxic effect, for further experiments. In contrast, compared with the controls, neither C3OG nor C3OR showed any clear evidence of cytotoxicity up to a concentration of 200 μM (Fig. 1B). Having established these effects, we subsequently examined the effects of C3OG and C3OR on blue light-induced cytotoxicity. Accordingly, we observed that both C3OG and C3OR dose-dependently reversed the blue light-induced cytotoxicity in HaCaT cells (Fig. 1C). This effect was also observed in crude extract of cherry fruit (Additional file 1: Fig S1).

Effects of C3OG and C3OR on blue lightinduced ROS production

Given that the cytotoxic effects of blue light are dependent on excessive ROS generation [1, 8], we sought to determine whether C3OG and C3OR can attenuate the blue light-induced generation of cellular ROS. To this end, we exposed HaCaT cells pre-treated with either C3OG or C3OR to blue light for 1 h and subsequently measured the cellular ROS production using H_2DCFDA staining. Compared with the controls, we observed that exposure to 20,000 lx blue light promoted ROS generation and that this response could be blocked by NAC (a ROS scavenger) treatment (Fig. 2). In line with

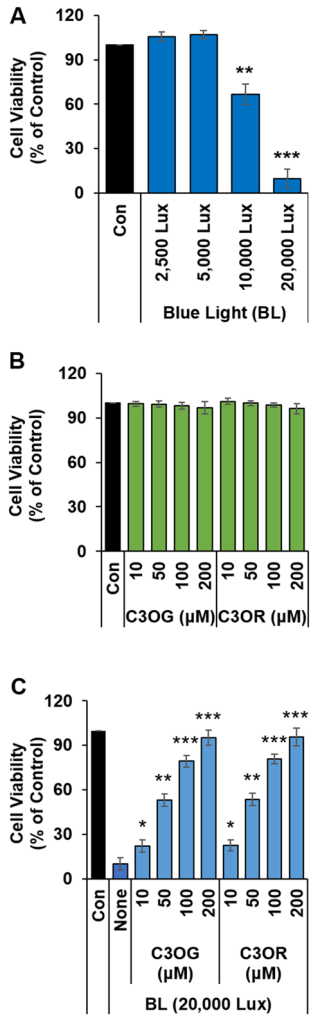

Fig. 1 Effects of C3OG and C3OR on the viability of HaCaT cells exposed to blue light. **A** HaCaT cells cultured in 96-well plates were exposed to blue light for 1 h. After 24 h, cell viability was quantified using an MTT assay ($n = 3$, ±SD). ** $p < 0.01$ and *** $p < 0.001$ vs. the control. **B** Cells were treated with or without the indicated concentrations of C3OG or C3OR for 24 h, and then cell viability was quantified using an MTT assay ($n = 3$, ±SD). **C** Cells were pre-treated with or without the indicated concentrations of C3OG or C3OR for 24 h prior to blue light exposure for 1 h. After 24 h, cell viability was quantified using an MTT assay ($n = 3$, ±SD). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. blue light (20,000 lx)

expectations, we found that pre-treatment with either C3OG or C3OR conferred a significant dose-dependent protective effect against blue light-induced cellular ROS production (Fig. 2).

Effects of C3OG and C3OR on FAK and MAPK activation in response to blue light exposure

We have recently reported that red, green, and blue light-induced ROS stimulates the activation of FAK and MAPKs in HaCaT cells [8], and numerous other studies have observed crosstalk between ROS and FAK or MAPKs [28]. Consequently, we evaluated the effects of C3OG and C3OR on FAK and MAPK activity in HaCaT keratinocytes by exposing cells to blue light for 30 min, and analyzing cell lysate using western blotting. We found that NAC and FAK inhibitor (FAK-i) treatments reduced the blue light-induced phosphorylation of FAK, MAPKs, or IKKα (Fig. 3). Treatment with C3OG or C3OR significantly reduced phosphorylation in a concentration-dependent manner (Fig. 3).

Effects of C3OG and C3OR on blue light-induced inflammation

Although the activation of ROS-induced MAPKs is reportedly an important process in the development of inflammation [29], the mechanisms underlying the blue light induction of these events have yet to be sufficiently studied. However, although we previously found that ROS generated by blue light activates MAPKs via the activation of FAK, we did not confirm whether this mechanism is associated with an inflammatory response [8]. In the present study, to investigate the effects of C3OG and C3OR on pro-inflammatory cytokine production in HaCaT cells, we pre-treated cells with C3OG or C3OR for 24 h, followed by exposure to blue light for 1 h, and then monitored the levels of TNF-α, IL-6, and IL-8 in cell culture supernatants.

The results of the respective ELISA assays revealed that blue light induced the expression of all three of these pro-inflammatory cytokines in HaCaT cells (Fig. 4). Moreover, we demonstrated that the expression of these cytokines was significantly inhibited by pretreatment with NAC or FAK-I. However, when administered individually, the effect of FAK-i in this regard was less pronounced than that of NAC. In addition, we found that pre-treatment with a combination of NAC and FAK-i had no clear synergistic effect (Fig. 4). These findings thus tend to indicate that FAK-mediated signaling is one of many ROS-regulated signaling pathways associated with the activation of an inflammatory response. Similarly, we found that both C3OG and C3OR significantly inhibited the blue light-induced expression of TNF-α, IL-6, and IL-8. The inhibitory effect was dose-dependent and the inhibitory effect of at the 200 μM concentration was superior to that obtained using FAK-i (Fig. 4).

