Review Article

Iron chelators may help prevent photoaging

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Summary

For years, cosmetic ingredients for anti-aging treatments have attracted consumers. Skin aging is accelerated by reactive oxygen species (ROS), generated by exposure to solar ultraviolet radiation (UVR), in a process known as photoaging. Because cutaneous iron catalyses ROS generation, it is thought to play a key role in photoaging. Iron is essential to almost all forms of life. However, excess iron is potentially toxic as its catalytic activity induces the generation of ROS. Iron-catalysed ROS generation is involved in numerous pathological conditions, including cutaneous damage.

When skin is directly exposed to UVR, cutaneous intracellular catalytic iron levels increase because of the release of iron from iron-binding proteins such as ferritin. Consequently, the subsequent ROS generation may overwhelm cutaneous defense systems such as the cellular iron sequestration and ROS scavenging capacity.

The harmful role of excess cutaneous iron implies that there may be a potential for topical iron chelator treatments. We now consider cutaneous photodamage skin photoaging as the result of iron-catalysed ROS generation and discuss preventative strategies based on iron chelators.

Keywords: antioxidant, Fenton reaction, free radical, iron, iron chelator, skin photoaging, ultraviolet light, radiation, reactive oxygen species

Introduction

For years, anti-aging cosmetics have attracted consumers with their expected ability to reduce wrinkles and other signs of skin aging. Retinol, α-hydroxy acid (AHA), and various sunscreen agents are components of anti-aging products in the current skin care market. Skin aging is accelerated by reactive oxygen species (ROS), generated by exposure to solar ultraviolet radiation (UVR), in a process known as photoaging.[1,2] Therefore, antioxidants, such as ascorbate and α-tocopherol, are often incorporated into anti-aging products. Recently, coenzyme Q10 (ubiquinone) has appeared on the market as a powerful antioxidant. Several antioxidants have been shown experimentally to reduce the various harmful effects induced by UVR.[3,4] Iron is thought to play a key role in the generation of ROS by UVR.[5,6] Iron is essential for critical life functions, including oxygen transport, energy metabolism and cell proliferation. However, it has been reported that iron causes oxidative stress in the body.[7] One of the deteriorative effects of iron is its catalysis of ROS generation. As shown in Fig. 1, iron participates in the Fenton reaction to catalyse the reduction of hydrogen peroxide to a hydroxyl radical (•OH), which is a highly reactive and largely indiscriminate oxidant[5,6] as follows:

$$H_2O_2 + Fe^{2+} \rightarrow OH + OH^- + Fe^{3+}$$

The iron is sequestered by iron-binding proteins, such as transferrin and ferritin, under normal conditions and does not participate in ROS generation.[8] However, iron is released from these proteins when the body is exposed to oxidative stress.[7] This free iron catalyses ROS generation by the mechanism described above (Fig. 1). Iron-catalysed ROS formation is involved in numerous pathological conditions, including UVR-induced skin damage,[9] coronary heart disease,[10] atherosclerosis[11] and Alzheimer's

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Accepted for publication April 4, 2004

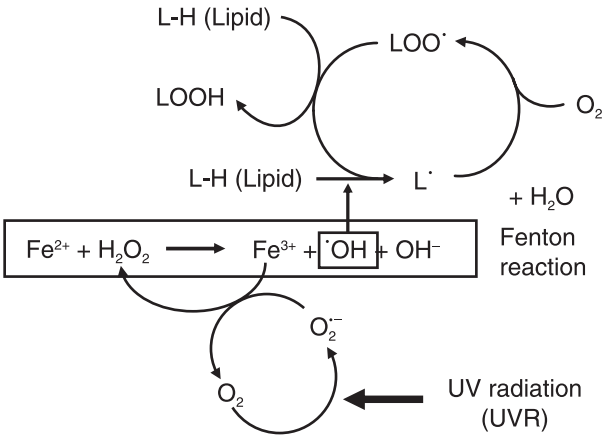

Figure 1 Role of iron in UV-induced reactive oxygen species (ROS) generation and lipid oxidation in cells and tissues.[5,6]

