Role of Anthocyanins in Skin Aging and UV Induced Skin Damage

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11 Role of Anthocyanins in Skin Aging and UV-Induced Skin Damage

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11.1 INTRODUCTION

The visible changes associated with chronological aging and chronic sun exposure, especially to the face, head, and neck areas, are particularly concerning for a significant percentage of the general population. This fact, along with the powerful influence of advertisement and the popular press, has led to an increasing demand for natural and efficient cosmetic ingredients that claim to reduce manifestations of skin aging (Baumann et al., 2009). More importantly, while skin cancers account for up to 40% of the newly diagnosed cancers in the United States (Afaq et al., 2005a), there are no natural preventive methods to avoid cutaneous malignancies associated with chronic sunlight exposure for individuals with pigmentary traits associated with high cancer risk (Zanetti et al., 1996). Consequently, new effective antiaging and chemopreventive agents are in high demand. Although many of the skin-protective claims attributed to botanical products still lack sufficient scientific evidence, the use of natural bioactives with potential antiaging and/or skin-protective properties continues to receive attention from consumers. During the last decade, a substantial body of knowledge has been produced in this area (Chiu and Kimball, 2003, Afaq et al., 2002, 2005a, Afaq, 2011).

Polyphenols (Afaq et al., 2005a, Afaq and Katiyar, 2011, Kao et al., 2007, Kim et al., 1998) and most recently, anthocyanins (Afaq et al., 2009, 2011, Lila, 2004,

Schmidt et al., 2008, Schreckinger et al., 2010, Tsoyi et al., 2008d) have been reported as potentially effective agents to prevent signs of skin aging and protect the skin from external injuries caused by ultraviolet (UV) radiation (Afaq et al., 2011, 2010, Schreckinger et al., 2010, Tsoyi et al., 2008c). A better understanding of the role of UV radiation, reactive oxygen species (ROS), inflammation, and extracellular matrix (ECM) remodeling in skin pathophysiology has allowed researchers to propose the specific molecular targets for anthocyanins and/or anthocyanin-rich extracts. Although some of the current research describes promising skin-protective effects for anthocyanins, most of the proposed dermatological applications still await clinical validation. This chapter reviews the current scientific literature on the potential of anthocyanins in preserving skin health and preventing skin aging.

11.2 SKIN AGING

Skin aging affects the dermis, epidermis, and hypodermis of the skin (Gomez and Berman, 1985, Giangreco et al., 2008). It not only makes the skin look different but also makes it more vulnerable to external injuries (Giangreco et al., 2008). The epidermis, the most external layer of the skin, is mainly composed of keratinocytes and is directly exposed to environmental aggressions (Figure 11.1). The dermis, rich in connective tissues (structural proteins), such as collagen and elastin, is under the epidermis and gives the young skin its characteristic strength, extensibility, and elasticity (Figure 11.1). Skin aging is an intrinsic biological process, which inevitably starts once a person reaches puberty (Farage et al., 2009) and is manifested by the appearance of skin wrinkles, dryness, thinning of the skin, loss of subcutaneous fat, and uneven pigmentation (Giacomoni, 2008). Each individual's genetic background