Effects of C3OG and C3OR on blue light-induced apoptosis

In our previous study, we found that high-intensity blue LED light induced apoptosis via the activation of caspase-3 activation and cleavage (inactivation) of FAK [8]. Consequently, in the present study, we examined

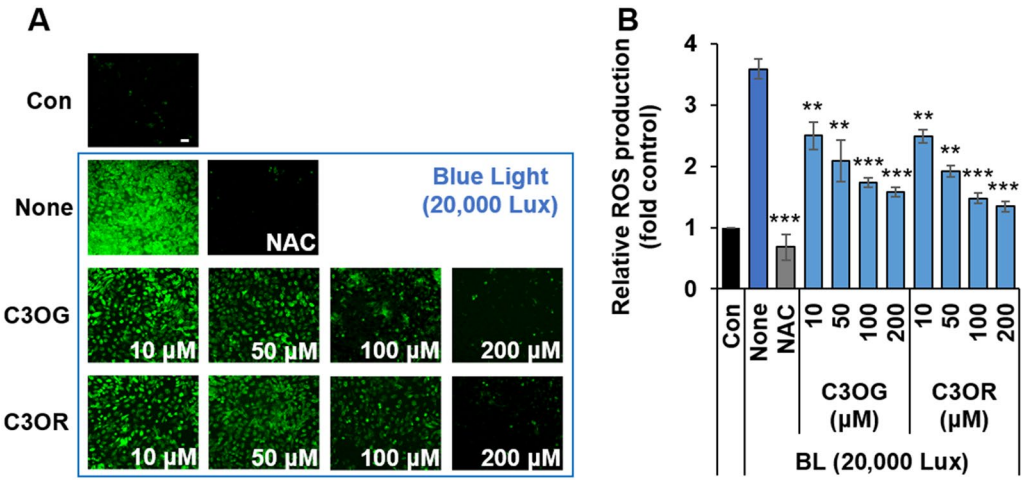

Fig. 2 Effects of C3OG and C3OR on ROS production in HaCaT cells exposed to blue light. HaCaT cells cultured in 96-well plates were exposed to blue light (20,000 lx) for 1 h and then stained with H_2DCFDA (**A, B**). HaCaT cells were pre-treated with the indicated concentrations of C3OG or C3OR for 24 h or with NAC (10 mM) for 1 h prior to exposure to blue light (**A, B**). The generation of ROS was visualized using a fluorescence microscope, Scale bar, 100 μm (**A**), or measured using a fluorescence plate reader (**B**). The bar graph presents the fold changes in ROS production in cells exposed to blue light relative to the unexposed control cells ($n = 3$, ±SD). ** $p < 0.01$ and *** $p < 0.001$ vs. blue light (20,000 lx)

the effects of C3OG and C3OR on blue light-induced apoptosis.

Based on a comparison of cell lysates prepared after 24 h following a 1-h exposure to blue light, we found that blue light increased the cleavage of PARP, caspase-3, and FAK and that pre-treatment with NAC or z-DEVD-fmk (a caspase-3 inhibitor) blocked the cleavage of these proteins (Fig. 5). In line with expectations, we observed that 200 μM C3OG or C3OR also markedly reduced the cleavage of FAK and PARP via caspase-3 activation (Fig. 5).

It has previously been shown that exposure to high-intensity blue light induces the activation of caspase-3, which in turn induces apoptosis and the cleavage of FAK [8], and subsequently anoikis [30]. In the present study, we observed that blue light could also reduce cell adhesion and increase cell detachment from the extracellular matrix (Fig. 6). However, these detrimental effects could be significantly reduced by treatment with C3OG, C3OR, z-DEVD-fmk, or NAC (Fig. 6).

Collectively, our findings indicate that C3OG or C3OR isolated from cherry fruits are effective suppressors of blue light-induced cytotoxic effects and inflammatory responses.

Discussion

The two major anthocyanins evaluated in this study, C3OG and C3OR, were isolated by cation exchange column chromatography and semi-preparative HPLC to yield 8 and 35 mg of pure compounds, respectively, from 1 L of fresh cherry juice, with an estimated total anthocyanins content of 428.6 ± 3.8 mg/L in fresh cherry juice. Having removed organic acids and polysaccharides from the crude juice by ion-exchange column chromatography, we succeeded in achieving a more than three-fold enrichment in the total content of anthocyanins in the eluate compared to the original juice and an overall 10% yield of purified C3OG and C3OR (Additional file 1: Fig. S2).

Blue light exposure can lead to cellular dysfunction via the generation of ROS in the skin [4], with high-level blue light-induced ROS causing damage to the skin barrier, leading to aging [1, 31], hyperpigmentation [2], inflammation [10], and melasma [32]. In this regard, although the antioxidant properties of anthocyanins are well established, their effects with respect to the detrimental effects of blue light are yet to be confirmed.

Especially, C3OG has already confirmed its effect in UVB-induced cytotoxicity studies. Cimino et al. observed that UVB-exposed cells showed increase of the translocation of transcription factors NF-kB and AP-1, overexpression of the proinflammatory cytokine IL-8, cleavage of procaspase-3, and DNA fragmentation and these effects inhibited by C3OG treatment [33]. Hu et al. reported that C3OG inhibit UVB-induced ROS production, DNA damage, and apoptosis [34]. He et al. demonstrated that C3OG could effectively prevent the UVB-induced apoptosis of HaCaT cells by the scavenging of ROS and the suppression of COX-2 expression by interaction with the MAPK and Akt signaling pathways [35]. Consistently with this, we found that positive effects of C3OG and C3OR on blue light-induced inflammation and apoptosis in HaCaT cells mediated via the generation of ROS. Although DNA damage by blue light irradiation