disease.[12] Therefore, the suppression of ROS generation should help to protect the skin against the aforementioned damage. One of the approaches to suppressing ROS generation is to inhibit the catalytic activity of free iron. Several iron chelators have been reported to suppress ROS generation and the ensuing pathological conditions.[13–15] These results have shown that the design of a suitable iron chelator to reduce oxidative stress will lead to protection against several types of oxidative damage including photoaging. Among them, we have focused our attention on the reduction of UV light-induced oxidative stress in skin by iron chelators. We now consider skin photoaging as the result of iron-catalysed ROS generation and discuss preventative strategies based on iron chelators.

Iron ion-induced damage in skin

UV-induced ROS generation

Skin is an organ that is directly exposed to both UVR and concentrated oxygen. Therefore, we can easily imagine ROS generation in skin irradiated by solar radiation containing UVR. Darr and Fridovich reviewed the influence of ROS generation with a focus on the skin.[16] For example, the hydroxyl radical, which is an extremely reactive species, is thought to be generated via the Fenton reaction coupled with cutaneous iron ions. Singlet oxygen seems to be generated by the excitation of an oxygen molecule in the presence of a cutaneous photosensitizer. Nitric oxide (NO) as well as ROS are formed in cutaneous cells and NOS is thought to be involved in signal transduction pathways in the skin.

Electron-spin resonance (ESR) is often used for real-time measurements of ROS generation. This technique revealed that a hydroxyl radical and a superoxide anion were generated by the UVB irradiation of murine keratinocyte homogenates.[5,17] In addition, a lipid peroxide radical has been detected at low temperature (−150 °C). The generation of these radicals is thought to be due to cutaneous iron ions, because the radicals disappeared with the addition of an iron chelator.

When intact or homogenized skin is exposed to UV irradiation, the cutaneous ascorbate is oxidized to an ascorbate radical via reactions with ROS and / or by direct oxidation. Therefore, the ascorbate radical is thought to be an indicator of oxidative stress in skin. Jurkiewicz and Buettner showed that the ascorbate radical was directly detectable by ESR and, using this technique, they investigated the ROS generation mechanism in skin exposed to UVR.[18–20] These investigators found that an iron chelator, desferrioxamine (DFO), suppressed the appearance of the ascorbate radical.[18–20] Using a murine skin homogenate exposed to UVR, we have shown that the addition of iron ions accelerated ascorbate oxidation.[21] These results suggest that the iron ion plays an important role in ascorbate radical generation in the skin.

The role of the iron ion which catalyses hydroxyl radical generation via the Fenton reaction is significant because the hydroxyl radical is the most reactive moiety among ROS. It reacts directly with biomolecules, such as lipids and DNA, and causes lipid peroxidation and DNA damage, followed by aberrant protein formation.[22,23] Thus, ROS and iron ions are deeply involved in the generation of skin damage.[24,25]

Cutaneous iron ions are usually stored in iron-binding proteins, such as ferritin and transferrin, and are thus in an inactive form in terms of their catalytic activity in ROS generation. However, various oxidative stresses, including UVR, seem to induce the release of iron ions from iron-binding proteins. The released iron causes ROS generation via the Fenton reaction.[26] In addition, the released iron also induces direct oxidation of the biomolecule via an equilibrium between the ferric and ferrous ions.[27]

Implication of iron ions in UVB-induced damage

Solar UVR is classified into UVA (320–400 nm) and UVB (290–320 nm). Many reports have shown that iron ions are involved in skin damage induced by UVR. Bissett *et al.* showed the deposition of free iron in the dermis in a histological study of UVB-irradiated hairless mouse skin. In addition, a human histological study revealed that the iron content of sun-exposed body sites, such as the face and neck, was ≈ 2–3 times higher than that of non-exposed sites, such as the buttock and thigh.[9]

Brenneisen *et al*. found that the iron chelator, DFO, and the hydroxyl radical scavengers, dimethylsulfoxide (DMSO) and Trolox, suppressed UVB-induced collagenase (matrix metalloprotease-1; MMP-1) expression in dermal fibroblasts.[28] Collagenase expression is thought to be involved in skin photoaging because dermal extracellular matrix components, such as collagen, are severely damaged in photoaged skin. These results indicate that iron is involved in UVB-induced ROS generation and the ensuing photoaging.

