Photochemistry and Photobiology, 2018, 94: 409–420

Invited Review

Photodegradation of Eumelanin and Pheomelanin and Its Pathophysiological Implications†

Shosuke Ito*[1], Kazumasa Wakamatsu[1] and Tadeusz Sarna[2]

[1]Department of Chemistry, Fujita Health University School of Health Sciences, Aichi, Japan
[2]Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Received 3 July 2017, accepted 21 August 2017, DOI: 10.1111/php.12837

ABSTRACT

Eumelanin is photoprotective for pigmented tissues while pheomelanin is phototoxic. In this review, we summarize current understanding of how eumelanin and pheomelanin structures are modified by ultraviolet A (UVA) and also by visible light and how reactive oxygen species participate in those processes. Alkaline hydrogen peroxide oxidation was employed to characterize eumelanin and benzothiazole-type pheomelanin, giving pyrrole-2,3,5-tricarboxylic acid (PTCA) and thiazole-2,4,5-tricarboxylic acid (TTCA), respectively. Reductive hydrolysis with hydroiodic acid gives 4-amino-3-hydroxyphenylalanine (4-AHP) from the benzothiazine moiety of pheomelanin. The results show that the photoaging of eumelanin gives rise to free PTCA (produced by peroxidation *in situ*) and pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA, produced by cross-linking). The TTCA/4-AHP ratio increases with photoaging, indicating the conversion of benzothiazine to the benzothiazole moiety. Analysis of those markers and their ratios show that both eumelanin and pheomelanin in human retinal pigment epithelium melanosomes undergo extensive structural modifications due to their lifelong exposure to blue light. Using synthetic melanins, we also found that singlet oxygen, in addition to superoxide anions, is photogenerated and quenched upon UVA irradiation. The (patho)physiological significance of those findings is discussed in relation to the tanning process, to melanomagenesis in the skin and to age-related macular degeneration in the eyes.

INTRODUCTION

Melanin pigment is believed to protect the skin and eyes against phototoxic reactions induced by ultraviolet (UV) or blue visible light (1–3). Exposure to UV radiation causes DNA damage, and thus, the accumulation of melanin can be considered an adaptive photoprotective response of melanocytes to prevent further DNA damage. The UV radiation that reaches the earth's surface consists mainly of 95% UVA (320–400 nm) and approximately 5% UVB (290–320 nm). Although both UVB and UVA lead to skin tanning following sun exposure, they induce different pigmentary responses that are mainly defined by the time kinetics, that is immediate pigment darkening and persistent pigment darkening as typical reactions to UVA (4), as well as delayed tanning that is induced by UVB (5). Our previous studies have clarified that delayed tanning is accompanied by an increase in melanin content (3,6). However, that increase is only slight, less than two-fold (3,6–8), and the observed several-fold increase in visible pigmentation is caused by additional contributions due to changes in the distribution and particle size of melanosomes (6,9).

UVA (>4 J cm^{-2}) elicits immediate pigment darkening within minutes, which is transient and fades away gradually and partially, while increasing UVA doses causes persistent pigment darkening within hours that persists for several days (4). The action spectrum of immediate pigment darkening has been reported to vary from 320 to 340 nm, that range being very close to the absorption maximum of 5,6-dihydroxyindole-2-carboxylic acid (DHICA; 321 nm). Persistent pigment darkening seems to be a distinct second phase of the tanning reaction. Interestingly, immediate pigment darkening typically appears gray to black while persistent pigment darkening is tan to brown (3). These darkening processes are thought to result from the oxidation and/or polymerization of existing melanin and/or melanogenic precursors (10). Reactive oxygen species (ROS) are considered to be responsible, at least in part, for these processes (11). In fact, our recent study (12) using DHICA-melanin confirmed that UVA radiation induces the oxidation of DHICA to indole-5,6-quinone-2-carboxylic acid, which is then cleaved to form a photodegraded, pyrrolic moiety and finally to pyrrole-2,3,5-tricarboxylic acid (PTCA; Fig. 1) in a free form. The possible involvement of superoxide anions and singlet oxygen during this photodegradation has been suggested, and the generation and quenching of singlet oxygen by DHICA-melanin was confirmed by direct measurements of singlet oxygen phosphorescence. This point was examined in more detail in a recent comparative study (11). In addition, the biological effects of UVA are now drawing much attention because of the recent finding that cyclobutane pyrimidine dimers are generated in melanocytes for >3 h after exposure to UVA ("dark CPDs"), involving UV-induced reactive oxygen and nitrogen species and melanin pigment (13).

UVB causes delayed tanning that takes several days or longer to develop (3). The main mechanism of skin tanning caused by UVB is considered to be the production of melanin as a result of the increased synthesis and activity of tyrosinase. Different

*Corresponding author email: sito@fujita-hu.ac.jp (Shosuke Ito)
†This article is a part of the Special Issue dedicated to the topics of Photopigmentation and Melanoma.
© 2017 The American Society of Photobiology

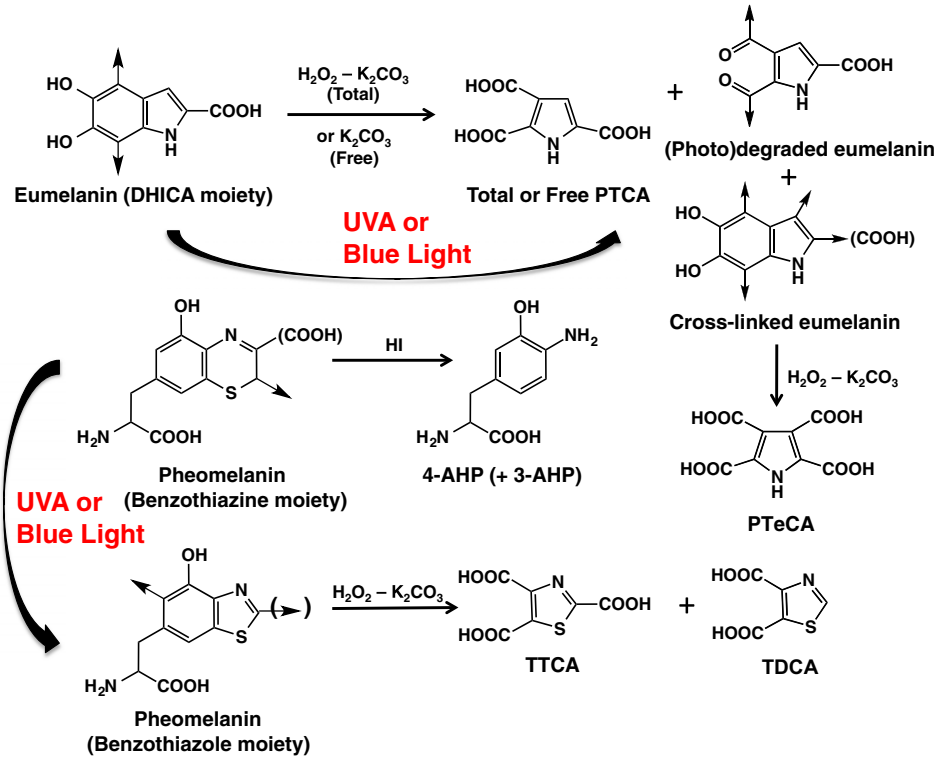

Figure 1. Photo-induced structural modifications of eumelanin and pheomelanin and their molecular markers. Eumelanin consists of DHI and DHICA moieties. However, for the sake of simplicity, only the DHICA moiety is depicted here. The DHICA moiety in eumelanin gives total PTCA upon alkaline hydrogen peroxide oxidation (40) while photo-induced oxidative degradation of the DHICA moiety liberates free PTCA and photodegraded eumelanin (diarylketone moiety). Reaction of the DHI moiety with the indolequinone moiety gives rise to DHI cross-linked at the C3 position (the C2 position being connected either to a carboxyl group or to an adjacent DHI moiety). This structure gives PTeCA upon alkaline hydrogen peroxide oxidation. The benzothiazine moiety in pheomelanin gives 4-AHP (and 3-AHP; not shown) upon reductive hydrolysis with hydroiodic acid (43), which originates from 5-*S*- and 2-*S*-cysteinyldopa. UVA induces conversion of the benzothiazine to the benzothiazole unit. The latter unit gives TTCA and TDCA upon alkaline hydrogen peroxide oxidation while the former gives much lower yields of TTCA and TDCA (40). Taken from Fig. 1 of reference 39 with minor modifications.

mechanisms are involved in the pigmentary responses of the skin to different types of UV. Repetitive exposure to UVB elicits dramatic increases in a large number of genes involved in pigmentation as well as in other cellular functions, whereas UVA has little or no effect on those genes (14).

