Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection

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Endogenous chromophores in human skin serve as photosensitizers involved in skin photocarcinogenesis and photoaging. Absorption of solar photons, particularly in the UVA region, induces the formation of photoexcited states of skin photosensitizers with subsequent generation of reactive oxygen species (ROS), organic free radicals and other toxic photoproducts that mediate skin photooxidative stress. The complexity of endogenous skin photosensitizers with regard to molecular structure, pathways of formation, mechanisms of action, and the diversity of relevant skin targets has hampered progress in this area of photobiology and most likely contributed to an underestimation of the importance of endogenous sensitizers in skin photodamage. Recently, UVA-fluorophores in extracellular matrix proteins formed posttranslationally as a consequence of enzymatic maturation or spontaneous chemical damage during chronological and actinic aging have been identified as an abundant source of light-driven ROS formation in skin upstream of photooxidative cellular stress. Importantly, sensitized skin cell photodamage by this bystander mechanism occurs after photoexcitation of sensitizers contained in skin structural proteins without direct cellular photon absorption thereby enhancing the potency and range of phototoxic UVA action in deeper layers of skin. The causative role of photoexcited states in skin photodamage suggests that direct molecular antagonism of photosensitization reactions using physical quenchers of photoexcited states offers a novel chemopreventive opportunity for skin photoprotection.

1 Introduction

The causative role of solar ultraviolet (UV) photons in skin photodamage is firmly established. Most of the solar UV energy incident on human skin derives from the deeply penetrating UVA region (>95% from 320 to 400 nm). An increasing body of experimental evidence supports a causative role of UVA irradiation in photoaging and carcinogenesis of human skin by photooxidative mechanisms[1–5] mediated by reactive oxygen species (ROS). The molecular consequences downstream of light-driven ROS production on skin structural integrity, signal transduction, gene expression and ultimately tumorigenic initiation and progression are widely studied, but the upstream molecular mechanisms linking UV-photon absorption with ROS production in skin have been elusive. The formation of ROS as mediators of photooxidative stress in UV-irradiated skin seems to be dependent on non-DNA chromophores acting as endogenous photosensitizers.[2,3,6] Here we review briefly the emerging causative role of endogenous skin chromophores involved in UVA photosensitization and summarize recent work on skin photodamage from photoexcited states of chromophores associated with endogenous skin structural proteins.[7,8] We will also review an approach to identify and design physical quenchers of photoexcited states (QPES) as small molecular antagonists targeting endogenous photosensitizers and provide evidence that QPES compounds may serve as chemopreventive agents in suppressing photooxidative pathways of photocarcinogenesis and photoaging.

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2 UVA-Photosensitization

2.1 Skin photodamage from sunlight

Upon interaction with skin, sunlight can be reflected, scattered, or absorbed. According to the first law of photochemistry (also referred to as the Grotthus–Draper law), light must be absorbed by an atom or molecule in order to initiate a physical or chemical process. Photosensitization after photon absorption occurs when a photoexcited chromophore does not return to the electronic ground state by mechanisms of energy dissipation such as heat generation or photon emission, but instead initiates chemical reactions leading to the formation of reactive intermediates and toxic photoproducts. This requires that the lifetime of the excited state of the chromophore is sufficiently long to allow interaction with target molecules. Thus, it is the physical nature of the incident solar photons and the chemical nature of the absorbing chromophore in skin that determine the biological effects of sun exposure.

Most of the solar UV energy incident on the skin is from the UVA region (>95% from 320–400 nm). The UVB (290–320 nm) content of total solar UV-flux on skin can be well below 2% depending on the solar angle, which determines the atmospheric light path length and thereby the degree of ozone-filtering and preferential Rayleigh scattering of short wavelength UV light. UVC (< 290 nm) is not present in the solar spectrum reaching the

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Earth's surface. Longer-wavelength UVA radiation is less affected than UVB by environmental variables and easily penetrates cloud cover and glass windows, because the short wavelength cutoff of glass is about 320 nm. Skin photon penetration is positively correlated with wavelength. Up to 50% of UVA can reach the depth of melanocytes and the dermal compartment, whereas only 14% of UVB reaches the lower epidermis[9,10] and it has been estimated that the total photon energy delivered into the lower epidermis and upper dermis is 100 fold higher in the UVA region than in the UVB region. Photobiological research has preferentially focused on the deleterious involvement of UVB and UVC radiation in skin photocarcinogenesis. However, the role of UVA and near visible solar irradiation in photodamage, photoaging and carcinogenesis and the need for effective UVA skin photoprotection are now rapidly emerging as important areas of skin photobiology.[11] Only recently several animal models of photocarcinogenesis have provided evidence that UVA plays a significant role in solar carcinogenesis in human skin[12–14] (and reviewed in ref. 11). After correction for differences in epidermal UV-transmission between mouse and human skin, the predicted UVA contribution to the induction of nonmelanoma skin cancer (NMSC) by sunlight for humans is substantial (10–20%).[15] In addition, data from animal models of UVA carcinogenesis, such as the xiphophorus fish and the monodelphis melanoma model, suggest that UVA is a causative factor in the induction of human malignant melanoma.[16,17] Increasing epidemiological evidence suggests a strong association between melanoma incidence and exposure to sunbeds where most of the exposures studied was from sunbeds emitting mainly UVA with only 0.1% to 2.1% total UV dose derived from UVB.[18] An increased long-term risk of melanoma is observed in patients treated with the combined action of the sensitizer psoralen and UVA irradiation for photochemotherapy of psoriasis (PUVA therapy)[19] Moreover, UVA is a potent inducer of a variety of physiological changes characteristic of photoaging including chronic inflammatory signaling, immunosuppression, epidermal thickening, dermal matrix degradation with solar elastosis, reduced skin barrier function, and breakdown in tissue homeostasis.[3,20–22]

2.2 Skin UVA damage from photooxidative stress

ptPhotooxidative mechanisms that depend on light-driven ROS formation now are widely accepted as contributors to skin photoaging and photocarcinogenesis.[23,24] UV-driven ROS production has been demonstrated in cultured human skin cells, skin homogenates and intact murine and human skin.[25–28] Consequently, much research has focused on the molecular events triggered

by ROS action on skin components. Solar irradiation-induced photooxidative damage effectively reaches through the upper layers of skin into the human dermis and dermal capillary system. The immediate (IPD) and persistent pigment darkening (PPD) responses of human skin to UVA irradiation are thought to be due to photooxidation of preexisting melanins (IPD) and its precursors (PPD), respectively.[29,30] Substantial protein and lipid oxidation occurs in human skin epidermis and dermis upon acute irradiation with solar simulated light together with a significant depletion of enzymatic and non-enzymatic antioxidants in the stratum corneum, epidermis and dermis.[31,32] There is now ample evidence that after UV exposure a rapid cellular antioxidant response is induced, since hemeoxygenase-1 (HO-1), ferritin, glutathione peroxidase, Cu–Zn-dependent superoxide dismutase (SOD1), manganese-dependent superoxide dismutase (SOD2), and catalase upregulation occurs after solar irradiation in cultured human skin cells and human skin.[33–36] UV irradiation activates cell surface growth factor and cytokine receptors on keratinocytes and fibroblasts in human skin, critical in the regulation of cell proliferation and survival.[37] UV-driven formation of H_2O_2 regulates the tyrosine kinase activity of the epidermal growth factor receptor (EGF–R) and emerging evidence suggests the inhibition of protein tyrosine phosphatases as a consequence of UV-induced ROS formation.[26,38,39] At the membrane-level, UVA irradiation also triggers the ceramide signaling cascade through oxidative phospholipid degradation by singlet oxygen ($^1O_2$), resulting in AP-2 activation and induction of intercellular adhesion molecule-1 expression in cultured normal human keratinocytes.[40] In addition, UV-driven generation of ROS appears to be critical for signal transduction cascades such as mitogen-activated protein (MAP) kinases (p38, ERK, JNK).[41] In human fibroblasts, UVA induced activation of p38 and c-Jun-N-terminal kinase (JNK) have been linked to the photosensitized formation of $^1O_2$ and physiological doses of UVB induce the formation of H_2O_2 with activation of extracellular regulated kinase 1 and 2 (ERK1/2).[42–44] Down-stream of MAPK and other signaling cascades, UVA irradiation of human skin cells has been shown to lead to the activation of transcription factors such as AP-1, NFκB and AP-2.[45–47] Recent studies indicate that $^1O_2$ is a primary effector in UVA radiation-induced gene expression in human keratinocytes and fibroblasts.[48] The UVA-induced expression of various genes including HO-1, matrix metalloproteinase-1, IL-1, IL-6, TNFα, intercellular adhesion molecule (ICAM-1), STAT3, AP-1 and AP-2 may be crucial for photocarcinogenesis, since proinflammatory, photoimmunosuppressive signaling, and the degradation of extracellular matrix proteins all favor tumorigenic progression and invasion.[44,49–51] The possibility of UV-driven production of significant amounts of ROS in skin has additional implications for carcinogenesis by modulation of endogenous mitogenic redox signaling. Mitogenic signaling in fibroblasts through both Ras and Rac is mediated by superoxide anion production with spontaneous H_2O_2 formation as reviewed in ref. 52. Various human tumor cells constitutively produce substantial amounts of superoxide anions and H_2O_2 from plasma membrane oxidoreductases such as NAD(P)H oxidoreductase.[52,53] This process has been interpreted as an autocrine maintenance of proliferative signaling in malignant melanoma cells where endogenous redox stress seems to be responsible for constitutive NF-κB activation.[52]

2.3 UVA-Photosensitization: a key mechanism of ROS formation in skin

Although ROS are widely known as mediators of UV photodamage, the exact mechanism of their generation in UV-irradiated skin is poorly understood. Many mechanisms likely contribute to ROS formation during UV-irradiation of skin, such as UV-enhanced electron leakage from the mitochondrial respiratory chain and UV-induced remodeling of plasma membrane lipid rafts.[54,55] However, photosensitization by endogenous non-DNA chromophores of skin appears to be a key mechanism of light-driven ROS production in human skin. Photosensitization occurs as a consequence of photon absorption by endogenous non-DNA chromophores responsible for solar light-driven ROS production. Light driven ROS production by sensitizers occurs by excited state chemistry.[3,7,56–58] After initial photon absorption, electronically excited singlet states can either relax to the ground state by energy dissipation with heat generation or light emission (fluorescence) or undergo intersystem crossing (ISC), the nonradiative transition between states of different multiplicity, with formation of highly reactive biradical triplet states. The long lived triplet state can either undergo photochemical reactions or relax to the ground state, for example by light emission (phosphorescence). Among many other factors, the photodynamic potency of a given sensitizer depends on the efficiency (quantum yield) of triplet state formation and triplet state lifetime. A classic example is provided by fluorescein-dyes, where fluorescein displays high fluorescence intensity and no capacity for singlet oxygen formation due to inefficient ISC into the triplet state, whereas halofluoresceins such as 4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein (Rose Bengal) with weak fluorescence intensity display high ISC efficiency into a long-lived triplet state associated with a high quantum yield of singlet oxygen production.[57] The photoexcited state, most often the triplet state of the sensitizer, is the key photoreactive intermediate and exerts skin photodamage by direct reaction with substrate molecules including DNA bases (type I photosensitization) or molecular oxygen (type II photosensitization) leading to ROS formation as shown in simplified form in Fig. 1.[56,58] The reaction between photoexcited sensitizer and other molecules can proceed by a multitude of mechanisms such as energy, electron, or hydrogen transfer reactions. Moreover, direct photoejection of electrons with formation of sensitizer radical cation and solvated electrons can occur.[59] Singlet oxygen ($^1O_2$), an excited state molecule and established mediator of skin photodamage,[40,42,51,60] is formed by direct energy transfer between the excited sensitizer and ground

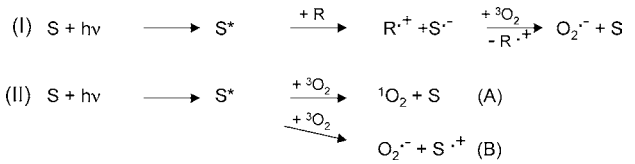

Fig. 1 Formation of ROS and organic free radicals by photosensitization reactions type I and type II. Photon absorption by sensitizer chromophores in the electronic ground state (S) induces formation of photoexcited states (S*). These can directly interact with substrate molecules (R), such as DNA bases (type I reaction) or activate molecular oxygen by electron or energy transfer reactions (type II reactions). Superoxide radical anions formed by type I or II mechanisms can then give rise to H_2O_2 formation by spontaneous dismutation.

state triplet oxygen (Fig. 1, reaction II A). The formation of superoxide radical anion (O_2^{-}) as a precursor of H_2O_2 occurs *via* electron transfer with production of a sensitizer radical cation (Fig. 1, reaction II B), or after intermediate reduction of the sensitizer by a substrate with subsequent single electron reduction of oxygen (Fig. 1, reaction I). H_2O_2 can then be formed by spontaneous or enzyme catalyzed dismutation of O_2^{-}. Additionally, toxic photoproducts such as lipid peroxides can be formed fueling secondary reaction pathways of cellular photodamage.[61,62] Photosensitization is terminated by spontaneous or quencher-induced return of the photoexcited sensitizer chromophore to the electronic ground state potentially followed by another cycle of photoexcitation. Irreversible termination occurs upon chemical sensitizer destruction (*e.g.* dye bleaching by $^1O_2$ attack), but the photooxidative formation of sensitizer reaction products with sustained photosensitizer activity is possible also.

2.4 DNA as a target of UV-photodamage by photosensitization

The mechanisms by which solar UV-irradiation cause skin photodamage are wavelength dependent[63,64] as classically exemplified by UV-induction of DNA photolesions as summarized in Fig. 2. DNA bases are important UVB skin chromophores. UVB is thought to cause direct structural damage to DNA in the form of epidermal cyclobutane pyrimidine dimers (CPD) and pyrimidine(6–4)pyrimidone dimers [(6–4)PD]. Indeed, more than 90% of human squamous-cell carcinomas contain mutations of the p53 tumor suppressor gene[65,66] and p53 gene mutations are most frequently C to T or CC to TT transitions at pyrimidine–pyrimidine sequences. CPD formation in DNA is thought to proceed by pyrimidine photoexcitation to a singlet state followed by intersystem crossing and subsequent reaction of the resulting triplet base with a second molecule in the ground state.[67] Thus, quantum yields of CPD formation are dramatically reduced when UV exposure is performed in the presence of specific

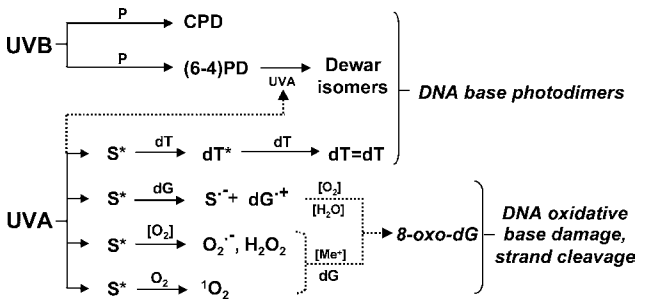

Fig. 2 Excited states of non-DNA chromophores and UVA-induced DNA damage. Direct UVB absorption by DNA bases induces cyclobutane pyrimidine dimers (CPD) from pyrimidine residues (P) and formation of pyrimidine(6–4)pyrimidone dimers [(6–4)PD]. UVA directly induces photoisomerization of (6–4)PDs to Dewar isomers. Photoexcited states of sensitizers (S*) are involved in UVA-induced DNA base photodimerization (dT = dT), particularly at deoxythymidine residues (dT) after triplet energy transfer from sensitizer to dT sites. Oxidative base damage can occur as a consequence of single electron transfer with formation of base radical cations ($dG^{\bullet+}$) and sensitizer radical anions ($S^{\bullet-}$). Most frequently, UVA-induced oxidative DNA damage occurs by sensitizer-induced formation of $^1O_2$ and other ROS, that directly or after metal ion catalyzed decay oxidize DNA bases, especially guanine residues (dG) with formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG).

triplet state quenchers such as isoprene and 2,4-hexadienol.[68] In contrast, [(6–4)PD] formation does not involve triplet excited states but rather proceeds *via* excited singlet state reactions. In contrast to UVB, UVA irradiation of isolated DNA results in little direct damage, since absorption of UVA photons by DNA bases is minimal, and photoexcitation of non-DNA chromophores acting as photosensitizers is critical for most UVA-induced DNA photodamage as summarized in Fig. 2. UVA photoexcitation of appropriate triplet sensitizers (S*) such as carbonyl compounds, *e.g.* benzophenone[69] and pyridopsoralens,[70] can lead to formation of photoexcited states capable of triplet–triplet energy reactions with DNA bases leading to CPDs (*e.g.* dT=dT formation in Fig. 2), if the photosensitizers are positioned in close physical proximity to DNA. Importantly, UVA-induced CPD formation has been recently demonstrated in cultured Chinese hamster ovary cells[71] and human skin keratinocytes and fibroblasts suggesting that these photoproducts may be involved in the genotoxic effects of solar UVA radiation.[72] Additionally, UVA exposure of [(6–4)PD] lesions in the absence of photosensitizers induces their phototransformation into their Dewar valence isomers, another class of prominent DNA photolesions. Oxidative base modification can occur by UVA photosensitization involving the triplet state sensitizer (S*) with formation of base radical cations by electron transfer reactions followed by water incorporation, a process involved in riboflavin-mediated base oxidation.[73] Most importantly, indirect oxidative modification of DNA bases results from the photosensitized formation of ROS such as H_2O_2 with subsequent hydroxyl radical generation and $^1O_2$ capable of direct base attack.[67,74]

The difference between UVB-induced direct DNA base damage and UVA-induced sensitized oxidative DNA base damage has been demonstrated by determining the UV action spectra for induction of DNA base damage in UV-irradiated fibroblasts.[2] Base photodimerization peaks at 300 nm, whereas oxidative DNA damage represented by the premutagenic signature lesion 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG) is extensive throughout the UVA region and reaches far into the near visible (440 nm). Indeed, recent experiments using laser capture microdissection of human skin lesions from actinic keratosis and squamous cell carcinoma (SCC) have demonstrated that in both conditions the basal epidermal layer harbors more UVA than UVB fingerprint mutations as revealed by p53 mutational analysis.[5,75] This epidermal layer selectivity was also observed using immunohistochemical detection of DNA photolesions, with only superficial localization of CPDs and abundance of 8-oxo-dG lesions in the basal layer suggesting a role for UVA-induced photosensitzation in human skin carcinogenesis. With regard to photoaging of human skin, it is interesting to note that the mitochondrial common deletion, a 4977-base pair signature mitochondrial DNA lesion observed during chronic exposure of human skin fibroblasts to UVA has been proposed as a biomarker of skin photoaging introduced by photooxidative mechanisms after sensitized formation of $^1O_2$.[76]

3 Photosensitizers in human skin

Photoexcited states of endogenous skin sensitizer chromophores are rapidly emerging as key intermediates of skin photooxidative damage operating upstream of ROS formation. Human skin is an abundant source of numerous chromophores with strong

absorption particularly in the UVA and blue visible region and endogenous photosensitizers include a multitude of chemical structures, pathways of formation, skin localization and photochemical mechanisms of action. The following section briefly reviews various classes of skin chromophores with established or likely roles as sensitizers of photooxidative stress.

