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Review

Photoaging from an oxidative standpoint

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Abstract

The free radical theory proposes that photoaging, which is both qualitatively and quantitatively different from chronological aging, may result from imperfect protection against cumulative stress of free radicals produced by chronic and repeated ultraviolet irradiation. Since the skin is always in contact with oxygen and is occasionally exposed to ultraviolet light, skin is one of the best target organs of environmental photo-oxidative stress. A growing body of evidence suggests that reactive oxygen species are generated by ultraviolet irradiation resulting in the structural and functional alteration of cutaneous components which should affect the photoaging process over a long period. The age-related alteration of cutaneous antioxidant defense capacity against cumulative effects of continual photo-oxidative stress to the skin may also affect the photoaging. Thus the possible use of antioxidants that attenuate photo-oxidative toxicity is believed to be an important strategy modulating photoaging. Several antioxidants have readily been proved to work in the experimental conditions. This paper reviews photoaging from a photo-oxidative standpoint and discusses the possible regulation of photoaging by antioxidants that is an important issue in the photo-dermatological field.

Keywords: Ultraviolet light; Antioxidant therapy; Oxidative stress; Cutaneous changes; Reactive oxygen species (ROS); Actinic damage; Photoaging

1. Introduction

Photoaging is a term that denotes the gross and microscopic cutaneous changes induced by chronic and repeated sun exposure [1]. It had long been believed that photoaging was an accelerated process of chronological aging leading to the older clinical appearance of outdoor workers by cumulative actinic damage. However, the distinction between intrinsic aging and photoaging is becoming evident, and these two conditions are both qualitatively and quantitatively quite different [2]. Since photoaging is extrinsic aging produced mainly by ultraviolet (UV) light, one may infer that photoaging could be prevented and regulated, which is significantly different from chronoaging.

Among the various insults brought by UV irradiation, reactive oxygen species (ROS) and lipid

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peroxides are one of the most reasonable candidates for explaining actinic injuries observed in photoaging. Because the skin is always in contact with oxygen in the presence of surface lipid, and is occasionally exposed to UV light, skin is one of the best target organs of environmental oxidative damage [3,4]. The free radical theory proposes that aging may result from imperfect protection against cumulative oxidative stress caused by free radicals produced in biological systems [5,6]. The UV-induced generation of ROS can result in the structural and functional alteration of cutaneous proteins, e.g. collagen, elastin and glycosaminoglycans (GAGs), which may contribute to photoaging [7]. In this review article, biochemical cutaneous changes of photoaging are reviewed and an attempt is made to explain them from a photo-oxidative standpoint in association with possible antioxidant therapy.

2. Photo-oxidative stress in skin

ROS produced by whatever stimuli including UV light play an important role in modulating the extent of inflammatory response and consequent tissue damage. There is now a growing body of evidence that ROS are generated in vivo by UV irradiation of skin [8]. There is more than enough energy in a photon of UV or visible light to elevate oxygen to the singlet state; however, it is now appreciated that not only singlet oxygen but other species of oxygen intermediates are generated in the epidermis under several conditions of UV radiation [9]. Products of lipid peroxidation, lipid radicals and other free radicals have all been noted in UV irradiated skin [10–12]. In senescence-accelerated mice, the serum and liver lipid peroxide levels rise significantly prior to the appearance of age-related clinical signs [13] and, furthermore, elevated serum lipid peroxide concentrations are transferred through the vascular wall into the skin tissue and provoke the skin symptoms involved in aging [14]. Sunburn cell formation, a hallmark of UVB-damaged epidermis, can be moderated by free radical scavengers [15,16], and contact photosensitization process is implicated to be partly attributable to UV-derived oxidants [17]. More importantly, there are many reports about the relationship between UV irradiation and skin antioxidants supporting the free radical hypothesis for UV light-induced cutaneous damage [18–24]. These reports demonstrate that antioxidant depletion and oxidative damage occur for some time after UV-induced insult in skin.