Epidermal (and follicular) melanocytes produce two types of melanin pigment, eumelanin and pheomelanin (15). It is generally agreed that eumelanin is photoprotective to pigmented tissues while pheomelanin is phototoxic. Eumelanin is a heterogeneous polymer consisting of 5,6-dihydroxyindole (DHI) and DHICA in various ratios, while pheomelanin is derived from the oxidative polymerization of 5-*S*- and 2-*S*-cysteinyldopa and consists of benzothiazine and benzothiazole units (16–18). The relative ratio of DHI to DHICA depends on the activity of dopachrome tautomerase (tyrosinase-related protein-2) and the availability of copper ions, which give a higher ratio of DHICA (19,20). That ratio appears important in providing eumelanin an antioxidant activity. DHICA-melanin was suggested to be a much more potent hydroxyl-radical scavenger compared to DHI-melanin (21). The superior antioxidant and free radical scavenging properties of DHICA-melanin relative to DHI-melanin were confirmed in other studies (22,23). However, caution should be exercised when synthetic DHI-melanin and DHICA-melanin are compared because their solubility is markedly different (DHI-melanin is completely insoluble in neutral buffers while DHICA-melanin is soluble), which makes direct comparison difficult and ambiguous. In the course of pheomelanin synthesis, structural modification proceeds gradually to convert benzothiazine to the benzothiazole moiety (24). The relative ratio of these two types of pheomelanin moieties appears important in determining whether pheomelanin acts as a pro-oxidant, whose activity is generally attributed to the benzothiazine moiety (23,25), although the (photo)reactivity of the benzothiazole moiety has not been examined thoroughly and thus remains to be studied. Until recently, it was considered that eumelanin accounts for more than 90% of total melanin both in unirradiated skin and in UV-irradiated skin and that both types of melanin increase in tandem after UVB irradiation (8,26). However, our recent study revealed that the melanin in human epidermis is comprised of approximately 74% eumelanin and 26% pheomelanin, regardless of the degree of pigmentation, the pheomelanin being mostly in the benzothiazole type (27). This fact points out the necessity to pay more attention to the role of pheomelanin in the photochemistry of the skin.

In contrast to extensive biosynthetic studies on melanin pigments, the biodegradation of eumelanin and pheomelanin remained poorly characterized until only several years ago. In this review, we summarize current understanding of how eumelanin and pheomelanin structures are modified by UVA and also by visible light and how ROS participates in those processes and then we briefly discuss the physiological significance of those findings. In this review, we do not discuss the role of UVB radiation on the modification of melanin structures even though UVB was reported to induce in keratinocytes ROS (28,29). This

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

is because UVB constitutes only 5% of UV radiation on the earth's surface and our irradiation experiments confirmed that UVB-induced changes in the melanin structure were much slower (ca. 1/3) than with UVA at a dose 20% that of UVA (30). In addition, unlike the generally accepted mechanism of photosensitized generation of ROS mediated by UVA (31), the UVB-induced generation of ROS is poorly understood and may involve such adverse processes as activation of the cellular catalase (28) or elevation of the intracellular Ca^{2+} levels (29).

PHOTODEGRADATION OF EUMELANIN AND PHEOMELANIN

UVA-induced degradation of melanin during immediate and persistent pigment darkening

UVA induces the modification of preexisting melanin through ROS production. Immediate pigment darkening results from UVA exposure for a short time, implying that it requires a relatively low dose of UVA. Eumelanin exists in two redox forms, a reduced 5,6-dihydroxyindole (DHI and DHICA) form and an oxidized 5,6-indolequinone (*o*-quinone form of DHI and DHICA) form in varying ratios. The structural modification of eumelanin that occurs initially should be the oxidation of dihydroxyindole to the indolequinone moiety. DHI and DHICA exhibit absorption maxima at 303 and 321 nm, respectively, while the corresponding indolequinones have maxima at 500 nm for 5,6-indolequinone and 420 and 560 nm for 5,6-indolequinone-2-carboxylic acid (32). Thus, we can expect that immediate pigment darkening results in an increase in absorbance around 400–600 nm, concomitant with a decrease around 300–350 nm. A similar spectral response was reported for a DOPA-melanin solution exposed to UVA (33). The *in vivo* UVA irradiation of human skin gave an apparent absorption spectrum showing a linear increase from 700 toward 400 nm and a sharp decrease in the UVA region (33). The oxidative polymerization of eumelanin precursors was suggested as an alternative mechanism for immediate pigment darkening (10). However, this is less likely to occur because the level of eumelanin precursors in the skin is extremely low compared to the level of eumelanin itself (26). Thus, it appears that during immediate pigment darkening an overall increase in visible absorption (with a decrease in UVA absorption) leads to a darkening of preexisting eumelanin without any change in its amount. In fact, our recent study using differential spectra of a UVA-exposed DHICA-melanin solution *vs* an unexposed solution confirms the above-mentioned spectral changes, which involves superoxide anion production (11,12). Earlier studies of the interaction of synthetic DOPA-melanin with oxidizing and reducing radicals generated by pulse radiolysis demonstrated that while the oxidation of melanin was accompanied by a distinct increase in its absorption in the visible region, the reduction of melanin led to its decreased absorption (34,35). The immediate pigment darkening may fade away through the reduction of indolequinone to the original dihydroxyindole moiety by endogenous reducing compounds such as ascorbic acid and NADH, although direct evidence for this process seems lacking.

Then how about persistent pigment darkening? This should be a more complex, irreversible process because it occurs during a longer period of time (a higher dose of UVA). This allows the chemical oxidative modification of melanin to proceed in hours. Our study has shown that following UVA irradiation, a human black hair melanin suspension (containing mostly eumelanin) undergoes oxidative cleavage of the indolequinone moiety to give free PTCA, a product of the peroxidation of DHICA, in a time (dose)-dependent manner, accompanied by a weak photobleaching as evidenced by the decrease in absorbance at 500 nm (A500) (30; Fig. 2). This reaction was confirmed with a synthetic DHICA-melanin solution (30). Our latest study (12) showed that singlet oxygen is generated and quenched during the UVA-induced oxidation of DHICA-melanin, which likely leads to cleavage of the indolequinone moiety to form free PTCA. Singlet oxygen is a highly reactive molecule that reacts with a variety of biomolecules, including aromatic amino acids and nucleotides. For example, histidine forms semistable cyclic endoperoxides through a Diels-Alder process (36,37). Endoperoxides are labile molecules that give rise to a number of stable end-products. Furthermore, it is known that *ortho*-quinones are highly reactive and undergo Diels-Alder cycloaddition reactions with a variety of nucleophiles (38). Thus, we proposed that the indolequinone moiety as an *ortho*-quinone produces an endoperoxide intermediate that gradually degrades to form free PTCA (12).

In our subsequent study (39), another type of oxidative modification of melanin was discovered. UVA irradiation of a synthetic eumelanin suspension leads to cross-linking of the dihydroxyindole moiety, which was proved by an increase in pyrrole-2,3,4,5-tetracarboxylic acid (PTeCA) upon alkaline hydrogen peroxide oxidation (to break down the eumelanin structure into its building blocks; 40). How PTeCA is produced in eumelanin by UVA (39) or by heat (41) has been studied in detail. The free PTCA production and the cross-linking occur in tandem, indicating that both events result from the oxidative modification of eumelanin. In the course of the reaction, the absorption of DHICA-melanin in the visible region (400–700 nm) increases in a UVA dose-dependent manner (12). These results suggest that persistent pigment darkening is a complex oxidative process in which the eumelanin structure undergoes oxidative cleavage and cross-linking of dihydroxyindole units simultaneously. The oxidative cleavage affords free PTCA from the terminus of the melanin polymer and the diarylketone moiety (Fig. 1) from its center accompanied by a robust increase in fluorescence (30). In support of this, a strong fluorescence is a unique feature of UV-exposed skin (42), and the diarylketone structure, such as benzophenone, is known to be highly fluorescent.

Pheomelanin also undergoes photodegradation during persistent pigment darkening. Pheomelanin can be detected as 4-amino-3-hydroxyphenylalanine (4-AHP), a specific degradation product of the benzothiazine moiety of pheomelanin upon HI reductive hydrolysis (43; Fig. 1). Thiazole-2,4,5-tricarboxylic acid (TTCA) and thiazole-4,5-dicarboxylic acid (TDCA) are degradation products derived from the benzothiazole moiety in pheomelanin upon alkaline hydrogen peroxide oxidation (40; Fig. 1). UVA irradiation induces the oxidative conversion of benzothiazine to the benzothiazole moiety, as indicated by a decrease in the 4-AHP/3-AHP ratio (30,39,44) and an increase in the TTCA/4-AHP ratio (30). Interestingly, the benzothiazine moiety derived from 5-*S*-cysteinyldopa yielding 4-AHP is more photolabile than one derived from 2-*S*-cysteinyldopa yielding 3-AHP (44). During UVA irradiation of human red hair melanin (pheomelanin-rich), the 4-AHP level progressively decreases with time, while the 3-AHP level decreases much slower than the 4-AHP level (Fig. 2). These results indicate that pheomelanin, especially the 5-*S*-cysteinyldopa-derived unit, is rather labile to