3.1 Porphyrins

Shortly after Raab's, Jodlbauer's, Jesionek's and von Tappeiner's seminal observations of the oxygen-dependent lethal effects of sunlight and fluorescent dyes on protozoa and skin carcinoma cells that was referred to as 'photodynamic', Meyer-Betz in 1913 noticed prolonged severe phototoxicity upon self-injection of sulfuric acid-extracted human blood and thereby established the potential photodynamic action of chromophores derived from human tissue, *i.e.* hematoporphyrin, on human skin.[57,77] Other early support for the role of porphyrins as potential photosensitizers came from a group of metabolic diseases associated with extreme photosensitivity caused by enzymatic deficiencies in the porphyrin synthesis pathway that can affect any of the eight enzymes involved in heme synthesis. Many porphyrins are potent photosensitizers, including uroporphyrins, coproporphyrins and protoporphyrin IX (PpIX **1**, Fig. 3), where six of the eight known porphyrias can effect cutaneous photosensitization. PpIX, a classical type II photosensitizer acting mainly by sensitization of $^1O_2$ formation, is the direct biosynthetic precursor of ferriprotoporphyrin IX (heme) that occurs in every skin cell. However, due to feedback inhibition of heme on 5-aminolevulinic acid (ALA) biosynthesis, concentrations of PpIX and other sensitizer porphyrins are kept to a minimum in human tissue under normal conditions. In contrast, in photosensitivity-associated porphyria cutanea tarda (PCT), caused by uroporphyrinogen decarboxylase deficiency, water soluble porphyrins with four and more carboxyl groups mostly accumulate in the skin. Erythropoietic protoporphyria (EPP) is caused by a defect in ferrochelatase, leading to the accumulation of PpIX in all cells, but predominantly in erythrocytes and hepatocytes, resulting in skin photosensitivity further enhanced by leaching of blood PpIX into the skin.[78]

Quenching of triplet state porphyrins by ground state oxygen with $^1O_2$ formation occurs with high quantum yield *via* electronic energy transfer mechanisms, and alternative pathways of oxygen activation such as superoxide formation by electron transfer mechanisms proceed generally with much lower efficiency.[79] However, UV-visible irradiation of porphyrins yields also H_2O_2, where the sensitization efficiency of H_2O_2 formation declines in the

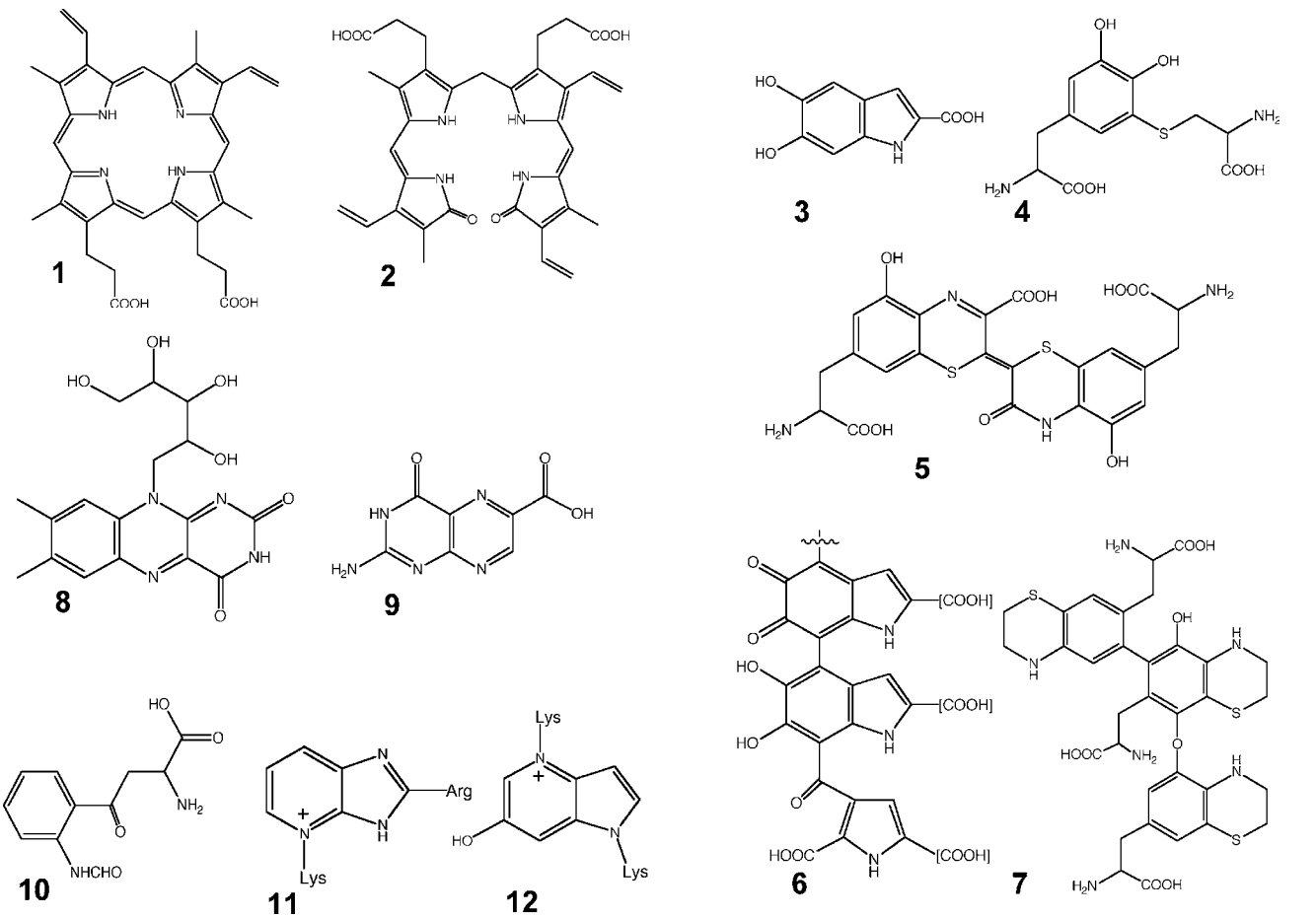

Fig. 3 UVA-photosensitizers with possible involvement in skin photodamage. Selected examples: protoporphyrin IX (**1**), (*Z*,*Z*)-bilirubin (**2**), 5,6-dihydroxyindole-2-carboxylate (**3**), 5-S-cysteinyldihydroxyphenylalanine (**4**), trichochrome C (**5**), eumelanin (**6**, representative structure from ref. 93), pheomelanin (**7**, representative structure, model trimer from ref. 94), riboflavin (**8**), 6-carboxypterin (**9**), *N*′-formylkynurenine (**10**), pentosidine (**11**), vesperlysine A (**12**).

order uroporphyrin > coproporphyrin > PpIX consistent with the clinical finding that the $^1O_2$ quencher beta-carotene reduces photosensitivity in PpIX-mediated EPP, but not in uroporphyrin-mediated PCT.[80] Porphyrin photoexcitation by visible photons is particularly pronounced in the region of Soret absorption (400–410 nm), but intense absorption throughout the UVA region suggests that porphyrins are powerful UVA photosensitizers, and photodynamic action of UVA-photoexcited PpIX has been demonstrated.[81] Massive intracellular PpIX formation is therapeutically induced after topical ALA application, used as a biosynthetic precursor drug for photodynamic therapy (PDT) of actinic keratosis and superficial basal cell carcinoma. ALA–PDT of human skin fibroblasts with variable excitation wavelengths revealed that UVA (320–400 nm) photoexcitation of protoporphyrin IX was 40-fold more efficient in producing photodynamic cell kill than ALA–PDT with commonly used red light. PpIX mediated UVA-photosensitization of skin fibroblasts has been shown to be dependent on $^1O_2$ formation and results in photodynamic activation of the p38 MAPkinase pathway.[81] PpIX photosensitization targets lipophilic cellular compartments, inducing free radical lipid peroxidation of plasma and mitochondrial membranes,[62] and self-sensitized bleaching and photodegradation of membrane-bound PpIX by chain lipid peroxidation. Interestingly, an oxidative chlorin-type photoproduct (photoprotoporphyrin, Ppp) formed from PpIX upon $^1O_2$ attack during cellular PDT is also a photodynamic sensitizer providing an example of the photooxidative transformation of one endogenous sensitizer into another one.[82] Although protein-bound heme is generally not considered to be a relevant photosensitizer, some hemoproteins such as catalase[83] and cytochromes[84] have been suggested as photosensitizers of UV and blue light, respectively. However, upon UVA irradiation of erythrocytes in dermal capillaries of human skin, a fluorescent photoproduct with photodynamic activity, thought to be bilirubin, is formed by photooxidative breakdown of heme from hemoglobin.[78]

3.2 Bilirubin

Bilirubin **2** (Fig. 3) is a lipophilic pigment originating from ubiquitous cellular heme catabolism catalyzed by hemeoxygenase and biliverdin reductase. Strong induction of fluorescence (emission at 508 nm) in erythrocytes under UVA exposure (excitation at 380 nm) is thought to originate from bilirubin formed by spontaneous heme photooxidation and has been suggested to occur constitutively during erythrocyte passage through capillaries of the papillary dermis in human skin.[78] Albumin-bound bilirubin is a suspected physiological $^1O_2$ quencher and bilirubin has been identified as a potent component of the endogenous antioxidant network involved in tissue protection against free radical damage.[85] The bichromophoric fluorescent molecule that exhibits a broad visible absorption at 450 nm comprises two dipyrrinone chromophores with the bridging double bond in *Z* configuration. Neonatal jaundice, caused by hyperbilirubinemia with accumulation of the unconjugated *Z*,*Z* isomer, is treated by phototherapy leading to configurational photoisomerization, photocyclization with lumirubin formation, and degradation by self-sensitized photooxygenation.[86] The phototherapy-induced *Z* to *E* photoisomerization is thought to enhance bilirubin secretion by increasing the water solubility of the molecule. Bilirubin photoreactivity and tendency for self-sensitized photodegradation are indicative of a potential activity as endogenous photosensitizer, and cellular phototoxicity of blue light activated bilirubin has been confirmed in numerous studies. Exposure of human fibroblasts to the combined action of visible light (420–490 nm) and bilirubin induced DNA strand breaks, and the effect was also observed when pre-irradiated bilirubin was added to cells kept in the dark.[87] Catalase protection suggested a causative involvement of H_2O_2 formed by bilirubin photosensitization. Similar experiments demonstrating bilirubin-sensitized induction of apoptosis and necrosis in mouse lymphoma cells at light doses (peak excitation at 450 nm) that are known to induce bilirubin photooxidation suggested the involvement of toxic bilirubin photoproducts acting in combination with H_2O_2.[88] Taken together, these data provide evidence for a potential role of bilirubin as a photosensitizer in skin photodamage by UVA and visible light.

3.3 Melanin and melanin precursors

The complex role of melanin as both skin photoprotector and photosensitizer has been the subject of numerous studies. Skin photoprotection by melanin has been attributed to the various functions of this biopolymer as UV filter, light scatterer, photon energy dissipator and excited state quencher, free radical trap, pseudoenzymatic and sacrificial antioxidant, and metal chelator.[89] On the other hand, melanin photoreactivity and involvement in light-driven formation of ROS and organic free radicals are well documented. Numerous studies suggest a role of melanin and particularly monomeric melanogenic precursors as potent endogenous sensitizers of skin photooxidative stress with particular relevance to UV-induction of melanoma skin cancer (MSC).[17,90,91] Clearly, the double-edged role of melanin and melanin precursors in skin photodamage originates from their heterogeneous chemical nature, unknown intracellular redox state and wavelength dependent photochemistry (pheomelanin *vs.* eumelanin, monomeric precursors *vs.* polymeric endproducts, constitutive *vs.* induced pigmentation, UVA *vs.* UVB) and is complicated by many other factors such as the complex physiology of melanosome morphology and trafficking and the availability of redox active transition metal ions.[92] The two types of melanin, eumelanin and pheomelanin, are derived from the common precursor dopaquinone that is formed by tyrosinase oxidation of tyrosine. 5,6-Dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHI2CA **3**, Fig. 3) units constitute the majority of the eumelanin polymer **6** (Fig. 3), while benzothiazine units derived from 5-*S*- and 2-*S*-cysteinyldopa (5-*S*-CD **4** (Fig. 3), 2-*S*-CD) compose the majority of the pheomelanin polymer **7** (Fig. 3).[93,94] Most melanin pigments in tissues appear to be present as mixtures or copolymers of eumelanin and pheomelanin. Dysplatic nevi contain pheomelanin in high proportion over eumelanin,[95] and trichochromes such as trichochrome C **5** (Fig. 3), dimeric pheomelanic pigments produced in small quantities concomitant with pheomelanin, can be detected together with 5-*S*-CD in urine of metastatic melanoma patients.[96,97] Melanocytes of light-skinned individuals exhibit a preference for pheomelanogenesis. The limited ability of pheomelanin to absorb UV radiation, extensive cellular thiol (cysteine and glutathione) depletion during pheomelanin synthesis, potential ROS leakage by single electron redox reactions during polymerization steps, and greater prooxidative photoreactivity

of pheomelanin may all contribute to enhanced photooxidative stress leading to an elevated risk of NMSC and MSC among fair-skinned individuals.[89] In red-haired subjects with equally fair skin, a positive correlation between hair eumelanin/pheomelanin ratio and minimal erythemal skin dose values has been established suggesting that the eumelanin/pheomelanin ratio could serve as a novel chemical parameter predictive of skin cancer risk.[98] Ample experimental evidence indicates a role of melanin and melanin precursors as photosensitizers of genotoxic oxidative stress.[91,99–104] In an attempt to reconcile the apparent contradictory photoprotective and phototoxic activities of melanin, it was recently suggested that melanin synthesis may sensitize melanocytes to oxidative DNA damage by UVA, yet protect melanocytes and other skin cells from direct DNA damage by UVB.[105] The hypothesis predicts that UVA or solar exposure of skin during high rates of melanin synthesis may be particularly harmful.

Sensitizer chromophores contained in melanin precursors clearly contribute to the potential phototoxicity of melanogenesis. The complex photochemistry of melanin precursors has only partly been elucidated and varies strongly between individual compounds.[106–110] Electron photoejection and photohomolytic generation of semiquinone free radicals with photoreductive formation of superoxide predominate upon laser flash photolysis of DOPA. Similar reactions following the initial formation of indoloxyl radicals occur upon UV (>300 nm) exposure of the eumelanic precursors DHI2CA, 5-methoxy-6-hydroxyindole-2-carboxylic acid (5M6HI2CA) and 5-hydroxy-6-methoxyindole-2-carboxylic acid (5H6MI2CA). Consistent with a higher photoreactivity of pheomelanic precursors, the KBr-quenchable photoexcited singlet state of cysteine-DOPA leads to photohomolytic formation of thiyl and carbon centered organic free radicals. Photolysis of isolated pheomelanins both with UV and visible light leads to rapid degradation of chromophores with electron ejection and superoxide formation. Trichochromes, pheomelanin constituents of defined benzothiazine-dimer structures, isolated from human red hair are likely, yet unexamined potential photosensitizers based on recent evidence of a long lived excited state species formed upon photoexcitation (540 nm) of decarboxytrichochrome c.[111] However, information about the actual cellular and extracellular skin levels of individual melanin precursors with suspected sensitizer activity is limited at this time, but indirect data such as the increased urinary excretion of cysteine-DOPA after solar exposure of individuals of Celtic origin suggest that skin levels may be appreciable and subject to photodamage-induced turnover. Interestingly, melanin precursors leak from melanosomes as a consequence of UV membrane damage thereby possibly extending the intracellular range of molecular targets hit by photosensitization. Melanin precursors also abound in human skin outside melanocytes as demonstrated by recent studies on skin persistent darkening in response to UVA radiation.[30] These results illustrate the broad extracellular distribution of photoreactive melanogenic precursors in skin suggesting that UVA photoactivation of extracellular melanin precursors potentially exerts skin photosensitization throughout large areas of the epidermis.

3.4 Flavins

The isoalloxazine chromophore contained in riboflavin **8** (vitamin B_2, RF, Fig. 3) and the related coenzymes flavin mononucleotide and flavin adenine dinucleotide have a complex photochemistry involved in blue light photoreception, DNA photorepair and cellular phototoxicity. Flavoproteins occur at many cellular sites such as plasma membranes (*e.g.* NAD(P)H oxidase and NAD(P)H quinone oxidoreductase) and mitochondria (*e.g.* the respiratory chain components electron transferring flavoprotein, NADH dehydrogenase and succinate dehydrogenase) positioning this ubiquitous photosensitizer throughout the skin cell. Pronounced RF-sensitization of photooxidative reactions upon UVA and blue visible photoexcitation of the 7,8-dimethylisoalloxazine ring occurs as a consequence of the high quantum yield for intersystem crossing (ISC) and the high oxidation potential of the triplet state.[112,113] RF photosensitization reactions proceed *via* type I and type II pathways. Photoexcitation of RF is known to produce ROS, both by energy transfer ($^1O_2$) or by complex electron transfer reactions (superoxide, H_2O_2). Triplet state RF mediates the photodegradation of free or protein-bound amino acids such as tryptophan and tyrosine[114,115] and initiates the photoinactivation of enzymes such as lysozyme. RF photosensitization has been implicated in protein photodamage during lens aging and cataractogenesis.[116] Interestingly, RF enhances UVA-induced collagen crosslinking with formation of dityrosine residues.[6,73,117] Immediate skin cell cytotoxicity of UVA-irradiated RF solutions[118] or RF containing cell culture media[119] seems to originate from sensitized formation of H_2O_2. An extensive literature describes RF photosensitization of DNA damage by covalent addition of photoproducts, introduction of strand breaks and base oxidation.[120] Photodynamic action of riboflavin (365 nm excitation) causes the formation of 8-oxo-dG in double stranded DNA specifically at the guanine residue located 5′ to another guanine through electron transfer to triplet excited RF (type I photosensitization) with intermediate formation of RF anion radical and guanine radical cation.[74,121] An oxygen independent reaction mechanism of 8-oxo-dG formation involving the hydration of the guanine radical cation was confirmed by the incorporation of the [^{18}O]-atom in isotopic experiments using [^{18}O]-H_2O.[73] An analogous mechanism was suggested to explain the formation of 8-oxo-dG in cellular DNA by photoirradiation of cultured mammalian cells in the presence of RF and a potential role of RF-photosensitization of DNA damage in skin carcinogenesis has been hypothesized.[122]

3.5 Pterins

Pterins (2-amino-4-hydroxypteridine derivatives) are highly fluorescent skin UV chromophores that potentially participate in photosensitization reactions. For pterin, 6-carboxypterin **9** (Fig. 3), 6-formylpterin, neopterin and biopterin $^1O_2$ production upon UVA excitation (367 nm) in aqueous solution occurs with high quantum yields[123] In contrast, the weakly fluorescent pterin derivative folic acid does not sensitize formation of $^1O_2$ efficiently, most probably due to an involvement of the *p*-aminobenzoylglutamate substituent in internal fluorescence quenching by radiationless deactivation of the singlet excited state leading to inefficient intersystem crossing. Pterins are documented photosensitizers of strand cleavage in isolated calf thymus and plasmid DNA.[124] More recently, an involvement of oxidative stress by pterin photosensitization has been demonstrated in the human skin depigmentation disorder vitiligo.[125] In this disorder, biopterin accumulation due to H_2O_2 oxidation of tetrahydroprecursors leads to the characteristic

fluorescence of the affected skin upon Wood's light examination (excitation at 351 nm). Subsequent photooxidation of biopterin by UVA/B induces formation of the potential photosensitizer 6-pterin-carboxylic acid and substantial amounts of H_2O_2 as an additional source of ROS in vitiligo. In contrast, rapid folate photolysis occurs upon UVA (360 nm) exposure of human plasma and folate serum levels drop dramatically in patients receiving PUVA photochemotherapy possibly contributing to photocarcinogenesis by nutrient depletion, but not photosensitization.[126] However, indirect skin photosensitization by folate cannot be ruled out as the potential $^1O_2$ sensitizer 6-formylpterin forms rapidly upon folate photolysis (excitation at 365 nm).[127] No information is available as to whether the *p*-aminobenzoic acid moiety, an established UVB-photosensitizer contained in early sunscreen preparations, could be liberated from folic acid under physiological conditions and then exert phototoxic activity.