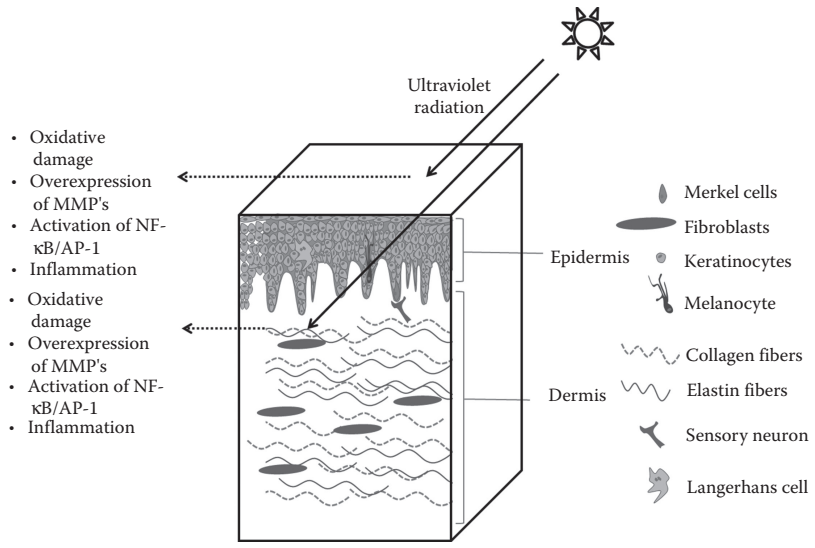


FIGURE 11.1 (**See color insert**.) Schematic representation of the skin architecture and mechanisms of skin damage.

dictates when and how quickly the so-called "intrinsic aging process" unfolds (Koehler et al., 2008). A number of factors can accelerate the intrinsic skin-aging process, which includes weakened deoxyribonucleic acid (DNA)-repairing mechanisms, alterations of the mitochondrial function, slower repair of the ECM, and alterations in cell cycle regulation. The most important extrinsic (accelerating) factor of skin aging is exposure to UV radiation, although diet and smoking can also play a key role in enhancing the appearance of signs of skin aging (Fazio et al., 1989, Fisher et al., 2002, Sakuraoka et al., 1996). It is well known that the acute exposure to high doses of UV radiation triggers the various inflammatory pathways and oxidative damage in the epidermis, dermis, and adnexal organs of the skin, especially UVB radiation (290–320 nm) (Afaq et al., 2005a, Fisher et al., 2002, Ting et al., 2003). The UVA radiation (320–400 nm) is less powerful than UVB, but can penetrate deeper into the skin. In addition, the chronic exposure to high levels of UV radiation can lead to accelerated skin aging (photoaging), hyperkeratosis or atrophy, and precancerous lesions, such as squamous cell carcinomas (Afaq et al., 2005a, Farage et al., 2009). One of the key molecular alterations associated with UV-induced skin damage is the overexpression of metalloproteinases (MMPs), a family of zinc-dependent endopeptidases capable of degrading proteins of the ECM, primarily collagen and elastin. MMPs play an important physiological role in skin regeneration and cell migration (adhesion/dispersion). However, repeated exposure to UV radiation induces the overexpression of specific MMPs (e.g., MMP-9 and MMP-2) leading to the degradation of skin collagen and elastin, incomplete repair of the ECM, loss of skin elasticity and resilience, and the appearance of skin wrinkles. UV radiation also triggers the increase in redox-sensitive transcription factors, including nuclear factor kappa-B, (NF-κB) and activator protein-1 (AP-1). Consequently, researchers are actively looking for natural compounds or mixtures capable of blocking UV radiation, suppressing UV-mediated oxidative damage, inhibiting UV-induced overexpression of MMPs, modulating NF-κB/AP-1 pathways, and decreasing skin inflammation (Figure 11.1).

11.3 ANTHOCYANINS AND SKIN PROTECTION

The known antioxidant power of anthocyanins has led researchers to study their potential in preventing noncommunicable chronic diseases (Cao et al., 2000, Chirinos et al., 2006, Grace et al., 2009, Prior and Wu, 2006, Rojo et al., 2012, Schreckinger et al., 2010). However, the potential of anthocyanins in preventing oxidative skin damage such as UV-induced erythema, skin cancer, and photoaging have received less attention and only a relatively limited number of reports has addressed this question (Afaq et al., 2005b, 2007, 2010, 2011). As the overexposure to UVB radiation is among the most relevant risk factors for oxidative damage to the skin, researchers have used various chemical and biological models to explore the potential of anthocyanins in preventing UVB-induced skin damage. A recent *in vitro* chemical study showed that a cosmetic formulation containing anthocyanins from TNG73 purple sweet potato, at a concentration of 0.61 mg/100 g of cream, could absorb up to 46% of the incident UV radiation (Chan et al., 2010). Although this study was not performed using cellular or animal models of skin damage and