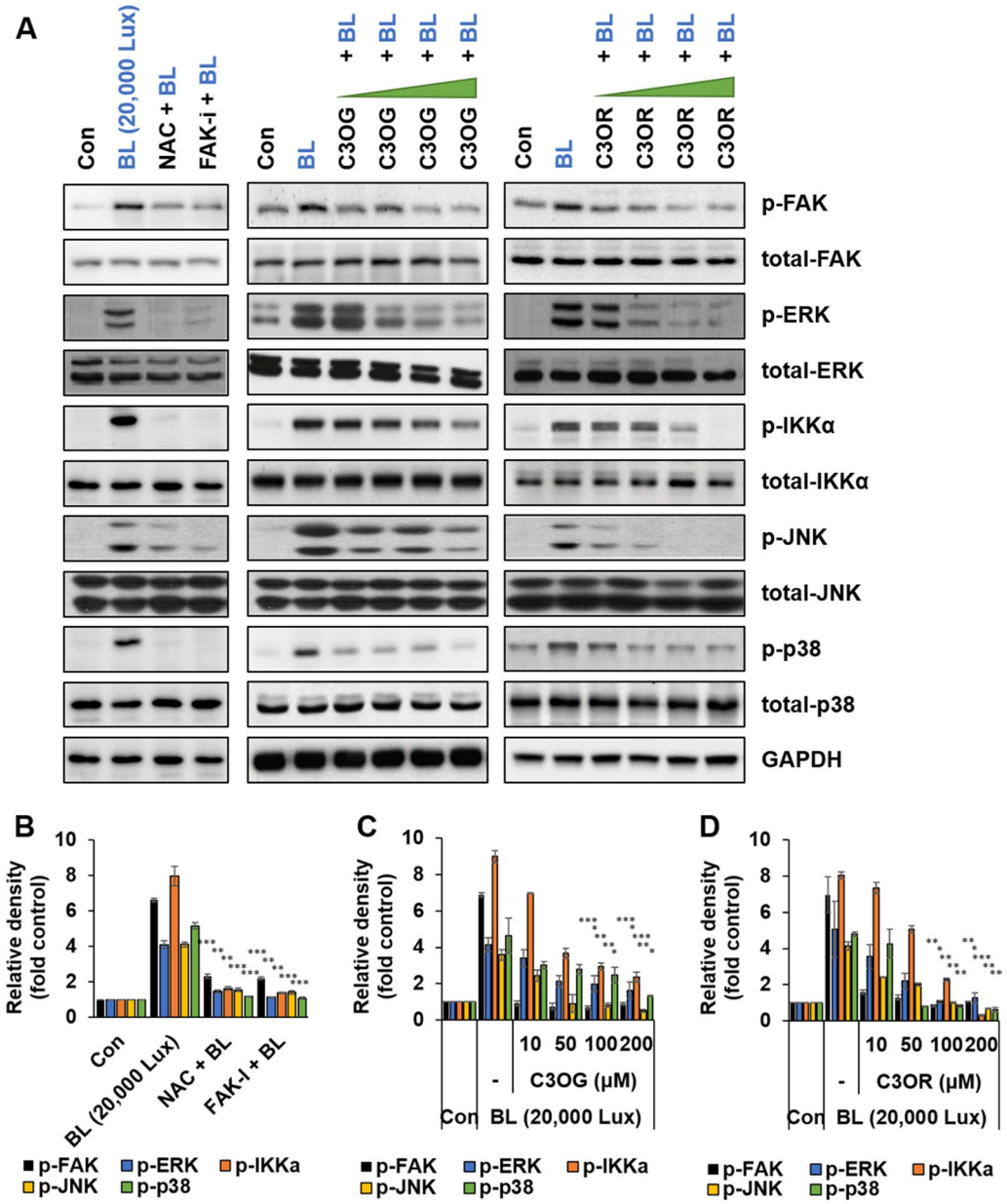

Fig. 3 Effects of C3OG and C3OR on the activation of FAK and MAPK induced by blue light. HaCaT cells in six-well plates were exposed to blue light (20,000 lx) for 30 min. Cells were pre-treated with or without NAC (10 mM) and FAK-i (1 μM) for 1 h or different concentrations (10, 50, 100, or 200 μM) of C3OG or C3OR for 24 h prior to blue light exposure (**A**). Immunoblots show pY397 FAK, p-IKKα, p-JNK, p-ERK, and p-p38, the corresponding total proteins, and GAPDH used as a loading control. **B–D** Calculated fold changes in pY397 FAK, p-IKKα, p-JNK, p-ERK, and p-p38 ($n = 3$, ±SD). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. blue light (20,000 lx)

was not confirmed in this study, it is expected that the results will not be different from those of UVB. However, in order to clarify the difference between UVB and blue light, confirmation is necessary.

We also established that FAK is an important upstream factor regulating the inflammatory responses induced by blue light (Fig. 4) and that C3OG and C3OR are effective inhibitors of this regulation (Fig. 7). Given that FAK is a major upstream signal molecule implicated in several pathophysiological mechanisms, C3OG and C3OR may potentially be used to treat various diseases characterized by FAK-associated mechanisms. Notably, 36 have reported that black rice-derived anthocyanins can inhibit breast cancer epithelial-mesenchymal transition-mediated metastasis by suppressing FAK activation in human breast cancer cells [36]. Although these authors demonstrated that