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

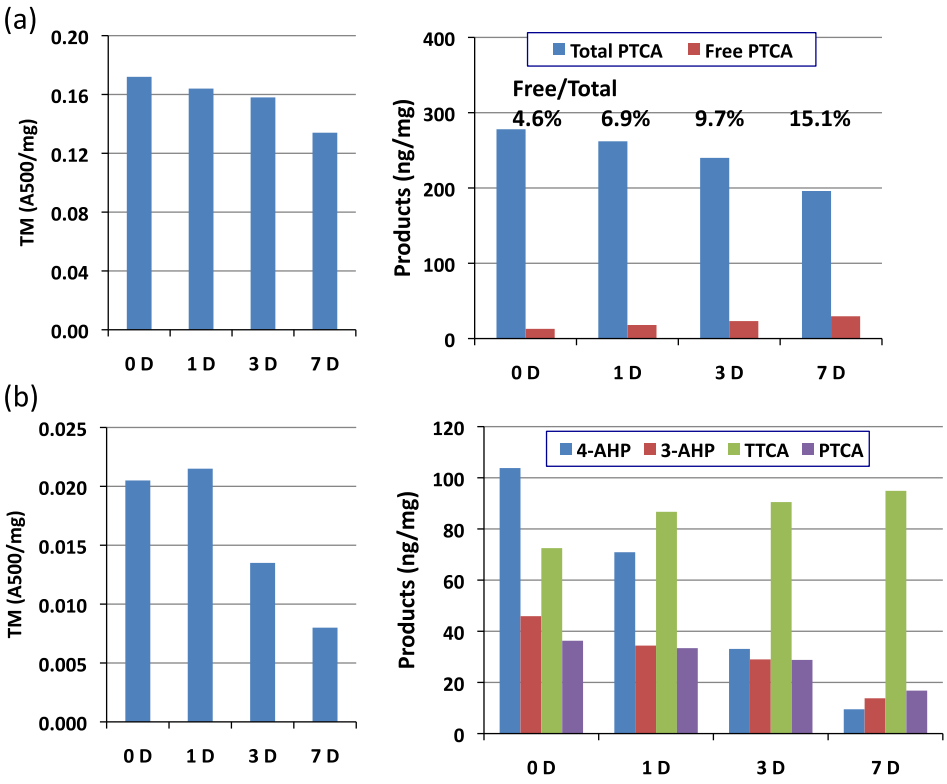

Figure 2. UVA-induced degradation of hair melanin. (a) human black hair. (b) human red hair. Suspensions of hair melanin (10 mg mL^{-1} water) were irradiated with 4 mW cm^{-2} UVA for the indicated time (days). Black hair was taken from a Japanese male, while red hair was from six German females and combined to obtain average values for the markers. The hair samples were taken from the base (0–4 cm) to avoid the influence from sun exposure (32). Marker analyses were performed in duplicate, and averages are shown. The irradiation experiments were repeated once with similar results to confirm the reproducibility. The accumulated dose per day is approximately 340 J cm^{-2}, which is higher than physiological doses. However, our other study (39) showed that even small doses of 20 J cm^{-2} or less of UVA induces significant changes in the ratios of PTeCA/PTCA, TTCA/4-AHP and TTCA/PTCA in human melanocyte cultures. Taken from Figs 4 and 5 of reference 32 with minor modifications.

UVA, giving rise to the conversion from benzothiazine to the benzothiazole structure. However, in terms of pigmentation in the skin, pheomelanin may not contribute so much because of its lower content (27) and paler color compared to eumelanin.

In sum, we propose that upon UVA irradiation, eumelanin undergoes immediate pigment darkening as a result of the ROS-dependent oxidation of dihydroxyindole to the indolequinone moiety accompanied by an increase in visible absorption and a decrease in UVA absorption. Immediate pigment darkening may fade away gradually to return to the original, reduced structure; however, with higher doses of UVA, persistent pigment darkening develops in several hours. Persistent pigment darkening is a more complex, irreversible chemical process involving oxidative cleavage of the indolequinone moiety and cross-linking of the dihydroxyindole moiety. With much higher doses of UVA, photobleaching may gradually develop. Pheomelanin also undergoes a rather extensive structural modification upon UVA irradiation. However, the precise process of the structural modification in pheomelanin remains to be studied.

Visible light-induced degradation of melanin

Then how about visible light? Until recently, it was generally believed that visible light does not affect skin pigmentation. However, recent studies have indicated that the effects of visible light are similar to those elicited by UVA (39,45–48). Liebel *et al.* (45) showed that visible light induces significant ROS production resulting in the release of pro-inflammatory cytokines and the expression of matrix metalloproteinases in the skin. Interestingly, a single exposure to visible light induces very little pigmentation whereas multiple exposures to visible light result in darker and sustained pigmentation (49).

In collaborative studies, we examined the effects of blue light on melanin isolated from bovine retinal pigmented epithelium (RPE) (40). The aerobic irradiation of melanosomes isolated from the RPE of bovine eyes with an intense short-wavelength visible light was viewed as an *in vitro* model for the photoaging of human RPE melanosomes (50,51). As shown in Fig. 3, irradiation with strong blue light (400–500 nm; 136 W cm^{-2}) for 70 h induced rather small changes in various melanogenic markers (except for 4-AHP). However, irradiation for 157 h did induce robust changes. Total melanin (TM, absorbance at 500 nm) and total PTCA (PTCA after alkaline hydrogen peroxide oxidation) values decreased to 49% and 44% of the control, respectively. The level of 4-AHP also showed a sharp decrease to 20%. On the other hand, PTeCA and free PTCA increased to 164% and 144%, respectively. As a result, various melanin marker ratios showed dramatic changes, including PTeCA/PTCA, free/total PTCA, 4-AHP/3-AHP and TTCA/PTCA (Fig. 3). The ratio of A650/A500 usually serves as a good indicator for the ratio of eumelanin and pheomelanin (52). However, the ratio in this case appears to indicate a modification of eumelanin structure such as oxidative cleavage of the benzene ring in dihydroxyindole units (30). This biochemical observation with isolated

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

RPE melanin was confirmed by *in vivo* observations using isolated RPE cells derived from nine human subjects of various ages. Figure 4 compares age-related changes in various melanogenic marker ratios. The PTCA/TM ratio showed a progressive decrease to 1/3 during 80 years of life, whereas the PTeCA/PTCA ratio increased linearly by 2.5-fold. The 4-AHP/3-AHP ratio decreased linearly to below 1.0, while the TTCA/PTCA ratio increased eight-fold. These results clearly indicate that both eumelanin and pheomelanin in human RPE tissue degrade very extensively during 80 years of life due to the continuous exposure to blue light (not UVA that does not reach the retina).

The effects of *in vitro* photoaging of bovine RPE melanosomes on their morphology, antioxidant properties and reactivity were recently examined employing atomic force microscopy and an array of complementary spectroscopic methods (53). That study demonstrated that moderately photobleached melanosomes exhibit a strongly modified surface morphology with clearly exposed melanin nanoaggregates. Compared to control untreated melanosomes, the photobleached melanosomes exhibited a reduced efficiency to protect unsaturated lipids against photosensitized oxidation and quenched singlet oxygen less efficiently. The increased photoreactivity of the experimentally photoaged bovine RPE melanosomes could be due to the augmented exposure of reactive melanin groups such as melanin free radicals.

Melanin-mediated photogeneration of ROS

Under aerobic conditions, the photoexcitation of both eumelanin and pheomelanin is accompanied by the consumption of oxygen (54,55) and by the formation of superoxide anions and hydrogen peroxide (56–58; Fig. 5). Can melanin photogenerate singlet oxygen, the most exemplary type of ROS? It was only recently that Chiarelli-Neto *et al.* (59) published the first report suggesting the generation and suppression of singlet oxygen in hair by the photosensitization of melanin. Subsequently, they showed that in the presence of eumelanin and pheomelanin, UVA and visible light can generate singlet oxygen and cause characteristic DNA damage (47). More recently, we studied the role of singlet oxygen (and superoxide anions) in the degradation of DHICA-melanin induced by irradiation with UVA in neutral buffer, using differential spectrophotometry and time-resolved near-infrared luminescence (12). That study unequivocally demonstrated that synthetic eumelanin photogenerates singlet oxygen even when excited with blue light. The melanin also effectively quenches singlet oxygen.

In our subsequent study (11), we examined the photoreactivity of synthetic eumelanins formed by the auto-oxidation of DOPA or by the enzymatic oxidation of DHICA as well as synthetic pheomelanins obtained by the enzymatic oxidation of 5-*S*-cysteinyldopa or a 1:1 mixture of DOPA and cysteine. Both superoxide anion and singlet oxygen were photogenerated by the synthetic melanins albeit with different efficiencies. At 450 nm, the quantum yield of singlet oxygen is very low (ca. 10^{-4}), but it strongly increases in the UV region. Melanins quench singlet oxygen efficiently, indicating that photogeneration and quenching of singlet oxygen may play an important role in the aerobic photochemistry of melanin pigment. Interestingly, DHICA-melanin exhibited the highest rate of oxygen photoconsumption when excited with short-wavelength visible light and photogenerated and quenched singlet oxygen several-fold more effectively than the other types of melanins. This is in contrast to DOPA-melanin, which photogenerated superoxide anion most effectively among the melanins tested. In contrast, in the short-wavelength part of UVA (320–360 nm), the efficacy of synthetic pheomelanins to photogenerate singlet oxygen is higher than those of eumelanins. It remains to be clarified which of benzothiazine and/or benzothiazole moiety is responsible for the photogeneration of singlet oxygen. In sum, the photochemical behaviors of eumelanin and pheomelanin are wavelength-dependent and subunits of each melanin would play an important role in determining which species of ROS is preferentially produced.

