

Melanocytes and Oxidative Stress

Christian Diehl*

Professor, University Marconi, Via Vittoria Colonna 11, Rome, Italy

Abstract

Melanocytes are the site of melanogenesis, a chemical reaction leading to the production of melanin. By its characteristics, melanogenesis submits the melanocytes to constant oxidative stress, which can be an essential causative factor for various pigmentary diseases such as vitiligo, melasma or melanoma. The same initial cause, oxidative stress, can lead to either hypopigmentation, or hyperpigmentation or even a carcinogenic process. Phototypes present different susceptibilities to each of these diseases, originally caused by a prooxidant/antioxidant imbalance.

In any case, administration of antioxidants appears to be helpful in their treatment.

Introduction

Oxidative stress is caused by an imbalance between the production of Reactive Oxygen Species (ROS) and a biological system's abilities to detoxify and prevent the resulting damage. It can result from the overexpression of pro-oxidant species in cells, and/or the reduction of their antioxidant capacities.

Free radicals may possess advantageous biological effects, such as the formation of the second messenger cGMP by stimulating the activation of guanylate cyclase under the influence of the superoxide anion [1], the increased production of interleukin-2 (IL-2) by T cells when they are activated by the superoxide anion [2] or the activation of the transcription nuclear factor κ B (NF- κ B) by hydrogen peroxide [3].

Meanwhile, most of their effects are deleterious for nucleic acids, lipids and proteins [4] and oxidative stress is thought to be involved in the development of numerous diseases like cancer [5], Parkinson's or Alzheimer's disease [6] or myocardial infarction [7]. With regards to dermatology, the role of ROS is more and more highlighted in various diseases such as melanoma [8], acne [9,10] or vitiligo [11].

The ROS production may be increased under pathological conditions such as inflammation or cancer, but also under the influence of external factors, especially UV-radiation [12]. Skin, because of its direct interface with the environment, is the major source of UV-induced ROS for the body. Owing not only to environmental UV-exposure but also to the pro-oxidant state generated during melanogenesis, melanocytes are both 'instigators and victims of oxidative stress [8].

Influence of the Melanogenesis on the Redox Status of Melanocytes

Melanocytes are dendritic cells located at the epidermis-dermis junction. They contain melanosomes; which feature membrane-bound cytoplasmic organelles. Melanocytes synthesize melanin in the melanosome before passing the melanosomes to the surrounding keratinocytes, giving the skin its color.

Melanogenesis is a complex process where the rate-limiting enzyme tyrosinase is synthesizing melanin by utilizing L-tyrosine, dihydroxyphenylalanine (L-DOPA) and 5,6-dihydroxyindole as substrates. First, L-tyrosine is hydroxylated to form L-DOPA, which is further oxidized to L-DOPAquinone which will then be further processed into eumelanin (black or brown pigment) and phaeomelanin (yellow or red pigment) [13]. DOPAquinone, once produced, generally forms eumelanin through spontaneous reactions involving cyclization, decarboxylation, oxidation, and polymerization. However, TRP-2 can generate 5,6-dihydroxyindole-2-carboxylic acid (DHICA) from DOPochrome and TRP-1 will catalyze the oxidation of DHICA to

indole-5,6-quinone carboxylic acid [14]. In the absence of thiols, DOPAquinone is immediately converted to DOPochrome and leads to eumelanin production. When glutathione (GSH) and cysteine are present, they can react with DOPAquinone intermediates to divert melanin pigment synthesis from eumelanin to phaeomelanin [15].

Immediate response of the melanocytes to UVA results in tanning, which is due to a combination of photo-oxidation of melanin, augmented dendricity and induction of melanin transfer from the melanocytes to the keratinocytes [16]. The delayed pigmentation, induced by both UVA and UVB, comprises of a proliferation of melanocytes, increased transfer of melanosomes to the keratinocytes and augmented melanin synthesis.

As it can be observed, melanogenesis consists of a sequence of oxidation reactions. It results in a continuous generation of ROS all along the process of melanogenesis: H_2O_2 during its early stages [17] but also during the redox cycling from indoles to quinones [18], hydroxyl radicals and superoxide anion due to the catalytic activity of tyrosinase [19]. In addition, oxidative intermediates including reactive quinones, which are cytotoxic to proteins and DNA in the cells, are generated during melanogenesis [15].