Implication of iron ions in UVA-induced damage

In terms of UVA-induced photodamage, reports have shown that singlet oxygen ($^1O_2$), but not a hydroxyl radical, plays a principal role in UVA-induced cytotoxicity.[29] UVA-induced photosensitization of porphyrin is a possible mechanism for singlet oxygen generation.[30]

Although the aforementioned report stressed the importance of the involvement of singlet oxygen, the involvement of iron ions in UVA damage has been described in several reports. Morlière *et al*. showed that the UVA-induced cytotoxicity of dermal fibroblasts increased with iron ion load. Incubation with DFO led to a protective effect on cells exposed to UVA.[31] Petersen *et al*. reported that the intracellular levels of H_2O_2 were increased in UVA-induced DNA damage of keratinocyte cell lines.[32] Petersen *et al*. demonstrated that DFO suppressed both H_2O_2 production and DNA damage, whereas $^1O_2$ scavengers, such as sodium azide and β-carotene, did not. Moreover, these forms of damage were stimulated by the addition of iron ions. Vile and Tyrrell reported that iron ion and $^1O_2$ were involved in the UVA-induced oxidation of lipids and proteins in dermal fibroblasts.[33] They demonstrated that the iron ion was released from the iron-binding protein by UVA. Pourzand *et al*. found that ferritin in dermal fibroblasts also decomposed upon UVA irradiation of the cells, and the amount of free iron increased.[34] These results indicate that both the Fenton reaction with iron ions and photosensitization with $^1O_2$ play key roles in UVA-induced damage. Thus, free iron ions have a harmful role in cutaneous photodamage induced by UVA as well as UVB.

The cutaneous defense system and its limitations

ROS scavenging by antioxidants

Cutaneous antioxidants play an important role in the skin's defense system against the oxidative stress caused by iron ions and UVR. They range from low molecular mass compounds, such as α-tocopherol, ascorbate, glutathione, and ubiquinone, to proteins, such as superoxide dismutase (SOD) and catalase. These antioxidants work by scavenging ROS and returning the oxidized species to their reduced state.[35] However, UV irradiation reduces the amounts of these antioxidants and hinders the cutaneous defense system.[36–38] Moreover, it has been reported that antioxidants may further exacerbate iron-induced damage (pro-oxidant effect). The ability of antioxidants to reduce ferric ions to ferrous ions promotes the generation of hydroxyl radicals via activation of the Fenton reaction.[39–42]

Sequestering iron ions by ferritin

In cells, the excess iron required for metabolism is stored in ferritin.[8] UVA-induced oxidative stress of human epidermal keratinocytes and dermal fibroblasts leads to increased levels of ferritin as well as iron ions.[43] Vile *et al*. showed that the induction of ferritin reduced cell membrane damage caused by UVA irradiation, suggesting that enhancement of the cellular iron sequestering capacity increased resistance to UVA stress.[44] Cai *et al*. found that ferritin induction suppressed UV-induced DNA strand breaks.[45] Vile and Tyrrell reported that the foregoing activation of heme oxygenase by UVA irradiation was closely associated with an increase in the cellular ferritin level.[46] Ferritin constitutes the storage for intracellular free iron via heme oxygenase activation, and suppresses various iron-catalysed harmful reactions. Ferritin induction also occurs in human skin *in vivo*. Immunohistochemical analyses revealed that ferritin is increased in both epidermal and dermal tissue.[47,48] Therefore, it is believed that ferritin induction plays a protective role in the skin.

However, as previously mentioned, UVA exposure caused ferritin to decompose and free iron to increase in dermal fibroblasts.[34] Therefore, it seems that ferritin photoprotection has limitations, especially with an excess of UVA.