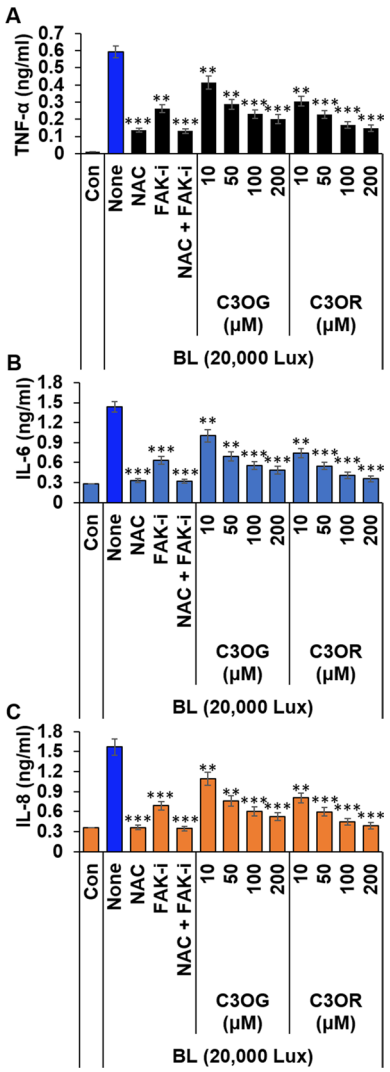

Fig. 4 Effects of C3OG and C3OR on the expression of inflammatory cytokines induced by blue light. HaCaT cells in six-well plates were exposed to blue light (20,000 lx) for 1 h. Cells were pretreated with or without NAC (10 mM), FAK-i (1 μM), or NAC + FAK-i for 1 h or different concentrations (10, 50, 100, or 200 μM) of C3OG or C3OR for 24 h prior to blue light exposure. After 24 h, supernatants were collected, and the amounts of TNF-α (**A**), IL-6 (**B**), and IL-8 (**C**) were determined by ELISA ($n = 3$, ±SD). ** $p < 0.01$ and *** $p < 0.001$ vs. blue light (20,000 lx)

phosphorylation of FAK is reduced by anthocyanin treatment, they did not elucidate the mechanisms by which these anthocyanins contribute to the regulation of FAK activity. In the present study, we showed that blue light-induced ROS production and FAK phosphorylation are reduced concentration-dependently by treatment with either C3OG or C3OR (Figs. 2 , 3). Moreover, we have previously established that ROS promotes the phosphorylation of FAK in both intact cells and a cell-free system [21]. Thus, it is conceivable that the antioxidant effects of anthocyanins may contribute to suppressing FAK activity in breast cancer cells.

As mentioned in the Introduction section, blue light-induced cytotoxicity and inflammation have detrimental effects on exposed skin. However, the effects of blue light tend to differ according to cell and tissue types. For example, in THP-1 cells, blue light has been demonstrated to inhibit the cytotoxic effects of low levels of lipopolysaccharide (LPS) and reduce the release of inflammatory cytokines. However, these effects were not observed at high LPS levels [37]. Blue light has also been shown to increase the expression of Nrf2 in A431 epidermoid carcinoma cells, thereby promoting an increase in the expression of heme oxygenase 1, an antioxidative factor [38]. However, whereas at high intensities, blue light (412–426 nm) appears to have toxic effects, at 453 nm, blue light irradiation has been demonstrated to be non-toxic up to an intensity of 500 J/cm^2 in primary human keratinocytes and immortalized skin-derived HMEC-1 cells [11]. Accordingly, these observations indicate that blue light may have beneficial effects at low intensities. However, these controversies due to the divergence of protocols used [39, 40] and the different mechanisms employed by cells to counter oxidative stress [41]. Accumulated evidence suggests that low levels of ROS are considered to have a protective role in cells, and excessive amounts cause damage to cells [42]. Concomitantly with this, our previous study [8] and this study showed low intensity of blue light make low level ROS and did not affect cell viability or slightly increased (Figs. 1 , 2).

Since this study used only skin cell lines, there are many limitations in explaining the clear effect of C3OG or C3OR on blue light in actual skin. Pure C3OG or C3OR is unstable even at room temperature and is easily oxidized or degradation by pH, light, oxygen, solvents, temperature and metal ions [24]. When oral uptake, it is broken down more rapidly and converted into various metabolites [43]. Therefore, even in animal experiments, the effect of C3OG or C3OR is likely to vary depending on the method of

(See figure on next page.)

Fig. 5 Effects of C3OG and C3OR on blue light-induced FAK cleavage and caspase-3 activation. HaCaT cells in six-well plates were exposed to blue light for 1 h and then treated with or without NAC (10 mM) or z-DEVD-fmk (200 μM) for 24 h. Cells were pre-treated with 3OG (200 μM) or C3OR (200 μM) for 24 h prior to blue light exposure. (**A**) Immunoblots of total-FAK, PARP, procaspase-3, active caspase-3 (cleaved), and the GAPDH loading control are shown. (**B**–**D**) Calculated fold changes of cleaved-FAK, -PARP, and -caspase-3 calculated ($n = 3$, ±SD). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. blue light (20,000 lx)