3.6 B_6 vitamers

Increased skin photosensitivity is a known consequence of vitamin B_6-overdosing in humans.[128] Phototoxicity of UV-irradiated pyridoxamine was reported as early as 1947 by Shwartzman and Fisher[129] followed by other reports thereafter as reviewed in ref. 130. Our own studies have identified 3-hydroxypyridine **13** (Fig. 4) and its more potent fluorescent analogue *N*-alkyl-3HP **14** (Fig. 4) as the miniumum phototoxic chromophores contained in B_6 vitamers **18–21** (Fig. 4).[130] This chromophore also occurs in other potential skin photosensitizer chromophores, such as the enzymatic collagen crosslink pyridinoline **15** (PYD) (Fig. 4) and various advanced glycation endproducts (AGEs) such as **16** contained in chronologically aged tissue (Fig. 4, *vide infra*). Human skin contains various B_6-vitamer forms in significant amounts (approximately 100 nmol per g protein[131]), with pyridoxal **20** (Fig. 4) and pyridoxal-5′-phosphate **21** (Fig. 4) being the predominant vitamers *in vivo*. Among the biogenic 3-HP derivatives tested, UVA-phototoxicity, measured by inhibition of skin proliferation and induction of apoptosis in cultured human skin keratinocytes and fibroblasts, increased in the order pyridinoline < pyridoxine < pyridoxamine < pyridoxal = pyridoxal-5-phosphate. Photosensitization induced intracellular oxidative stress and was inhibited by thiol-antioxidants, but NaN_3 did not exert photoprotection suggesting that $^1O_2$ is not involved in vitamin B_6-photosensitized induction of cellular apoptosis. Peptide photooxidation was examined by solar simulated light-irradiation of the model peptide melittin in the presence or absence of pyridoxine. Pyridoxine photosensitization induced the formation of a reaction product with a detected mass increase of 32 u by MALDI-TOF mass spectrometry providing clear evidence for sensitized introduction of molecular oxygen into the target peptide that was not observed in the absence of pyridoxine and again not suppressed by NaN_3. Interestingly, exposure of unirradiated cultured human skin cells to pre-irradiated B_6-vitamers exerts cellular toxicity measured as inhibition of proliferation that can not be reversed by co-treatment with antioxidants suggesting the formation of an unknown toxic, stable B_6-derived photoproduct.[130,132] Generation of endoperoxide- and hydroperoxide-pyridoxine intermediates *via* rapid chemical quenching of $^1O_2$ has been reported recently.[133] Thus, formation of B_6-peroxides during photosensitization reactions by additional routes independent of $^1O_2$ formation may be involved in the generation of toxic photoproducts during UVA irradiation of B_6 vitamers observed in our studies. The phototoxic role of B_6-vitamers in human skin suggested by these experiments strongly contrasts but does not contradict a protective role of pyridoxine against $^1O_2$ damage observed recently in a phytotoxic fungus.[134]

3.7 Vitamin K

Pronounced UVA-photosensitization of thymidine has been observed with menadione (2-methyl-1,4-napthoquinone, vitamin K_3)

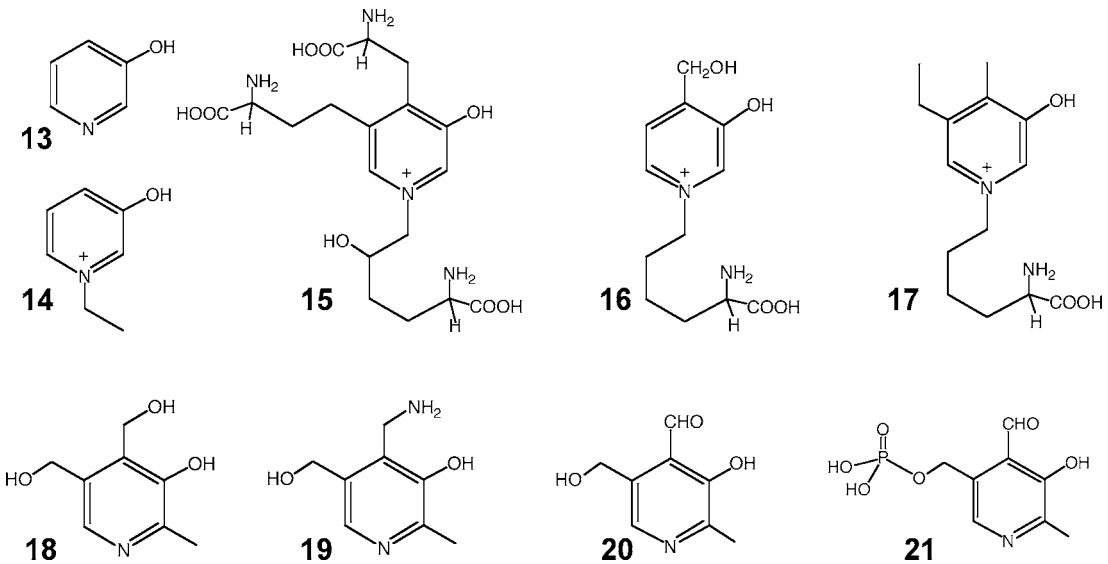

Fig. 4 The 3-hydroxypyridine (3-HP) class of endogenous UVA-photosensitizers with possible involvement in skin photodamage. Selected structures of the 3-HP class of endogenous photosensitizers are shown, which comprise synthetic molecules such as 3-HP (**13**) and *N*-ethyl-3-hydroxypyridinium salt (**14**), and biomolecules, such as enzymatically generated matrix protein crosslinks [pyridinoline (**15**)], nonenzymatically generated AGEs [glycol–aldehyde–pyridine (**16**)], lipid peroxidation lysine adducts (**17**), and the B_6 vitamers pyridoxine (**18**), pyridoxamine (**19**), pyridoxal (**20**) and pyridoxal-5-phosphate (**21**).

and formation of the photooxidation product 5,6-dihydroxy-5,6-dihydrothymidine occurred after single electron transfer reactions between triplet state menadione and thymidine.[69] However, little information is available on vitamin K as a potential UVA photosensitizer in human skin.

3.8 NAD(P)H

NAD(P)H is an abundant fluorophore in skin that strongly contributes to skin autofluorescence upon UVA photoexcitation (λ_{ex} = 340 nm). Based upon limited evidence from photochemical studies, NADH has been suggested as a potential skin photosensitizer. NAD(P)H exposure to full spectrum solar and monochromatic (290, 334, 365, 405 nm) radiation induces single electron transfer reactions leading to superoxide formation with only moderate quantum yields.[6,135,136] On the contrary, accumulating evidence supports a role of NAD(P)H as a potent cellular inhibitor of photosensitization acting as a metabolically regenerated sacrificial target of $^1O_2$. Importantly, $NAD(P)^+$ formation occurs upon aerobic exposure of NAD(P)H to UVA with intermediate formation of a $NAD^\bullet$ free radical.[137] NAD(P)H oxidation to $NAD(P)^+$ results from interaction with $^1O_2$ upon visible excitation of haematoporphyrin,[138,139] and rapid depletion of tissue NADH occurs during PDT.[140] Recent experimental evidence obtained in intact mammalian cells indicates that NAD(P)H is a primary target of $^1O_2$ in mitochondria.[141] Single electron transfer reactions of NAD(P)H may deactivate $^1O_2$ and thereby inhibit cellular photosensitization.[142] Ultimately, NADP(H) deactivates $^1O_2$ by reductive formation of superoxide with subsequent enzymatic detoxification, *e.g.* by the mitochondrial SOD/GSH peroxidase cascade, and metabolic regeneration of NADH, an antioxidant mechanism reminiscent of glutathione-mediated detoxification of organic free radicals by superoxide formation followed by enzymatic regeneration of glutathione.[143] Moreover, studies in human skin demonstrate that raising skin $NAD(P)^+/NAD(P)H$ by application of NAD^+ liposomes or nicotinic acid prodrugs did not increase photosensitivity, but rather decreased the erythemal response to UV radiation.[144,145] In light of the emerging evidence supporting a photoprotective action by sacrificial quenching and metabolic regeneration, NAD(P)H does not seem to qualify as a relevant endogenous photosensitizer in human skin.

3.9 *trans*-Urocanic acid

trans-Urocanic acid (3-(4-imidazolyl)acrylic acid, t-UCA) is a histidine derivative formed by histidase-catalyzed enzymatic conversion after proteolytic degradation of filaggrin during stratum corneum maturation. t-UCA substantially contributes to stratum corneum dry weight and is involved in skin pH control, anti-microbial defense, immunomodulation, hydration and photoprotection.[146,147] t-UCA strongly absorbs UVB and exerts potent UVB photoprotection of skin cells acting by UV filtering. Pronounced *cis-trans* photoisomerization occurs upon UVB exposure dependent on pH and solvent polarity. Due to strong triplet quencher activity related to *cis-trans* photoisomerization reactions t-UCA has been proposed to act as an endogenous 'triplet state sink' in human skin contributing to UV-photoprotection.[148] However, recent studies indicate that UVA photoexcitation of t-UCA induces the population of a long-lived triplet state with subsequent formation of $^1O_2$.[149,150] Based on similarities between the action spectra for UVA-induced $^1O_2$ formation by t-UCA and photo-sagging of mouse skin, t-UCA skin photosensitization has been implicated in photoaging.[151] The potential for sensitization of $^1O_2$ by t-UCA, a UVB chromophore, at UVA wavelengths provides an interesting example where photobiologically relevant photochemical reactions of biochromophores may be induced at excitation wavelengths that are distant from wavelengths of maximal absorption. Importantly, the photoisomer *cis*-UCA has been recognized as a mediator of skin photo-immunosuppression likely involved in photocarcinogenesis.[146,147] A role of UCA as an endogenous sensitizer of photooxidative stress remains controversial, since dual activities as triplet state sensitizer/quencher as well as $^1O_2$-sensitizer/quencher and powerful hydroxyl radical scavenger have been observed.

3.10 Tryptophan and tryptophan photooxidation products

The photochemistry associated with biogenic indole derivatives is complex and dual activity as both UV-photosensitizers as well as potent physical and chemical quenchers of $^1O_2$ have been described. For example, protein-bound tryptophan quenches $^1O_2$ by physical and chemical reaction pathways with high rate constants of $2–7 \times 10^7$ and 3×10^7 M^{-1} s^{-1}, respectively as reviewed in ref. 60. However, UV-photooxidation of L-tryptophan has long been known to be associated with formation of cytotoxic products, including superoxide and H_2O_2.[152,153] A prominent photooxidation product of L-tryptophan, *N*′-formylkynurenine **10** (NFK, Fig. 3), is a strong UVA photosensitizer providing an example of sensitizer formation by photooxidation of a biogenic precursor molecule.[154] Photooxidative formation of NFK has been observed in sun light exposed human lens protein, serum albumin and lysozyme suggesting the actinic introduction of potent protein-bound endogenous photosensitizers suspected to be involved in lens and possibly skin photoaging.[155,156] Interestingly, the human lens also contains a family of tryptophan-derived UV filter compounds with maximum absorption around 365 nm, of which 3-hydroxykynurenine *O*-β-D-glucoside is present at the highest concentrations. Spontaneous adduction of lens proteins by tryptophan-derived filter compounds is thought to mediate lens photosensitization during chronological aging,[157] and calf lens protein-bound kynurenine has recently been identified as a potent UVA photosensitizer of $^1O_2$ and peroxide formation leading to subsequent oxidative protein modification with introduction of dityrosine and 3,4-dihydroxyphenylalanine.[158] Moreover, tryptophan photooxidation products such as 6-formylindolo[3,2-*b*]carbazole are potent agonists at the aryl hydrocarbon receptor (AhR).[159] AhR activation by L-tryptophan photooxidation products from visible, UVA, UVB, or UVC irradiation mediate induction of cytochromes P450 1A1 and 1B1 in HaCaT keratinocytes suggesting that tryptophan photooxidation products in human skin may act as AhR agonists with obvious implications for skin photocarcinogenesis.

Tryptophan occurs in skin keratin, but is completely absent from skin collagen and elastin. Little is known about tryptophan-related photochemistry and keratin photoreactions in human skin.[160] Our own experiments testing sensitization of UV-driven H_2O_2 formation revealed no photodynamic activity of keratin isolated from cultured human keratinocytes.[8] However, earlier work has examined the complex photochemistry of wool

keratin, which closely resembles keratin from human skin.[161] These photobiological studies on wool keratin demonstrated (a) UVB-driven electron transfer reactions originating from tyrosine and tryptophan residues, (b) $^1O_2$ formation by interaction of ground state oxygen with triplet state species formed upon UVA (366 nm) photoexcitation and, (c) photooxidative introduction of the potential photosensitizer chromophore NFK. On the other hand, tryptophan excited state luminescence studies with UV photoexcitation (<300 nm) performed on wool keratin suggest that cystine-mediated non-radiative fluorescence and triplet state quenching is very effective and limits the potential harmful photoreactivity of keratin tryptophan residues photoexcited by UVB.[162] Therefore, it is possible that skin keratin may contribute to epidermal photoprotection where keratin UV absorption would be followed by rapid chromophore deexcitation and energy dissipation, a role compatible with the established induction of epidermal thickening during skin photoaging. However, elucidation of the complex keratin photoreactivity in sun-exposed human skin awaits future studies.

3.11 Advanced glycation endproducts (AGEs), reactive carbonyl species (RCS) and lipofuscin

Recent research findings suggest that during chronological and actinic aging, as well as under pathological conditions, skin proteins accumulate significant chemical damage with formation of chromophore-epitopes that can act as UV photosensitizers. AGEs, crosslink chromophores formed by non-enzymatic amino-carbonyl reactions (glycation) between reactive carbonyl species (RCS) and protein-bound amino-groups, accumulate on long-lived skin proteins such as dermal collagen, elastin, laminin and fibronectin inducing marked changes in extracellular matrix architecture.[163,164] RCS are glycation intermediates that comprise a diverse group of carbonyl compounds including reducing sugars (hexoses, pentoses and trioses), ascorbic acid and its oxidation products and dicarbonyl compounds originating from metal catalyzed sugar autoxidation (*e.g.* glucosone), sugar degradation (*e.g.* 1- and 3-deoxyosones), glycolysis (*e.g.* the glycolytic byproduct methylglyoxal), and lipid peroxidation (*e.g.* malondialdehyde and glyoxal).[165] RCS are the chemical precursors of AGEs that form on protein-bound lysine and arginine residues. Importantly, skin levels of AGEs are elevated under conditions of increased carbonyl stress such as actinic and chronological aging,[163,164] cutaneous malignant melanoma,[166] and diabetes.[163] AGEs comprise a heterogeneous group of molecular structures including poorly defined macromolecular yellow-brown pigments called melanoidins,[167] structurally defined non-fluorescent and fluorescent chromophores (*e.g.* pentosidine **11** (Fig. 3), vesperlysine A **12** (Fig. 3) and argpyrimidine) and simple alkylation adducts such as N^{ε}-carboxymethyl-L-lysine (CML).[168]

The phototoxic activity of AGEs extracted from skin collagen and ocular lens crystallin is well documented,[169,170] and recent experimental evidence suggests that protein-bound AGEs act as UV-sensitizers of photooxidative stress in sun exposed human tissues.[7] Generation of reactive oxygen species (ROS), superoxide anion and H_2O_2 in particular, during UVA-irradiation of AGE-proteins and isolated AGEs has been demonstrated.[170–172] AGEs are strong UVA photosensitizers of tryptophan and ascorbic acid degradation. For the established photosensitizer-AGEs, pentosidine and vesperlysine A (also called LM-1), UVA-driven formation of $^1O_2$ has been demonstrated using the NaN_3-inhibitable *N*,*N*-dimethyl-4-nitrosoaniline (RNO) bleaching assay.[169] Consequently, AGE-sensitization has been implicated in photodamage of glycated lens protein and chronologically aged human skin. Photosensitization of skin cell photooxidative stress by UVA-irradiation of AGE-modified proteins has been demonstrated in cultured human skin fibroblasts and keratinocytes[7,172] using glycated bovine serum albumin and skin collagen. H_2O_2 has been identified as the primary mediator of UVA-photosensitization of skin cells and complete cell protection was achieved when AGE-photosensitization was performed in the presence of the antioxidants catalase or D-penicillamine. Due to the extensive accumulation of skin AGEs during chronological and actinic aging, involvement of AGE-photosensitization in skin photooxidative stress may contribute considerably to UVA-induced photoaging and carcinogenesis. Of particular importance, AGE-induced photooxidative stress may be part of a UVA driven vicious cycle of sensitized skin photodamage, in which ROS, formed from AGE-photosensitization, induce membrane lipid peroxidation with formation of more RCS-type lipid breakdown products. RCS then induce the formation of more AGE-sensitizer epitopes on skin proteins now available for further photodynamic action upon light activation as detailed in section 4 and illustrated in Fig. 5. This mechanism proposes a novel synergism between skin chronological and actinic aging, in which chronological skin aging by glycation leads to the accumulation of UVA-photosensitizers that drive photoaging.

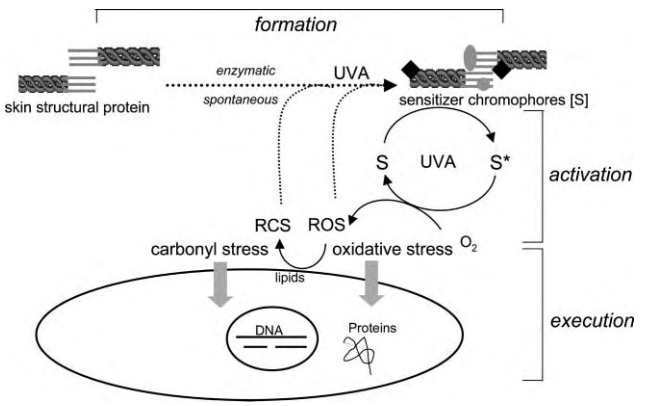

Fig. 5 A three-step model of skin photodamage by endogenous photosensitizers. Photosensitization by three subsequent reaction steps that form a vicious cycle leading to skin photodamage: (step I) photosensitizer (S) formation by enzymatic and spontaneous reaction pathways leading to the accumulation of photosensitizer chromophores (gray and black ellipsoids and diamonds), particularly on long-lived proteins of the extracellular matrix; (step II) photosensitizer activation by absorption of solar photons with formation of photoexcited states (S*) and reactive molecular species, such as ROS; (step III) execution of photosensitization by photooxidative chemical modification of target molecules, such as proteins, lipids, and DNA inducing structural and functional changes characteristic of skin photodamage. Among the reaction products formed as a consequence of chemical reactions in step III are photosensitizer chromophores, which can undergo light-driven activation thereby closing the vicious cycle of skin photodamage by endogenous photosensitizers.