Applications of iron ion chelators

Strategy of molecular design

The harmful role of free iron ions and the limitations of the defense systems against cutaneous photodamage indicate the value of topical iron chelator treatments. That is, sequestering iron using chelating agents is an effective approach to prevent the generation of deleterious ROS that cause UV-induced damage. There are six binding

sites in iron ions. In general, when all the sites are occupied by a ligand molecule (chelator) the iron complex no longer catalyses the Fenton reaction. Therefore, the chelators need to be able to form a stable complex with the iron ions. An effective stability constant, log K_{eff}, was a suitable parameter to estimate the stability under physiological conditions. Shalev and Hebbel showed that chelators with a high value of log K_{eff}, such as DFO, effectively sequestered cellular iron ions.[49]

The formation of a stable iron complex is necessary, but not sufficient, for the inactivation of iron ions. The toxicity of Fe^{2+} is far higher than that of Fe^{3+}, because Fe^{2+} acts directly as a catalyst for the Fenton reaction. The Fe^{3+}/Fe^{2+} equilibrium in a chelator–iron complex is determined by the reduction–oxidation (redox) potential of the complex. In general, a complex with a low redox potential reduces the ratio of the Fe^{2+} concentration.[60] The DFO–iron complex shows a fairly low redox potential (−440 mV *vs*. normal hydrogen electrode).

These suitable physical properties of DFO, with respect to the redox potential as well as the stability constant, seem to lead to the suppression of iron-induced damage.[51] The various efficacies of DFO are described below. In contrast, EDTA, which is a typical iron chelator, is disadvantageous because the redox potential of the EDTA–iron complex is high (120 mV). Actually, there are many reports of the pro-oxidative effects of EDTA. For example, EDTA accelerates ascorbate oxidation in the presence of iron ions.[52]

Efficacy and limitations of DFO

As mentioned above, ROS are generated in human or mouse skin exposed to UVR, and the ROS signals can be detected by measuring the ESR. It has been reported that the ESR signals are diminished by the addition of DFO.[18,20] DFO, like ferritin, may also have a protective role against UVA-induced damage. When dermal fibroblasts were exposed to UVA, heme oxygenase-1 (HO-1) was induced, followed by an increase in the ferritin level, as previously described.[46] However, the addition of DFO to the cells did not enhance the ferritin level, suggesting that DFO sequestered the iron ions, like ferritin.[46]

In contrast to its aforementioned efficacy, DFO has various limitations in terms of its clinical use. It is known that DFO treatment is accompanied by several side effects, such as septicaemia.[53,54] The toxicity of DFO has also been reported in reviews.[55,56] These unfavorable aspects seem to be due to its extremely high affinity for iron ions. Indeed, DFO not only traps free iron but also removes stored iron from iron-storage proteins.[57–59]

Efficacy of iron chelators *in vitro*

Dean and Nicholson investigated the efficacies of typical iron chelators, such as DFO and EDTA, using non-cellular ROS generation systems.[60] Galey *et al*. assessed whether the chelators showed a suppressive effect on iron-induced damage, using dermal fibroblasts. They found that *N*,*N*′-bis-dibenzyl ethylenediaminediacetic acid (DBED) scavenged the hydroxyl radical and was converted to *N*-2-hydroxybenzyl-*N*′-benzyl ethylenediaminediacetic acid (HBBED).[61] HBBED formed a stable iron complex and remarkably decreased the catalytic properties of the iron.[61] HBBED also reduced the cytotoxicity induced by peroxidation.[62] Galey *et al*. advanced the study using structural modifications, and discovered the high efficacy of the esterified derivative of *N*,*N*′-bis(3,4,5-trimethoxybenzyl)ethylenediamine-*N*,*N*′-diacetic acid (OR10141).[63]

The authors have designed amino acid derivatives that mimic the iron ion binding site of iron-binding proteins: *N*-(2-hydroxy-1-naphthol) amino acids and *N*-(2-hydroxybenzyl) amino acids.[64–66] For example, *N*-(2-hydroxybenzyl)-L-serine (HBSer) suppressed UVB-induced cytotoxicity in murine dermal fibroblasts. In contrast, DFO had no effect because of its cytotoxicity.