There are conflicting reports about the role of melanin and melanin intermediates as pro-oxidants or antioxidants. While melanin can exhibit a photoprotective effect, it's pro-oxidant and antioxidant activity appears to depend on the redox state of the melanocytes [20], on the relative eumelanin/phaeomelanin contents, the levels of melanin intermediates [21], and the composition and concentration of reactive metal ions in the melanosome microenvironment [22]. Generation of H_2O_2 in response to UV correlates inversely with melanin content of the skin, suggesting an antioxidant property of the latter [23] while cultures of human melanocytes with high melanin content were reported to be more vulnerable to UVA- induced oxidative DNA damage than melanocytes with lower melanin content [24]. Still in cultured human melanocytes, UVA- stimulation appears to increase DNA damage most likely through phaeomelanin and/or melanin intermediates [25].

*Corresponding author: Christian Diehl, Professor, University Marconi, Via Vittoria Colonna 11, Rome, Italy, Tel: 41763992637; E-mail: chdiehl@hotmail.com

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As it was seen; during the process of melanogenesis, oxidative insults responsible for melanocyte damage can be caused not only by various ROS, namely H_2O_2 , hydroxyl radicals and superoxide anion, but also by intermediates in melanin synthesis.

Antioxidant Defense System of Melanocytes

The pool of endogenous antioxidants in the melanocytes comprises enzymatic substances such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GR), and Thioredoxin Reductase (TR). Non-enzymatic antioxidants include ascorbic acid, Glutathione (GSH) or α -tocopherol [26]. Obviously, enzymatic and non-enzymatic antioxidants work together to maintain an optimal redox balance in the melanocytes, and prevent oxidative damage at this stage. As key players in the homeostasis of epidermal cells, in particular melanocytes, paracrine factors are influencing the redox status of the latter. Endothelin-1 (ET-1) produced by the keratinocytes is a melanogenic factor reducing H_2O_2 generation in melanocytes [27]. ACTH, which is further processed by amidation and acetylation to α -MSH, has been identified in both melanocytes and keratinocytes [28]. Most of the actions of α -MSH in the skin are *via* the melanocortin-1 receptor (MC-1R) expressed on the cell surface of melanocytes. α -MSH stimulation increases both levels and activity of catalase and reduces UV-induced H_2O_2 expression [27,29] reducing oxidative DNA damage induced by the same [23]. Further, activation of MC-1R by α -MSH regulates intracellular redox status by up regulating the expression of various antioxidant genes (heme oxygenase-1 [HO-1], ferritin and peroxiredoxin-1) [23,30], Mitf, and APE-1 [27].

The Redox Status of Melanocytes Depends on the Skin Color

Variations of the skin and hair color are genetically determined and depend on the relative content of pheomelanin and eumelanin. While eumelanin could be considered as the 'normal' end product of melanogenesis, some conditions may divert melanin pigment synthesis from eumelanin to pheomelanin. It was discovered in 1995 that specific mutations at the (MC-1R) gene were responsible for the switch from eumelanin to pheomelanin: impaired receptor activity produces pheomelanin, whereas high MC-1R activity leads to the production of eumelanin [31]. Chemically, this diversion from eumelanin to pheomelanin is explained by the fact that when Glutathione (GSH) and its precursor cysteine are present, they react preferably with DOPAquinone intermediates and lead to pheomelanin production through cysteinylDOPA. The consequence is that the consumption of the two intracellular cell protectors GSH and cysteine lowers the cellular defense mechanisms against oxidative stress in the melanocytes [32,33]. Although cysteinylDOPA was suggested to have a role in scavenging ROS [34], this is only an intermediary in the chemical reaction, and pheomelanin would behave as a unique 'living' polymer and biocatalyst that may grow by simple exposure to monomer building blocks and then trigger autooxidative processes [33]. This increased risk of oxidative damage with increased pheomelanin production could potentially put nevus cells, recognized precursors of melanoma, at risk of oxidative damage [35,36]. To date, there is no clear view about whether eumelanin has a pro-oxidant or antioxidant capacity and this issue remains controversial. In contrast, there is growing evidence that pheomelanin is a potent pro-oxidant. In both barn owls and eagles, the degree of black and reddish pigmentation, directly correlated to eumelanin/pheomelanin balance, was positively and negatively correlated, respectively, with resistance to oxidative stress [37,38].

Role of Oxidative Stress in Various Pigmentation Disorders

With the growing understanding in both skin physiology and oxidative stress mechanisms, the role of the latter is better defined in the occurrence of more and more pigmentation disorders.