The potential role of AGEs as important sensitizers of skin photooxidative stress is substantiated by current research that examines the structure activity relationship of novel phototoxic

AGE-epitopes in human tissue. We have recently identified 3-HP **13** (Fig. 4) as the minimum phototoxic chromophore contained in many skin biomolecules.[130] Fig. 4 presents important members of the 3-HP class of endogenous photosensitizers comprising B_6 vitamers **18–21** (Fig. 4), enzymatic collagen crosslinks of the pyridinoline type **15** (Fig. 4), AGEs **16**—from human tissue (Fig. 4) and amino acid adducts **17** of RCS derived from lipid peroxidation products (Fig. 4). UVA photosensitization of human skin cells by 3-HP-derivatives occurs in the lower micromolar range with potent induction of apoptosis, intracellular oxidative stress, and p38 MAPkinase activation. *N*-Alkyl-3HP cationic derivatives are formed during glycation and lipid peroxidation under physiological conditions, such as glyceraldehyde-derived pyridinium compound (GLAP)[173] and 3-hydroxy-4-hydroxymethyl-1-(5-amino-5-carboxypentyl) pyridinium cation **16** [glycol–aldehyde–pyridine, (Fig. 4)] isolated from glycated human atherosclerotic lesions.[174] Moreover, lys-hydroxytriosidines, another protein crosslink of the *N*-alkyl-3-HP-type was isolated from human cornea collagen exposed to the artificial tanning agent and RCS dihydroxyacetone[175] and is thus expected to form in appreciable amounts in human skin exposed to this compound. Chemical tanning of human skin therefore may place a suspected AGE-type photosensitizer in direct proximity to sensitive targets and adds to the increasing health concerns associated with the cosmetic use of dihydroxyacetone preparations[176]

In addition to extracellular glycation events, intracellular accumulation of AGEs has been described as a result of cellular oxidative and carbonyl stress, mediated by RCS such as methylglyoxal originating from the glycolytic sugar phosphate glyceraldehyde-3-phosphate. Recently, nuclear glycation events targeting chromatin components including histones and high mobility group proteins, have been described by several groups as reviewed in ref. 170. Specifically, accumulation of histone-bound carbonyl groups as a consequence of nuclear carbonyl stress has been suggested and modification of nuclear proteins in various mammalian tissues and cells by fluorescent and non-fluorescent AGEs has been demonstrated. The intranuclear accumulation of protein-bound AGEs places a potential photosensitizer in close proximity to DNA. Thus, intranuclear photosensitization by AGEs, if operative in skin, may have severe consequences for genomic integrity. Using a standard plasmid DNA cleavage assay for the assessment of photosensitized DNA damage, we have recently demonstrated that AGE-proteins are photosensitizers of DNA damage by oxygen-dependent and independent pathways.[170] A multistep scenario of AGE-sensitized genotoxicity in skin cells could be envisioned to proceed as follows: First, accumulation of AGEs in skin cell nuclei occurs as a result of chronic glycoxidative stress mediated by reactive carbonyl species. Solar irradiation, especially the deeply penetrating UVA portion, induces photoexcitation of chromatin-bound AGEs in close proximity to DNA, and AGE-sensitized DNA damage occurs by direct redox interaction between excited AGEs and DNA, by intermediate formation of ROS, or by direct energy transfer mechanisms potentially leading to triplet state derived base photodimers. Thus, it is tempting to speculate that photoexcited triplet states of nuclear AGEs may be the unidentified causative agents that mediate the UVA-driven DNA base photodimerization recently demonstrated in mammalian cells.[72] However, since only AGE-photosensitization of DNA cleavage has been demonstrated in our earlier study, future experiments using skin cells with elevated nuclear protein glycation damage should test the proposed involvement of AGE-photosensitization in DNA base photooxidation and photodimerization.

It should be noted that various biogenic RCS have interesting electronic excited state properties derived from carbonyl singlet and triplet states. Thus, in addition to the emerging role of AGEs in skin photosensitization, endogenous RCS of the α-dicarbonyl type such as methylglyoxal, glyoxal and 1- and 3-deoxyosones could qualify as another class of emerging triplet state sensitizers involved in skin photodamage. Importantly, earlier work has demonstrated that the α-dicarbonyl compound 2,3-butanedione is a powerful photosensitizer of protein damage.[177] The n,π*-excited triplet state of 2,3-butanedione (Nd–YAG laser excitation at 355 nm) has been involved in $^1O_2$ formation as evidenced by infrared phosphorescence at 1270 nm.[178] Early work has demonstrated that dihydroxyacetone and many other RCS are potent triplet sensitizers of DNA pyrimidine photodimerization.[179] Using acetone as a triplet sensitizer carbonyl compound (308 nm excitation) with high quantum yield of intersystem crossing and high triplet state energy content, efficient triplet state energy transfer onto all DNA base mononucleotides with lower triplet state energies was demonstrated ('downhill' quenching reaction), but quenching of triplet state ketone sensitizers with lower triplet state energy such as benzophenone occurred by electron and proton transfer from purines ('uphill' quenching reaction) leading to ketyl radical formation.[180] In plasmid DNA, acetone photoexcitation strongly induced triplet state sensitized cyclobutylpyrimidine dimer formation as observed earlier with α-dicarbonyl compounds.

Lipofuscins are another class of endogenous polymeric fluorescent pigments that show considerable structural and functional overlap with AGEs, since both originate in part from spontaneous amino carbonyl reactions between proteins and lipid peroxidation derived RCS.[181,182] Lipofuscin accumulates primarily in lysosomes of postmitotic cells such as cardiac myocytes and neuronal cells as a result of cellular oxidative stress and chronological aging. Lysosomal lipofuscin granules consist of undegradable oxidatively modified proteins and lipids with less than 2% metal ions, including Fe, Cu, Al, Zn, Ca and Mn. and display a characteristic broad spectrum autofluorescence upon excitation at blue (450–490 nm) and green (510–560 nm) wavelengths. Because of the difficulties inherent in the analysis of lipofuscin fluorophores, their nature and composition have not been fully defined, but ene-aminal-Schiff bases, 1,4-dihydropyridines and 2-hydroxy-1,2-dihydropyrrol-3-ones have been implicated as chromophores causing the autofluorescent properties of natural lipofuscin as reviewed in ref. 181. A particular lipofuscin fluorophore, the pyridinium bis-retinoid A2E, has been identified in retinal pigment epithelial cells and implicated in blue light photosensitization of ROS formation in these cells.[183] In contrast to an emerging role of lipofuscin as an ocular photosensitizer in retinal photodamage and age-related macular degeneration, occurrence and photosensitizer activity of lipofuscins in human skin have not been described. However, since lipofuscin accumulates in lysosomes of presenescent cultured skin fibroblasts under oxidative stress conditions,[184] it is tempting to speculate that senescent fibroblasts in photoaged skin may accumulate lipofuscin as a potential photosensitizer contributing to photooxidative stress.

3.12 Chromophores associated with skin extracellular matrix proteins

The most abundant and therefore likely primary targets of photons in human skin are chromophores associated with skin structural proteins such as keratin, collagen and elastin. The potential role of keratin photochemistry in skin photosensitization has already been discussed (section 3.10). In human dermis, collagen is estimated to contribute approximately 70% of dry weight. Upon UVA and blue visible photoexcitation, human skin emits a strong tissue autofluorescence indicative of excited singlet state emission, attributed to the presence of specific protein-bound fluorophores.[185,186] The remarkable autofluorescence of skin structural proteins upon UVA/visible excitation is thought to originate from three distinct classes of skin fluorophores: (a) AGEs, (b) photooxidation products of protein-bound amino acids, and (c) enzymatically formed amino acid crosslinks. The role of glycation as a key mechanism of fluorophore formation during chronological and actinic aging of skin, and the potential role of AGEs as endogenous photosensitizers has already been discussed above. Photooxidation products of protein-bound amino acids may be formed upon prolonged UV exposure of skin structural proteins. Photooxidative[187] and glycoxidative[188] formation of ortho-dityrosine and 3,4-dihydroxy-L-phenylalanine (DOPA) from tyrosine residues has been described for skin collagen. Tryptophan and tryptophan-oxidation products are not relevant to photosensitization reactions involving collagen and elastin photosensitization, since this amino acid is absent from these proteins in human skin.

Enzymatically formed amino acid crosslinks are post-translational modifications introduced during collagen and elastin maturation by enzymes such as lysyl oxidase and lysyl hydroxylase. Our recent work on skin UVA photodamage suggests a causative role of these abundant dermal fluorophores as potent endogenous sensitizers of skin photooxidative stress.[7,8,170] Our earlier studies on the photodynamic properties of AGE-modified collagen revealed the unexpected result that even collagen with very low or negligible AGE-modification displayed photodynamic activity with significant sensitization of UVA-driven formation of superoxide and H_2O_2. A detailed examination of extracellular matrix (ECM)-proteins from various sources and human skin revealed their remarkable photodynamic properties. Collagen and elastin are active as sensitizers of solar simulated light-driven generation of H_2O_2, and oxidative protein damage, as obvious from protein carbonylation, occurred concomitant with light-driven ROS formation, suggesting a light driven electron transfer process in which collagen acts as a donor and molecular oxygen as an electron acceptor. Similar results were obtained with elastin samples. Importantly, collagen phototoxicity induced skin cell intracellular oxidative stress, and DNA damage is fully reversed by catalase treatment during UV exposure.[8] In all cases, H_2O_2 was identified as the stable, diffusible cytotoxic mediator of collagen phototoxicity. Importantly and consistent with the sensitized formation of a stable diffusible cytotoxic factor, collagen pre-irradiation was sufficient to exert similar phototoxic effects on unirradiated cells suggesting a mode of cellular phototoxicity that does not require the direct light exposure of skin cells. The demonstration of photodynamic activity of non-glycated collagen has led to the conclusion that non-AGE UVA/UVB chromophores in collagen must be responsible for the observed phototoxic effects. Enzymatically formed fluorophores occur in high abundance in skin structural proteins, but only a few structures have been identified such as the collagen crosslinks pyridinoline **15** (PYD, Fig. 4) and deoxypyridinoline or cyclopentenosine recently isolated from elastin. As already detailed above phototoxicity studies on the isolated 3-hydroxypyridinium derivative PYD and its fluorescent structural synthetic analogue *N*-ethyl-3HP salt **14** (Fig. 4) demonstrated a strong photodynamic activity of this member of the 3-HP class of endogenous photosensitizers.[130] UVA-photosensitization by PYD induced a dose dependent formation of H_2O_2 with inhibition of skin cell proliferation that was completely suppressed by antioxidant intervention. PYD therefore qualifies as an endogenous candidate photosensitizer contained in skin dermal protein. Photolysis of PYD during extended exposure to solar simulated UV radiation was observed suggesting the sensitizer activity of unkown photolysis products. Skin collagen pyridinoline content is generally low (16 mmol/mol of collagen) but increases dramatically during conditions of wound healing, scar formation, and sclerotic disorganization as referenced in ref. 8. It is tempting to speculate that a significant increase in pyridinoline and deoxypyridinoline content in skin collagen characteristic of scar tissue and sclerotic skin diseases introduces an endogenous UVA sensitizer that may contribute to the known predisposition of scar tissue to photocarcinogenesis,[130] a hypothesis to be explored in the future.

4 Skin photodamage by endogenous photosensitizers: a three-step model

From the foregoing discussion of potential skin photosensitizer candidates, an understanding of the mechanistic involvement of photosensitization in skin photoaging and carcinogenesis is complicated by the diversity of sensitizer structures, pathways of formation, mechanisms of action, and relevant skin targets. However, a unifying model of skin photosensitization by endogenous sensitizers involves three successive reaction phases that are part of a vicious cycle leading to skin photodamage as shown in Fig. 5:

I Photosensitizer formation

Photosensitizers occur in human skin as constitutive structural and functional cellular components, such as mitochondrial respiratory coenzymes (*e.g.* flavins and porphyrins) and pigments (melanins and precursors). Other potential photosensitizers are introduced as a result of enzymatic posttranslational modification of skin structural proteins (*e.g.* the collagen crosslink PYD), but photosensitizers may also accumulate as a result of photooxidative and carbonyl stress by spontaneous chemical modification of intra- and extracellular precursor molecules (*e.g.* AGE formation on protein-lysine residues, oxidative formation of protein-bound NFK from tryptophan, and photooxidation of folate to 6-formylpterin).

II Photosensitizer activation

Photoexcited states of sensitizer chromophores are then generated by absorption of solar photons (mostly in the UVA and blue visible regions), and photochemical reaction cascades available to the particular photosensitizer are initiated as determined by the

conditions of local pH, solute concentrations, water activity, oxygen partial pressure, and surrounding target molecules. Photoactivation of endogenous sensitizers potentially occurs throughout human skin, ranges from nuclear to extracellular compartments, and will depend on localization of a particular sensitizer and tissue transmission of the corresponding excitation wavelengths.

III Execution of photosensitization

After sensitizer formation and activation, repetitive cycles of light-driven formation of reactive intermediates such as ROS and organic free radicals induce skin photodamage by chemical modification of target molecules, such as proteins, lipids and DNA. Moreover, toxic photoproducts formed by photooxidation of target molecules or sensitizer can accumulate and stress-induced cellular responses may be initiated by oxidative modification of redox sensitive components of signaling cascades and transcription factors. Secondary reaction cascades are initiated after ROS attack on sensitive targets such as membrane lipids[61,62] leading to RCS formation and enhanced mitochondrial leakage of metabolically generated ROS. Dependent on the proximity of photosensitizer to nuclear DNA, introduction of DNA photolesions occurs by direct energy/electron transfer reactions or are mediated by ROS that can act over short ($^1O_2$) or long distances (H_2O_2).

Importantly, the three phases of skin photosensitization may form part of a vicious cycle. As indicated in Fig. 5, ROS and RCS can initiate the chemical modification of cellular components with formation of novel photosensitizer chromophores such as AGEs, thereby closing the light-driven vicious cycle of skin photodamage by endogenous photosensitizers.[7]

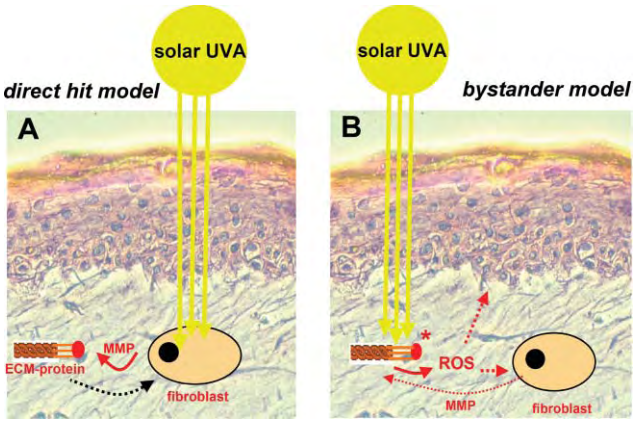

Fig. 6 Direct hit *vs.* bystander model of skin cell photodamage. (A) Current model, in which cellular components are the primary targets of solar photons (direct hit model) inducing signaling and gene expression with changes in the surrounding dermal matrix as downstream effects after MMP induction and secretion. (B) Alternative model of skin cell phototoxicity, in which skin cell damage occurs after initial photoexcitation of dermal (and possibly epidermal) structural proteins (bystander effect model). Photoexcitation of dermal extracellular matrix protein chromophores by solar light in the presence of oxygen sensitizes the formation of diffusible ROS that enter target skin cells in the dermis and basal epidermis with downstream induction of intracellular oxidative stress. This bystander model of sensitized skin photodamage only requires photoexcitation of sensitizers contained in structural proteins and therefore does not depend on cellular photon absorption. Cross sections of paraffin-embedded, H & E stained full thickness human skin reconstructs with differentiated stratum corneum (SC), epidermis (E) and dermis (D) are shown.

5 Skin photosensitization by dermal extracellular matrix proteins: a bystander model of cellular photodamage

ECM proteins are currently perceived as downstream targets of skin photooxidative stress, but not as crucial mediators of skin photosensitization. The role of skin proteins as chemical targets of photooxidative stress is firmly established. Earlier work has described collagen insolubilization and resistance to proteolytic cleavage as consequences, and recent work has demonstrated that skin proteins are important targets of chemical photooxidative damage *in vitro*[6] with degradation of enzymatic imidazolium collagen crosslinks (histidinohydroxylysinonorleucine) by $^1O_2$ attack and protein carbonylation. Accumulation of oxidatively modified proteins was found specifically within the upper dermis of photoaged skin during human photoaging *in vivo*.[32] Moreover, UV-dependent induction of matrix metalloproteinases (MMPs) leads to the increased enzymatic degradation of dermal collagen, often used as a major hallmark of skin photodamage,[189–191] with possible implications for tumor cell invasion and metastasis. Recent work indicates a more functional role of MMP-generated collagen fragments as important mediators of photosuppression of fibroblast collagen synthesis,[192] and elastin fragments have been implicated in melanoma cell invasion by upregulation of MMP-2 activity. However, in all of these events, direct UV-photon action on skin cells is perceived as the initial trigger for downstream ECM effects ('direct hit model of skin cell photodamage'). This current view is depicted in Fig. 6(A), in which cellular components are the primary targets of solar photons and changes in the surrounding dermal matrix occur as downstream effects after MMP induction and secretion.

Our recent results suggest an alternative mechanism of skin cell phototoxicity, in which skin cell damage occurs after initial photoexcitation of dermal (and possibly epidermal) structural proteins which initiate the functional and causative events in skin photodamage as summarized in Fig. 6(B) ('bystander model of skin cell photodamage'). In this model, photo-excitation of dermal ECM-protein chromophores by solar light (mainly by the deeply penetrating UVA-portion) in the presence of oxygen sensitizes the formation of ROS, with concomitant oxidative damage to the sensitizing protein itself. Subsequently, reactive species of sufficient stability, such as H_2O_2, other peroxides and organic free radicals, enter target skin cells causing subsequent oxidative cell damage presumably by Fenton-type chemistry after transition metal ion-catalyzed peroxide decomposition. The resultant intracellular oxidative stress is expected to severely impair skin cell structure and function, as demonstrated by our results on suppression of skin cell proliferation and accumulation of chromosomal DNA damage. According to this 'bystander' mechanism of skin cell photodamage, UVA chromophores positioned in ECM proteins are responsible for UV absorption, excited states of UVA-photosensitizer are the initial phototoxic agents, ROS are the diffusible toxic mediators, and skin cell membranes, proteins and DNA are the ultimate molecular targets in unirradiated bystander cells.