Recently, Creighton-Gutteridge and Tyrrell found that HBSer sequestered intracellular iron, but its influence on the intracellular states was different from that of DFO.[67] It is known that iron chelation by DFO induces a hypoxic response, for example, the activation of hypoxia-inducible factor-1 (HIF-1).[68] In their study, HBSer was similar to iron-binding proteins in that it sequestered iron without activating HIF-1. These results may be due to iron sequestration by HBSer, in the same way as iron-binding proteins.

Efficacy of iron chelators *in vivo*

Assessments using hairless mice are used widely to investigate the *in vivo* efficacy of materials against photoaging. UV irradiation of the mouse induces various forms of skin damage, such as wrinkle formation, denaturation of dermal matrix components, lipid peroxidation, free iron deposition and so on. It is thought that these changes correspond to those occurring in human skin photoaging.[69,70]

Bissett *et al*. were the first to show the efficacy of the iron chelator using this technique. Wrinkle formation in mouse skin, caused by UVB radiation, was delayed by the application of 2,2′-dipyridyl, 1,10-phenanthroline and 2,2′-dipyridylamine (DPA), which possess iron-binding properties. Skin histological observations revealed that the chelators suppressed UVB-induced epidermal hypertrophy. Bissett *et al*. concluded that these photoprotective

properties were due to sequestration of the iron ion, because DPA significantly suppressed the increase in free iron content in the skin.[9] Later, Bissett and McBride showed the efficacy of a topical iron chelator, 2-furildioxime (FDO).[71] FDO exerted inhibitory effects on UV-induced erythema and epidermal hypertrophy in human skin.[72]

Using hairless mice, we found that *N*-(4-pyridoxy/methylene)-L-serine (PYSerine) delayed UVB-induced wrinkle formation and dermal hypertrophy.[73] The redox potential of the PYSerine–iron complex is −34 mV, which indicates better stabilization of Fe^{3+} by PYSerine compared with EDTA (120 mV). Actually, PYSerine suppressed the Fenton reaction as well as HBSer in an *in vitro* assay.

Kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone), which is widely used as a whitening reagent in Japan, has an iron-chelating function.[74] Recently, kojic acid has been shown to have anti-wrinkle properties and to suppress the deposition of glycosaminoglycan in the dermis of hairless mice irradiated with UVB.[75]

Thus, the protective potential of iron chelators suggests that they could be valuable as anti-aging cosmetic ingredients. The validity of this reasoning should be assessed by testing the efficacy of the iron chelators in clinical human skin studies.

Future directions

Recent advances in molecular biological analyses have revealed several gene expression pathways involved in oxidative damage. In this respect, it has been shown that transcription factors, such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), are regulated by ROS generation and are involved in skin photoaging.[76–78] It has been reported that iron plays an important role in these pathways, and that DFO-modulated NF-κB and AP-1 activation by ROS and UVR.[79–81] We found that one of the aforementioned amino acid-based iron chelators, *N*-(2-hydroxybenzyl)glycine (HBGly), suppressed UVB-induced NF-κB activation in human keratinocytes.[82] These results suggest that photoprotection by iron chelators favorably influences gene expression pathways. A better understanding of these beneficial properties might provide further effective cosmetic products based on iron chelators.

Conclusions

Iron participates in UV-induced skin damage, such as photoaging. The participation of iron involves ROS generation by its catalytic activity. Excess UV exposure to cutaneous cells releases catalytic iron from ferritin and overwhelms its iron-sequestering capacity. These results indicate the value of topical iron chelator treatments. From this viewpoint, the protective potential of iron chelators, in both *in vitro* and *in vivo* assays, has been discussed in this article. These beneficial properties hold great promise for practical applications to prevent chronic photoaging. We wish to make further progress toward the clinical and/or cosmetic utilization of iron chelators.

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