Another piece of important information suggesting that photoaging may have a free radical component is the age-related alteration of cutaneous antioxidant defense mechanisms. We determined skin superoxide dismutase (SOD) activities of aged people [25] and aged guinea pigs [26] and found no difference with non-aged subjects. However, under the various kinds of oxidative stress, SOD activity is defective in induction in aged subjects indicating that the capacity to play a protective role in defending against overall oxidative damage to cells and augment antioxidant activity under a variety of oxidative stress conditions appears to be subject to age-related alterations. This concept should be compatible with the recent work suggesting that skin aging is not accelerated in old age due to a general decrease in the antioxidant capacity of the tissue [27].

Taking these results into consideration, it is challenging to speculate that the age-related alteration of cutaneous antioxidant defense capacity against cumulative effects of continual photo-oxidative stress to the skin may affect the photoaging process over a long period. It should be answered if changes seen in vivo in photoaged skin can be reproduced in vitro with repeated UV irradiation and/or exposure to free radical generating systems.

3. Experimental animal models of photoaging

Since it becomes increasingly apparent that the characteristics of photoaging differ significantly from those of chronologically aged skin, the effects of chronic exposure to UV light and the multifavious aspects of photoaging are now the subject of intense research. The development of the hairless mouse model of photoaging greatly contributed to the histological and biochemical investigation [1,28,29] confirming the earlier findings in humans [30,31]. Histochemical studies revealed wavelength-dependent elastosis, collagen damage

and increased GAGs [32]. Ultrastructural studies revealed the deformed configuration of elastic fibers by UVB [33], and UVA induced different ultrastructural changes from UVB [34].

Since the biochemical changes associated with the photoaging process are not fully understood, the theory that accumulated photo-oxidative cellular injury contributes significantly to the photoaging process in skin still remains open to debate. Experiments using photoaging model mice present circumstantial evidence that, after chronic UV irradiation, both glutathione peroxidase and SOD activities are elevated; however, continued UV irradiation resulted in a steady decline in SOD and lipid soluble antioxidants, suggesting that SOD and lipid soluble antioxidants in the skin may be involved in protecting it from UV damage and deteriorate with chronic irradiation [35]. We have also demonstrated that SOD activity was increased by repeated UVB irradiation and gradually returned to the control levels but unaffected by UVA irradiation. In contrast, catalase activity was suppressed by UVA irradiation, indicating that the skin SOD and catalase activities are not coordinately regulated by long-term UV irradiation [36]. In any event, the SOD activity, which has been reported to decrease after acute actinic injury, is induced by repeated exposures to UVB, presumably in response to the chronic photo-oxidative stress, and a similar phenomenon has been observed by low dose X-irradiation [37]. However, continual cumulative stress may overwhelm the capacity of this system with resultant photoaging symptoms.

UV radiation induces definite alterations in connective tissue including accumulation of elastotic material into the upper and middle parts of the dermis and increased amounts of GAGs [38]. However, biochemical studies have not disclosed marked alterations in collagen and elastin [39], although a recent study demonstrates increased elastin mRNA levels in photoaged skin [40]. We have investigated the alteration of main disaccharide units of GAGs from hairless mice skin after chronic and repeated UV radiation using high performance liquid chromatography [41] by our newly developed method [42]. We found that the total amount of main disaccharide units increased by UVA irradiation at the 36th week. UVA significantly increased three main disaccharide units, hyaluronic acid-derived ΔDi-HA (HA), dermatan sulfate-derived ΔDi-4S (DS) and chondroitin-sulfate derived ΔDi-4S (CS). UVB also increased the amount of each main disaccharide as compared with control, although less significantly than UVA did. Concerning the changes in the disaccharide composition after 36 weeks' repeated exposure, ΔDi-HA (HA) decreased and ΔDi-4S (DS) increased in the order of control, UVA- and UVB-irradiated groups. These results, for the first time, demonstrate the precise alterations of GAGs both in total amount and composition, confirming the previous histochemical findings [30].