Both types of melanin pigment, eumelanin and pheomelanin, are redox-active and rapidly and repeatedly redox cycle between the oxidized and reduced states. This concept of melanin being a redox buffer has been known for years. However, a study by Kim *et al.* (60) recently reinforced this concept. Melanin molecules can act as a reductant that transfers electrons to acceptors such as oxygen molecules generating superoxide anions while it can act as an oxidant accepting electrons from donors such as reduced glutathione (GSH) yielding oxidized glutathione (Fig. 5). Both eumelanin and pheomelanin exist in fully reduced and oxidized melanin subunits with a so-called comproportionation equilibrium through the inducible melanin radicals. Extrinsic melanin radicals are induced by many factors, including radiation, that can shift the equilibrium toward their free radical form (61). The

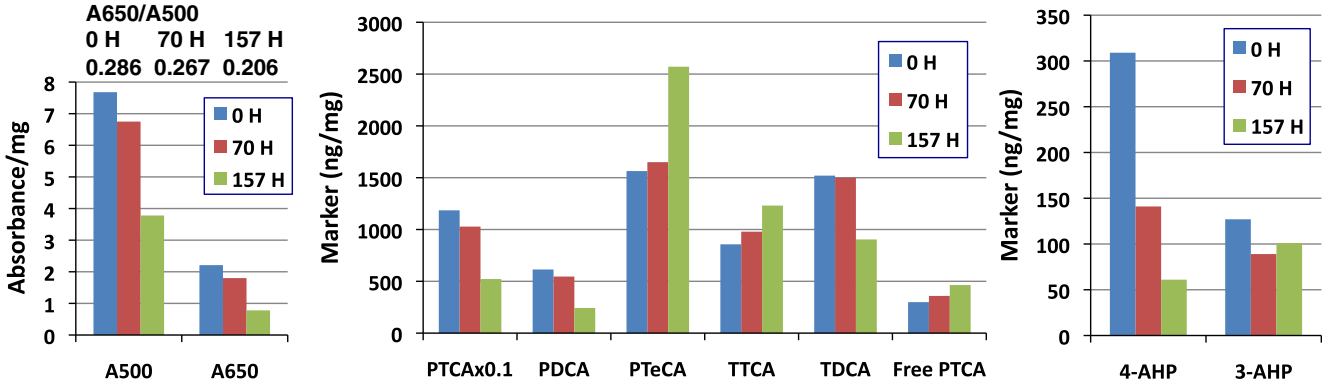

Figure 3. Blue light–induced degradation of melanin in bovine RPE melanosomes. The sample was irradiated with strong blue light (136 mW cm^{-2}) for the indicated time (h). Marker analyses were performed in duplicate and averages are shown. The accumulated dose of blue light at 157-h irradiation was 76 900 J cm^{-2}. For the sake of clarity, the PTCA values shown are × 0.1 of actual ones. Prepared from Table 1 of reference 39.

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

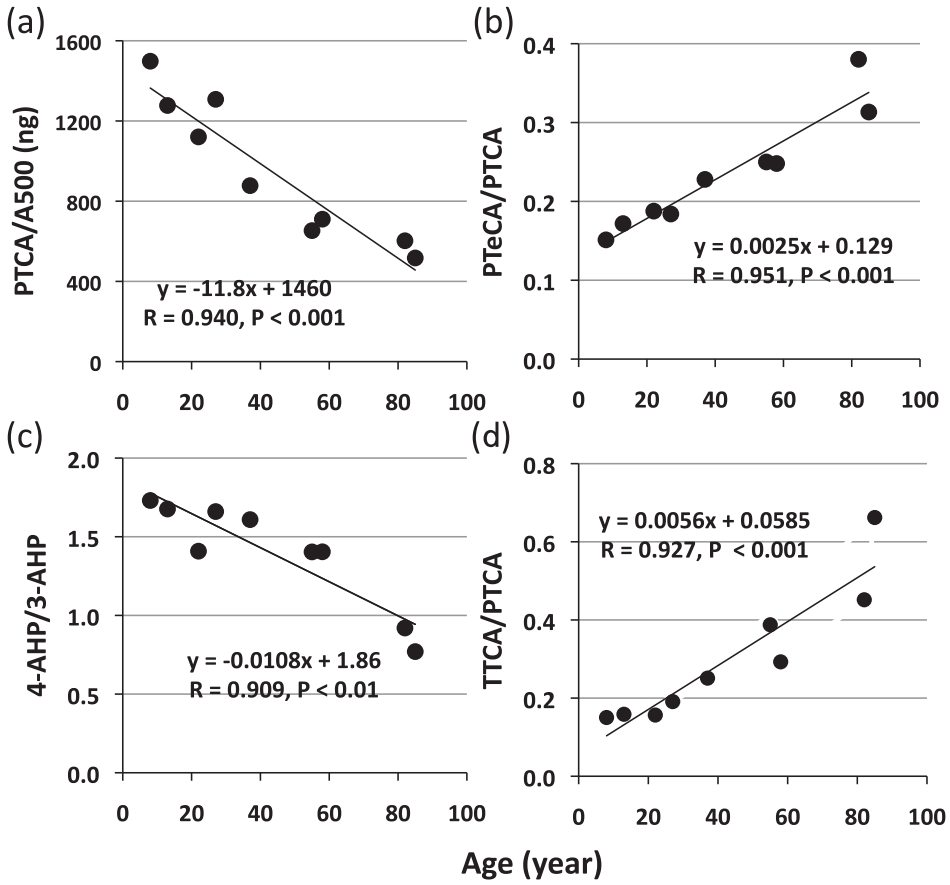

Figure 4. Correlation of various melanin marker ratios in human RPE cells derived from donors of different ages. (a) PTCA/TM ratio. (b) PTeCA/PTCA ratio. (c) 4-AHP/3-AHP ratio. (d) TTCA/PTCA ratio. Samples of human RPE cells were obtained from nine donors of various ages (between 9 and 85 years old). Taken from Figure 4 of reference 39 with minor modifications.

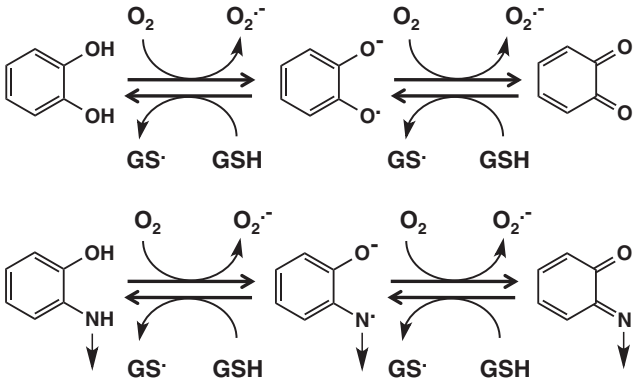

Figure 5. Redox buffering by melanin pigment. During aerobic (photo) reaction, melanin pigment undergoes redox-cycling, giving rise to superoxide anion production and oxidation of reducing agents such as glutathione (GSH). GS· radicals immediately dimerize to give oxidized glutathione (GSSG). Only *o*-diphenol and *o*-aminophenol moieties of eumelanin and pheomelanin structure are shown.

generated superoxide anions undergo spontaneous dismutation or react with the oxidized and reduced moieties of melanin (62). Both pulse radiolysis (34) and electrochemical (63) studies indicated that the interaction of superoxide anions with eumelanin was predominantly an electron transfer from the superoxide to melanin with the formation of molecular oxygen and melanin radicals. Of course, the minor reaction, that is the oxidation of melanin by superoxide, will lead to the formation of hydrogen peroxide (58).

The generation of superoxide anions from both eumelanin and pheomelanin is wavelength-dependent and is progressively faster at shorter wavelengths (56–58). In this regard, pheomelanosomes from red human hair exhibited a lower ionization potential with respect to eumelanosomes with the photoionization threshold of pheomelanosomes at 326 nm and of eumelanosomes at 280 nm (64). An increased rate of oxygen photoconsumption was observed between 338 and 323 nm. Nevertheless, the recent finding of UVA-independent melanomagenesis in yellow pheomelanic mice by Mitra *et al.* (65) suggested that a mechanism(s) should exist in which pheomelanin promotes melanoma development not involving UVA radiation. Subsequently, Panzella *et al.* (66) demonstrated that the purified pigment from red human hair markedly accelerates the auto-oxidation of two important cellular antioxidants and reducing agents, GSH and NAD(P)H, compared to purified black human eumelanin. It was argued that the accelerating effect of pheomelanin is due to its capacity to serve as a redox catalyst accepting H-atoms from the substrates and transferring electrons to oxygen (25). In this connection, it should be noted that pheomelanin rich in carboxylated benzothiazine units generates more superoxide anions than pheomelanin containing mostly noncarboxylated units following irradiation with UVA or visible light (67), whereas the opposite trend was observed for

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the rate of GSH depletion (23). It would thus be worthwhile to examine how the presence of this carboxyl group affects the course of the photodegradation of pheomelanin.

The UV-independent production of superoxide anions and the depletion of cellular antioxidants (pro-oxidant activity) certainly open a new avenue of research in melanin chemistry and biochemistry (for example, 60,68). However, it is likely that this UV-independent pro-oxidant activity can be greatly accelerated by UVA radiation, considering the greater photoconsumption of oxygen at shorter wavelengths (64). Comparative studies examining the pro-oxidant activity of both eumelanin and pheomelanin with/without UVA radiation are certainly required, and a study toward this goal is in progress in our laboratory.

PATHOPHYSIOLOGICAL IMPLICATIONS

This review has summarized the photodegradation of melanin pigment induced by UVA or by blue light irradiation. With lower doses, DHI units in eumelanin are oxidized to indolequinone units, a process observed in immediate pigment darkening. It is likely that dihydrobenzothiazine units in pheomelanin are also oxidized to benzothiazine units, although direct evidence for this is lacking. With higher doses, the eumelanin structure undergoes cleavage of the benzene ring and cross-linking, as evidenced by the increase in free PTCA and PTeCA. Likewise, the pheomelanin structure undergoes conversion of benzothiazine to the benzothiazole moiety, as proved by the decrease in 4-AHP and the increase in TTCA and TDCA. These modifications of melanin chromophores are accompanied by decreases in absorbance in the visible region (A500) and in the ratio of A650/A500 in "Aged" melanin (Fig. 6). At the same time, ROS, especially superoxide anions and singlet oxygen, are generated directly through the interaction of the excited state of melanin molecules with molecular oxygen. Hydrogen peroxide and hydroxyl radicals are then generated with the participation of superoxide dismutase and iron ions, respectively. Thus, the production of "Aged" melanin and the generation of ROS are two major events that take place during UVA or blue light irradiation.