Radiation-induced bystander effects, the occurrence of radiation damage in non-irradiated neighbors of irradiated cells, are now well established for various forms of ionizing radiation, such as α-particles and X-rays.[193] The bystander cells may be immediately adjacent to or some distance away from the exposed cell. Radiation-induced bystander effects include changes in gene expression, induction of genetic effects such as mutations and chromosomal alterations, DNA damage, cell killing, and malignant transformation.[193] Bystander effects may significantly contribute to radiation-induced carcinogenesis, especially at low doses where only a limited number of cells in a population are directly hit, since the bystander effect tends to amplify low dose effects by communicating damage from irradiated to non-irradiated cells. For example, the targeting of a single cell in nonconfluent human fibroblast cultures with a precise number of α-particles led to substantial chromosomal alterations in neighboring bystander cells not directly hit by radiation.[194] While the existence of the radiation-induced bystander effects has been well established, the underlying mechanisms are still largely unknown. Reactive oxygen species, stable free radicals, and cytokine release from the irradiated cells were suggested to be involved in transferring damage signals from irradiated to unirradiated cells. In striking analogy to ionizing radiation-induced bystander effects, skin UVA photosensitization by ECM proteins could exert photooxidative damage in areas that have not been hit by photons. The bystander model of sensitized skin photodamage only requires photoexcitation of sensitizers contained in structural proteins and therefore does not depend on cellular photon absorption. The proposed bystander mechanism of skin photodamage may be operative in parallel with mechanisms initiated by skin cell photon absorption and recent evidence suggests that skin cell bystander damage can also be initiated by other classes of skin photosensitizers, such as melanin and melanin precursors.[104] Based on the bystander mechanism of skin photodamage it might be hypothesized that ECM-protein phototoxicity enhances the genotoxicity of a given dose of UV-irradiation, if cells are irradiated in the presence of ECM-proteins. A striking example of UVA-dose potentiation by collagen-photosensitization is depicted in Fig. 7, in which cultured human skin fibroblasts, seeded on dishes either in the absence of collagen or with collagen-embedment in a dermal equivalent, were exposed to the same dose of UVA radiation. Cytotoxicity was assessed 24 h later by flow cytometric analysis of

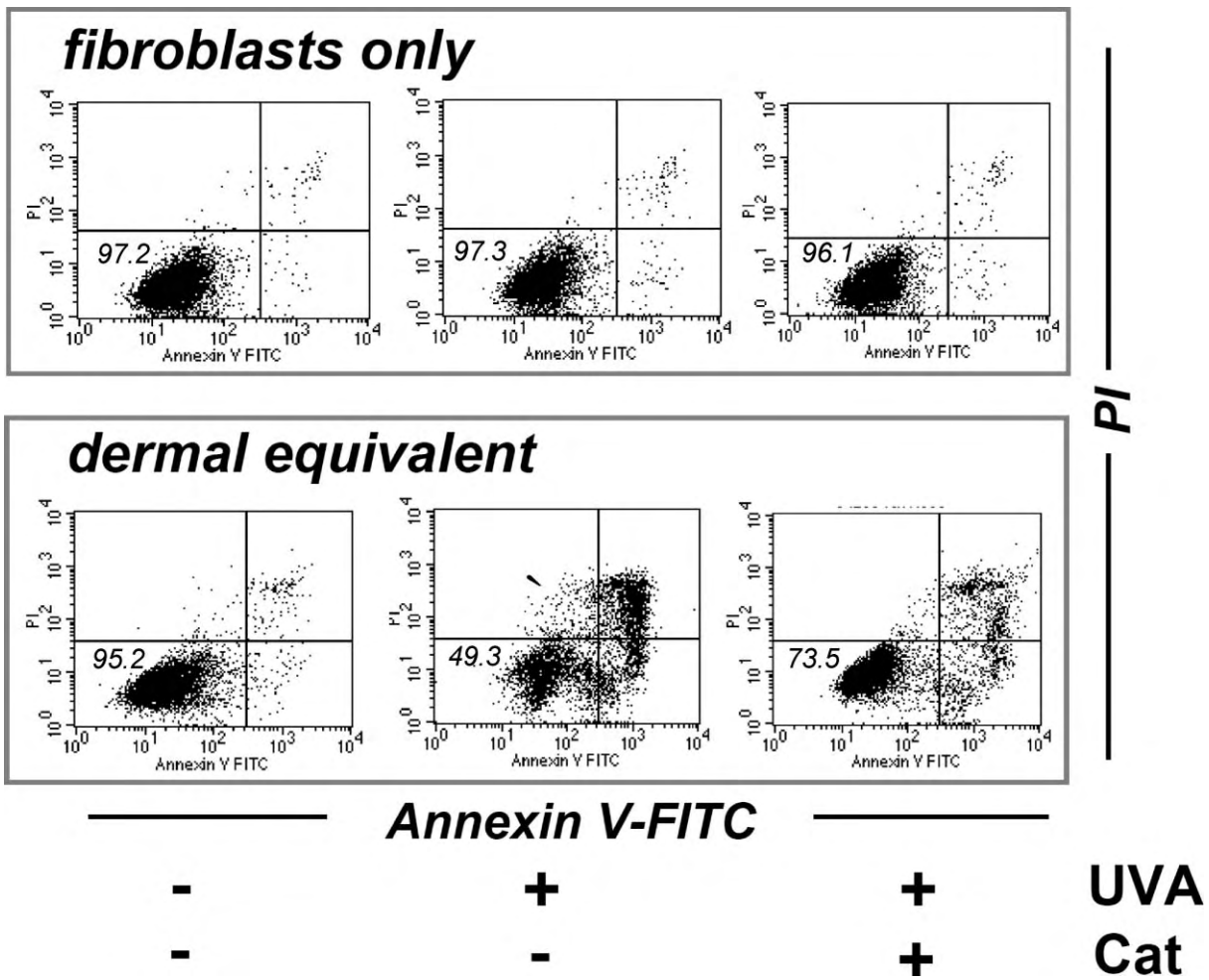

Fig. 7 Example of the bystander mechanism of matrix photosensitization: dermal sensitization of UVA-induced fibroblast apoptosis/necrosis. Human skin fibroblasts were seeded on 35 mm culture dishes as either isolated cells ('fibroblasts only') or embedded in type I collagen ('dermal equivalent').[198] Dermal equivalents were also created with inclusion of catalase (Cat, 400 units per mL acid soluble collagen). Cells were exposed 24 h later to UVA (10 J cm^{-2}). At 24 h after irradiation cells were harvested by collagenase/trypsin treatment and cell viability was assayed by flow cytometry after annexinV-FITC/propidium iodide staining. Numbers indicated express viable cells (lower left quadrant) as a percentage of total cells.

annexinV-FITC/propidium iodide stained cells. Remarkably, fibroblasts irradiated in the absence of collagen were completely resistant to the cytotoxic effects of UVA, whereas fibroblasts irradiated in the collagen matrix were driven into apoptosis and necrosis. Catalase inclusion during dermal reconstruction strongly reduced fibroblast UVA-photodamage consistent with collagen-sensitized formation of H_2O_2 as key mediator of the observed phototoxic effects. Therefore, photobiological studies performed on isolated cells in culture in the absence of a three-dimensional ECM-matrix and the photosensitizers contained therein may significantly underestimate UVA phototoxicity, and biologically relevant UV action spectra may be biased towards UVB contributions.[2] Moreover, this may be a relevant mechanism for the induction of genotoxic effects in cells situated in deeper layers of skin receiving little UV-exposure due to prior absorption by the epidermis and upper dermis. Thus, the bystander mechanism of ECM-protein-sensitized skin cell photodamage could be involved in the pronounced accumulation of oxidative DNA base modifications (8-oxo-dG) that are confined to the basal cell layers (positioned adjacent to basement membrane and dermal collagen) of AK and SCC as reported recently.[5,75] Dermal photooxidative stress therefore may contribute to mutational carcinogenesis of the lower layers of the epidermis, which contain the stem cells and transient amplifying cells from which tumors are thought to arise. It may also be speculated that dermal photosensitization provides a source of ROS that creates a stromal environment conducive to dermal tumor invasion by cancer cells originating from the epidermis.[52] In summary, the bystander mechanism of sensitized photooxidative stress may contribute significantly to skin photoaging and carcinogenesis, a hypothesis to be rigorously tested by future experiments using model systems that allow qualitative and quantitative control over dermal photosensitizers and cellularity such as full thickness human skin tissue reconstructs.

6 Photoexcited states of skin chromophores as molecular targets for photoprotection

Based on the causative involvement of photochemically reactive excited states in skin photodamage, it is intuitive that molecular antagonism of photoexcited states offers a potential therapeutic opportunity for skin photoprotection. Importantly, photoexcited states in skin occur downstream of photon absorption but upstream of ROS formation. Endogenous sensitizers and their photoexcited states are therefore promising targets for molecular chemoprevention of skin photodamage. Three routes of early molecular intervention in the process of skin photooxidative stress by targeting photosensitization can be envisioned as described in sections 6.1, 6.2 and 6.3.

6.1 Inhibition of skin photosensitization by sunscreens

Protection of human skin DNA against direct UVB photodamage by topical sunscreens is firmly established. However, UVA photoprotection is chemically more difficult to achieve and few photostable chromophores for complete broadband UVA filtering are available.[11,195] In addition, skin photosensitization can be induced by visible solar photons, which are not filtered by UVA/B sunscreens. Photoisomerization and photodegradation due to uncontrolled excited state chemistry has been described for many UVA filters[196,197] and some UVA/B sunscreens and inorganic sunblockers act as potent triplet state photosensitizers with light-driven formation of ROS and genotoxic consequences to human skin cells as reviewed in ref. 198. Thus, significant progress is still needed to develop adequate protection against skin photosensitization in the UVA and visible region of sunlight.

6.2 Inhibition of skin photosensitization by antioxidants

Antioxidant intervention does not directly interfere with photosensitization but suppresses photooxidative stress in skin downstream of the formation of highly reactive ROS. Moderate skin photoprotection by topical application of antioxidants has been demonstrated in many experiments on animal and human skin, and inhibition of mouse skin photocarcinogenesis by these agents has been observed as reviewed in ref. 198. The therapeutic effectiveness of topical antioxidants is limited by their sacrificial depletion and redox chemistry.[199] Also of concern are recent reports of antioxidant enhanced carcinogenesis observed in transgenic mice with upregulated antioxidant response.[200] Due to these limitations, antioxidant intervention can only play an adjuvant role in skin photoprotection against photosensitization.

6.3 Inhibition of skin photosensitization by quenchers of photoexcited states

Compounds capable of inactivating photoexcited states by direct chemical and/or physical interaction are called quenchers of photoexcited states (QPES). For the sake of simplicity, we define chemical quenching as the sacrificial reaction of a target molecule (chemical quencher) with the excited state leading to formation of chemical products. Many antioxidants are capable of sacrificial quenching of $^1O_2$ at high reaction rates followed by enzymatic regeneration of the oxidized chemical quencher, a situation that would be part of a photoprotective antioxidant network in skin. On the other hand, sacrificial quenching can also occur with skin constituents, which are irreversibly depleted by the reaction, *e.g.* histidine residues or thiol compounds. The reaction products can be toxic and induce harmful secondary reactions, such as thiyl radicals, endoperoxides and peroxides formed upon reaction of $^1O_2$ with thiol groups, imidazole groups and unsaturated fatty acids, respectively. In contrast, physical quenching of photoexcited states occurs by a variety of dissipative mechanisms that lead to the complete regeneration of the reaction partners in the electronic ground state, now available for another round of excited state deactivation directed to triplet states or $^1O_2$ as depicted in Fig. 8. A prime example of biologically relevant photoprotection by physical quenching of excited states is provided by the role of carotenoids in photosynthetic organisms. Chlorophyll is a potent photosensitizer and singlet state photoexcited chlorophyll easily undergoes intersystem crossing into the triplet state with subsequent formation of $^1O_2$. Carotenoids are powerful physical quenchers of the chlorophyll triplet state and $^1O_2$.[201] Moreover, recent research demonstrates that under conditions of light harvesting in excess light, feedback deexcitation of chlorophyll singlet excited states occurs by reversible charge separation with transient carotenoid cation formation during the xanthophyll cycle of thermal energy dissipation.[202] Physical quenchers of photoexcited states that can undergo repetitive cycles of excited state

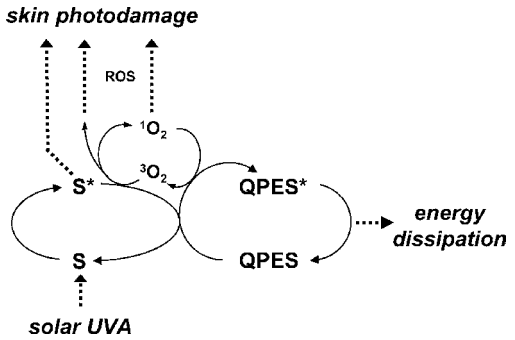

Fig. 8 Physical quenchers of photoexcited states: effective dissipators of excitation energy. Solar excitation of endogenous skin sensitizers (S) leads to the formation of excited states (S*) with possible formation of $^1O_2$. Interaction between physical quenchers of photoexcited states (QPES) and S* in skin enables harmless return to the electronic ground state. The scheme indicates energy dissipation by energy transfer mechanisms with intermediate formation of electronically excited quencher (QPES*), but many other routes of quenching may be operative. Importantly, the QPES compound returns to the ground state without chemical depletion and can thus catalyze repetitive cycles of S* detoxification.

quenching without chemical depletion or the need for metabolic regeneration represent a very attractive class of quenchers for skin photoprotection from photosensitization.[198]

6.4 Physical QPES: mechanisms of action

A detailed discussion of the multiple physical mechanisms that are involved in the inactivation of photoexcited states by quencher substances is beyond the scope of this article. However, the principal pathways of excited state quenching by physical mechanisms will be briefly reviewed focusing on $^1O_2$ quenching given the importance of this photoexcited species as an established mediator of skin photodamage,[51] the abundance of studies that have examined the photochemical aspects of $^1O_2$ quenching,[203,204] and the general importance of these mechanisms for the molecular design of QPES compounds as chemopreventive agents for skin photoprotection.

6.5 Physical singlet oxygen quenchers

Paradoxically, ground state molecular oxygen [$O_2(^3\Sigma_g^-)$] is a potent quencher of triplet photoexcited states. Triplet state quenching by molecular oxygen induces the formation of $^1O_2$ [$O_2(^1\Delta_g)$, the lowest excited singlet state, which is of utmost photobiological relevance], a relatively long-lived, highly reactive species that chemically attacks other singlet state molecules overcoming the spin restrictions associated with the triplet state of ground state molecular oxygen. Therefore triplet state quenching by molecular oxygen with $^1O_2$ formation provides an example for the unsuccessful detoxification of a photoexcited state. Physical deactivation of $^1O_2$ can occur by three major pathways.[203,204]

6.5.1 Electronic-vibrational deactivation. Collisional deactivation proceeds *via* conversion of the electronic excitation energy of $O_2(^1\Delta_g)$ into vibrational energy of terminal bonds of a deactivating collision partner. This spin-forbidden and therefore relatively slow deactivation (3.1 μs lifetime in H_2O) occurs with rate constants that increase exponentially with the energy of the stretching vibration of the deactivating bond (C–F < C–D < O–D < C–H < O–H) leading to a pronounced solvent dependence of the $^1O_2(\Delta g)$ lifetime. Therefore, enhancement of a sensitized photobiological effect in D_2O over H_2O is commonly regarded as an indicator of $^1O_2$ involvement.

6.5.2 Charge transfer (CT) quenching. The CT-induced deactivation is observed with quenchers of high triplet energies and low oxidation potentials. A good example is provided by physical quenching of $^1O_2$ by organic amines, *e.g.* polyamines, such as spermine,[205] leading to the formation of ground state $^3O_2$ and unaltered ground state amine.[198] According to the accepted mechanism the radiationless deactivation of the initially formed singlet encounter complex is enhanced by the formation of a singlet exciplex, which is stabilized by the transfer of electric charge from the quencher to the oxygen molecule. This species decays mainly by intersystem crossing to a triplet CT-ground state complex, which finally dissociates to the ground state amine and ground state $^3O_2$ without charge separation in most cases. However, a chemical reaction may also occur from singlet exciplexes, particularly in the case of sterically hindered amines (such as TEMP) leading to the formation of nitroxide free radical reaction products (such as TEMPO) as a result of irreversible CT reactions between quencher and $^1O_2$.[206] The discrimination between these different pathways of physical and chemical quenching will depend on amine ionization potential, hydrogen bond donating activity and substituent effects.[207] Other important CT-quenchers are the widely used $^1O_2$-probes DABCO and NaN_3, metal complexes, and many biomolecules such as hydroxycinnamic acids, vitamin E and ascorbate as reviewed in ref. 204.

6.5.3 Electronic energy transfer (EET) quenching. If the triplet state energy of the quencher is smaller than the excitation energy of $^1O_2(\Delta_g)$ (94 kJ mol^{-1}), deactivation by electronic energy transfer can occur with almost diffusion-controlled rates leading to formation of an excited state of the quencher that quickly returns to the ground state by nonradiative processes. Numerous carotenoids, *e.g.* lycopene[208] and xanthophylls (zeaxanthin and lutein), large polyheterocyclic aromatics such as naphthalocyanines and phthalocyanines,[209] and some azomethine dyes[210] are potent EET-quenchers possessing low triplet energies.

6.6 Physical triplet state quenchers

High quantum yields of triplet state formation are a hallmark of potent photosensitizers and excited triplet states of skin photosensitizers are therefore the ultimate molecular targets for photoprotective intervention by physical QPES compounds. The involvement of triplet states in pyrimidine base photodimerization reactions was studied by earlier researchers demonstrating the possibility of suppressing the quantum yield of photodimerization by irradiation in the presence of conjugated diene triplet state quenchers, (*e.g.* isoprene and 2,4-hexadien-1-ol).[68] Many energy acceptors such as piperylene, 1-methylnapthalene, 1,3-cyclohexadiene, sorbic acid,[211] and the naphthalene derivative naproxen[212] are efficient triplet state quenchers, but singlet state (fluorescence) quenching is also observed with some of these substances. Low energy triplet quenchers such as 3,3,4,4-tetramethyl-1,2-diazetine-1,2-dioxide, devoid of fluorescence (singlet state) quenching activity, have been proposed as general molecular probes to examine the involvement of triplet states in photochemical reactions.[213] A reversible electron transfer reaction between triplet state eosin and

p-phenylendiamine with regeneration of sensitizer ground state and unaltered quencher has been proposed to explain inhibition of eosin-sensitized photooxidation of trypsin.[214] The structure activity relationship of many physical quencher substances is difficult to predict, especially under physiological conditions. Although some general requirements must be fulfilled to allow physical quenching, *e.g.* the triplet energy of the acceptor must be below the energy of the sensitizer triplet state, only very limited information is available that would allow the rational design of efficient triplet state quenchers for photoprotection of human skin.[215]

6.7 Functional screening of QPES compounds for skin photoprotection

The complex photochemistry and heterogeneous, mostly ill-defined structure activity relationships of known QPES compounds complicate the molecular design and identification of agents that would predictably inactivate photoexcited states of endogenous skin photosensitizers under *in vivo* conditions. High quencher activity of a given test compound observed under test tube conditions may not be relevant to the *in vivo* situation due to cellular toxicity profile of the compound, insufficient tissue distribution, characteristics of intracellular localization and pH/solvent effects. Conversely, administration of high concentrations of weak quenchers may overcome moderate rate constants that might disqualify test compounds during screening *in vitro*. Thus, compounds may be photoprotective under test tube conditions but completely ineffective in a tissue situation. QPES compounds designed for skin photoprotection must fulfill the following minimal criteria: (1) inactivation of photoexcited states of relevant skin photosensitizers by physical (nonsacrificial) pathways, (2) appropriate skin delivery and tissue saturation, (3) absence of toxicity, even in high concentrations, and (4) proven photoprotection of cultured human skin cells and tissue equivalents against actinic damage. Based on these four criteria we have designed a simple activity-based screening assay for the rapid identification of physical QPES compounds as experimental chemopreventive agents for skin photoprotection.[198] The screening procedure is based on (a) activity screening of compound libraries for inhibition of AGE-sensitized plasmid DNA cleavage under aerobic and anaerobic conditions, (b) $^1O_2$ quenching without chemical depletion of the test compound, and (c) skin cell protection against $^1O_2$-induced apoptosis. (d) Finally, substances are tested for photoprotection of full thickness human skin reconstructs against actinic damage from solar simulated light. The screen does not reveal the mechanism of excited state deactivation, but it clearly distinguishes between physical and chemical quenchers and identifies compounds that interfere with AGE-photosensitization, deactivate $^1O_2$ and protect cells against photosensitization without undergoing chemical depletion or displaying dark or light toxicity. Using this screening procedure, secondary amines of the proline alkylester type were identified as novel photoprotective QPES compounds.