has not been clinically confirmed, the results suggest that the topical application of anthocyanins from TNG73 purple sweet potato at very low doses may prevent UV-induced skin damage by decreasing the amount of UVB radiation reaching the epidermis. This mechanism of skin protection is not unexpected, considering that anthocyanins also attenuate UV damage in plants (Woodall and Stewart, 1998, Harvaux and Kloppstech, 2001). Anthocyanins absorb strongly in the visible and UV spectrums, with maximum absorbances in the ranges of 500–550 and 280–320 nm (Harborne, 1958). The UV absorption capacity of anthocyanins varies depending on their specific aglycones, sugar conjugation, and acylation patterns. Consequently, in some colored plant species, other C6–C3–C6 flavonoids, but not anthocyanins, are responsible for the UV protection (Woodall and Stewart, 1998). More importantly, for anthocyanin-rich UV-blocking formulations, it has been reported that acylated anthocyanins containing coumaric acid, caffeic acid, and ferulic acid display the enhanced adsorption of UVB radiation (Harborne, 1958). Chan et al. (2010) also concluded that acidic ethanol-extracted anthocyanins have better radical scavenging ability, higher total phenolic content, and stronger reducing ability than acidic water-extracted anthocyanins from TNG73 purple sweet potato.

A variety of cellular and animal models have been used to elucidate the pharmacological mechanism by which anthocyanins prevent UV-induced damage to the skin (Table 11.1). A recent study using the reconstituted human skin (EpiD5erm(TM) FT-200) showed that pomegranate-derived extracts and juices rich in anthocyanins prevented UVB-induced damage to the dermal structures (Afaq et al., 2009). In this study, the pomegranate-derived products were applied to reconstituted human skin 1 h prior to a 12-h UVB (60 mJ/cm^2) irradiation period. The pomegranate-derived products significantly inhibited protein oxidation, elevation of cyclobutane pyrimidine dimers (CPD), and 8-dihydro-2'-deoxyguanosine (8-OHdG), suggesting the protective effects against the oxidative damage to proteins and DNA. According to the authors, anthocyanin-rich products from pomegranate also protected the ECM of the skin by ameliorating the UVB-induced overexpression of various MMPs, such as collagenase (MMP-1), gelatinase (MMP-2, MMP-9), stromelysin (MMP-3), marilysin (MMP-7), and elastase (MMP-12). Similarly, another study showed that an extract from the blueberry (*Vaccinium uliginosum* L.), rich in cyanidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, and delphinidin-3-glucoside, prevented UVB-induced overexpression of MMPs and upregulated the UVB-induced suppression of collagen synthesis in human fibroblasts (Bae et al., 2009). These results suggest that anthocyanins from the blueberry may offer protection against photoaging.

Another report by Cimino et al. (2006) showed that the anthocyanin cyanidin-3-*O*-glucoside (C3G) inhibited UV-induced translocation of the transcription factors NF-κB and AP-1 and other inflammatory responses in keratinocytes. According to these data, C3G could provide multifaceted protection against skin damage since NF-κB and AP-1 are the key modulators of several cellular survival programs of skin cells, including the synthesis of inflammatory mediators, and effectors of both innate and adaptive immunity. C3G was also found to prevent the UV-induced overexpression of IL-8, caspase-3 activation, and DNA fragmentation in human keratinocytes (Cimino et al., 2006). This evidence points to a potential protective role of C3G-rich extracts, not only against UVB accumulative skin damage, but also against