In the skin

Then what would be the pathophysiological implications of these events? Among the various types of UV radiation, UVB has long been considered to be carcinogenic. However, evidence has accumulated in recent years suggesting that UVA also causes skin cancers, in particular, melanomas. Wood *et al.* (69) used a genetically melanoma-susceptible *Xiphophorus* fish model to show that the action spectrum of melanin photosensitized oxidant production is identical to that for melanoma induction in a wavelength range of 300–400 nm. That study suggested that the presence of melanin plays an important role in inducing melanoma. Noonan *et al.* (70) used a mouse model to show that melanoma induction by UVA requires the presence of melanin pigment and is associated with oxidative DNA damage within melanocytes. In contrast, UVB radiation initiates melanoma in a pigment-independent manner that is associated with direct UVB DNA damage. Their study used C57BL/6-HGF mice that produce mostly (>90%) eumelanin in the skin. Surprisingly, neonatal mice were irradiated only with a single dose of UVA at 150 kJ m^{-2} (15 J cm^{-2}), which is a relatively low and physiological dose (70). In the nuclei of pigmented melanocytes in UV-irradiated skin, 8-oxodGuo was detected, which is considered the most abundant oxidative product in UVA-induced DNA damage. Interestingly, the black C57BL/6-HGF mice produced spontaneous melanomas without UV exposure, although UVA tumors arose more rapidly than spontaneous tumors. Spontaneous melanomas were not found in their albino counterparts. Melanin pigment, most likely eumelanin in these studies, is likely to induce melanoma development, and the process is accelerated by UVA irradiation.

How about the role of pheomelanin in inducing melanoma development? Pheomelanin is phototoxic, amplifying the UV-induced generation of ROS (64,67,71,72). Individuals with pale

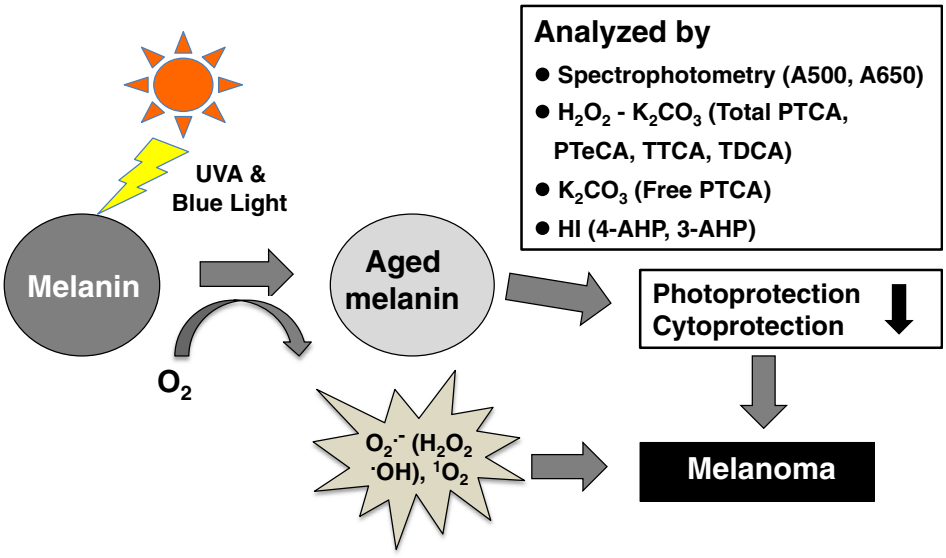

Figure 6. Photo-induced "aging" of melanin and its possible consequences. How the process of "aging" can be evaluated is also summarized. This figure is based on Fig. 3 of reference 25 with some modifications.

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

skin, red hair, freckles and an inability to tan (the red hair–fair skin phenotype) are at the highest risk of developing melanoma, compared to all other pigmentation types (73). Genetically, this phenotype is the product of inactivating polymorphisms in the melanocortin 1 receptor (*MC1R*) gene, which encodes MC1R, a cyclic AMP-stimulating G-protein-coupled receptor that controls pigment production. Minimal MC1R receptor activity produces pheomelanin, as occurs in the red hair–fair skin phenotype, whereas increasing MC1R activity stimulates the production of eumelanin (74). Mitra *et al.* (65) demonstrated an UV radiation-independent pathway to melanoma carcinogenesis in the red hair–fair skin background using a mouse model. Using a conditional, melanocyte-targeted allele of the melanoma oncoprotein, $BRAF^{V600E}$, in red-haired mice carrying an inactivating mutation in the *Mc1r* gene, a high incidence of invasive melanomas was observed without additional gene aberrations or UV radiation exposure. The selective absence of pheomelanin in albino mice was protective against melanoma development. These data suggest that the pheomelanin pigment pathway produces UV radiation-independent carcinogenic contributions to melanomagenesis. In a subsequent study (75), two mechanisms by which the pheomelanin pathway could mediate oxidative stress and melanomagenesis were postulated: (1) it might generate ROS that cause oxidative DNA damage, and (2) it might consume cellular antioxidant stores and make cells more vulnerable to elevated ROS levels. These studies were followed by studies on the UV-independent pro-oxidant activity of pheomelanin (see above, 25,66).

These studies using mouse models (65,70) raised at least two fundamental issues: (1) Is eumelanin and/or pheomelanin more likely to induce melanoma development? And (2) is UVA radiation not required in this process? In connection to the first issue, pigment in human epidermis is comprised of approximately 75% eumelanin and 25% pheomelanin, regardless of the degree of pigmentation, the pheomelanin being mostly of the benzothiazole type (27). Our study using 60 different human melanocyte cell lines showed that eumelanin is the predominant pigment irrespective of *MC1R* loss-of-function mutations (76). Therefore, the issue of how much pheomelanin contributes to melanoma development in humans seems to depend on the photochemistry of benzothiazole-type pheomelanin, which is mostly unknown at present. Regarding the second issue, the previous studies do not necessarily exclude the possible participation of UVA in accelerating melanoma development. Our new finding (10) that synthetic eumelanins and pheomelanins photogenerate singlet oxygen suggests that the potentially damaging effects of singlet oxygen occur in human skin exposed to UVA. More studies are needed to address these issues.

Irradiation with UVA or visible light induces the photodegradation of eumelanin and pheomelanin. Eumelanin is the major pigment determining the color of human skin (27,76). During irradiation, eumelanin undergoes a progressive photodegradation to produce "aged" eumelanin, which leads to decreased absorbance in the UVA region (12,33) despite the increase in visible absorbance. This raises a serious health concern that the persistent pigment darkening induced by UVA may deteriorate the photoprotective function of (eu)melanin because of its decreased ability as a sunscreen against UV light. In fact, Miyamura *et al.* (77) showed that when human skin was repeatedly exposed to suberythemal doses of UVA and/or UVB over 2 weeks after which a challenge dose of UVA/UVB was given, UVA tanning contributed essentially no photoprotection, although all types of UV-induced tanning resulted in DNA and cellular damage. These studies (77,78) and those by Noonan *et al.* (70) suggest that minimally protected melanocytes in fair skin are vulnerable not only to UVB but, stimulated to carry out melanin synthesis, are also vulnerable to UVA-induced melanoma. These results are in line with an epidemiological observation that artificial UVA tanning is a significant melanoma risk, particularly for young women. Women younger than 30 years were six times more likely to develop melanomas than the control group if they tanned indoors (79).

In the eyes

In the eyes, melanin acts as a cellular antioxidant and may protect RPE cells against the oxidative stress elicited by visible light or by redox-active metal ions such as iron (80,81). The oxidation of melanin and its irreversible bleaching can be induced by the aerobic irradiation of melanin with UV or visible light (82). This process is accompanied by the production of superoxide anions and hydrogen peroxide (54,56,71). Examining RPE cells from human donors of different ages, Sarna *et al.* (82) showed that the content of melanin in RPE cells undergoes an age-related loss and changes in its composition and antioxidant properties may be partly responsible for the dysfunction of RPE cells.

Then, what would be the possible consequence of the structural modifications observed with RPE melanin as summarized above (Figs 3 and 4)? RPE eumelanin is believed to exert cytoprotection through the scavenging of ROS produced by visible light or nonphotoic stress (80,81) and by sequestrating redox-active metal ions, especially iron (83). The scavenging action of eumelanin against superoxide anions appears to depend on the dihydroxyindole and/or indolequinone moiety (34). Thus, the loss of this structural integrity in the photodegraded eumelanin (Fig. 1) would lead to a lowered capacity to scavenge ROS. The chelating action of eumelanin against redox-active metal ions, in particular iron, also depends on the dihydroxyindole moiety (84). The cross-linking of the eumelanin structure at the C2 and C3 positions would give rise to a more crowded three-dimensional structure. It can be speculated that this structural modification would result in a lowered capacity to chelate metal ions. In fact, the photodegradation of synthetic eumelanin and RPE melanosomes induced by visible light (and by UV light) result in a reduced capacity to bind iron ions and a loss of an inhibitory effect on iron-mediated oxidation (50,51,85). In this connection, there is another consequence of the photoaging of RPE melanin, that is its increased photochemical reactivity, particularly melanin's ability to photogenerate superoxide anions and photoconsume oxygen (86). It was demonstrated that RPE melanosomes from older human donors after phagocytosis by ARPE-19 cells exhibited cytotoxicity upon irradiation with blue light (87). Increased phototoxicity and photochemical reactivity was also observed in the case of experimentally photoaged bovine RPE melanosomes (50,88).