6.8 Therapeutic and chemopreventive potential of QPES skin photoprotection

Based on the causative involvement of photosensitization reactions in skin photodamage the following chemopreventive applications of topical or systemic QPES compounds as molecular antagonists of skin photoexcited states can be anticipated and deserve further experimental evaluation:

6.8.1 Inhibition of photoaging and photocarcinogenesis. QPES, that interfere with formation of reactive photoexcited states and ROS in sun exposed human skin, could reduce photomutagenesis suppressing both formation of oxidative DNA base damage and potentially photodimers, if triplet excited states and photosensitization are involved in their formation as indicated by recent work.[23,64,72,74] Furthermore, later stages of skin tumor promotion and invasion may be attenuated by QPES-interference, since photooxidative stress is involved in these processes.[24] Similarly, QPES-suppression of photoxidative activation of stress signaling pathways and direct oxidative modification of skin structural targets could interfere with skin photoaging and skin cell senescence.[32] When tested in a hairless mouse animal model of chronic skin photodamage, significant suppression of UVB-photocarcinogenesis and photoaging by topical administration of the triplet state quencher 2,4-hexadien-1-ol has been demonstrated.[216] UV screening was excluded as a mechanism of action. Similarly, cell protection against photodynamic inactivation using a cyanine dye as triplet state quencher has been reported.[215] Cell protection against $^1O_2$-damage is documented for numerous potential QPES compounds such as mycosporine glycine,[217] N-substituted 2,5-dimethylpyrroles,[218] carotenoids,[219] and curcumin,[220] many of which deserve further experimental evaluation as chemopreventive agents for skin photoprotection.[221] Using the prototype QPES compounds L-proline and L-proline methylester, we observed strong photoprotection of cultured human skin cells and reconstructed full thickness human skin.[198]

6.8.2 Inhibition of skin photohypersensitivity. QPES could be used as adjuvant agents in pathological situations associated with overproduction of endogenous photosensitizers, such as porphyria-related skin photohypersensitivity. A photoprotective benefit of QPES compounds may be anticipated based on the partial effectiveness of oral carotenoid administration on porphyrin-sensitized skin photodamage in erythropoietic protoporphyria patients as reviewed in ref. 219. Moreover, QPES-suppression of generalized skin photosensitivity after systemic administration of photosensitizers for PDT or photochemotherapy (PUVA) applications may be possible as suggested by earlier work[222] and our recent demonstration of QPES protection of HaCaT keratinocytes against PUVA-induced cell.[198]

6.8.3 Photostabilization of suncreen agents. Some UV screens have been reported to exert triplet state quencher activity independent of their UV screening activity. In an *in vitro* study of photosensitizing properties of bergamot oil, the cinnamate sunscreen 2-ethylhexyl-4-methoxycinnamate decreased the photoactivity of bergamot oil *via* triplet state quenching processes, which were unrelated to UV-filtering activity.[223] Cinnamate sunscreens (*E* isomers) are efficient quenchers of 5-methoxypsoralen, 8-methoxypsoralen and 5-geranoxypsoralen (bergamottin) triplet states.[224] Quenching most likely occurs by triplet energy transfer with *E* to *Z* photoisomerization. In a recent study, photostabilization of the sunscreen 4-*tert*-butyl-4′-methoxydibenzoylmethane (avobenzone) was achieved by addition of bis-ethylhexyloxyphenolmethoxyphenyltriazine, a novel

sunscreen capable of triplet–triplet energy transfer reactions and optimized for energy dissipative reactions by reversible photoisomerization and intramolecular hydrogen transfer.[225] Therefore QPES may be envisioned as combinatorial agents used together with existing sunscreen formulations to exert photoprotection by antagonizing excited states not only of endogenous skin photosensitizers but also UV filters.

7 Epilogue: targeting novel mechanisms of skin photodamage

Much progress has been made over recent decades leading to a better understanding of the role of endogenous photosensitizers in skin photodamage. However, the molecular identity and mechanism of action of key skin photosensitizers remain elusive, and recent experimental findings have only offered a first glance on what might be called the 'terra incognita' of photoexcited states in human skin photodamage. Rigorous molecular characterization of key chromophores and their chemical action, particularly in intranuclear and extracellular matrix-derived photooxidative stress, will reveal novel molecular mechanisms that reach beyond our current understanding of how skin photodamage occurs. Further progress in validating the dermal bystander mechanism of skin photodamage by matrix protein photosensitization will depend on advanced techniques of skin reconstruction that allow the controlled introduction of particular candidate sensitizer chromophores. These novel insights into the mechanistic importance of skin photoexcited states will translate hopefully into the development of specific molecular antagonists (quenchers of photoexcited states) for topical chemoprevention of skin photoaging and carcinogenesis.

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References

1 R. M. Tyrrell, Ultraviolet radiation and free radical damage to skin, *Biochem. Soc. Symp.*, 1995, **61**, 47–53.
2 E. Kvam and R. M. Tyrrell, Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation, *Carcinogenesis*, 1997, **18**, 2379–2384.
3 K. Scharffetter-Kochanek, M. Wlaschek, P. Brenneisen, M. Schauen, R. Blaudschun and J. Wenk, UV-induced reactive oxygen species in photocarcinogenesis and photoaging, *Biol. Chem.*, 1997, **378**(11), 1247–1257.
4 F. R. de Gruijl, Photocarcinogenesis: UVA *vs* UVB, *Methods Enzymol.*, 2000, **319**, 359–366.
5 N. S. Agar, G. M. Halliday, R. S. Barnetson, H. N. Ananthaswamy, M. Wheller and A. M. Jones, The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis, *PNAS*, 2004, **101**(14), 4954–4959.
6 Carbonare M. Dalle and M. A. Pathak, Skin photosensitizing agents and the role of reactive oxygen species in photoaging, *J. Photochem. Photobiol. B.*, 1992, **14**(1–2), 105–124.
7 G. T. Wondrak, M. J. Roberts, M. K. Jacobson and E. L. Jacobson, Photosensitized growth inhibition of cultured human skin cells: mechanism and suppression of oxidative stress from solar irradiation of glycated proteins, *J. Invest. Dermatol.*, 2002, **119**(2), 489–498.
8 G. T. Wondrak, M. J. Roberts, D. Cervantes-Laurean, M. K. Jacobson and E. L. Jacobson, Proteins of the Extracellular Matrix Are Sensitizers of Photo-oxidative Stress in Human Skin Cells, *J. Invest. Dermatol.*, 2003, **121**(3), 578–586.
9 K. H. Kaidbey, P. P. Agin, R. M. Sayre and A. M. Kligman, Photoprotection by melanin–a comparison of black and Caucasian skin, *J. Am. Acad. Dermatol.*, 1979, **1**(3), 249–260.
10 W. A. Bruls, H. van Weelden and J. C. van der Leun, Transmission of UV-radiation through human epidermal layers as a factor influencing the minimal erythema dose, *Photochem. Photobiol.*, 1984, **39**(1), 63–67.
11 F. P. Gasparro, Sunscreens, skin photobiology, and skin cancer: the need for UVA protection and evaluation of efficacy, *Environ. Health. Perspect.*, 2000, **108**(Suppl 1), 71–78.
12 H. J. Sterenborg and J. C. van der Leun, Tumorigenesis by a long wavelength UV-A source, *Photochem. Photobiol.*, 1990, **51**(3), 325–330.
13 G. Kelfkens, F. R. de Gruijl and J. C. van der Leun, Tumorigenesis by short-wave ultraviolet A: papillomas *versus* squamous cell carcinomas, *Carcinogenesis*, 1991, **12**(8), 1377–1382.
14 A. de Laat, J. C. van der Leun and F. R. de Gruijl, Carcinogenesis induced by UVA (365-nm) radiation: the dose-time dependence of tumor formation in hairless mice, *Carcinogenesis*, 1997, **18**(5), 1013–1020.
15 G. Kelfkens, F. R. de Gruijl and J. C. van der Leun, Ozone depletion and increase in annual carcinogenic ultraviolet dose, *Photochem. Photobiol.*, 1990, **52**(4), 819–823.
16 R. B. Setlow, E. Grist, K. Thompson and A. D. Woodhead, Wavelengths effective in induction of malignant melanoma, *Proc. Natl. Acad. Sci. USA*, 1993, **90**(14), 6666–6670.
17 S. Q. Wang, R. Setlow, M. Berwick, D. Polsky, A. A. Marghoob, A. W. Kopf and R. S. Bart, Ultraviolet A and melanoma: a review, *J. Am. Acad. Dermatol.*, 2001, **44**(5), 837–846.
18 J. Westerdahl, H. Olsson, A. Masback, C. Ingvar, N. Jonsson, L. Brandt, P. E. Jonsson and T. Moller, Use of sunbeds or sunlamps and malignant melanoma in southern Sweden, *Am. J. Epidemiol.*, 1994, **140**(8), 691–699.
19 R. S. Stern, The risk of melanoma in association with long-term exposure to PUVA, *J. Am. Acad. Dermatol.*, 2001, **44**(5), 755–761.
20 L. H. Kligman, F. J. Akin and A. M. Kligman, The contributions of UVA and UVB to connective tissue damage in hairless mice, *J. Invest. Dermatol.*, 1985, **84**(4), 272–276.
21 R. Lavker and K. Kaidbey, The spectral dependence for UVA-induced cumulative damage in human skin, *J. Invest. Dermatol.*, 1997, **108**(1), 17–21.
22 M. Wlaschek, I. Tantcheva-Poor, L. Naderi, W. Ma, L. A. Schneider, Z. Razi-Wolf, J. Schuller and K. Scharffetter-Kochanek, Solar UV. irradiation and dermal photoaging, *J. Photochem. Photobiol. B.*, 2001, **63**(1–3), 41–51.
23 C. Nishigori, Y. Hattori and S. Toyokuni, Role of reactive oxygen species in skin carcinogenesis, *Antioxid. Redox Signal.*, 2004, **6**(3), 561–570.
24 C. S. Sander, F. Hamm, P. Elsner and J. J. Thiele, Oxidative stress in malignant melanoma and non-melanoma skin cancer, *Br. J. Dermatol.*, 2003, **148**(5), 913–922.
25 J. Nishi, R. Ogura, M. Sugiyama, T. Hidaka and M. Kohno, Involvement of active oxygen in lipid peroxide radical reaction of epidermal homogenate following ultraviolet light exposure, *J. Invest. Dermatol.*, 1991, **97**(1), 115–119.
26 D. Peus, R. A. Vasa, A. Meves, M. Pott, A. Beyerle, K. Squillace and M. R. Pittelkow, H2O2 is an important mediator of UVB-induced EGF-receptor phosphorylation in cultured keratinocytes, *J. Invest. Dermatol.*, 1998, **110**(6), 966–971.
27 H. Yasui and H. Sakurai, Chemiluminescent detection and imaging of reactive oxygen species in live mouse skin exposed to UVA, *Biochem. Biophys. Res. Commun.*, 2000, **269**(1), 131–136.
28 S. Kang, J. H. Chung, J. H. Lee, G. J. Fisher, Y. S. Wan, E. A. Duell and J. J. Voorhees, Topical N-Acetyl Cysteine and Genistein Prevent Ultraviolet-Light-Induced Signaling That Leads to Photoaging in Human Skin *in vivo*, *J. Invest. Dermatol.*, 2003, **120**(5), 835–841.
29 C. Routaboul, A. Denis and A. Vinche, Immediate pigment darkening: description, kinetic and biological function, *Eur. J. Dermatol.*, 1999, **9**(2), 95–99.

30 K. Maeda and M. Hatao, Involvement of photooxidation of melanogenic precursors in prolonged pigmentation induced by ultraviolet A, *J. Invest. Dermatol.*, 2004, **122**(2), 503–509.
31 J. J. Thiele, C. Schroeter, S. N. Hsieh, M. Podda and L. Packer, The antioxidant network of the stratum corneum, *Curr. Probl. Dermatol.*, 2001, **29**, 26–42.
32 C. S. Sander, H. Chang, S. Salzmann, C. S. Muller, S. Ekanayake-Mudiyanselage, P. Elsner and J. J. Thiele, Photoaging is associated with protein oxidation in human skin *in vivo*, *J. Invest. Dermatol.*, 2002, **118**(4), 618–625.
33 L. A. Applegate, C. Scaletta, R. Panizzon and E. Frenk, Evidence that ferritin is UV inducible in human skin: part of a putative defense mechanism, *J. Invest. Dermatol.*, 1998, **111**(1), 159–163.
34 C. Meewes, P. Brenneisen, J. Wenk, L. Kuhr, W. Ma, J. Alikoski, A. Poswig, T. Krieg and K. Scharffetter-Kochanek, Adaptive antioxidant response protects dermal fibroblasts from UVA-induced phototoxicity, *Free Radical Biol. Med.*, 2001, **30**(3), 238–247.
35 M. T. Leccia, M. Yaar, N. Allen, M. Gleason and B. A. Gilchrest, Solar simulated irradiation modulates gene expression and activity of antioxidant enzymes in cultured human dermal fibroblasts, *Exp. Dermatol.*, 2001, **10**(4), 272–279.
36 R. M. Tyrrell, Solar ultraviolet A radiation: an oxidizing skin carcinogen that activates heme oxygenase-1, *Antioxid. Redox Signal.*, 2004, **6**(5), 835–840.
37 L. Rittie and G. J. Fisher, UV-light-induced signal cascades and skin aging, *Ageing. Res. Rev.*, 2002, **1**(4), 705–720.
38 S. Gross, A. Knebel, T. Tenev, A. Neininger, M. Gaestel, P. Herrlich and F. D. Bohmer, Inactivation of protein-tyrosine phosphatases as mechanism of UV-induced signal transduction, *J. Biol. Chem.*, 1999, **274**(37), 26378–26386.
39 Y. S. Wan, Z. Q. Wang, J. Voorhees and G. Fisher, EGF receptor crosstalks with cytokine receptors leading to the activation of c-Jun kinase in response to UV irradiation in human keratinocytes, *Cell. Signal.*, 2001, **13**(2), 139–144.
40 S. Grether-Beck, G. Bonizzi, H. Schmitt-Brenden, I. Felsner, A. Timmer, H. Sies, J. P. Johnson, J. Piette and J. Krutmann, Non-enzymatic triggering of the ceramide signalling cascade by solar UVA radiation, *EMBO J.*, 2000, **19**(21), 5793–5800.
41 M. Karin, Mitogen-activated protein kinase cascades as regulators of stress responses, *Ann. N. Y. Acad. Sci.*, 1998, **851**, 139–146.
42 L. O. Klotz, C. Pellieux, K. Briviba, C. Pierlot, J. M. Aubry and H. Sies, Mitogen-activated protein kinase (p38-, JNK-, ERK-) activation pattern induced by extracellular and intracellular singlet oxygen and UVA, *Eur. J. Biochem.*, 1999, **260**(3), 917–922.
43 D. Peus and M. R. Pittelkow, Reactive oxygen species as mediators of UVB-induced mitogen-activated protein kinase activation in keratinocytes, *Curr. Probl. Dermatol.*, 2001, **29**, 114–127.
44 M. A. Bachelor, A. L. Silvers and G. T. Bowden, The role of p38 in UVA-induced cyclooxygenase-2 expression in the human keratinocyte cell line, HaCaT, *Oncogene*, 2002, **21**(46), 7092–7099.
45 G. F. Vile, A. Tanew-Ilitschew and R. M. Tyrrell, Activation of NF-kappa B. in human skin fibroblasts by the oxidative stress generated by UVA radiation, *Photochem. Photobiol.*, 1995, **62**(3), 463–468.
46 S. Grether-Beck, S. Olaizola-Horn, H. Schmitt, M. Grewe, A. Jahnke, J. P. Johnson, K. Briviba, H. Sies and J. Krutmann, Activation of transcription factor AP-2 mediates UVA radiation- and singlet oxygen-induced expression of the human intercellular adhesion molecule 1 gene, *Proc. Natl. Acad. Sci. USA*, 1996, **93**(25), 14586–14591.
47 H. Nakano, F. P. Gasparro and J. Uitto, UVA-340 as energy source, mimicking natural sunlight, activates the transcription factor AP-1 in cultured fibroblasts: evidence for involvement of protein kinase-C, *Photochem. Photobiol.*, 2001, **74**(2), 274–282.
48 L. O. Klotz, N. J. Holbrook and H. Sies, UVA and singlet oxygen as inducers of cutaneous signaling events, *Curr. Probl. Dermatol.*, 2001, **29**, 95–113.
49 M. S. Matsui and V. A. DeLeo, Longwave ultraviolet radiation and promotion of skin cancer, *Cancer Cells*, 1991, **3**(1), 8–12.
50 T. Schwarz, Photoimmunosuppression, *Photodermatol. Photoimmunol. Photomed.*, 2002, **18**(3), 141–145.
51 L. O. Klotz, K. D. Kroncke and H. Sies, Singlet oxygen-induced signaling effects in mammalian cells, *Photochem. Photobiol. Sci.*, 2003, **2**(2), 88–94.
52 S. S. Brar, T. P. Kennedy, A. B. Sturrock, T. P. Huecksteadt, M. T. Quinn, T. M. Murphy, P. Chitano and J. R. Hoidal, NADPH oxidase promotes NF-kappaB activation and proliferation in human airway smooth muscle, *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 2002, **282**(4), L782–L795.
53 T. P. Szatrowski and C. F. Nathan, Production of large amounts of hydrogen peroxide by human tumor cells, *Cancer Res.*, 1991, **51**(3), 794–798.
54 R. Gniadecki, T. Thorn, J. Vicanova, A. Petersen and H. C. Wulf, Role of mitochondria in ultraviolet-induced oxidative stress, *J. Cell. Biochem.*, 2000, **80**(2), 216–222.
55 R. Gniadecki, N. Christoffersen and H. C. Wulf, Cholesterol-rich plasma membrane domains (lipid rafts) in keratinocytes: importance in the baseline and UVA-induced generation of reactive oxygen species, *J. Invest. Dermatol.*, 2002, **118**(4), 582–588.
56 C. S. Foote, Definition of type I and type II photosensitized oxidation, *Photochem. Photobiol.*, 1991, **54**(5), 659.
57 J. Paczkowski, J. J. Lamberts, B. Paczkowska and D. C. Neckers, Photophysical properties of rose bengal and its derivatives (XII), *J. Free Radical Biol. Med.*, 1985, **1**(5–6), 341–351.
58 C. S. Foote, Mechanisms of photosensitized oxidation. There are several different types of photosensitized oxidation which may be important in biological systems, *Science*, 1968, **162**(857), 963–970.
59 F. Elisei, L. Latterini, G. G. Aloisi, U. Mazzucato, G. Viola, G. Miolo, D. Vedaldi and F. Dall'Acqua, Excited-state properties and in vitro phototoxicity studies of three phenothiazine derivatives, *Photochem. Photobiol.*, 2002, **75**(1), 11–21.
60 M. J. Davies, Reactive species formed on proteins exposed to singlet oxygen, *Photochem. Photobiol. Sci.*, 2004, **3**(1), 17–25.
61 A. W. Girotti, Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms, *J. Photochem. Photobiol. B.*, 2001, **63**(1–3), 103–113.
62 G. D. Ouedraogo and R. W. Redmond, Secondary reactive oxygen species extend the range of photosensitization effects in cells: DNA damage produced *via* initial membrane photosensitization, *Photochem. Photobiol.*, 2003, **77**(2), 192–203.
63 M. J. Peak, J. G. Peak and B. A. Carnes, Induction of direct and indirect single-strand breaks in human cell DNA by far- and near-ultraviolet radiations: action spectrum and mechanisms, *Photochem. Photobiol.*, 1987, **45**(3), 381–387.
64 C. Kielbassa, L. Roza and B. Epe, Wavelength dependence of oxidative DNA damage induced by UV and visible light, *Carcinogenesis*, 1997, **18**(4), 811–816.
65 D. E. Brash, J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin and J. Ponten, A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma, *Proc. Natl. Acad. Sci. USA*, 1991, **88**(22), 10124–10128.
66 A. Ziegler, D. J. Leffell, S. Kunala, H. W. Sharma, M. Gailani, J. A. Simon, A. J. Halperin, H. P. Baden, P. E. Shapiro and A. E. Bale, *et al.*, Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers, *Proc. Natl. Acad. Sci. USA*, 1993, **90**(9), 4216–4220.
67 J. Cadet, C. Anselmino, T. Douki and L. Voituriez, Photochemistry of nucleic acids in cells, *J. Photochem. Photobiol. B.*, 1992, **15**(4), 277–298.
68 A. A. Lamola and J. P. Mittal, Solution photochemistry of thymine and uracil, *Science*, 1966, **154**(756), 1560–1561.
69 T. Delatour, T. Douki, C. D'Ham and J. Cadet, Photosensitization of thymine nucleobase by benzophenone through energy transfer, hydrogen abstraction and one-electron oxidation, *J. Photochem. Photobiol. B.*, 1998, **9**, 105–116.
70 A. Moysan, A. Viari, P. Vigny, L. Voituriez, J. Cadet, E. Moustacchi and E. Sage, Formation of cyclobutane thymine dimers photosensitized by pyridopsoralens: quantitative and qualitative distribution within DNA, *Biochemistry*, 1991, **30**(29), 7080–7088.
71 T. Douki, A. Reynaud-Angelin, J. Cadet and E. Sage, Bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation, *Biochemistry*, 2003, **42**(30), 9221–9226.
72 S. Courdavault, C. Baudouin, M. Charveron, A. Favier, J. Cadet and T. Douki, Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells, *Mutat. Res.*, 2004, **556**(1–2), 135–142.
73 H. Kasai, Z. Yamaizumi, F. Yamamoto, T. Bessho, S. Nishimura, M. Berger and J. Cadet, Photosensitized formation of 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in DNA by riboflavin, *Nucleic. Acids. Symp. Ser.*, 1992, (27), 181–182.
74 K. Ito and S. Kawanishi, Site-specific DNA. damage induced by UVA radiation in the presence of endogenous photosensitizer, *Biol. Chem.*, 1997, **378**(11), 1307–1312.

