Melatonin and human skin aging

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Like the whole organism, skin follows the process of aging during life-time. Additional to internal factors, several environmental factors, such as solar radiation, considerably contribute to this process. While fundamental mechanisms regarding skin aging are known, new aspects of anti-aging agents such as melatonin are introduced. Melatonin is a hormone produced in the glandula pinealis that follows a circadian light-dependent rhythm of secretion. It has been experimentally implicated in skin functions such as hair cycling and fur pigmentation, and melatonin receptors are expressed in many skin cell types including normal and malignant keratinocytes, melanocytes and fibroblasts. It possesses a wide range of endocrine properties as well as strong antioxidative activity. Regarding UV-induced solar damage, melatonin distinctly counteracts massive generation of reactive oxygen species, mitochondrial and DNA damage. Thus, there is considerable evidence for melatonin to be an effective anti-skin aging compound, and its various properties in this context are described in this review.

Introduction

Melatonin (N^1-acetyl-5-methoxytryptamine) was initially isolated from bovine pineal tissue.[1] Subsequently, it was documented that in mammals the nocturnal increase in blood levels of melatonin is almost exclusively a result of its nighttime synthesis and secretion from the pineal gland.[2]

Considering chronobiological aspects of melatonin, it regulates the circadian day-night-rhythm and seasonal bio-rhythms,[3,4] and independent of that, melatonin has been shown in the mammalian system to modulate immune defense responses,[5,6] body weight and reproduction[4] and to exert tumor growth-inhibitory and anti-jet lag effects.[7-10] Additionally, melatonin serves as a direct, receptor-independent potent antioxidant,[11-13] a chemotoxicity-reducing agent,[14-16] a putative general anti-aging substance[17,18] and an anti-cancer agent.[19]

For decades, investigations concerning occurrence of melatonin in different body compartments revealed that significantly high concentrations are found in the bile fluid,[20] bone marrow,[21] cerebrospinal fluid,[22] ovary,[23] eye,[24] lymphocytes[5] or skin[25] and is differentially distributed in subcellular organelles.[21,26-28] It was reported that melatonin levels in organs mentioned above may

be 10- to 1000-fold higher than in the plasma.[27,28] High concentrations of melatonin across different organs suggest an ubiquitous, biologically highly relevant existence of tissue-specific, local melatoninergic systems which have the biological role of counteracting specific tissue-related regional stressors exactly at the place where they occur.[20,21,25,27] In the skin, a melatoninergic antioxidative system (MAS) has been recently discovered in a highly differentiated manner regulating skin homeostasis and—very importantly—having the potential to prevent the harmful consequences of UV solar skin damage, i.e., skin aging and skin cancer.[25,29,30]
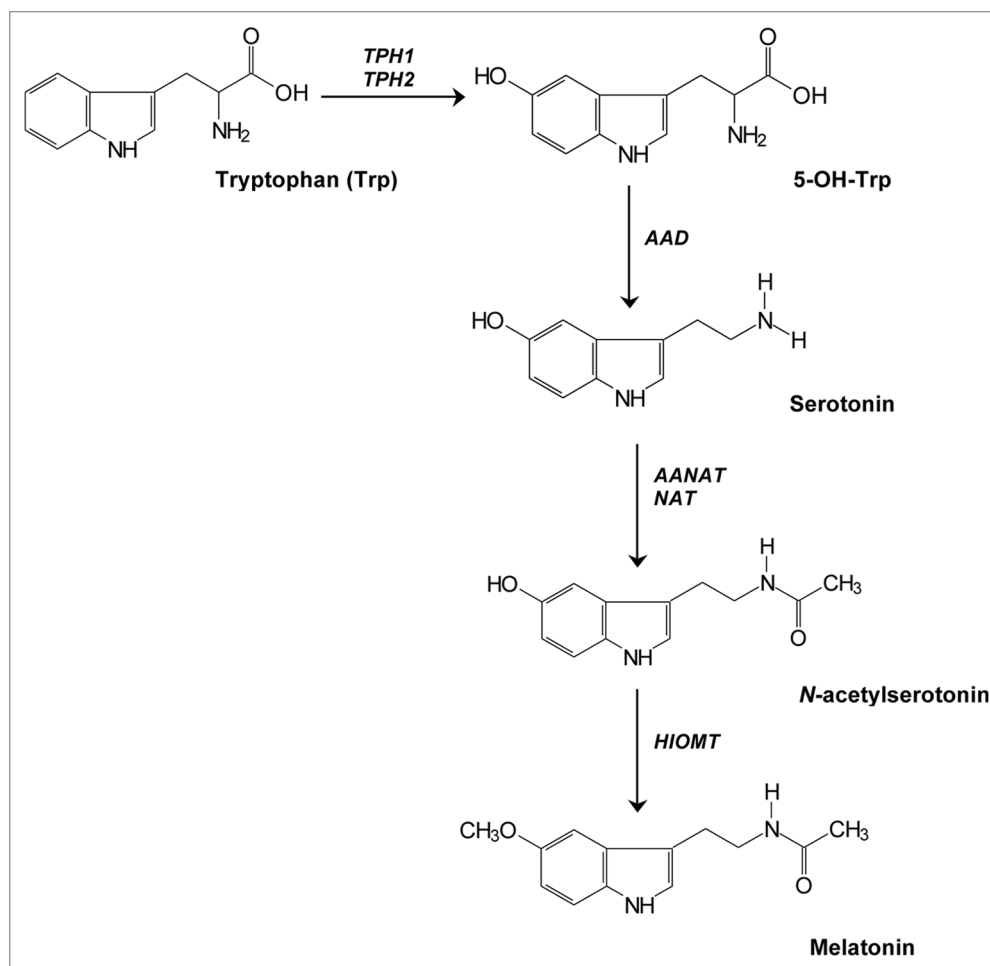
Cutaneous Synthesis of Melatonin