Human RPE melanin examined in our study (39) consists of mainly eumelanin and in a minor part benzothiazole-type pheomelanin (40) as judged from a high level of PTCA (2860 ng mg^{-1}) and low levels of TTCA (438 ng mg^{-1}) and 4-AHP (110 ng mg^{-1}) at birth. The TTCA/4-AHP, 4-AHP/3-AHP and TTCA/PTCA ratios serve as markers for the photo-induced degradation of (pheo)melanin. Figure 4 indicates that the 4-AHP/3-AHP ratio in donors >80 years old was below 1.0, indicating

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

nearly complete degradation of the benzothiazine moiety to benzothiazole at this age. Nevertheless, photobleached porcine and bovine RPE melanosomes exhibit distinct pro-oxidant and phototoxic properties (51,88,89). These results are not compatible with the general belief that it is the benzothiazine units that are photoreactive and pro-oxidant in the pheomelanin structure (25). Thus, the photochemical behaviors of the benzothiazole structure in pheomelanin need to be studied in comparison with the structure of benzothiazine.

It can be speculated that the age-related degradation of melanin in the human RPE may be partly responsible for the dysfunction of this important retina-supporting tissue and, therefore, could contribute to the development of age-related macular degeneration, the primary cause of blindness in people over 60 (90).

CONCLUSIONS

Upon UVA irradiation, eumelanin undergoes immediate pigment darkening as a result of the ROS-dependent oxidation of DHI to *o*-indolequinone, accompanied by an increase in visible absorption and a decrease in UVA absorption. This deteriorates the ability of eumelanin to serve as a sunscreen. With higher (continuing) doses of UVA, persistent pigment darkening develops in several hours. Persistent pigment darkening is a complex, irreversible process involving the oxidative cleavage of indolequinone and cross-linking. With much higher doses of UVA, photobleaching may proceed. During the photoaging of RPE melanin caused by lifelong exposure to blue light, eumelanin is progressively degraded. The decrease in the quality of eumelanin would lead to a deterioration of its ability to protect RPE cells against ROS-induced oxidative stress. Pheomelanin also undergoes light-induced conversion from benzothiazine to the benzothiazole moiety. However, the physiological significance of this process remains to be clarified.

Acknowledgements—This work was partially supported by a Japan Society for the Promotion of Science (JSPS) grant (Nos 23591659 and 26461705) given to SI and KW. TS thanks to the support from the National Science Centre of Poland (2013/08/A/NZ1/00194).

REFERENCES

1. Brenner, M. and V. J. Hearing (2008) The protective role of melanin against UV damage in human skin. *Photochem. Photobiol.* **84**, 539–549.
2. Hu, D. N., J. D. Simon and T. Sarna (2008) Role of ocular melanin in ophthalmic physiology and pathology. *Photochem. Photobiol.* **84**, 639–644.
3. Miyamura, Y., S. G. Coelho, R. Wolber, S. A. Miller, K. Wakamatsu, B. Z. Zmudzka, S. Ito, C. Smuda, T. Passeron, W. Choi, J. Batzer, Y. Yamaguchi, J. Z. Beer and V. J. Hearing (2007) Regulation of human skin pigmentation and responses to ultraviolet radiation. *Pigment Cell Res.* **20**, 2–13.
4. Routaboul, C., A. Denis and A. Vinche (1999) Immediate pigment darkening: Description, kinetic and biological function. *Eur. J. Dermatol.* **9**, 95–99.
5. Honigsmann, H. (2002) Erythema and pigmentation. *Photodermatol. Photoimmunol. Photomed.* **18**, 75–81.
6. Tadokoro, Y., N. Kobayashi, B. S. Zmudzka, S. Ito, K. Wakamatsu, Y. Yamaguchi, K. S. Korossy, S. A. Miller, J. Z. Beer and V. J. Hearing (2003) UV-induced DNA damage and melanin content in human skin differing in radical/ethnic origin and photosensitivity. *FASEB J.* **17**, 1177–1179.
7. Alaluf, S., D. Atkins, K. Barrett, M. Blount, N. Carter and A. Heath (2002) Ethnic variation in melanin content and composition in photoexposed and photoprotected human skin. *Pigment Cell Res.* **15**, 112–118.
8. Hennessy, A., C. Oh, B. Diffey, K. Wakamatsu, S. Ito and J. L. Rees (2005) Eumelanin and pheomelanin concentrations in human epidermis before and after UVB irradiation. *Pigment Cell Res.* **18**, 220–223.
9. Tadokoro, T., Y. Yamaguchi, J. Batzer, S. G. Coelho, B. Z. Zmudzka, S. A. Miller, R. Wolber, J. Z. Beer and V. J. Hearing (2005) Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation. *J. Invest. Dermatol.* **124**, 1326–1332.
10. Maeda, K. and M. Hatao (2004) Involvement of photooxidation of melanogenic precursors in prolonged pigmentation induced by ultraviolet A. *J. Invest. Dermatol.* **122**, 503–509.
11. Szewczyk, G., A. Zadlo, M. Sarna, S. Ito, K. Wakamatsu and T. Sarna (2016) Aerobic photoreactivity of synthetic eumelanins and pheomelanins: Generation of singlet oxygen and superoxide anion. *Pigment Cell Melanoma Res.* **29**, 669–678.
12. Ito, S., M. Kikuta, S. Koike, G. Szewczyk, M. Sarna, A. Zadlo, T. Sarna and K. Wakamatsu (2016) Roles of reactive oxygen species in UVA-induced oxidation of 5,6-dihydroxyindole-2-carboxylic acid melanin as studied by differential spectrophotometric method. *Pigment Cell Melanoma Res.* **29**, 340–351.
13. Premi, S., S. Wallisch, C. M. Mano, A. B. Weiner, A. Bacchiocchi, K. Wakamatsu, E. J. Bechara, R. Halaban, T. Douki and D. E. Brash (2015) Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science* **347**, 842–847.
14. Choi, W., Y. Miyamura, R. Wolber, C. Smuda, W. Reinhold, H. Liu, L. Kolbe and V. J. Hearing (2010) Regulation of human skin pigmentation *in situ* by repetitive UV exposure: Molecular characterization of responses to UVA and/or UVB. *J. Invest. Dermatol.* **130**, 1685–1696.
15. Thody, A. J., E. M. Higgins, K. Wakamatsu, S. Ito, S. A. Burchill and J. M. Marks (1991) Pheomelanin as well as eumelanin is present in human epidermis. *J. Invest. Dermatol.* **97**, 340–344.
16. Ito, S. and K. Wakamatsu (2008) Chemistry of mixed melanogenesis – pivotal roles of dopaquinone. *Photochem. Photobiol.* **84**, 582–592.
17. d'Ischia, M., K. Wakamatsu, A. Napolitano, S. Briganti, J. C. Garcia-Borron, D. Kovacs, P. Meredith, A. Pezzella, M. Picardo, T. Sarna, J. D. Simon and S. Ito (2013) Melanins and melanogenesis: Methods, standards, protocols. *Pigment Cell Melanoma Res.* **26**, 616–633.
18. d'Ischia, M., K. Wakamatsu, F. Cicoira, E. Di Mauro, J. C. Garcia-Borron, S. Commo, I. Galván, G. Ghanem, K. Koike, P. Meredith, A. Pezzella, C. Santato, T. Sarna, J. D. Simon, L. Zecca, F. A. Zucca, A. Napolitano and S. Ito (2015) Melanins and melanogenesis: From pigment cells to human health and technological applications. *Pigment Cell Melanoma Res.* **28**, 520–544.
19. Tsukamoto, K., I. J. Jackson, K. Urabe, P. M. Montague and V. J. Hearing (1992) A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPAchrome tautomerase. *EMBO J.* **11**, 519–526.
20. Ito, S., N. Suzuki, S. Takebayashi, S. Commo and K. Wakamatsu (2013) Neutral pH and copper ions promote eumelanogenesis after the dopachrome stage. *Pigment Cell Melanoma Res* **26**, 817–825.
21. Jiang, S., X. M. Liu, X. Dai, Q. Zhou, T. C. Lei, F. Beermann, K. Wakamatsu and S. Z. Xu (2010) Regulation of DHICA-mediated antioxidation by dopachrome tautomerase: Implication for skin photoprotection against UVA radiation. *Free Radic. Biol. Med.* **148**, 1144–1151.
22. Panzella, L., G. Gentile, G. D'Errico, N.F. Della Vecchia, M.E. Errico, A. Napolitano, C. Carfagna and M. d'ischia (2013) Atypical structural and π-electron features of a melanin polymer that lead to superior free-radical-scavenging properties. *Angew. Chem. Int. Ed.* **52**, 12684–12687.
23. Micillo, R., L. Panzella, K. Koike, G. Monfrecola, A. Napolitano and M. d'Ischia (2016) "Fifty shades" of black and red or how carboxyl groups fine tune eumelanin and pheomelanin properties. *Int. J. Mol. Sci.* **17**, e746.
24. Wakamatsu, K., K. Ohtara and S. Ito (2009) Chemical analysis of late stages of pheomelanogenesis: Conversion of dihydrobenzothiazine to a benzothiazole structure. *Pigment Cell Melanoma Res.* **22**, 474–486.