TABLE 11.1
Skin-Protective Effects Reported for Anthocyanins

Anthocyanin(s) Tested	Reported Mode of Action	Type of Study	Associated Skin Disease	Reference
Anthocyanins (+ reduced glutathione)	Reduction of erythema after radiation therapy in patients with breast cancer	Clinical/ human	Radiation of dermatitis, discomfort associated with breast irradiation	Enomoto et al. (2005)
Cyanidin-3-*O*-β-glucopyranoside	Protection against UVA-induced oxidative stress in human keratinocytes	*In vitro*	Photoaging, hyperkeratosis, skin atrophy, precancerous lesions, and skin cancer	Tarozzi et al. (2005)
C3G	Reduction of UVB-induced translocation of NF-κ B and AP-1, overexpression of the cytokines IL-8, apoptosis, and DNA fragmentation in cultured human keratinocytes.	*In vitro*	Photoaging, UV-induced erythema	Cimino et al. (2006)
Anthocyanins (+ proanthocyanidin) from Jacquez grapes	Reduction of IL-1α and PGE2, malondialdehyde/4-hydroxynonenal, protein carbonyl groups, and oxidized glutathione, in human reconstructed dermis	*In vitro*	Photoaging, UV-induced erythema	Tomaino et al. (2006)
Delphinidin	Protection of human HaCaT keratinocytes and mouse skin against UVB-mediated oxidative stress and apoptosis	*In vitro* and *in vivo*	Photoaging and skin cancer	Afaq et al. (2007)
Anthocyanin-rich extract from red orange	Reduction of UVB-induced translocation of NF-κ B and AP-1, anti-inflammation in cultured human keratinocytes.	*In vitro*	Photoaging, UV-induced erythema	Cimino et al. (2007)
Black soybean seed anthocyanins	Prevention of UVB-induced apoptotic cell death, inflammation, COX-2, and PGE2. Decreased production of NF-κ B and inhibition of phosphatidylinositol 3-kinase/ Akt pathway	*In vitro* and *in vivo*	Photoaging, hyperkeratosis, skin atrophy, precancerous lesions, and skin cancer	Tsoyi et al. (2008)
Blueberry anthocyanins	Amelioration of UVB-induced damage to human dermal fibroblasts	*In vitro*	Photoaging, precancerous lesions, and skin cancer	Bae et al. (2009)

(*continued*)

TABLE 11.1 (continued)
Skin-Protective Effects Reported for Anthocyanins

Anthocyanin(s) Tested	Reported Mode of Action	Type of Study	Associated Skin Disease	Reference
Bilberry anthocyanins	Reduction of UVA-stimulated oxidative damage to keratinocytes	*In vitro*	Photoaging, hyperkeratosis, skin atrophy, precancerous lesions, and skin cancer	Bae et al. (2009)
Anthocyanins from TNG73 purple potato	Absorption of 46% incident UV radiation (0.61 mg/100 g of cream)	*In vitro*	Sun burns, photoaging, and UV-induced erythema	Chan et al. (2010)

psoriasis, characterized by hyperactive NF-κB in keratinocytes. A similar effect was documented using anthocyanins from bilberry and human keratinocytes as a model of dermal UV-induced damage (Svobodova et al., 2008). This latter study showed that anthocyanins from bilberry reduce UVA-stimulated ROS formation and lipid peroxidation.

Analogous skin-protective mechanisms were documented in two separate publications from the same research group (Tsoyi et al., 2008a,b). According to these studies, anthocyanins from black soybean coats may offer protection against UV-induced damage not only to cultured keratinocytes, but also *in vivo* to hairless mice skin. At least two different modes of action were identified in these reports: (i) a reduction of UVB-induced elevation of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE(2)) through a NF-κB-dependent pathways (Tsoyi et al., 2008b) and (ii) the prevention of apoptotic cell death by inhibiting caspase-3 activation and reduction of proapoptotic Bax protein levels (Tsoyi et al., 2008a). Delphinidin, an ubiquitous anthocyanin, commonly found in edible berries (Escribano-Bailon et al., 2006, Rojo et al., 2012, Schreckinger et al., 2010) has also shown the protective effect to human HaCaT keratinocytes and mouse skin against UVB-mediated oxidative stress and apoptosis (Afaq et al., 2009). Similarly, another anthocyanin, cyanidin-3-*O*-β-glucopyranoside, was found to prevent UVA-induced damage to human keratinocytes (Tarozzi et al., 2005).

11.4 CURRENT AND FUTURE WORK

It is well known that oxidative damage, inflammation, apoptotic cell death, and overexpression of MMPs play a key role in skin aging and certain forms of UV-induced skin damage. The accumulated scientific evidence suggests that anthocyanins may offer the protection against UV-induced precancerous lesions and possibly delay the appearance of signs of skin aging (Table 11.1). The protective effect of anthocyanins and mode of action have been partially described in several *in vitro* and *in vivo*

models of skin damage. However, the current preclinical evidence is seemingly insufficient to conclude that anthocyanins are solely responsible for the skin-protective properties observed *in vitro* and *in vivo* (Table 11.1) because various polyphenols that are different from anthocyanins may be present in the test materials used for these studies.