Most of investigations regarding the different aspects of melatonin confirm that, both, biosynthetic and biodegradative pathways of melatonin are observed in whole human and rodent skin and in the major cutaneous cell populations.[31] The most important compound for intracutaneous synthesis of melatonin (**Fig. 1**) is an amino acid, tryptophan (Trp) which is converted by tryptophan hydroxylase (TPH1, TPH2) to 5-OH-Trp and further to serotonin by activity of aromatic amino acid decarboxylase (AAD). In fact, serotonin is essential in the melatonin biosynthesis pathway, nevertheless it has independent biological actions by itself and enters degradation independently of melatonin.[32] Subsequently, occurring acetylation of serotonin mediates formation of N-acetylserotonin (NAS) catalyzed by either arylalkylamine N-acetyltransferase (AANAT) and/or arylamine N-acetyltransferase (NAT). Finally, NAS produced in the skin may be released into the circulation or stay in the cutaneous tissue and thereafter could be transformed into melatonin after active hydroxyindole-O-methyltransferase (HIOMT).[32,33] Expression of these enzymes has been consistently demonstrated in human skin cells.[32]

Melatonin Receptors

The hypothesis that all actions of melatonin are mediated via specific receptors in cellular membranes has also been markedly modified in recent years. Previous studies indicated that the receptors for melatonin were primarily located within cells in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus.[34] While the cells of the SCN contain large numbers of membrane receptors, they have also been found to be more widely distributed not only in the brain but in many other organs as well.[35] This implies evidently that the actions of melatonin are extremely widespread. Phenotypic effects of

Figure 1. Synthesis of melatonin in the skin. It is involved sequential transformation of tryptophan in a cascade of enzymatic reactions catalyzed by tryptophan hydroxylase (TPH1, TPH2), amino acid decarboxylase (AAD), arylalkylamine N-acetyltransferase (AANAT), arylamine N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT).

melatonin can be mediated through interaction with the G protein-coupled membrane bound MT1 and MT2 receptors[36] or with nuclear receptors of RZR/ROR subfamily of orphan receptors.[37,38] The melatonin receptor type 3 (MT3) has been identified to be the enzyme quinone reductase II (NQO2)[39,40] however, an alternative explanation of its role could be the function of melatonin as a co-factor or regulator of the enzyme NQO2.[31] Expression of membrane-bound cell surface MT receptors in the skin is variable, depending on the species. For example, skin from the C57BL/6 mouse predominantly or exclusively expresses MT2,[41] while human skin expresses both receptors, although with a strong bias toward MT1 (the predominant form found in both whole skin and cultured cells) (**Table 1**).[42,43] Immunocytochemical studies on the human skin showed cell-type- and compartment-dependent expression of MT1 and MT2 proteins (**Table 2**).[31]