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

25. Napolitano, A., L. Panzella, G. Monfrecola and M. d'Ischia (2014) Pheomelanin-induced oxidative stress: Bright and dark chemistry bridging red hair phenotype and melanoma. *Pigment Cell Melanoma Res.* **27**, 721–733.
26. Wolber, R., K. Schlenz, K. Wakamatsu, C. Smuda, Y. Nakanishi, V. J. Hearing and S. Ito (2008) Pigmentation effects of solar-simulated radiation as compared with UVA and UVB radiation. *Pigment Cell Melanoma Res.* **21**, 487–491.
27. Del Bino, S., S. Ito, J. Sok, Y. Nakanishi, P. Bastien, K. Wakamatsu and F. Bernerd (2015) Chemical analysis of constitutive pigmentation of human epidermis reveals constant eumelanin to pheomelanin ratio. *Pigment Cell Melanoma Res.* **28**, 707–717.
28. Heck, D. E., A. M. Vetrano, T. M. Mariano and J. D. Laskin (2003) UVB light stimulates production of reactive oxygen species. Unexpected role for catalase. *J. Biol. Chem.* **278**, 22432–22436.
29. Masaki, H., Y. Izutsu, S. Yahagi and Y. Okano (2009) Reactive oxygen species in HaCaT keratinocytes after UVB irradiation are triggered by intracellular Ca^{2+} levels. *J. Invest. Dermatol. Symp. Proc.* **14**, 50–52.
30. Wakamatsu, K., Y. Nakanishi, N. Miyazaki, L. Kolbe and S. Ito (2012) UVA-induced oxidative degradation of melanins: Fission of indole moiety in eumelanin and conversion to benzothiazole moiety in pheomelanin. *Pigment Cell Melanoma Res.* **25**, 434–445.
31. Kozinska, A., T. Oles and T. Sarna (2012) Photoactivation and detection of photoexcited molecules and photochemical products. *Isr. J. Chem.* **52**, 745–756.
32. Edge, R., M. d'Ischia, E. J. Land, A. Napolitano, S. Navaratnam, L. Panzella, A. Pezzella, C. A. Ramsden and P. A. Riley (2006) Dopaquinone redox exchange with dihydroindole and dihydroxyindole carboxylic acid. *Pigment Cell Res.* **19**, 443–450.
33. Ou-Yang, H., G. Stamatas and N. Kollias (2004) Spectral responses of melanin to ultraviolet A irradiation. *J. Invest. Dermatol.* **122**, 492–496.
34. Sarna, T., B. Pilas, E.J. Land and T.G. Truscott (1986) Interaction of radicals from water radiolysis with melanin. *Biochim. Biophys. Acta* **883**, 162–167.
35. Różanowska, M., T. Sarna, E. J. Land and T. G. Truscott (1999) Free radical scavenging properties of melanin interaction of eu- and pheo-melanin models with reducing and oxidizing radicals. *Free Radic. Biol. Med.* **26**, 518–525.
36. Au, V. and S. A. Madison (2000) Effects of singlet oxygen on the extracellular matrix protein collagen: Oxidation of the collagen cross-link histidinohydroxylysinonorleucine and histidine. *Arch. Biochem. Biophys.* **384**, 133–142.
37. Méndez-Hurtado, J., R. López, D. Suárez and M. I. Menéndez (2012) Theoretical study of the oxidation of histidine by singlet oxygen. *Chem. Eur. J.* **18**, 8437–8447.
38. Nair, V., R. S. Menon, A. T. Biju and K. G. Abhilash (2012) 1,2-Benzoquinones in Diels-Alder reactions, dipolar cycloadditions, nucleophilic additions, multicomponent reactions and more. *Chem. Soc. Rev.* **41**, 1050–1059.
39. Ito, S., A. Pilat, W. Gerwat, C. M. B. Skumatz, M. Ito, A. Kiyono, A. Zadlo, Y. Nakanishi, L. Kolbe, J. M. Burke, T. Sarna and K. Wakamatsu (2013) Photoaging of human retinal pigment epithelium is accompanied by oxidative modifications of its eumelanin. *Pigment Cell Melanoma Res.* **26**, 357–366.
40. Ito, S., Y. Nakanishi, R. K. Valenzuela, M. H. Brilliant, L. Kolbe and K. Wakamatsu (2011) Usefulness of alkaline hydrogen peroxide oxidation to analyze eumelanin and pheomelanin in various tissue samples: Application to chemical analysis of human hair melanins. *Pigment Cell Melanoma Res.* **24**, 605–613.
41. Ito, S., K. Wakamatsu, K. Glass and J. D. Simon (2013) High-performance liquid chromatography estimation of cross-linking of dihydroxyindole moiety in eumelanin. *Anal. Biochem.* **434**, 221–225.
42. Elleder, M. and J. Borovanský (2001) Autofluorescence of melanins induced by ultraviolet radiation and near ultraviolet light. A histochemical and biochemical study. *Histochem. J.* **33**, 273–281.
43. Wakamatsu, K., S. Ito and J. L. Rees (2002) The usefulness of 4-amino-3-hydroxyphenylalanine as a specific marker of pheomelanin. *Pigment Cell Res.* **15**, 225–232.
44. Greco, G., K. Wakamatsu, L. Panzella, S. Ito, A. Napolitano and M. d'Ischia (2011) Isomeric cysteinyldopas provide a (photo)degradable bulk component and a robust structural element in red human hair pheomelanin. *Pigment Cell Melanoma Res.* **22**, 319–327.
45. Liebel, F., S. Kaur, E. Ruvolo, N. Kollias and M. D. Southall (2012) Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. *J. Invest. Dermatol.* **132**, 1901–1907.
46. Kolbe, L. (2012) How much sun protection is needed? Are we on the way to full-spectrum protection. *J. Invest. Dermatol.* **132**, 1756–1757.
47. Chiarelli-Neto, O., A. S. Ferreira, W. K. Martins, C. Pavani, D. Severino, F. Faião-Flores, S. S. Maria-Engler, E. Aliprandini, G. R. Martinez, P. Di Mascio, M. H. Medeiros and M. S. Baptista (2014) Melanin photosensitization and the effect of visible light on epithelial cells. *PLoS ONE* **9**, e113266.
48. Duteil, L., N. Cardot-Leccia, C. Queille-Roussel, Y. Maubert, Y. Harmelin, F. Boukari, D. Ambrosetti, J. P. Lacour and T. Passeron (2014) Differences in visible light-induced pigmentation according to wavelengths: A clinical and histological study in comparison with UVB exposure. *Pigment Cell Melanoma Res.* **27**, 822–826.
49. Randhawa, M., I. Seo, F. Liebel, M. D. Southall, N. Kollias and E. Ruvolo (2015) Visible light induces melanogenesis in human skin through a photoadaptive response. *PLoS ONE* **10**, e0130949.
50. Zaręba, M., G. Szewczyk, T. Sarna, L. Hong, J. D. Simon, M. M. Henry and J. M. Burke (2006) Effects of photodegradation on the physical and antioxidant properties of melanosomes isolated from retinal pigment epithelium. *Photochem. Photobiol.* **82**, 1024–1029.
51. Zadlo, A., M. B. Rozanowska, J. M. Burke and T. J. Sarna (2007) Photobleaching of retinal pigment epithelium melanosomes reduces their ability to inhibit iron-induced peroxidation of lipids. *Pigment Cell Res.* **20**, 52–60.
52. Ozeki, H., S. Ito, K. Wakamatsu and A. J. Thody (1996) Spectrophotometric characterization of eumelanin and pheomelanin in hair. *Pigment Cell Res.* **9**, 265–270.
53. Zadlo, A., G. Szewczyk, M. Sarna, A. Kozinska, A. Pilat, K. Kaczara and T. Sarna (2016) Photoaging of retinal pigment epithelium melanosomes: The effect of photobleaching on morphology and reactivity of the pigment granules. *Free Radic. Biol. Med.* **97**, 320–329.
54. Sarna, T. and R. C. Sealy (1984) Photoinduced oxygen consumption in melanin systems. Action spectra and quantum yields for eumelanin and synthetic melanin. *Photochem. Photobiol.* **39**, 69–74.
55. Sarna, T., I. A. Menon and R. C. Sealy (1984) Photoinduced oxygen consumption in melanin systems – II. Action spectra and quantum yields for pheomelanins. *Photochem. Photobiol.* **39**, 805–809.
56. Felix, C. C., J. S. Hyde, T. Sarna and R. C. Sealy (1978) Melanin photoreactions in aerated media: Electron spin resonance evidence for production of superoxide and hydrogen peroxide. *Biochem. Biophys. Res. Commun.* **84**, 335–341.
57. Chedekel, M. R., P. P. Agin and R. M. Sayre (1980) Photochemistry of pheomelanin: Action spectrum for superoxide production. *Photochem. Photobiol.* **31**, 553–555.
58. Korytowski, W., B. Pilas, T. Sarna and B. Kalyanaraman (1987) Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanin. *Photochem. Photobiol.* **45**, 185–190.
59. Chiarelli-Neto, O., C. Pavani, A. S. Ferreira, A. F. Uchoa, D. Severino and M. S. Baptista (2011) Generation and suppression of singlet oxygen in hair by photosensitization of melanin. *Free Radic. Biol. Med.* **51**, 1195–1202.
60. Kim, E., L. Panzella, R. Micillo, W. E. Bentley, A. Napolitano and G. F. Payne (2015) Reverse engineering applied to red human hair pheomelanin reveals redox-buffering as a pro-oxidant mechanism. *Sci. Rep.* **5**, 18447.
61. Felix, C. C., J. S. Hyde, T. Sarna and R. C. Sealy (1978) Interactions of melanin with metal ions. Electron spin resonance evidence for chelate complexes of metal ions with free radicals. *J. Am. Chem. Soc.* **100**, 3922–3930.
62. Korytowski, W. B., I. A. Kalyanaraman, T. Sarna Menon and R. C. Sealy (1986) Reaction of superoxide anions with melanins: Electron spin resonance and spin trapping studies. *Biochim. Biophys. Acta* **882**, 145–153.
63. Korytowski, W., P. Hintz, R. C. Sealy and B. Kalyanaraman (1985) Mechanism of dismutation of superoxide produced during autoxidation of melanin pigments. *Biochem. Biophys. Res. Commun.* **131**, 659–665.
64. Ye, T., L. Hong, J. Garguilo, A. Pawlak, G. S. Edwards, R. J. Nemanich, T. Sarna and J. D. Simon (2006) Photoionization thresholds of melanins obtained from free electron laser-photoelectron emission microscopy, femtosecond transient absorption spectroscopy