Additional work is needed to address another important "innovation gap"; the development of chemically stable and clinically effective anthocyanin-rich formulations for dermatological applications. Only one clinical study was available at the time this chapter was written. It reported that a multicomponent formulation containing anthocyanins and glutathione significantly reduced skin erythema after radiation therapy in patients with breast cancer (Enomoto et al., 2005). Unfortunately, the report provided very scarce information regarding the specific group of anthocyanins and doses used for topical applications.

Our research group has recently reported that anthocyanins, along with other polyphenols, can be efficiently separated from highly polar carbohydrates, bound, concentrated, and stabilized into protein-rich, food matrixes, such as defatted soybean flour (DSF) and soy protein isolate (SPI), while preserving their pharmacological effects (Roopchand et al., 2012). The stability of anthocyanins captured in this type of protein-rich matrices was verified up to 50 weeks (Figure 11.2) at 37°C. This form of stabilized anthocyanins opens challenging avenues to develop stable

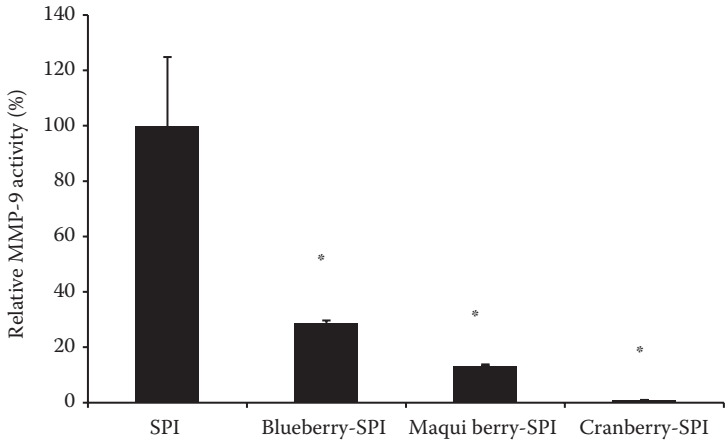
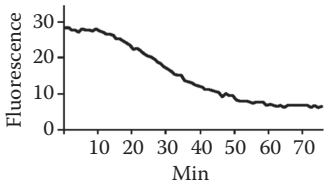
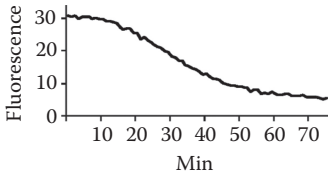
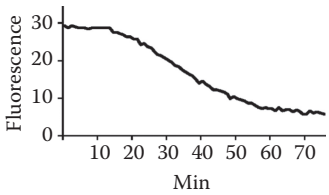
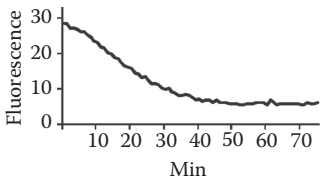


FIGURE 11.2 Effect of anthocyanin-rich protein matrix on MMP-9 activity *in vitro*. The polyphenols including anthocyanins from different sources were concentrated and stabilized in SPI. The concentrations of anthocyanins bound to SPI are shown in Table 11.2. The SPI-enriched matrices were suspended in water mixed with recombinant human MMP-9 (1 μg/mL in PBS) and dye-quenched (DQ) gelatin (1 mg/mL in PBS), a fluorescent quenched substrate of MMP-9. Anthocyanin-enriched matrices (1.5 mg/mL) were incubated with 0.4 μg/mL MMP-9, and 50 μg/mL DQ gelatin at 37°C for 30 min, centrifuged to precipitate solids, and the supernatant was transferred to a 96-well plate to measure MMP-9 activity. The data are reported as the percentage of the inhibition of MMP-9 activity relative to control (SPI). The values correspond to the mean of the three replicates ± SD (*), $P < 0.05$, and *t*-test.