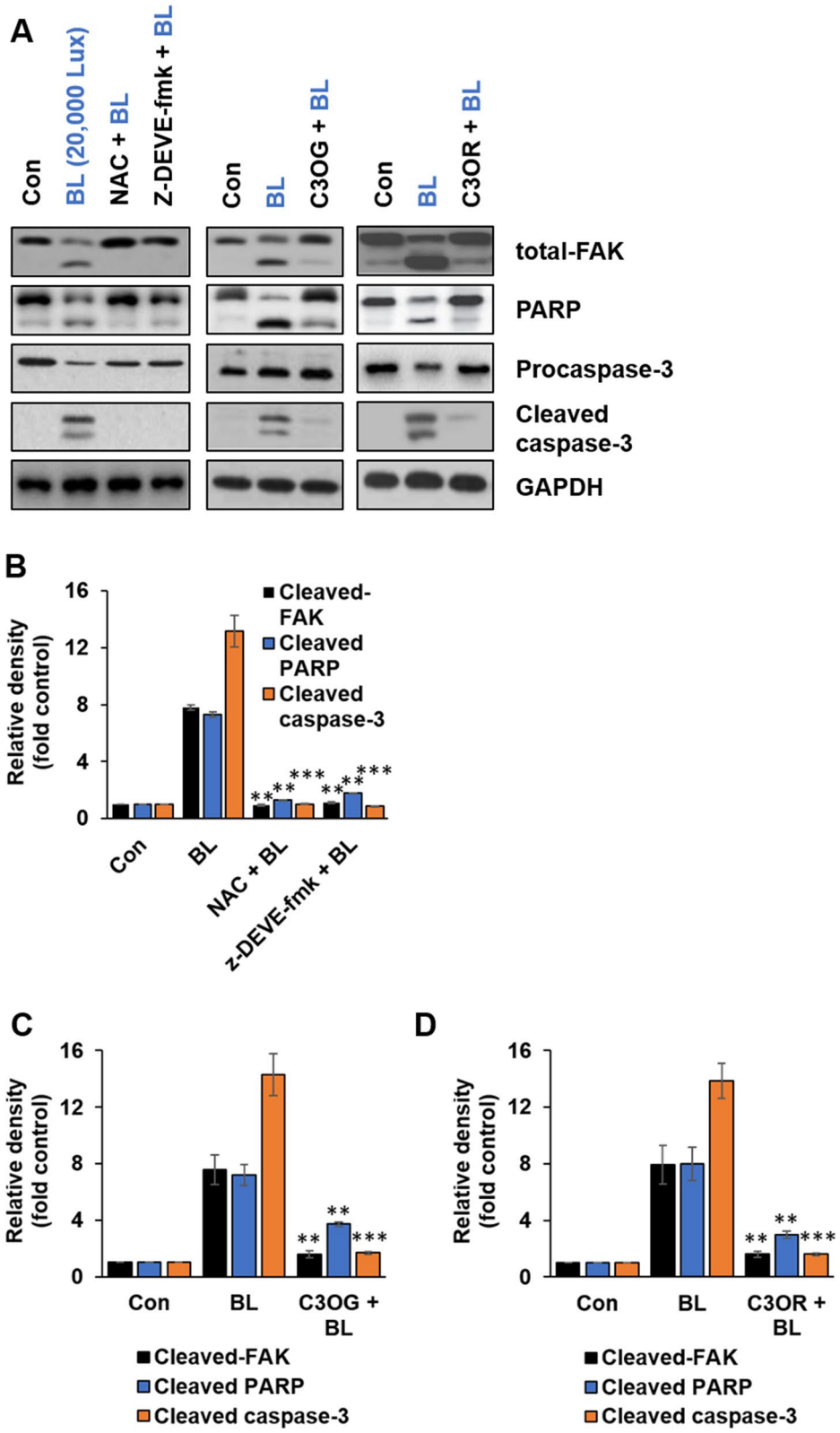

Fig. 5 (See legend on previous page.)

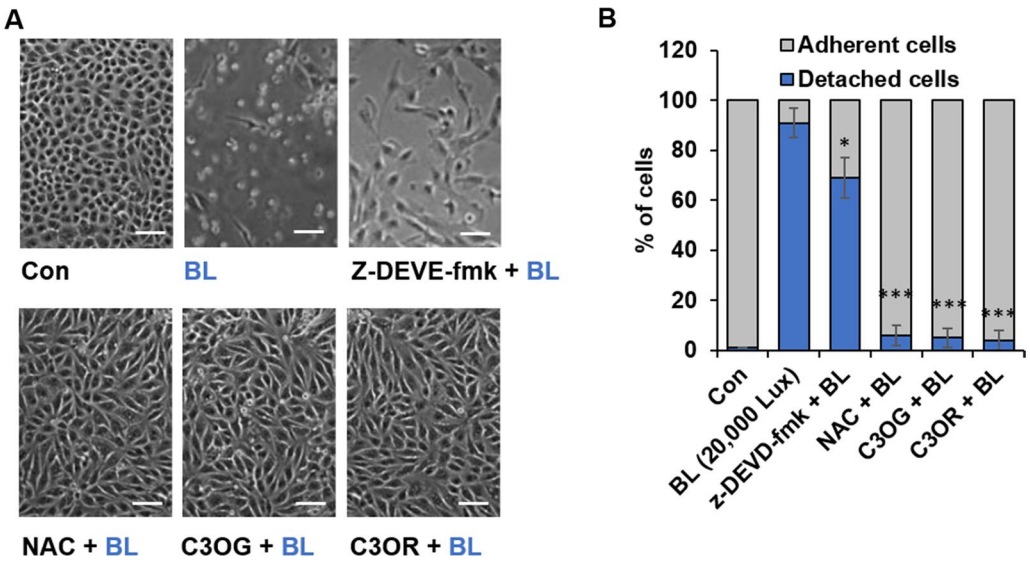

Fig. 6 Effects of C3OG and C3OR on blue light-induced cell detachment. HaCaT cells in six-well plates were exposed to blue light for 1 h and then treated with or without NAC (10 mM) or z-DEVD-fmk (200 μM) for 24 h. Cells were pre-treated with C3OG (200 μM) or C3OR (200 μM) for 24 h prior to blue light exposure (**A**, **B**). Representative phase-contrast images are shown (**A**), and the percentages of cell adhesion and detachment relative to the control (**B**) were quantified ($n = 3$, ±SD). Scale bar, 100 μm. * $p < 0.01$ and *** $p < 0.001$ vs. blue light (20,000 lx)

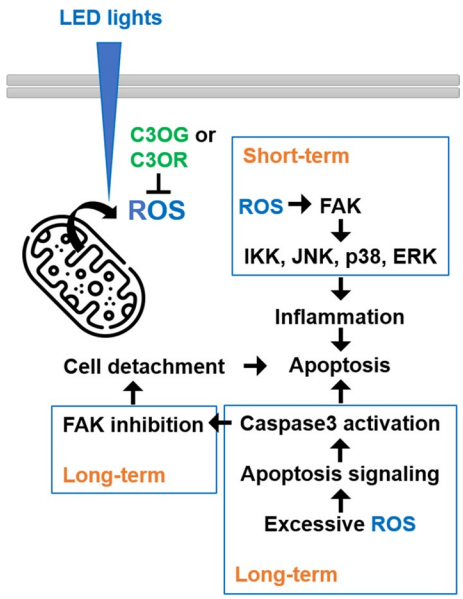

Fig. 7 C3OG and C3OR block blue light-induced cytotoxicity. Exposure to blue light initiates FAK-related inflammation signaling events via ROS, triggering apoptotic signaling. Treatment with the anthocyanins C3OG and C3OR effectively suppress the detrimental effects of blue light via a reduction in the generation of ROS.

direct application to the skin and oral feeding. For commercial use of C3OG or C3OR, new methods preparing much more stable anthocyanins needs to be further explored.