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and electron paramagnetic resonance measurements of oxygen photoconsumption. *Photochem. Photobiol.* **82**, 733–737.
65. Mitra, D., X. Luo, A. Morgan, J. Wang, M. P. Hoang, J. Lo, C. R. Guerrero, J. K. Lennerz, M. C. Mihm, J. A. Wargo, K. C. Robinson, S. P. Devi, J. C. Vanover, J. A. D'Orazio, M. McMahon, M. W. Bosenberg, K. M. Haigis, D. A. Haber, Y. Wang and D. E. Fisher (2012) An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature* **491**, 449–453.
66. Panzella, L., L. Leone, G. Greco, G. Vitiello, G. D'Errico, A. Napolitano and M. d'Ischia (2014) Red human hair pheomelanin is a potent pro-oxidant mediating UV-independent contributory mechanisms of melanomagenesis. *Pigment Cell Melanoma Res.* **27**, 244–252.
67. Panzella, L., G. Szewczyk, M. d'Ischia, A. Napolitano and T. Sarna (2010) Zinc-induced structural effects enhance oxygen consumption and superoxide generation in synthetic pheomelanins on UVA/visible light irradiation. *Photochem. Photobiol.* **86**, 757–764.
68. Ito, S., M. Okura, K. Wakamatsu and T. Yamashita (2017) The potent pro-oxidant activity of rhododendrol-eumelanin induces cysteine depletion in B16 melanoma cells. *Pigment Cell Melanoma Res.* **30**, 63–67.
69. Wood, S. R., M. Berwick, R. D. Ley, R. B. Walter, R. B. Setlow and G. S. Timmins (2006) UV causation of melanoma in Xiphophorus is dominated by melanin photosensitized oxidant production. *Proc. Natl Acad. Sci. USA* **103**, 4111–4115.
70. Noonan, F. P., M. R. Zaidi, A. Wolnicka-Glubisz, M. R. Anver, J. Bahn, A. Wielgus, J. Cadet, T. Douki, S. Mouret, M. A. Tucker, A. Popratiloff, G. Merlino and E. C. De Fabo (2012) Melanoma induction by ultraviolet A but not ultraviolet B radiation requires melanin pigment. *Nat. Commun.* **3**, e884.
71. Chedekel, M. R., S. K. Smith, P. W. Post, A. Pokora and D. L. Vessell (1978) Photodestruction of pheomelanin: Role of oxygen. *Proc. Natl Acad. Sci. USA* **75**, 5395–5399.
72. Wenczl, E., G. P. Van der Schans, L. Roza, R. M. Kolb, A. J. Timmerman, N. P. Smit, S. Pavel and A. A. Schothorst (1998) (Pheo) melanin photosensitizes UVA-induced DNA damage in cultured human melanocytes. *J. Invest. Dermatol.* **111**, 678–682.
73. Rhodes, A. R., M. A. Weinstock, T. B. Fitzpatrick, M. C. J. Mihm and A. J. Sober (1987) Risk factors for cutaneous melanoma. A practical method of recognizing predisposed individuals. *J. Am. Med. Assoc.* **258**, 3146–3154.
74. Valverde, P., E. Healy, I. Jackson, J. L. Rees and A. J. Thody (1995) Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* **11**, 328–330.
75. Morgan, A. M., J. Lo and D. E. Fisher (2013) How does pheomelanin synthesis contribute to melanomagenesis?: Two distinct mechanisms could explain the carcinogenicity of pheomelanin synthesis. *BioEssays* **35**, 672–676.
76. Wakamatsu, K., R. Kavanagh, A. L. Kadekaro, S. Terzieva, R. A. Sturm, S. Leachman, Z. Abdel-Malek and S. Ito (2006) Diversity of pigmentation in cultured human melanocytes is due to differences in the type as well as quantity of melanin. *Pigment Cell Res.* **19**, 154–162.
77. Miyamura, Y., S. G. Coelho, K. Schlenz, J. Batzer, C. Smuda, W. Choi, M. Brenner, T. Passeron, G. Zhang, L. Kolbe, R. Wolber and V. J. Hearing (2011) The deceptive nature of UVA tanning versus the modest protective effects of UVB tanning on human skin. *Pigment Cell Melanoma Res.* **24**, 136–147.
78. Coelho, S. G., L. Yin, C. Smuda, A. Mahns, L. Kolbe and V. J. Hearing (2015) Photobiological implications of melanin photoprotection after UVB-induced tanning of human skin but not UVA-induced tanning. *Pigment Cell Melanoma Res.* **28**, 210–216.
79. Lazovich, D., R. I. Vogel, M. A. Weinstock, H. H. Nelson, R. L. Ahmed and M. Berwick (2016) Association between indoor tanning and melanoma in younger men and women. *JAMA Dermatol.* **152**, 268–275.
80. Sarna, T. (1992) Properties and function of the ocular melanin – a photo-biophysical view. *J. Photochem. Photobiol., B* **12**, 215–258.
81. Burke, J. M., P. Kaczara, C. M. B. Skumatz, M. Zareba, M. W. Raciti and T. Sarna (2011) Dynamic analyses reveal cytoprotection by RPE melanosomes against non-photic stress. *Mol. Vis.* **17**, 2864–2877.
82. Sarna, T., J. M. Burke, W. Korytowski, M. Różanowska, C. M. B. Skumatz, A. Zaręba and M. Zaręba (2003) Loss of melanin from human RPE with aging: Possible role of melanin photooxidation. *Exp. Eye Res.* **76**, 89–98.
83. Kaczara, P., M. Zaręba, A. Herrnreiter, C. M. Skumatz, A. Żądło, T. Sarna and J. M. Burke (2012) Melanosome-iron interactions within retinal pigment epithelium-derived cells. *Pigment Cell Melanoma Res.* **25**, 804–814.
84. Hong, L. and J. D. Simon (2007) Current understanding of the binding sites, capacity, affinity, and biological significance of metals in melanin. *J. Phys. Chem. B.* **111**, 7938–7947.
85. Różanowski, B., J. M. Burke, M. E. Boulton, T. Sarna and M. Różanowska (2008) Human RPE melanosomes protect from photosensitized and iron-mediated oxidation but become pro-oxidant in the presence of iron upon photodegradation. *Invest. Ophthalmol. Vis. Sci.* **49**, 2838–2847.
86. Różanowski, B., W. Korytowski, B. Różanowski, C. Skumatz, M.E. Boulton, J. M. Burke and T. Sarna (2002) Photoreactivity of aged human RPE melanosomes: A comparison with lipofuscin. *Invest. Ophthalmol. Vis. Sci.* **43**, 2088–2096.
87. Różanowski, B., J. Cuenco, S. Davies, F. A. Shamsi, A. Zadlo, P. Dayhaw-Barker, M. Różanowski and T. Sarna (2008b) The phototoxicity of aged human retinal melanosomes. *Photochem. Photobiol.* **84**, 650–657.
88. Zaręba, M., T. Sarna, G. Szewczyk and J. M. Burke (2007) Photobleaching of melanosomes from retinal pigment epithelium: II. Effects on the response of living cells to photic stress. *Photochem. Photobiol.* **83**, 925–930.
89. Burke, J. M. and M. Zareba (2009) Sublethal photic stress and the motility of RPE phagosomes and melanosomes. *Invest. Ophthalmol. Vis. Sci.* **50**, 1940–1947.
90. Hahn, P., A. H. Milam and J. L. Dunaief (2003) Maculas affected by age-related macular degeneration contain increased chelatable iron in the retinal pigment epithelium and Bruch's membrane. *Arch. Ophthalmol.* **121**, 1099–1105.

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

AUTHOR BIOGRAPHIES

Shosuke Ito, PhD, is an Emeritus Professor at Department of Chemistry, Fujita Health University School of Health Sciences. He has been engaged in the chemistry of melanin and melanin-related metabolites since early 1970s. In 1985, he developed a methodology for analyzing two major types of melanin, eumelanin and pheomelanin, in melanin-containing tissue samples such as hair, skin, and cultured melanocytes. Applying this methodology, he has published about 350 peer-reviewed articles including those from collaborative studies with other laboratories. His current interest is focused on (photo)degradation of eumelanin and pheomelanin.

Kazumasa Wakamatsu is a Professor at Department of Chemistry, Fujita Health University School of Health Sciences. His main research interests are focused on chemical studies of the biological pigment melanins and their roles in skin, hair, feather, eye, and brain of human, bird, mouse, amphibian, and reptiles et al. As one of the leading scientists in this field, he has been engaged chemically in melanin research since 1987. He has been a member of Japanese Society for Pigment Cell Research since 1988. He is now a President of Japanese Society for Pigment Cell Research and a council member of International Federation of Pigment Cell Societies.

Tadeusz (Tad) Sarna is a Professor of Biophysics and Head of the Laboratory of Photobiophysics at the Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland. He has been a member of ASP since 1982. Between 1997 and 1999 he served as President of the European Society for Photobiology. His main research interests are biophysics of melanin pigments, phototoxic reactions of the age pigment lipofuscin, mechanisms of photoprotection, role of free radicals and singlet oxygen in photodynamic phenomena mediated by selected photosensitizers.

17511097, 2018, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/php.12837 by Cochrane Poland, Wiley Online Library on [24/11/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License