Although this disaccharide analysis should provide a useful method for examining the biochemical changes of skin GAGs in photoaging, it is necessary to repeat the experiments in vitro to evaluate the effect of agents that may regulate the photoaging process. For this purpose, we have developed the three-dimensional culture system supplemented with L-ascorbic acid 2-phosphate (Asc 2-P) using dermal fibroblasts [43]. The addition of Asc 2-P rendered fibroblasts to the organization of the dermis-like three-dimensional structure in vitro without any pretreatments of the plastic dish. Multilayered fibroblasts surrounded by extracellular matrices were readily observed and the amounts of both main disaccharide units and hydroxyproline are significantly increased. Furthermore, the composition revealed more similarity to that of dermis when compared with a monolayer culture system. Using this culture system, it should be possible to investigate the biochemical changes of collagen produced by dermal fibroblasts obtained from photoaged skin, and the effects of ascorbic acid on proliferation and collagen synthesis have been recently reported [44].

Collagen composes 70–80% of the dry weight of the dermis and it is well known that solar irradiation induces an accumulation of abnormal elastotic materials [45]. The total collagen content of sun-damaged skin was 20% less than non-solar-exposed skin, presumably due to increased degradation, and it seems likely that the elastin masks the presence of collagen fibers in the dermis

of sun-damaged skin [46] and that there is insufficient collagen in photoaged skin to support the increasing mass of the tissue [47] with resultant skin wrinkling [48]. Of greater importance is the diminished solubility of collagen due to cross-linking [49]. Chronic sunlight exposure may be associated with an impediment to normal maturation of dermal collagen, resulting in collagen cross-linking which differs from chronologic aging of skin [50]. Other available information indicates that direct stimulation of collagenase synthesis by skin fibroblasts by UVA radiation may contribute to the connective tissue damage [51] and that elastotic degeneration could be the consequence of a defect in either degradation or synthesis of metabolic turnover of elastic fibers resulting in abnormal and excessive polypeptide chains extremely rich in disulfide bonds [52].

Further studies are required to correlate these biochemical changes in photoaged skin with events induced by chronic photo-oxidative stress in an in vitro culture system because photochemically generated ROS can induce protein denaturation and cross-links in collagen and are thought to contribute to the accelerated process of photoaging [53].

4. Possible strategies of antioxidant therapy for photoaging

To survive the insults of actinic damage resulting from sun exposure, human skin has evolved several defensive mechanisms including the compact horny layer, which absorbs, filters and scatters UV radiation, the melanin, which acts as a UV-absorbing optical filter as well as a free radical scavenger, and various kinds of antioxidants such as carotenoid, SOD and a glutathione peroxidase-reductase enzyme system in the epidermis acting as selective scavengers for ROS produced by UV radiation and protecting the membrane lipoproteins from photodamage [54]. Yet, as excessive amounts of UV radiation can be damaging to our skin, it is necessary to minimize or prevent the harmful effects of UV light on skin by supplementing artificial defenses. The most effective and appropriate photoprotection seems to be physical protection by clothes and physicochemical protection by sunscreens. There has been great progress in the development of new sunscreens for both UVB and UVA protection [55,56]. UVA is harmful to skin and is abundant in solar radiation reaching the dermis and thus making fibroblasts and the dermal collagen component more vulnerable to photoaging. Irradiation of cultured human skin fibroblasts with UVA leads to a decrease in the membrane fluidity, which may be related to lipid peroxidation [57] and the damaging effects appear to be due to a sensitized reaction with riboflavin [58] or pyridoxine [59]. Of course, UVA-absorbing chemicals are quite effective and sunscreens offer considerable protection for free radical defense against both UVA and UVB [60], but these chemicals may induce allergic reactions or further possible free radical reactions. Taken together, these points indicate that there is good reason to develop other photoprotective agents as another strategy against photoaging.