The nuclear receptor RORα (retinoid-related orphan receptor α) contains at least four splicing variants: RORα1, RORα2, RORα3 and RZRα (RORα4).[31,37,38,43] All of the tested skin cells expressed at least one of three RORα isoforms while RORα3 was consistently absent (**Table 3**).[10,31]

Melatonin as an Activity Enhancer of Antioxidative Enzymes

Melatonin was discovered to be a free radical scavenger two decades ago.[11] However, the data documenting its ability to overcome oxidative stress has accumulated at a rapid pace and is now abundant.[44-46] The efficacy of melatonin in functioning in this capacity relates to its direct free radical scavenging actions.[44] The chemical formula of melatonin allows to interact with various forms of free radicals such as H_2O_2, $^•OH$, singlet oxygen ($^1O_2$), superoxide anion ($^•O_2^-$), peroxynitrite anion ($ONOO^-$) and peroxyl radical ($LOO^•$).[12] It should be noted that melatonin's antioxidant mechanism of action implies a free radical scavengers cascade in case of UV irradiation. Metabolites of melatonin degradation are formed such as N^1-acetyl-5-methoxykynuramine (AMK) or N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), the main photoproducts and simultaneously potent antioxidants.[25,47] Moreover, investigations using cell-free melatonin containing systems exposed to UV radiation (UVB: 60%, UVA: 30%) revealed four metabolites identified by HPLC and LC-MS: 2-OH-melatonin, 4-OH-melatonin, 6-OH-melatonin

and AFMK.[25] Since these metabolites are partly or fully potent antioxidants, this may suggests that melatonin metabolites, unlike classic antioxidants, do not induce prooxidant reactions.

Melatonin acts as a potent antioxidative agent also by indirect effects through enhancing activity of antioxidative enzymes.[29] Of note, not only enzyme activity, but also gene transcription of antioxidant enzymes such as manganese superoxide dismutase (Mn-SOD), copper-zinc superoxide dismutase (Cu/Zn-SOD), GPx and gamma-glutamylcysteine synthetase (γ-GCS), the rate-limiting enzyme of glutathione (GSH) synthesis,[48-50] was upregulated by melatonin during porphyrin-induced cell damage in rat brain cortex and in neuronal cell lines.[48] This prolonged elevation suggests a possible involvement of membrane and/or nuclear melatonin receptor activation with signal transduction on transcriptional mRNA level to modify the regulation of antioxidant enzymes by melatonin following outer signals to stress.[51,52]

Currently, there are some proposals that melatonin-mediated expression of antioxidant enzymes is dependent of signal transduction pathways related to membrane, cytosolic and nuclear receptors,[53] but this hypothesis still needs to be elucidated.

Melatonin as a Protector Against UV-Induced Skin Aging

Because of its broad antioxidant and radical scavenger properties,[12] melatonin may act as a protective agent against UV-mediated damage in the skin.[25,29] Clinical studies indicated that melatonin is able to prevent sun damage only when it is administrated before UV irradiation to be present in relevant concentrations directly at the irradiation site upon UV exposure.[55-57] These melatonin protective effects against UV-induced damage have strong experimental support from in vitro studies.[58-63]

Melatonin increased cell viability in UV-irradiated fibroblasts by counteracting the formation of polyamine levels,[58] and accumulation of malondialdehyde while decreasing apoptosis cells.[59] According to investigations performed by Ryoo et al.[59] in UV-exposed fibroblasts, only 56% of the cells survived upon UV exposure (140 mJ/cm^2), while cells preincubated with 1 nM melatonin revealed a cell survival rate of 92.50% which was paralleled by significant decrease of lipid peroxidation and cell death. Comparative experiments using UV-treated fibroblasts showed similar correlation in cell viability in presence of 100 nM melatonin.[58]