Melasma

Melasma is a common pigmentary disorder whose pathogenesis is currently far from being perfectly understood. Unfortunately there is a lack of reliable reports about the prevalence of melasma, as most studies are conducted in dermatology clinics which may indicate some ascertainment bias [39]. A randomized study in a Hispanic female population in Texas reported a prevalence of 8,8% [40] while in Southwest Asia the prevalence has been reported as high as 40% in females and 20% in males [41]. It is important to highlight that all phototypes do not have an equal risk of developing melasma: a multicenter survey of females from nine countries revealed that Fitzpatrick skin phototypes III and IV were more commonly affected than lighter ones [42]. Melasma might be caused by the presence of more biologically active melanocytes in the affected skin, rather than an increase in melanocytes number [39]. While a genetic component is probably involved in melasma, sun exposure and hormonal changes/imbalance are exacerbating factors but not causative ones. As there were no studies evaluating the role of oxidative stress in melasma, it was recently reported for the first time that the balance between oxidants and antioxidants was disrupted and oxidative stress was increased in melasma [43]. Prior to this finding, various antioxidants had been reported in the past as improving melasma. Topical and oral melatonin have been found to improve the redox balance along the treatment, and as a consequence, Melasma Area Severity Index (MASI) score experienced significant reduction in those patients [44].

An 8-week course of oral procyanidin+vitamins A, C and E was also proved to be effective in Filipino women with epidermal melasma [45]. Silymarin, a natural polyphenolic flavonoid, used as a cream during 4 weeks, was shown to give significant improvement in pigmentation and lesion size reduction in melasma patients [46]. More commonly used in daily practice, vitamin C is also an effective and safe option for treating melasma. Ascorbic acid is the most plentiful antioxidant in human skin. As it is water soluble, it functions in the aqueous compartment of the cell and protects it from oxidative stress by sequentially donating electrons to neutralize the free radicals [47]. Ascorbic acid can protect against UVA-dependent melanogenesis through the improvement of antioxidant defense capacity of melanocytes, and inhibition of NO production through down-regulation of eNOS and iNOS mRNA [48]. Its clinical efficacy in a 25% cream was assessed in melasma, based on the MASI and Mexameter scores [49].

Vitiligo

Vitiligo is an acquired depigmentation disorder featuring circumscribed depigmented macules due to the destruction of melanocytes from the lesional skin responding to an autoimmune mechanism [50]. However, the early death of vitiligo melanocytes was shown to be provoked by their increased sensitivity to oxidative stress, impacting tyrosinase activity and eumelanin synthesis [51,52]. It was also demonstrated that there were very high levels of H_2O_2 and concomitant reduced activity of catalase in the epidermis of patients with vitiligo [53], reducing the levels of methionine sulfoxide reductase A and B and thioredoxin/thioredoxin reductase which triggers oxidative stress,

leading to melanocyte death [54]. These high levels of H_2O_2 are also logically oxidizing both ACTH and α -MSH. All these consequences of high levels of H_2O_2 can be reversed by treatment with catalase [27]. On the other hand, in vitiligo the levels of various enzymatic antioxidants, namely catalase, glutathione peroxidase and glutathione reductase were also found to be altered, explaining abnormally high levels of H_2O_2 [55]. It was recently suggested that oxidative stress appeared to be the initial triggering factor of vitiligo, being further exacerbated by autoimmune factors and oxidative stress [56].

Whilst vitiligo is classically reported as affecting 0.5-1% of the worldwide population, here too is appearing a disparity between phototypes. A Brazilian study [57] conducted in 669 patients with vitiligo reports that the most frequent phototype in vitiligo patients was phototype III. Although this trial had been conducted in Sao Paulo, there was still a risk for some ascertainment bias in the absence of demographic data of phototype prevalence in this area. However, an Estonian study [58] has some relevancy, as the majority of Estonian phototypes are I and II. Among 155 patients with vitiligo the majority (65.8%) had phototype III, twice more than patients with phototype II (32.9%).

This data strongly suggests that higher phototypes are more likely to be affected by vitiligo than lighter ones, which appears to be in complete contradiction with the report that UV-stimulated production of H_2O_2 inversely correlates with the melanin content of the skin [23].

Based on the aforementioned evidence, treatment of vitiligo with antioxidants makes sense and reported trials sustain the interest of using CAT/SOD creams alone [59] or combined with NB-UVB [60]. A novel gel formulation containing phenylalanine, *Cucumis melo* extract and N-acetylcysteine was also showing a good efficacy and promising results [61]. Oral intake of antioxidants was giving good results in term of repigmentation in mice (vitamin A, C, E+selenium+zinc) [62] and also in humans (vitamin E) [63].