Consequently, our findings indicate that high-intensity blue light induces high levels of ROS production, inflammation, and apoptosis. The anthocyanins C3OG and C3OR are effective in suppressing the detrimental effects of blue light on HaCaT cells.

Materials and methods

Cells and reagents

Human skin keratinocytes (HaCaT cells) were obtained from the American Type Culture Collection (USA) and maintained in Dulbecco's modified essential medium containing 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, 100 units/mL penicillin, and 100 μg/mL streptomycin. *N*-acetyl-l-cysteine (NAC), *z*-DEVD-fmk, and cell proliferation kit I (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA); FAK inhibitor (FAKi; PF-562,271) was obtained from MedKoo (Chapel Hill, NC, USA); and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) was purchased from Invitrogen (Carlsbad, CA, USA). Antibodies against FAK (Millipore, MA, USA), pY397-FAK (Invitrogen), extracellular signal-regulated kinase (ERK), p-ERK, c-Jun N-terminal kinase (JNK), p-JNK, p38, p-p38, IKKα, p-IKKα, procaspase-3, cleaved caspase-3, PARP (Cell Signaling, Danvers, MA, USA), and GAPDH (Millipore, MA, USA) were obtained as indicated.

Preparation of Cyanidin-3-*O*-glucoside (C3OG) and cyanidin 3-*O*-rutinoside from cherry fruits (*Prunus serrulata* L. var. *tomentella* Nakai)

Cyanidin-3-*O*-glucoside (C3OG) and cyanidin-3-*O*-rutinoside (C3OR) were extracted from cherry fruits collected in June 2019 from trees in Jeungpyeong Gun, Chungbuk, South Korea (Fig. 1 A). 1.0 L of 95% ethanol was added to 0.20 kg of the fresh cherry fruits, grinded and squeezed to produce a purple juice, which was filtered through a vacuum filtration device to yield crude extract that was used for further preparation of anthocyanin-enriched extracts. The crude extract was purified by column chromatography through a Dowex ECR-S resin. The preparation thus obtained was washed with acidified water (0.1% HCl) to remove naturally-occurring sugars and acids contained in the fruits. Thereafter, the resulting preparation was eluted with acidified methanol (0.1% HCl), concentrated, and freeze-dried to yield an anthocyanin-rich powder [44]. The two target anthocyanins, C3OG and C3OR, were isolated by semi-preparative HPLC using a YMC Triart C18 column (250 × 10 mm, 5 μm, 12 nm), yielding 8 and 35 mg of pure anthocyanin compound, respectively, from 1 L of cherry juice (200 g of fresh cherry fruits). We included anthocyanins content of extracts in Additional file data (Additional file 1: Fig. S2).

High performance liquid chromatography (HPLC-DAD)

All HPLC analyses were performed using a Youngrin YL9100 HPLC system equipped with a photodiode array (PDA) detector. Samples were analyzed using a YMC ODS-A C18 column (250 × 4.6 mm, 5 μm, 12 nm), for which we used a gradient mobile phase comprising solvent A (H_2O containing 0.1% trifluoroacetic acid) and solvent B (acetonitrile). The gradient elution program used was as follows: an initial A to B ratio of 90:10, followed by an increase in B from 10 to 15% in 40 min at a flow rate of 1.0 mL/min, and a subsequent increase in B from 15 to 80% in 10 min. The retention time of compound 1 was 27.4 min, whereas compound 2 was eluted at 30.0 min. Based on comparing the chromatogram peaks obtained for isolated compounds and those of standard compounds, compounds 1 and 2 were identified as C3OG and C3OR, respectively (Fig. 8B and D). The results of full-wave scanning (Fig. 8 C and E) revealed that both C3OG and C3OR have two maximum absorption peaks at approximately 281 and 518 nm and 279 and 515 nm, respectively, which is consistent with the previously reported characteristics of these anthocyanins. We also further confirmed the chemical structures of two major anthocyanin compounds using NMR spectroscopy (Additional file 1: Figs. S3, S4) [45, 46].

Cell viability assay

Cell viability was determined using the thiazolyl blue tetrazolium bromide (MTT) assay. HaCaT cells were cultured in 96-well plates (clear-bottomed, dark-sided 96-well microplate; Thermo Fisher Scientific, Rochester, NY, USA) at a density of 1×10^4 cells/well and pretreated with or without NAC (10 mM) for 1 h, and C3OG (10–200 μM) or C3OR (10–200 μM) for 24 h prior to exposure to 2,500–20,000 lx blue LED light for 1 h. After 24 h, the cells were incubated with MTT (0.25 mg/mL) at 37 °C in a CO_2 incubator for 4 h. The resulting MTT formazan products were dissolved in DMSO, and absorbances were measured at 570 nm using a microplate reader (Bio-Tek Instruments Inc., Santa Clara, CA, USA).

ROS measurement

HaCaT cells were cultured in 96-well plates (clear-bottomed, dark-sided) at a density of 1×10^4 cells/well and pretreated with or without NAC (10 mM) for 1 h and C3OG (10–200 μM) or C3OR (10–200 μM) for 24 h prior to exposure to 20,000 lx blue LED light for 1 h. Thereafter, the cells were stained with 10 μM H_2DCFDA for 30 min at 37 °C and subsequently washed with phosphate-buffered saline prior to analysis using a fluorescence microplate reader or visualized under a fluorescence microscope.