dermatological and cosmetic anthocyanin-rich formulations with the application in cosmetics and food products. We also evaluated whether the SPI with electrostatically bound and concentrated anthocyanins and other polyphenols from maqui berry, blueberry, and cranberry retains the antioxidant capacity and human MMP-9 inhibitory activity of its components. According to our results, these anthocyanin-rich matrices not only displayed a powerful antioxidant capacity (Table 11.2), but also inhibited collagen degradation by human MMP-9 (Figure 11.3), a collagenase known to participate in UV-induced skin damage. The molecular mechanisms by

TABLE 11.2
ORAC Antioxidant Capacity of Different Anthocyanin- Rich Soy Protein Matrices from Fruits

	Anthocyanins (mg/g)	Phenolics (mg/g)	ORAC[a] (Trolox Equivalents (μmol/g)	Representative Kinetic Curve of AAPH[b]-induced Fluorescence Decay
Maqui berry–SPI	32	80	1090 ± 130	
Blueberry–SPI	29	251	1380 ± 360	
Cranberry–SPI	16	290	1550 ± 200	
SPI	—	—	230 ± 110	

Note: SPI, Soy protein isolate.

[a] ORAC, Oxygen radical absorbance capacity.

[b] AAPH, 2,2′-Azobis (2-amidinopropane) hydrochloride.

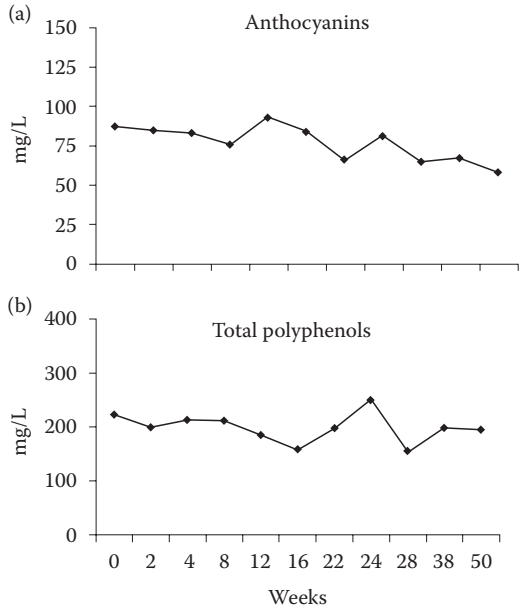


FIGURE 11.3 Stability of blueberry anthocyanins and polyphenols bound to DSF. The concentration of (a) monomeric anthocyanins and (b) total polyphenols are eluted from blueberry polyphenol-enriched DSF after the indicated number of weeks postincubation at 37°C. Polyphenolic compounds were eluted from DSF with 75% methanol, 20% water, 5% acetic acid solution, and the quantifications were done as described elsewhere. (From Roopchand, D. et al. 2012. *Food Chemistry*, 131, 1193–1200.)

which polyphenols bound to SPI inhibit MMP-9 activity remain to be elucidated; our hypothesis is that the specific and nonspecific mechanisms may explain this inhibition and are worthy of further investigations.

11.5 CONCLUSIONS

The previous publications and the presented data suggest that anthocyanins from plants can prevent skin aging and UV-induced skin damage, particularly in formulations that enhance their stability to temperature, pH, and light (Gironés-Vilaplana et al., 2012, Roopchand et al., 2012, Schreckinger et al., 2010). For example, acylated anthocyanins have shown increased stability to pH offering a natural and safer alternative to synthetic dyes for food and cosmetics (Giusti and Wrolstadb, 2003). However, the skin-protective properties of acylated anthocyanins from plants are scarcely studied (Schreckinger et al., 2010). Other authors have addressed this problem by stabilizing anthocyanins from *Aristotelia chilensis* in beverages using lemon juice (Gironés-Vilaplana et al., 2012). Stabilizing anthocyanins by electrostatically binding them to protein matrices may provide another strategy for protecting their structural integrity, function, and color (Roopchand et al., 2012). We hope that in the

future, the intense and beautiful colors of free or stabilized anthocyanins may offer a possibility for developing naturally colored cosmetics with skin-protecting and antiaging properties.

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