Melanoma

Melanoma is less common than other skin cancers. Globally its incidence tends to increase. Many European countries are showing an annual increase in incidence higher than 2% [64], although in some regions it tends to reach a plateau [65]. This increase may be at least partly attributed to better health education in the population and among the practitioners, with more and more popular concern for this problem; leading to a better and wider detection.

Sun exposure has long been mentioned as the major risk factor for melanoma, but to date it appears to be at least controversial. This apparent association between sun exposure and risk of melanoma was based on comparative odds between Queensland (Australia) and the UK, two countries with quite comparable populations in term of genetics and phototypes, exposed to very different sun conditions. In that case, the odds ratio was reported as four times higher in the former than in the latter [66]. However, a metaanalysis published in 2005 supported the hypothesis that there was a positive association between the risk of melanoma and intermittent sun exposure, and an inverse association with high continuous pattern of sun exposure, suggesting that the relation with sunshine was not dose-dependent [67]. Obviously sunlight is a major promotor of ROS in the skin, both by inducing high levels of ROS and impairing the physiological antioxidant defense mechanisms. Exposing melanocytes to UV results in a rapid generation of H_2O_2 and corresponding decrease in catalase activity [68] and reduced Ho-1 expression [27]. Disregarding the role of UV exposure, there is increasing evidence for the responsibility of oxidative

stress in both initiation and progression of melanoma [8], based on the finding that the activating $V600E$ BRAF mutation, commonly expressed in nevi and melanoma, and could be induced by oxidative stress [69]. Melanoma risk was also found to be increased by MC-1R variant alleles [31,70] suggesting that ROS would be a driver of melanomagenesis [71]. Higher sensitivity of melanocytes from melanoma lesions to oxidizing agents was long demonstrated [71] as well as increased levels of markers of oxidative stress such as O_2^- [72] or NOS [73].

Based on our phototype, we are not created equal for confronting the risk of melanoma. Melanomas are 10-20 times more frequent in white, Caucasian people than in non-white people [65]. Persons with fair skin, a poor ability to tan and a freckled complexion double their risk for melanoma [74]. In those persons with fair skin and red hair, specific mutations at the MC1R gene are responsible for the switch from eumelanin to pheomelanin, inducing increased oxidative stress and higher risk of melanoma [35,36]. In a similar manner, it was demonstrated that recessive yellow mice with loss-of-function MC1R were developing more invasive melanoma than their albino counterparts [75].

Beneficial effects of antioxidants on individuals with melanoma susceptibility and under stress environment against development of melanoma seem warranted [14]. N-acetylcysteine (NAC) was found to inhibit tumor formation and delay the onset of UV-induced melanoma in HGF mice [76] through inhibition of oxidative DNA damage. In the same manner, some phytochemicals could be helpful. Gallic acid, commonly found in gallnuts, witch hazel, tea leaves or oak bark, was capable of exhibiting protective effects on human melanoma cells through improvement of their antioxidant defenses [77]. Genistein, a soy-isoflavon was also exerting growth inhibitory activities on human melanoma cells, mainly due to its antioxidant properties [78,79]. Quercetin, a dietary flavonoid, is also exerting anti-melanoma activities by inhibiting STAT3 signaling and modulating oxidative stress-induced melanogenesis [80,81]. A recent, promising paper was describing the melanogenesis alteration effect of *Achillea millefolium* essential oil in melanoma cells *via* suppression of oxidative stress [82].

Conclusion

As the whole melanogenesis process develops inside the melanocytes, they will constantly be exposed to oxidative stress by the chemical characteristics of the melanin production, which is a chain of oxidizing reaction, and due to the production of intermediary oxidative species or end products such as pheomelanin which possess prooxidant properties. While pheomelanin appears to be clearly prooxidant, eumelanin is more likely considered as a scavenger of ROS [83], which means that the ratio eumelanin/pheomelanin will be crucial for assessing the prooxidant vs. antioxidant properties of melanin. This is probably an explanation for the controversy still existing with regards to the exact behavior of melanin on oxidative stress.

Along this brief but incomplete review, a prominent role could be attributed to oxidative stress in the pathogenesis of various dermatological diseases related with melanogenesis, namely melasma, vitiligo or melanoma.

However, it can be observed that oxidative stress can lead to either hypopigmentation (vitiligo) or hyperpigmentation (melasma) or even carcinogenic process following initial hyperpigmentation (melanoma).

On the other hand, phototype makes us unequal in front of the occurrence of vitiligo, melasma or melanoma. This is probably worth further investigation.

Finally, in any case antioxidants will have a place in our armamentarium for treating melanogenesis-related disorders.

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