LED light exposure

Direct sunlight can reach a light intensity of up to 100,000 lx and 25,000 lx in full daylight. Comparatively, indoor light intensities are considerably lower, with standard office lighting typically not exceeding 500 lx. During the day, light levels are determined by the presence of clouds and haze. They can vary to differing degrees and durations, depending on factors such as the prevailing cloud cover and atmospheric turbidity. Consequently, in the present study, we simulated overcast (2500 lx) and full-daylight (20,000 lx) conditions for 1 h, as was used in a previous study [8].

As a source of illumination, we used LED (GT-P25G6: blue, 460–470 nm, 9 W, GT-P25WB; Shenzhen Getian Opto-Electronics Co., Ltd., Shenzhen, China), which were attached to a fan and a heat sink to reduce the transfer of heat to samples. To eliminate unwanted thermal effects caused by the LED light, we monitored that the temperature of the incubator and cell culture dish was maintained at 37 °C in all experiments performed. HaCaT cells were seeded into 96-well cell culture plates (for MTT assays or ROS measurement) or six-well cell culture plates (for western blotting or adhesion assays). After 24 h, the cells were exposed to blue (450 nm) LED light at a unified illuminance of 2,500–20,000 lx for 1 h in an incubator equipped with an LED box. Illuminance was

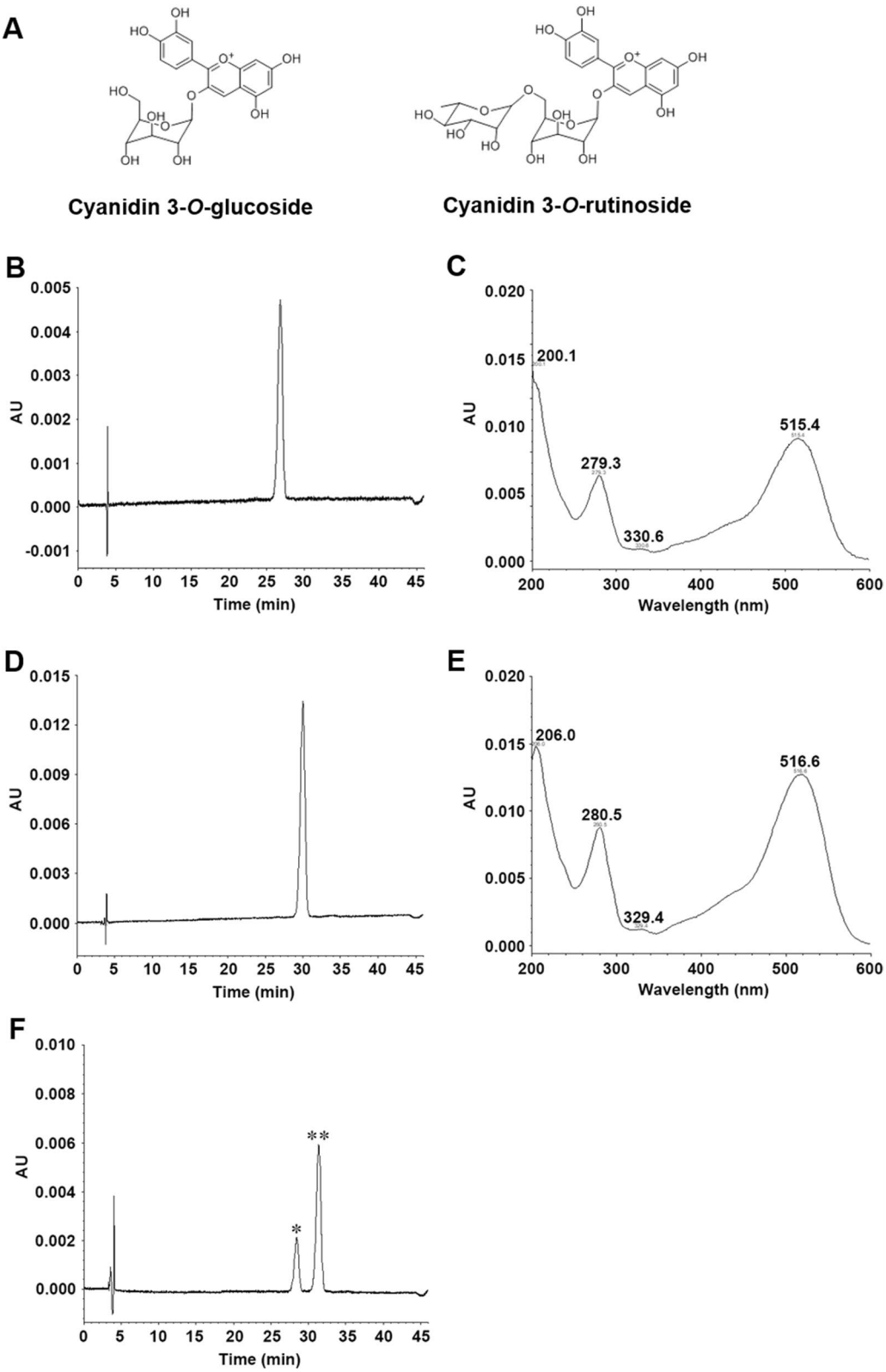

Fig. 8 Chemical structures of the anthocyanins cyanidin 3-*O*-glucoside (C3OG) and cyanidin 3-*O*-rutinoside (C3OR) (**A**). The 280 nm wavelength scan used to determine the purity of compounds 1 and 2 (**B** and **D**). A full-wave scan of C3OG and C3OR (**C** and **E**). An HPLC **c**hromatogram of anthocyanin standard compounds (**F**): cyanidin 3-*O*-glucoside (*) and cyanidin 3-*O*-rutinoside (**)

measured and adjusted using a Hioki 3423 lx HiTester lux meter (Hioki E. E. Corporation, Japan) on the sample surface. As a control, a similar plate of cells was incubated in an unilluminated incubator. To avoid any potential bias, the cells used for the control and light irradiation treatments cells were derived from the same stock.