75 G. M. Halliday, N. S. Agar, R. S. Barnetson, H. N. Ananthaswamy and A. M. Jones, UV-A fingerprint mutations in human skin cancer, *Photochem. Photobiol.*, 2005, **81**(1), 3–8.
76 M. Berneburg, S. Grether-Beck, V. Kurten, T. Ruzicka, K. Briviba, H. Sies and J. Krutmann, Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion, *J. Biol. Chem.*, 1999, **274**(22), 15345–15349.
77 J. Moan and Q. Peng, An outline of the hundred-year history of PDT, *Anticancer Res.*, 2003, **23**(5A), 3591–3600.
78 L. Kaestner, A. Juzeniene and J. Moan, Erythrocytes-the 'house elves' of photodynamic therapy, *Photochem. Photobiol. Sci.*, 2004, **3**(11–12), 981–989.
79 F. Ricchelli, Photophysical properties of porphyrins in biological membranes, *J. Photochem. Photobiol. B*, 1995, **29**(2–3), 109–118.
80 I. A. Menon, M. A. Becker, S. D. Persad and H. F. Haberman, Quantitation of hydrogen peroxide formed during UV-visible irradiation of protoporphyrin, coproporphyrin and uroporphyrin, *Clin. Chim. Acta.*, 1990, **186**(3), 375–381.
81 D. P. Buchczyk, L. O. Klotz, K. Lang, C. Fritsch and H. Sies, High efficiency of 5-aminolevulinate – photodynamic treatment using UVA irradiation, *Carcinogenesis*, 2001, **22**(6), 879–883.
82 L. Ma, S. Bagdonas and J. Moan, The photosensitizing effect of the photoproduct of protoporphyrin IX, *J. Photochem. Photobiol. B.*, 2001, **60**(2–3), 108–113.
83 D. E. Heck, A. M. Vetrano, T. M. Mariano and J. D. Laskin, UVB light stimulates production of reactive oxygen species: unexpected role for catalase, *J. Biol. Chem.*, 2003, **278**(25), 22432–22436.
84 A. King, E. Gottlieb, D. G. Brooks, M. P. Murphy and J. L. Dunaief, Mitochondria-derived reactive oxygen species mediate blue light-induced death of retinal pigment epithelial cells, *Photochem. Photobiol.*, 2004, **79**(5), 470–475.
85 S. Dore, M. Takahashi, C. D. Ferris, R. Zakhary, L. D. Hester, D. Guastella and S. H. Snyder, Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury, *Proc. Natl. Acad. Sci. USA*, 1999, **96**(5), 2445–2450.
86 L. Cheng and D. A. Lightner, A new photoisomerization of bilirubin, *Photochem. Photobiol.*, 1999, **70**(6), 941–948.
87 B. S. Rosenstein, J. M. Ducore and S. W. Cummings, The mechanism of bilirubin-photosensitized DNA strand breakage in human cells exposed to phototherapy light, *Mutat. Res.*, 1983, **112**(6), 397–406.
88 T. Christensen, E. B. Roll, A. Jaworska and G. Kinn, Bilirubin- and light induced cell death in a murine lymphoma cell line, *J. Photochem. Photobiol. B.*, 2000, **58**(2–3), 170–174.
89 *Melanin: Its role in human photoprotection*, ed. L. Zeise, M. R. Chedekel and T. B. Fitzpatrick, Valdenmar Publishing Company, Overland Park, KS, 1995, pp.1–320.
90 J. Moan, A. Dahlback and R. B. Setlow, Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation, *Photochem. Photobiol.*, 1999, **70**(2), 243–247.
91 A. Yoneta, T. Yamashita, H. Y. Jin, S. Kondo and K. Jimbow, Ectopic expression of tyrosinase increases melanin synthesis and cell death following UVB irradiation in fibroblasts from familial atypical multiple mole and melanoma (FAMMM) patients, *Melanoma. Res.*, 2004, **14**(5), 387–394.
92 F. L. Meyskens, Jr., P. Farmer and J. P. Fruehauf, Redox regulation in human melanocytes and melanoma, *Pigm. Cell. Res.*, 2001, **14**(3), 148–154.
93 Y. Liu, L. Hong, K. Wakamatsu, S. Ito, B. Adhyaru, C. Cheng, C. R. Bowers and J. D. Simon, Comparison of structural and chemical properties of black and red human hair melanosomes, *Photochem. Photobiol.*, 2005, **81**, 135–144.
94 A. Napolitano, Donato P. Di and G. Prota, New regulatory mechanisms in the biosynthesis of pheomelanins: rearrangement *vs.* redox exchange reaction routes of a transient 2*H*-1,4-benzothiazine-o-quinonimine intermediate, *Biochim. Biophys. Acta.*, 2000, **1475**(1), 47–45.
95 T. G. Salopek, K. Yamada, S. Ito and K. Jimbow, Dysplastic melanocytic nevi contain high levels of pheomelanin: quantitative comparison of pheomelanin/eumelanin levels between normal skin, common nevi, and dysplastic nevi, *Pigm. Cell. Res.*, 1991, **4**(4), 172–179.
96 G. Agrup, C. Lindbladh, G. Prota, H. Rorsman, A. M. Rosengren and E. Rosengren, Trichochromes in the urine of melanoma patients, *J. Invest. Dermatol.*, 1978, **70**(2), 90–91.
97 T. Horikoshi, S. Ito, K. Wakamatsu, H. Onodera and H. Eguchi, Evaluation of melanin-related metabolites as markers of melanoma progression, *Cancer*, 1994, **73**(3), 629–636.
98 M. R. Vincensi, M. d'Ischia, A. Napolitano, E. M. Procaccini, G. Riccio, G. Monfrecola, P. Santoianni and G. Prota, Phaeomelanin *vs.* eumelanin as a chemical indicator of ultraviolet sensitivity in fair-skinned subjects at high risk for melanoma: a pilot study, *Melanoma Res.*, 1998, **8**(1), 53–58.
99 I. A. Menon, S. Persad, N. S. Ranadive and H. F. Haberman, Effects of ultraviolet-visible irradiation in the presence of melanin isolated from human black or red hair upon Ehrlich ascites carcinoma cells, *Cancer Res.*, 1983, **43**(7), 3165–3169.
100 E. Kvam and R. M. Tyrrell, The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells, *J. Invest. Dermatol.*, 1999, **113**(2), 209–213.
101 C. Kipp and A. R. Young, The soluble eumelanin precursor 5,6-dihydroxyindole-2-carboxylic acid enhances oxidative damage in human keratinocyte DNA after UVA irradiation, *Photochem. Photobiol.*, 1999, **70**(2), 191–198.
102 L. Marrot, J. P. Belaidi, J. R. Meunier, P. Perez and C. Agapakis-Causse, The human melanocyte as a particular target for UVA radiation and an endpoint for photoprotection assessment, *Photochem. Photobiol.*, 1999, **69**(6), 686–693.
103 E. Wenczl, G. P. Van der Schans, L. Roza, R. M. Kolb, A. J. Timmerman, N. P. Smit, S. Pavel and A. A. Schothorst, (Pheo)melanin photosensitizes UVA-induced DNA. damage in cultured human melanocytes, *J. Invest. Dermatol.*, 1998, **111**(4), 678–682.
104 S. Takeuchi, W. Zhang, K. Wakamatsu, S. Ito, V. J. Hearing, K. H. Kraemer and D. E. Brash, Melanin acts as a potent UVB photosensitizer to cause an atypical mode of cell death in murine skin, *Proc. Natl. Acad. Sci. USA*, 2004, **101**(42), 15076–15081.
105 E. Kvam and J. Dahle, Melanin synthesis may sensitize melanocytes to oxidative DNA damage by ultraviolet A radiation and protect melanocytesfrom direct DNA damage by ultraviolet B radiation, *Pigm. Cell. Res.*, 2004, **17**(5), 549–550.
106 E. J. Land, A. Thompson, T. G. Truscott, K. V. Subbarao and M. R. Chedekel, Photochemistry of melanin precursors: dopa, 5-S-cysteinyldopa and 2,5-S,S′-dicysteinyldopa, *Photochem. Photobiol.*, 1986, **44**(6), 697–702.
107 W. H. Koch and M. R. Chedekel, Photoinitiated DNA. damage by melanogenic intermediates in vitro, *Photochem. Photobiol.*, 1986, **44**(6), 703–710.
108 B. Pilas, C. C. Felix, T. Sarna and B. Kalyanaraman, Photolysis of pheomelanin precursors: an ESR-spin trapping study, *Photochem. Photobiol.*, 1986, **44**(6), 689–696.
109 W. Korytowski, B. Pilas, T. Sarna and B. Kalyanaraman, Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanins, *Photochem. Photobiol.*, 1987, **45**(2), 185–190.
110 K. Jimbow, K. Reszka, S. Schmitz, T. Salopek and P. Thomas, Distribution of eu- and pheomelanins in human skin and melanocytic tumors, and their photoprotective *vs.* phototoxic properties, in *Melanin: Its role in human photoprotection*, ed. L. Zeise, M. R. Chedekel and T. B. Fitzpatrick, Valdenmar Publishing Company, Overland Park, KS, 1995, pp. 155–175.
111 T. Ye, L. E. Lamb, K. Wakamatsu, S. Ito and J. D. Simon, Ultrafast absorption and photothermal studies of decarboxytrichochrome C in solution, *Photochem. Photobiol. Sci.*, 2003, **2**(7), 821–823.
112 A. M. Edwards, C. Bueno, A. Saldano, E. Silva, K. Kassab, L. Polo and G. Jori, Photochemical and pharmacokinetic properties of selected flavins, *J. Photochem. Photobiol. B.*, 1999, **48**(1), 36–41.
113 R. M. Kowalczyk, E. Schleicher, R. Bittl and S. Weber, The photoinduced triplet of flavins and its protonation states, *J. Am. Chem. Soc.*, 2004, **126**(36), 11393–11399.
114 E. Silva, M. Jopia, A. M. Edwards, E. Lemp, J. R. De la Fuente and E. Lissi, Protective effect of Boldo and tea infusions on the visible light-mediated pro-oxidant effects of vitamin B2, riboflavin, *Photochem. Photobiol.*, 2002, **75**(6), 585–590.
115 D. R. Cardoso, D. W. Franco, K. Olsen, M. L. Andersen and L. H. Skibsted, Reactivity of bovine whey proteins, peptides, and amino acids toward triplet riboflavin as studied by laser flash photolysis, *J. Agric. Food Chem.*, 2004, **52**(21), 6602–6606.
116 R. Ugarte, A. M. Edwards, M. S. Diez, A. Valenzuela and E. Silva, Riboflavin-photosensitized anaerobic modification of rat lens proteins. A correlation with age-related changes, *J. Photochem. Photobiol. B.*, 1992, **13**(2), 161–168.

117 Y. Kato, K. Uchida and S. Kawakishi, Aggregation of collagen exposed to UVA in the presence of riboflavin: a plausible role of tyrosine modification, *Photochem. Photobiol.*, 1994, **59**(3), 343–349.
118 K. Sato, H. Taguchi, T. Maeda, H. Minami, Y. Asada, Y. Watanabe and K. Yoshikawa, The primary cytotoxicity in ultraviolet-a-irradiated riboflavin solution is derived from hydrogen peroxide, *J. Invest. Dermatol.*, 1995, **105**(4), 608–612.
119 A. Mahns, I. Melchheier, C. V. Suschek, H. Sies and L. O. Klotz, Irradiation of cells with ultraviolet-A (320–400 nm) in the presence of cell culture medium elicits biological effects due to extracellular generation of hydrogen peroxide, *Free Radical Res.*, 2003, **37**(4), 391–397.
120 W. T. Speck, C. C. Chen and H. S. Rosenkranz, In vitro studies of effects of light and riboflavin on DNA and HeLa cells, *Pediatr. Res.*, 1975, **9**(3), 150–153.
121 K. Ito, S. Inoue, K. Yamamoto and S. Kawanishi, 8-Hydroxydeoxyguanosine formation at the 5′ site of5′-GG-3′ sequences in double-stranded DNA by UV radiation with riboflavin, *J. Biol. Chem.*, 1993, **268**(18), 13221–13227.
122 F. Yamamoto, S. Nishimura and H. Kasai, Photosensitized formation of 8-hydroxydeoxyguanosine in cellular DNA by riboflavin, *Biochem. Biophys. Res. Commun.*, 1992, **187**(2), 809–813.
123 A. H. Thomas, C. Lorente, A. L. Capparelli, C. G. Martinez, A. M. Braun and E. Oliveros, Singlet oxygen ($^1\Delta_g$) production by pterin derivatives in aqueous solutions, *Photochem. Photobiol. Sci.*, 2003, **2**(3), 245–250.
124 K. Ito and S. Kawanishi, Photoinduced hydroxylation of deoxyguanosine in DNA by pterins: sequence specificity and mechanism, *Biochemistry*, 1997, **36**(7), 1774–1781.
125 H. Rokos, W. D. Beazley and K. U. Schallreuter, Oxidative stress in vitiligo: photo-oxidation of pterins produces H(2)O(2) and pterin-6-carboxylic acid, *Biochem. Biophys. Res. Commun.*, 2002, **292**(4), 805–811.
126 R. F. Branda and J. W. Eaton, Skin color and nutrient photolysis: an evolutionary hypothesis, *Science*, 1978, **201**(4356), 625–626.
127 G. Cremer-Bartels and I. Ebels, Pteridines as nonretinal regulators of light-dependent melatonin biosynthesis, *Proc. Natl. Acad. Sci. USA*, 1980, **77**(5), 2415–2418.
128 Y. Murata, K. Kumano, T. Ueda, N. Araki, T. Nakamura and M. Tani, Photosensitive dermatitis caused by pyridoxine hydrochloride, *J. Am. Acad. Dermatol.*, 1998, **39**(2 Pt 2), 314–317.
129 G. Shwartzman and A. Fisher, Studies on antibacterial properties of irradiated pyridoxamine, *J. Biol. Chem.*, 1947, **167**, 345–362.
130 G. T. Wondrak, M. J. Roberts, M. K. Jacobson and E. L. Jacobson, 3-hydroxypyridine chromophores are endogenous sensitizers of photooxidative stress in human skin cells, *J. Biol. Chem.*, 2004, **279**(29), 30009–30020.
131 S. P. Coburn, A. Slominski, J. D. Mahuren, J. Wortsman, L. Hessle and J. L. Millan, Cutaneous metabolism of vitamin B-6, *J. Invest. Dermatol.*, 2003, **120**(2), 292–300.
132 K. Sato, H. Taguchi, T. Maeda and K. Yoshikawa, Pyridoxine toxicity to cultured fibroblasts caused by near-ultraviolet light, *J. Invest. Dermatol*, 1993, **100**(3), 266–270.
133 B. K. Ohta and C. S. Foote, Characterization of endoperoxide and hydroperoxide intermediates in the reaction of pyridoxine with singlet oxygen, *J. Am. Chem. Soc.*, 2002, **124**(41), 12064–12065.
134 P. Bilski, M. Y. Li, M. Ehrenshaft, M. E. Daub and C. F. Chignell, Vitamin B6 (pyridoxine) and its derivatives are efficient singlet oxygen quenchers and potential fungal antioxidants, *Photochem. Photobiol.*, 2000, **71**(2), 129–134.
135 M. L. Cunningham, N. I. Krinsky, S. M. Giovanazzi and M. J. Peak, Superoxide anion is generated from cellular metabolites by solar radiation and its components, *J. Free Radical Biol. Med.*, 1985, **1**(5–6), 381–385.
136 T. G. Burchuladze and G. Fraikin, [Mechanism of NADH-sensitized formation of DNA breaks during irradiation with near UV light], *Mol. Biol. (Moscow)*, 1991, **25**(4), 955–959.
137 B. Czochralska, W. G. B. Kawczynski and D. Shugar, Oxidation of excited state NADH and NAD dimer in aqueous medium: involvement of O_2^- as a mediator in the presence of oxygen, *Biochim. Biophys. Acta.*, 1984, **801**, 403–409.
138 R. S. Bodaness and P. C. Chan, Singlet oxygen as a mediator in the hematoporphyrin-catalyzed photooxidation of NADPH to NADP+ in deuterium oxide, *J. Biol. Chem.*, 1977, **252**(23), 8554–8560.
139 V. Kirveliene, R. Rotomskis, A. Pugzlys, G. Slekys, V. Krasauskas, A. Piskarskas and B. Juodka, Fluence-rate-dependent photosensitized oxidation of NADH, *J. Photochem. Photobiol. B.*, 1993, **21**(1), 53–60.
140 B. W. Pogue, J. D. Pitts, M. A. Mycek, R. D. Sloboda, C. M. Wilmot, J. F. Brandsema and J. A. O'Hara, *In vivo* NADH fluorescence monitoring as an assay for cellular damage in photodynamic therapy, *Photochem. Photobiol.*, 2001, **74**(6), 817–824.
141 F. Petrat, S. Pindiur, M. Kirsch and H. de Groot, NAD(P)H, a primary target of $^1O_2$ in mitochondria of intact cells, *J. Biol. Chem.*, 2003, **278**(5), 3298–3307.
142 M. Kirsch and Groot H. De, NAD(P)H, a directly operating antioxidant?, *FASEB J.*, 2001, **15**(9), 1569–1574.
143 C. C. Winterbourn, Free radical toxicology and antioxidant defence, *Clin. Exp. Pharmacol. Physiol.*, 1995, **22**(11), 877–880.
144 M. K. Jacobson, B. Tastoush, D. L. Coyle, M. Kim, H. Kim and E. L. Jacobson, Elevation of Skin Cell NAD Content by Topical Application of Pro-NAD Compounds, *J. Invest. Dermatol.*, 2000, **114**, 849.
145 E. L. Jacobson, P. U. Giacomoni, M. J. Roberts, G. T. Wondrak and M. K. Jacobson, Optimizing the energy status of skin cells during solar radiation, *J. Photochem. Photobiol. B.*, 2001, **63**(1–3), 141–147.
146 T. Mohammad, H. Morrison and H. HogenEsch, Urocanic acid photochemistry and photobiology, *Photochem. Photobiol.*, 1999, **69**(2), 115–135.
147 C. M. Chuong, B. J. Nickoloff, P. M. Elias, L. A. Goldsmith, E. Macher, P. A. Maderson, J. P. Sundberg, H. Tagami, P. M. Plonka, K. Thestrup-Pederson, B. A. Bernard, J. M. Schroder, P. Dotto, C. M. Chang, M. L. Williams, K. R. Feingold, L. E. King, A. M. Kligman, J. L. Rees and E. Christophers, What is the 'true' function of skin?, *Exp. Dermatol.*, 2002, **11**(2), 159–187.
148 H. Morrison, D. Avnir, C. Bernasconi and G. Fagan, Z/E Photoisomerization of urocanic acid, *Photochem. Photobiol.*, 1980, **32**, 711–714.
149 N. Haralampus-Grynaviski, C. Ransom, T. Ye, M. Rozanowska, M. Wrona, T. Sarna and J. D. Simon, Photogeneration and quenching of reactive oxygen species by urocanic acid, *J. Am. Chem. Soc.*, 2002, **124**(13), 3461–3468.
150 E. L. Menon and H. Morrison, Formation of singlet oxygen by urocanic acid by UVA irradiation and some consequences thereof, *Photochem. Photobiol.*, 2002, **75**(6), 565–569.
151 K. M. Hanson and J. D. Simon, Epidermal *trans*-urocanic acid and the UV-A-induced photoaging of the skin, *Proc. Natl. Acad. Sci. USA*, 1998, **95**(18), 10576–10578.
152 J. P. McCormick, J. R. Fischer, J. P. Pachlatko and A. Eisenstark, Characterization of a cell-lethal product from the photooxidation of tryptophan: hydrogen peroxide, *Science*, 1976, **191**(4226), 468–469.
153 J. Craggs, S. H. Kirk and S. I. Ahmad, Synergistic action of near-UV and phenylalanine, tyrosine or tryptophan on the inactivation of phage T7: role of superoxide radicals and hydrogen peroxide, *J. Photochem. Photobiol. B.*, 1994, **24**(2), 123–128.
154 P. Walrant and R. Santus, N-formyl-kynurenine, a tryptophan photooxidation product, as a photodynamic sensitizer, *Photochem. Photobiol.*, 1974, **19**(6), 411–417.
155 A. Pirie, Formation of *N′*-formylkynurenine in proteins from lens and other sources by exposure to sunlight, *Biochem. J.*, 1971, **125**(1), 203–208.
156 L. I. Grossweiner, Photochemistry of proteins: a review, *Curr. Eye Res.*, 1984, **3**(1), 137–144.
157 J. A. Aquilina and R. J. Truscott, Identifying sites of attachment of UV filters to proteins in older human lenses, *Biochim. Biophys. Acta.*, 2002, **1596**(1), 6–15.
158 N. R. Parker, J. F. Jamie, M. J. Davies and R. J. Truscott, Protein-bound kynurenine is a photosensitizer of oxidative damage, *Free Radical Biol. Med.*, 2004, **37**(9), 1479–1489.
159 R. K. Sindhu, F. E. Wagner and Y. Kikkawa, Induction of cytochrome p450 1A1 and 1B1 by photooxidized tryptophan in transformed human keratinocytes, *Adv. Exp. Med. Biol.*, 2003, **527**, 297–306.
160 J. J. Thiele, S. N. Hsieh, K. Briviba and H. Sies, Protein oxidation in human stratum corneum: susceptibility of keratins to oxidation in vitro and presence of a keratin oxidation gradient *in vivo*, *J. Invest. Dermatol.*, 1999, **113**(3), 335–339.
161 G. J. Smith, New trends in photobiology: photodegradation of keratin and other structural proteins, *J. Photochem. Photobiol. B.*, 1995, **27**, 187–198.