Moreover, human keratinocytes, the main target cell population in epidermal photodamage, irradiated at increasing doses (10, 25, 50 and 100 mJ/cm^2) were investigated for proliferation, colony formation and induction of apoptosis (TUNEL positivity), respectively. Here, melatonin at 10^{-3} and 10^{-4} M significantly protected keratinocytes against UV-mediated apoptosis, thus ensuring cell survival.[60] Melatonin was also determined as a crucial agent that downregulates expression of genes playing an important role in the execution of UV-induced skin photodamage: aldehyde dehydrogenase 3 type A1, interstitial collagenase (MMP-1), stromelysin 1 (MMP-3) or stromelysin 2 (MMP-10).[31] Additionally, melatonin was described as an effective anti-apoptotic compound that inhibits mitochondria-dependent (intrinsic) apoptosis through inhibition of caspase 9 and caspase 3, but does not inhibit the receptor-dependent (extrinsic) pathway of apoptosis mediated by caspase 8.[61] It reduces dissipation of mitochondrial transmembrane potential, cleavage of caspases or activation of poly(ADP-ribose) polymerase (PARP), a key DNA-repair-mediating enzyme.[61] All these events are supposedly caused by UV-induced mitochondrial ROS (mROS) generation which are effectively reduced by melatonin at the concentrations of 10^{-6}, 10^{-4} and 10^{-3} M.[61] On a cellular level, melatonin also significantly reduced UV-induced detachment of keratinocytes in culture representative for apoptotic cells. These observations underline the direct and potent protective actions of melatonin in vitro related to molecular and cellular consequences of UV-induced apoptosis.[29]

Clinical investigations with patients were performed on 20 healthy volunteers who were treated with 0.6 mg/cm^2 melatonin or vehicle either 15 min before or 1, 30 or 240 min after UV exposure.[54] The irradiation was performed with solar spectrum, i.e., UVA and UVB light at a wavelength range 290–390 nm. UV-mediated erythema was assessed 24 h post-UV by visual score and chromametric method. It was reported that melatonin application 15 min prior to UV-irradiation significantly suppressed erythema compared to treatment with vehicle alone. Interestingly,

Table 1. Expression of membrane receptors for melatonin regarding tissue and cell type (adapted from Slominski, et al.[42])

Tissue/cell type	MT1	MT2
Brain (whole tissue)	+	+
Pituitary	+	abberant
Adrenal gland	+	-
Skin normal	+	-
Skin with basal cell carcinoma	+	-
Epidermal keratinocytes	+	-
Hair follicles keratinocytes	+	-
Neonatal keratinocytes	+	+
HaCaT keratinocytes	-	abberant
Epidermal melanocytes	+	-
Hair follicles melanocytes	-	-
Dermal fibroblasts	+	-
Hair follicles papilla fibroblasts	+	abberant

+, positive; -, negative abberant, alternatively spliced isoform.

Table 2. Localization of membrane MT1 and MT2 receptors for melatonin in human scalp skin (adapted from Slominski, et al.[31])

Localization	MT1	MT2
Epidermis	++ (Stratum granulosum) + (Stratum spinosum)	-
Eccrine glands	+++	+++
Blood vessels	+++ (Endothelium)	++ (Endothelium)
Hair follicles	+ (Upper outer root sheath) + (Inner root sheath)	+ (Inner root sheath)

+, weak; ++, moderate; +++, strong; -, negative.

Table 3. Gene expression of cytosolic melatonin-binding site (MT3/NQO2) and nuclear receptors RORα including selected isoforms RORα1 and RORα4/ RZR that are relevant in normal skin cells and malignant melanoma cells (adapted from Fischer, et al.[10]; Slominski, et al.[31])

Cells		Species	Detection	Cytosolic melatonin- binding site	Nuclear receptor	Nuclear receptor splicing variants	
				MT3/NQO2	RORα	RORα1	RORα4 (RZRα)
Normal skin cells	Adult epidermal keratinocytes	Human	RT-PCR	+	+	-	+
	Neonatal epidermal melanocytes		RT-PCR	+	+	-	+
	Adult dermal fibroblasts		RT-PCR	+	+	+	+
Melanoma cell lines	SKMEL-188	Human	RT-PCR	-	-	-	-
	WM 164		RT-PCR	+++	+	-	+
	WM 98		RT-PCR	++	+	-	+
	SBCE2		RT-PCR	+	+	-	+

+, present; -, absent.

treatment with melatonin at any time point after UV irradiation did not suppress UV erythema.[54] Dose-response investigations revealed that 0.5% melatonin was the most potent suppressive concentration against UV-erythema.[55]