Immunoblotting

To observe cellular canonical signaling, HaCaT cells in six-well plates were pre-treated with NAC (10 mM) and PF-271 (1 μM) for 1 h or C3OG (10–200 μM) or C3OR (10–200 μM) for 24 h prior to exposure to blue LED illumination (20,000 lx) for 30 min, and thereafter immediately lysed (Fig. 4). Similarly, to determine the activation of caspase-3, HaCaT cells in six-well plates were pretreated with NAC (10 mM) and DEVD-fmk (200 μM) for 1 h or C3OG (200 μM) or C3OR (200 μM) for 24 h prior to exposure to blue LED illumination (20,000 lx) for 1 h, followed by cell lysis (Fig. 6). Clarified lysates were run on 4–12% NuPAGE Tris-Bis gels (Invitrogen). The separated proteins were transferred to polyvinylidene fluoride membranes, blocked with 3% bovine serum albumin, and incubated overnight with primary AT antibodies at 4 °C. The following day, the membranes were washed with Tris-buffered saline containing 0.1% Tween® 20 detergent and then incubated with horseradish peroxidase-conjugated secondary antibodies. Bound antibodies were visualized using Enhanced chemiluminescence in conjunction with a ChemiDoc MP Imaging System (Bio-rad, Hercules, CA, USA).

Cell detachment assay

HaCaT cells in six-well plates were pretreated with NAC (10 mM) and DEVD-fmk (200 μM) for 1 h or C3OG (200 μM) or C3OR (200 μM) for 24 h prior to being exposed to blue LED illumination (20,000 lx) for 1 h. After 24 h, floating (detached cells in the supernatant) and adherent cells (obtained by trypsinization) were harvested separately. Cell counts were performed using a hemocytometer and the trypan blue exclusion method, with all viable (unstained) and dead (stained) cells being counted. Using the count data thus obtained, we calculated the percentages of cell adhesion and detachment using the following equations 8:

Percentageadherent cells = (number of adherent cells/total number of cells) × 100%

Percentage detached cells = (number of floatingcells/total number of cells) × 100%

Statistical analysis

Statistical significance between experimental groups was determined using the Student's *t*-test or two-way analysis of variance (ANOVA) in conjunction with the Sidak multiple comparisons test (Prism software, v7.0d; GraphPad Software, La Jolla, CA, USA).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00767-5.

Additional file 1: Figure S1. Effects of cherry fruitextract (CFE) on the viability of HaCaT cells exposed to blue light. (**A**)HaCaT cells were treated with or without the indicated concentrations of CFEfor 24 h, and then cell viability was quantified using an MTT assay (n =3, ±SD). (**B**) Cells were pre-treated with or without the indicatedconcentrations of CFE for 24 h prior to blue light exposure for 1 h. After 24h, cell viability was quantified using an MTT assay (n = 3, ±SD). * p< 0.05, ** p < 0.01, and *** p < 0.001 vs. blue light(20,000 Lux). **Figure S2.** Total anthocyanine content of the crude extract (1)and the anthocyanine enriched extract (2) from ion exchange chromatography. 1;428.6 ± 3.8mg/L, 2; 1185.6 ± 3.4mg/L. **Figure S3.** NMR spectra of cyanidin 3-*O*-glucoside (C3OG). A; ^{1}H-NMR, B; ^{13}C-NMR. ^{1}H-NMR(400 MHz, D_2O) 8.08 (d, 1H, *J*=10.4 Hz), 7.28 (s, 1H), 6.94(d, 1H, *J*=15.6Hz), 6.29 (t, 1H, *J*=10.2Hz), 6.16 (t, 2H, *J*=8.6Hz), 5.0 (s, 1H),3.93 (d, 1H, *J*=12.0Hz), 3.79 (m, 1H), 3.58 ~ 3.51 (m, 4H). ^{13}C-NMR(100 MHz, D_2O) 168.7, 158.8, 156.5, 154.6, 153.3, 144.6, 142.9,132.9, 126.3, 118.4, 116.1, 111.2, 102.4, 101.2, 100.3, 94.7, 75.7, 75.5, 72.5,71.8, 70.3, 69.8, 69.1, 68.5, 66.3, 16.4. **Figure S4.** NMR spectra of cyanidin 3-*O*-rutinoside (C3OR). A; ^{1}H-NMR, B; ^{13}C-NMR. ^{1}H-NMR(400 MHz, D_2O) 8.10 (s, 1H), 7.40 (d, 1H, *J*=8.4 Hz), 7.13 (s,1H), 6.45 (d, 1H, *J*=8.8Hz), 6.27 (d, 2H, *J*=8.0Hz), 4.95 (d, 1H, *J*=7.2Hz),3.98 (d, 1H, , *J*=10.8Hz), 3.83 (s, 1H), 3.53 (m, 8H), 3.28 (t, 1H, , *J*=9.6Hz),1.05 (d, 3H, , *J*=6.0Hz). ^{13}C-NMR (100 MHz, D_2O) 168.7,158.8, 156.5, 154.6, 153.3, 144.6, 142.9, 132.9, 126.3, 118.4, 116.1, 111.2,102.4, 101.2, 100.3, 94.7, 75.7, 75.5, 72.5, 71.8, 70.3, 69.8, 69.1, 68.5,66.3, 16.4.

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Author contributions

JS KIM and HY LEE designed the research, performed the experiments, analyzed data, interpreted data, and wrote the manuscript.

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Availability of data and materials

The datasets used in this study are available from the corresponding authors upon request.

Declarations

Competing interests

The authors declare that they have no competing interest.

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