162 G. J. Smith and W. H. Melhuish, Relaxation and quenching of the excited states of tryptophan in keratin, *J. Photochem. Photobiol. B.*, 1993, **17**, 63–68.
163 D. G. Dyer, J. A. Dunn, S. R. Thorpe, K. E. Bailie, T. J. Lyons, D. R. McCance and J. W. Baynes, Accumulation of Maillard reaction products in skin collagen in diabetes and aging, *J. Clin. Invest.*, 1993, **91**(6), 2463–2469.
164 C. Jeanmaire, L. Danoux and G. Pauly, Glycation during human dermal intrinsic and actinic ageing: an *in vivo* and in vitro model study, *Br. J. Dermatol.*, 2001, **145**(1), 10–18.
165 G. T. Wondrak, D. Cervantes-Laurean, M. J. Roberts, J. G. Qasem, M. Kim, E. L. Jacobson and M. K. Jacobson, Identification of alpha-dicarbonyl scavengers for cellular protection against carbonyl stress, *Biochem. Pharmacol.*, 2002, **63**(3), 361–373.
166 R. Abe, T. Shimizu, H. Sugawara, H. Watanabe, H. Nakamura, H. Choei, N. Sasaki, S. Yamagishi, M. Takeuchi and H. Shimizu, Regulation of human melanoma growth and metastasis by AGE-AGE receptor interactions, *J. Invest. Dermatol.*, 2004, **122**(2), 461–467.
167 R. Tressl, G. T. Wondrak, R. P. Kruger and D. Rewicki, New Melanoidin-like Maillard Polymers from 2-Deoxypentoses, *J. Agric. Food Chem.*, 1998, **46**(1), 104–110.
168 S. R. Thorpe and J. W. Baynes, Maillard reaction products in tissue proteins: new products and new perspectives, *Amino. Acids.*, 2003, **25**(3–4), 275–281.
169 B. J. Ortwerth, M. Prabhakaram, R. H. Nagaraj and M. Linetsky, The relative UV sensitizer activity of purified advanced glycation endproducts, *Photochem. Photobiol.*, 1997, **65**(4), 666–672.
170 G. T. Wondrak, E. L. Jacobson and M. K. Jacobson, Photosensitization of DNA damage by glycated proteins, *Photochem. Photobiol. Sci.*, 2002, **1**, 355–363.
171 H. Masaki, Y. Okano and H. Sakurai, Generation of active oxygen species from advanced glycation end-products (AGEs) during ultraviolet light A (UVA) irradiation and a possible mechanism for cell damaging, *Biochim. Biophys. Acta*, 1999, **1428**(1), 45–56.
172 Y. Okano, H. Masaki and H. Sakurai, Pentosidine in advanced glycation end-products (AGEs) during UVA irradiation generates active oxygen species and impairs human dermal fibroblasts, *J. Dermatol. Sci.*, 2001, **27**(Suppl 1), S11–S18.
173 T. Usui, S. Shizuuchi, H. Watanabe and F. Hayase, Cytotoxicity and oxidative stress induced by the glyceraldehyde-related Maillard reaction products for HL-60 cells, *Biosci. Biotechnol. Biochem.*, 2004, **68**(2), 333–340.
174 R. Nagai, C. M. Hayashi, L. Xia, M. Takeya and S. Horiuchi, Identification in human atherosclerotic lesions of GA-pyridine, a novel structure derived from glycolaldehyde-modified proteins, *J. Biol. Chem.*, 2002, **277**(50), 48905–48912.
175 F. J. Tessier, V. M. Monnier, L. M. Sayre and J. A. Kornfield, Triosidines: novel Maillard reaction products and cross-links from the reaction of triose sugars with lysine and arginine residues, *Biochem. J.*, 2003, **369**(Pt 3), 705–719.
176 A. B. Petersen, H. C. Wulf, R. Gniadecki and B. Gajkowska, Dihydroxyacetone, the active browning ingredient in sunless tanning lotions, induces DNA damage, cell-cycle block and apoptosis in cultured HaCaT keratinocytes, *Mutat. Res.*, 2004, **560**(2), 173–186.
177 H. Inano, H. Ohba and B. Tamaoki, Photochemical inactivation of human placental estradiol 17 beta-dehydrogenase in the presence of 2,3-butanedione, *J. Steroid Biochem.*, 1983, **19**(5), 1617–1622.
178 W. M. Nau and J. C. Scaiano, Qxygen quenching of excited aliphatic ketones and diketones, *J. Phys. Chem.*, 1996, **100**, 11360–11367.
179 A. Kornhauser and M. A. Pathak, Studies on the mechanism of the photosensitized dimerization of pyrimidines, *Z. Naturforsch., Teil B*, 1972, **27**(5), 550–553.
180 I. G. Gut, P. D. Wood and R. W. Redmond, Interaction of triplet photosensitizers with nucleotides and DNA in aqueous solution at room temperature, *J. Am. Chem. Soc.*, 1996, **118**, 2366–2373.
181 U. T. Brunk and A. Terman, Lipofuscin: mechanisms of age-related accumulation and influence on cell function, *Free Radical Biol. Med.*, 2002, **33**(5), 611–619.
182 M. Boulton, M. Rozanowska, B. Rozanowski and T. Wess, The photoreactivity of ocular lipofuscin, *Photochem. Photobiol. Sci.*, 2004, **3**(8), 759–764.
183 L. E. Lamb and J. D. Simon, A2E: a component of ocular lipofuscin, *Photochem. Photobiol.*, 2004, **79**(2), 127–136.
184 N. Sitte, K. Merker, T. Grune and T. von Zglinicki, Lipofuscin accumulation in proliferating fibroblasts in vitro: an indicator of oxidative stress, *Exp. Gerontol.*, 2001, **36**(3), 475–486.
185 N. Kollias, R. Gillies, M. Moran, I. E. Kochevar and R. R. Anderson, Endogenous skin fluorescence includes bands that may serve as quantitative markers of aging and photoaging, *J. Invest. Dermatol*, 1998, **111**(5), 776–780.
186 L. Brancaleon, G. Lin and N. Kollias, The *in vivo* fluorescence of tryptophan moieties in human skin increases with UV exposure and is a marker for epidermal proliferation, *J. Invest. Dermatol.*, 1999, **113**(6), 977–982.
187 J. M. Menter, G. D. Williamson, K. Carlyle, C. L. Moore and I. Willis, Photochemistry of type I acid-soluble calf skin collagen: dependence on excitation wavelength, *Photochem. Photobiol.*, 1995, **62**(3), 402–408.
188 S. Fu, M. X. Fu, J. W. Baynes, S. R. Thorpe and R. T. Dean, Presence of dopa and amino acid hydroperoxides in proteins modified with advanced glycation end products (AGEs): amino acid oxidation products as a possible source of oxidative stress induced by AGE proteins, *Biochem. J.*, 1998, **330**(Pt 1), 233–239.
189 M. J. Petersen, C. Hansen and S. Craig, Ultraviolet A. irradiation stimulates collagenase production in cultured human fibroblasts, *J. Invest. Dermatol.*, 1992, **99**(4), 440–444.
190 G. Herrmann, M. Wlaschek, T. S. Lange, K. Prenzel, G. Goerz and K. Scharffetter-Kochanek, UVA irradiation stimulates the synthesis of various matrix-metalloproteinases (MMPs) in cultured human fibroblasts, *Exp. Dermatol.*, 1993, **2**(2), 92–97.
191 G. J. Fisher, S. C. Datta, H. S. Talwar, Z. Q. Wang, J. Varani, S. Kang and J. J. Voorhees, Molecular basis of sun-induced premature skin ageing and retinoid antagonism, *Nature*, 1996, **379**(6563), 335–339.
192 J. Varani, D. Spearman, P. Perone, S. E. Fligiel, S. C. Datta, Z. Q. Wang, Y. Shao, S. Kang, G. J. Fisher and J. J. Voorhees, Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro, *Am. J. Pathol.*, 2001, **158**(3), 931–942.
193 T. K. Hei, R. Persaud, H. Zhou and M. Suzuki, Genotoxicity in the eyes of bystander cells, *Mutat. Res.*, 2004, **568**(1), 111–120.
194 B. Ponnaiya, G. Jenkins-Baker, A. Bigelow, S. Marino and C. R. Geard, Detection of chromosomal instability in alpha-irradiated and bystander human fibroblasts, *Mutat. Res.*, 2004, **568**(1), 41–48.
195 H. Maier, G. Schauberger, K. Brunnhofer and H. Honigsmann, Change of ultraviolet absorbance of sunscreens by exposure to solar-simulated radiation, *J. Invest. Dermatol.*, 2001, **117**(2), 256–262.
196 J. M. Allen, C. J. Gossett and S. K. Allen, Photochemical formation of singlet molecular oxygen in illuminated aqueous solutions of several commercially available sunscreen active ingredients, *Chem. Res. Toxicol.*, 1996, **9**(3), 605–609.
197 N. Serpone, A. Salinaro, A. V. Emeline, S. Horikoshi, H. Hidaka and J. Zhao, An in vitro systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents, *Photochem. Photobiol. Sci.*, 2002, **1**(12), 970–981.
198 G. T. Wondrak, M. K. Jacobson and E. L. Jacobson, Identification of quenchers of photoexcited states as novel agents for skin photoprotection, *J. Pharmacol. Exp. Ther.*, 2005, **312**(2), 482–491.
199 A. Meves, S. N. Stock, A. Beyerle, M. R. Pittelkow and D. Peus, Vitamin C. derivative ascorbyl palmitate promotes ultraviolet-B-induced lipid peroxidation and cytotoxicity in keratinocytes, *J. Invest. Dermatol.*, 2002, **119**(5), 1103–1108.
200 Y. P. Lu, Y. R. Lou, P. Yen, H. L. Newmark, O. I. Mirochnitchenko, M. Inouye and M. T. Huang, Enhanced skin carcinogenesis in transgenic mice with high expression of glutathione peroxidase or both glutathione peroxidase and superoxide dismutase, *Cancer. Res.*, 1997, **57**(8), 1468–1474.
201 Y. Koyama, Structures and functions of carotenoids in photosynthetic systems, *J. Photochem. Photobiol. B.*, 1991, **9**, 265–280.
202 N. E. Holt, D. Zigmantas, L. Valkunas, X. P. Li, K. K. Niyogi and G. R. Fleming, Carotenoid cation formation and the regulation of photosynthetic light harvesting, *Science*, 2005, **307**(5708), 433–436.
203 S. Beutner, B. Bloedorn, T. Hoffmann and H. D. Martin, Synthetic singlet oxygen quenchers, *Methods Enzymol.*, 2000, **319**, 226–241.
204 C. Schweitzer and R. Schmidt, Physical mechanisms of generation and deactivation of singlet oxygen, *Chem. Rev.*, 2003, **103**, 1685–1757.

205 A. U. Khan, Y.-H. Mei and T. Wilson, A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 11426–11427.
206 Y. Lion, M. Delmelle and A Van De Vorst, New method of detecting singlet oxygen production, *Nature*, 1976, **263**, 442–443.
207 E. L. Clennan, L. J. Noe, T. Wen and E. Szneler, Solvent Effects on the Ability of Amines to Physically Quench Singlet Oxygen As Determined by Time-Resolved Infrared Emission Studies, *J. Org. Chem.*, 1989, **54**, 3581–3584.
208 Mascio P. Di, S. Kaiser and H. Sies, Lycopene as the most efficient biological carotenoid singlet oxygen quencher, *Arch. Biochem. Biophys.*, 1989, **274**(2), 532–538.
209 A. A. Krasnovsky, Jr., M. A. Rodgers, M. G. Galpern, B. Rihter, M. E. Kenney and E. A. Lukjanetz, Quenching of singletmolecular oxygen by phthalocyanines and naphthalocyanines, *Photochem. Photobiol.*, 1992, **55**(5), 691–696.
210 P. Douglas, J. D. Thomas, H. Strohm, C. Winscom, D. Clarke and M. S. Garley, Triplet energies and the singlet oxygen quenching mechanism for 7H-pyrazolo[5,1-c]-1,2,4-triazole azomethine dyes, *Photochem. Photobiol. Sci.*, 2003, **2**(5), 563–568.
211 G. Cosa, L. J. Martinez and J. C. Scaiano, Influence of solvent polarity and base concentration on the photochemistry of ketoprofen: independent singlet and triplet pathways, *Phys. Chem. Chem. Phys.*, 1999, **1**, 3533–3537.
212 M. C. Cuquerella, F. Bosca and M. A. Miranda, Photonucleophilic aromatic substitution of 6-fluoroquinolones in basic media: triplet quenching by hydroxide anion, *J. Org. Chem.*, 2004, **69**, 7256–7261.
213 E. F. Ullman and P. Singh, 3,3,4,4-Tetramethyl-1,2-diazetine-1,2-dioxide, a useful low-energy triplet quencher, *J. Am. Chem. Soc.*, 1972, **94**, 5077–5078.
214 F. Rizzuto and J. D. Spikes, Mechanisms involved in the chemical inhibition of the eosin-sensitized photooxidation of trypsin, *Radiat. Environ. Biophys.*, 1975, **12**(3), 217–232.
215 P. D. Sima and J. R. Kanofsky, Cyanine dyes as protectors of K562 cells from photosensitized cell damage, *Photochem. Photobiol.*, 2000, **71**(4), 413–421.
216 D. L. Bissett, S. Majeti, J. J. Fu, J. F. McBride and W. E. Wyder, Protective effect of topically applied conjugated hexadienes against ultraviolet radiation-induced chronic skin damage in the hairless mouse, *Photodermatol. Photoimmunol. Photomed.*, 1990, **7**(2), 63–67.
217 H. J. Suh, H. W. Lee and J. Jung, Mycosporine glycine protects biological systems against photodynamic damage by quenching singlet oxygen with a high efficiency, *Photochem. Photobiol.*, 2003, **78**(2), 109–113.
218 J. M. Gaullier, M. Bazin, A. Valla, M. Giraud and R. Santus, Amino acid-pyrrole N-conjugates; a new class of antioxidants II. Effectiveness of singlet oxygen quenching by luminescence measurements, *J. Photochem. Photobiol. B.*, 1995, **30**, 195–200.
219 H. Sies and W. Stahl, Nutritional Protection Against Skin Damage From Sunlight, *Annu. Rev. Nutr.*, 2004, **24**, 173–200.
220 W. H. Chan and H. J. Wu, Anti-apoptotic effects of curcumin on photosensitized human epidermal carcinoma A431 cells, *J. Cell. Biochem.*, 2004, **92**(1), 200–212.
221 T. A. Dahl, W. R. Midden and P. E. Hartman, Some prevalent biomolecules as defenses against singlet oxygen damage, *Photochem. Photobiol.*, 1988, **47**(3), 357–362.
222 M. Rougee, R. V. Bensasson, E. J. Land and R. Pariente, Deactivation of singlet molecular oxygen by thiols and related compounds, possible protectors against skin photosensitivity, *Photochem. Photobiol.*, 1988, **47**(4), 485–489.
223 P. Morliere, G. Huppe, D. Averbeck, A. R. Young, R. Santus and L. Dubertret, In vitro photostability and photosensitizing properties of bergamot oil. Effects of a cinnamate sunscreen, *J. Photochem. Photobiol. B.*, 1990, **7**(2–4), 199–208.
224 P. Morliere, O. Avice, T. S. Melo, L. Dubertret, M. Giraud and R. Santus, A study of the photochemical properties of some cinnamate sunscreens by steady state and laser flash photolysis, *Photochem. Photobiol.*, 1982, **36**(4), 395–399.
225 E. Chatelain and B. Gabard, Photostabilization of butyl methoxydibenzoylmethane (Avobenzone) and ethylhexyl methoxycinnamate by bis-ethylhexyloxyphenol methoxyphenyl triazine (Tinosorb S.), a new UV broadband filter, *Photochem. Photobiol.*, 2001, **74**(3), 401–406.