This observation was confirmed by another series of experiments showing that melatonin, but also other antioxidants (vitamin E and vitamin C) have no effect on UV-erythema when administered after UV-irradiation, irrespective of the time-course of application.[57] Taking into account that the UV-induced free radical formation in the skin is an immediate event directly upon UV irradiation, the oxidative stress leading to all known consecutive damaging events in the skin can obviously only be antagonized by antioxidants such as melatonin that are already present at the target sites at the time point of UV-exposure.[29,54-56,61]

Besides, there is clear evidence that the protective effects of melatonin against photobiological disturbances are mediated by the strong antioxidative properties of this compound. It was shown that melatonin has a higher reduction potential (0.73 V) than vitamin C (0.23 V).[62] Interestingly, formation of highly toxic hydroxyl radicals occurred in the presence of certain concentrations of vitamin C, while to date melatonin has not demonstrated such pro-oxidant properties. Considering that UV-induced ROS generation is tightly connected with photodamage, it was shown that melatonin is a significantly stronger scavenger of free radicals compared with vitamin C or Trolox, a vitamin E analog.[63]

UV-Enhanced Melatonin and Metabolites Production in Skin Cells

To date, the described protective effects of melatonin against UV induced solar skin damage were exclusively shown from investigations with exogenously added melatonin to skin cells. Since in 2002 Slominski et al.[32] reported that skin cells of multiple types (normal and immortalized skin keratinocytes, hair follicle keratinocytes, fibroblasts from dermis and hair follicle dermal papilla, melanocytes, melanoma cells and squamous cell carcinoma cell lines) and cutaneous tissue samples from benign as

well as malignant skin phenotypes (skin of basal cell carcinoma) express the essential enzymes for melatonin synthesis such as TPH, AANAT and HIOMT, it seemed very likely that melatonin can be produced in the skin as an extrapineal site of melatonin synthesis.[32]

Systematic investigations in keratinocytes under different experimental conditions of non-melatonin and melatonin supplemented keratinocyte cultures revealed time- and UV-dependent modifications of melatonin production or metabolization to specific melatonin degradants and—of note—definite melatonin detection in keratinocyte cell extracts at defined measurable levels.[25] Specifically, it was shown by HPLC of keratinocyte cell extracts that "naïve" (i.e., non-melatonin pre-incubated) keratinocytes showed intracellular melatonin levels of 146.0 pmoles/ 3×10^6 cells, thus indicating autonomous melatonin synthesis by human keratinocytes at a level of approximately 11 fg per single keratinocyte. Given that the cell volume of a HaCaT keratinocyte is approximately 1.43×10^{-6} μl,[64] the calculated intracellular melatonin concentration per keratinocyte, can be appreciated to be around 34 μM. Evaluation of melatonin levels after 24 h of keratinocyte proliferation in culture showed decrease of melatonin down to 65.0 pmoles/3×10^6 cells, while there was a time-parallel increase of the melatonin metabolites 2-hydroxymelatonin from 7.8 pmoles/3×10^6 to 20.4 pmoles/3×10^6 cells and AFMK from 17.4 pmoles/3×10^6 to 33.6 pmoles/3×10^6 cells, thus indicating a 24 h consumption of melatonin with metabolism to above mentioned melatonin metabolites.[25] AFMK is also known to have anti-inflammatory properties, e.g., reduction of LPS-induced COX-2 upregulation, decrease of inducible nitric oxide synthase (iNOS) and prostaglandin E2 (PGE2).[65] Moreover, AFMK inhibits 5-aminolevulinic acid-induced DNA damage[66] and prevents protein destruction,[67] therefore representing a potent antioxidative and anti-inflammatory agent against many different types of UV-induced solar damage.

These observations have also to be seen in the context of the study setting in which they were generated. While earlier studies had investigated melatonin metabolites only in UV wavelengths
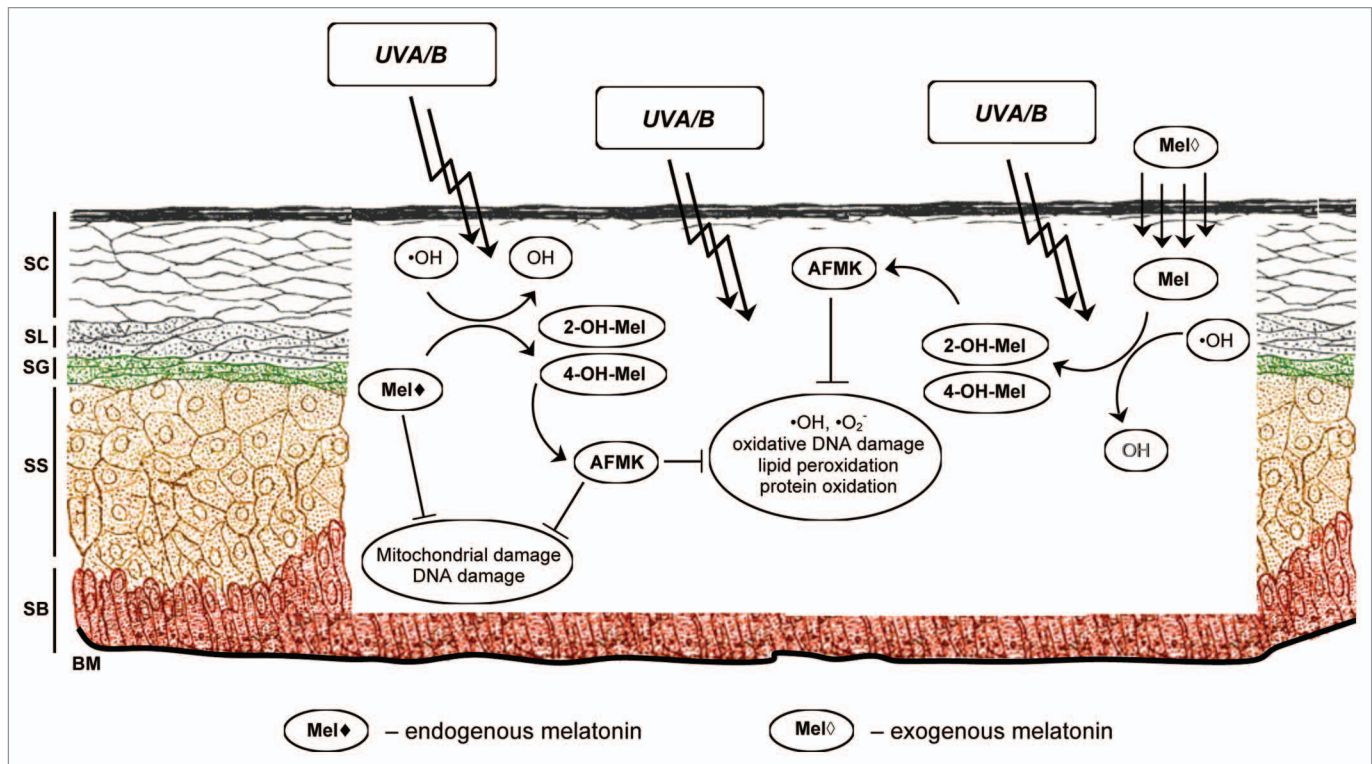
Figure 2. The melatoninergic antioxidative system in human skin. Parallel to directly scavenging UVB-induced reactive oxygen species (ROS), namely hydroxyl radicals, melatonin is transformed to 2-hydroxymelatonin, 4-hydroxymelatonin and consecutively to AFMK. All metabolites are strong antioxidants themselves creating a potent antioxidative cascade that reduces lipid peroxidation, protein oxidation, mitochondrial and DNA damage. SC, stratum corneum; SL, stratum lucida; SG, stratum granulosum; SS, stratum spinosum; SB, stratum basale; BM, basement membrane.

that are not essentially related to UV induced stress in cutaneous biology in naturam (300–575 nm, UV-visible light),[68] the above mentioned and more recent studies have developed a human skin related approach by using relevant UV wavelengths within UVB/UVA range and also a mixed UV-source (UVB 60%; UVA 30%) that is closer to the naturally occurring solar irradiation.[25]

While earlier studies with detection of melatonin metabolites were performed in cell-free systems,[69,70] the recent studies with melatonin provided the first evidence for UV-enhanced photolytic and/or enzymatic melatonin metabolism in cultured human keratinocytes.[25] Therefore, it can be concluded with almost certainty, that human keratinocytes, and likely the skin and its appendages in vivo themselves as well, are not only targets for protective exogenous melatonin treatment, but also an extrapineal site of melatonin synthesis and of fully functioning local autonomous melatonin metabolism.[25,32,42] Since cutaneous local synthesis and metabolism are inducible by UV-irradiation,[25] it can be further postulated that the skin provides itself a self-regulated protective system that is switched on by environmental stressors such as UVR and ionizing radiation, and also by inflammation.[54,55,57,60,70-74]

The Melatoninergic Antioxidative System (MAS) of the Skin

Melatonin is alleged as a major pineal hormone with activity of neurotransmitter, cytokine, antioxidant and global regulator of

circadian clock.[74] As mentioned before, synthesis of melatonin is not restricted to the pineal gland, but extends also to various other organs including the skin.[5,20-25] Since UV-exposed skin mediates melatonin metabolism leading to the generation of antioxidant melatonin metabolites in human keratinocytes that exert strong antioxidative properties themselves, an antioxidative cascade can also be postulated for the skin in analogy to previously described melatonin-related antioxidative cascades in chemical or other tissue homogenate systems.[47,69,75] This cascade has been defined as the melatoninergic antioxidative system (MAS) of the skin (**Fig. 2**) which would protect the skin as an important barrier organ against UV-induced oxidative stress-mediated damaging events on nuclear, subcellular, protein and cell morphology level.[25] Regarding the chemical structure, melatonin as well as all metabolites are strongly lipophilic, an important fact which would render them able to diffuse easily in every skin and cell compartment, therefore extending the MAS beyond the epidermis, namely to the dermis and the hair follicle[31,76,77] where it represents a defense mechanism against the multifaceted threats of environmental stress, especially UVR, to which the skin is life-long exposed.[31,70,78,79]

One of the most reactive radicals, the hydroxyl radical, occurs under UV irradiation in the skin and reacts directly with melatonin.[11,78,80,81] Melatonin is either autonomously produced in epidermal and/or hair follicle keratinocytes where it engages in intracrine signaling/interactions or is released into

the extracellular space to regulate auto-, para- or endocrine signaling.[25,76,82] The reaction of melatonin with hydroxyl radical induces the formation of 2-OH-melatonin and 4-OH-melatonin which are then further metabolized to AFMK and by arylamine formamidase or catalase to AMK.[69,83] The effective scavenging of hydroxyl radicals indirectly or directly mediates the reduction of lipid peroxidation, protein oxidation, mitochondrial damage and DNA damage. This makes the melatoninergic antioxidative cascade very potent in reducing the extensive amounts of free radicals occurring under UV solar radiation and therefore represents a very promising strategy to protect the skin against this main environmental stressor and causative factor for skin aging and tumor promotion.

Conclusions

Aging of skin is a complex process, taking place over the many years of a human life-span. In addition to endogenous factors, several environmental factors contribute to this process and sometimes accelerate aging. Therefore, numerous investigations within dermatological science and especially dermato-endocrinology are having the aim to develop effective anti-aging agents and one of the very highly promising candidates is the indol melatonin. For years and in many investigations, great and convincing evidence revealed that melatonin with its strong antioxidative properties shows a tremendously wide spectrum of action such as bioregulatory as well as pluripotent and essential protective effects in many cells, tissues and compartments of unicells, animals and the human body.[47] The predominant feature of melatonin therein is that of a potent cyto- and tissue-protective substance on multiple molecular and cellular damage levels and mechanisms, both in physiological and pharmacological concentrations.[27,28,47,82]

The fact that the essential enzymes for melatonin synthesis are expressed in skin cells and cutaneous tissue at a great variety, and that skin cells are able to produce autonomously melatonin and to develop metabolism with the generation of metabolites with strong antioxidative properties, renders the skin a major extrapineal site of melatonin production and activity.[25,32,42] Moreover, solar UV irradiation is one of the main environmental skin stressors and it is significantly counteracted or modulated by melatonin in the context of a complex intracutaneous melatoninergic antioxidative system of the skin.[25,29,30,60,61]

Regarding clinical application, exogenous melatonin should rather be used topically than orally, since orally administered melatonin appears in rather low levels in the blood due to prominent first-pass degradation in the liver, thus limiting skin access.[84] Topical application might be meaningful, since melatonin can penetrate into the stratum corneum and build there a depot due to its distinct lipophilic chemical structure.[85] Therefore, endogenous intracutaneous melatonin production, together with topically applied exogenous melatonin or metabolites can be expected to represent one of the most potent antioxidative defense systems against UV-induced skin aging.[25,29-31]

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