Available information indicates that topical anti-inflammatory agents such as hydrocortisone, ibuprofen and naproxen are very effective [61]. If the free radical theory is applicable to the photoaging process, it is reasonable to administer antioxidants for this purpose and recent advances in free radical research have opened the door to clinical application of therapeutic antioxidants. Regulation of the antioxidant capacity includes the maintenance of adequate levels of antioxidant and the localization of antioxidant compounds and enzymes [62]. Various approaches can be taken to augment antioxidant effectiveness: (1) enhancement of intracellular, or plasma, antioxidant levels, (2) incorporation of lipophilic antioxidants into membranes and lipids, (3) extracellular targeting of drug antiradical interventions or of antioxidants, and (4) scavenging superoxide [63]. Drug development first focused on SOD and the clinical effect of both systemic and topical SOD was confirmed by several experiments with a ischemia-reperfusion injury model using skin flaps [64–66] and with experimental burns [67] as far as the dermatological field is concerned. As for the photoprotective effect of SOD, both systemic [18] and topical administration of SOD [68] revealed the protective effect on decreased levels of cutaneous SOD activities, confirming that exogenous-

ly administered antioxidants can respond adaptively to changes in the activity of the detoxication process under certain oxygen stress preventing photo-oxidative damage to the skin. However, topical SOD treatment could not successfully reduce erythematous sunburn reaction indicating that topical SOD protects skin from photo-oxidative stress without affecting erythema response. Thus from a practical standpoint, sunscreen agents seem better at present for daily sunprotection. SOD is no longer a target for development against cutaneous actinic damage. The reason for this includes not only the results mentioned above, but also the following points: (1) the effective defense against ROS may require balanced increments in antioxidant enzymes and cannot necessarily be improved by increases in the activity of single enzymes, and (2) an improved drug delivery system should be considered, including chemical modification for prolonged half-life time, better tissue penetration and accumulation to lesions.

Based on these points, low molecular weight antioxidants and other substances reducing photo-oxidative damage are now under experimental trials. The newcomer to this field is iron chelators. Since transition metals such as iron and copper can act as catalysts in the formation of ROS [69] and chronic exposure of murine skin to sub-erythemal doses of UVB results in an increased skin level of non-heme iron, the elevated iron content of UV-exposed skin increases the potential for iron-catalysed radical production. Bissett et al. demonstrated that topical application of certain iron chelators dramatically delayed the onset of UVB radiation-induced skin photodamage but that non-chelating analogs provided no significant protection confirming the proposed mechanism of chelator photoprotection [70].

It is a well-known fact that β-carotene quenches photochemical reactions in the epidermis involving singlet oxygen and oxygen radicals that occur when epidermis is exposed to UVB [71] and β-carotene has been successfully used in the treatment of various photodermatoses. Vitamin E is also considered to be an antioxidant because vitamin E-depleted animals are more susceptible to oxidative stress than the supplemented group, although increasing evidence indicates that the antioxidant function of vitamin E is interrelated to and complemented by many enzymatic and non-enzymatic antioxidant systems [72]. The vitamins E, C and reduced glutathione system may play a prophylactic role in the defense of skin and eye tissue from a number of serious light-induced conditions that are mediated by photo-oxidative damage to cell membranes [73]. Even topical administration of a 1% solution of vitamin E has been reported to be effective in protecting the epidermis against the early damage induced by UV radiation [74]. UVB-induced sunburn reactions are significantly reduced by topical application of tocopherol acetate even after the exposure has occurred [75].

Ascorbic acid functions as a biological co-factor and antioxidant due to its reducing properties and vitamin C levels of the skin can be severely depleted after UV irradiation. It has been reported that vitamin C protects porcine skin from UVA-mediated phototoxic reactions (PUVA) [76] and prevents common cellular degenerative diseases associated with lipid peroxidation [77]. Other antioxidants reported to provide photoprotective effects are flavopherol [78], conjugated hexadienes [79], propyl gallate and Trolox [80]. Natural products such as green tea [81] and certain herbs [82] also seem to be promising.

Another important approach to regulate photo-oxidative damage is to induce endogenous antioxidant activities by non-antioxidants. Hanada et al. showed protective effects of cadmium chloride against UVB injury in mouse skin presumably via cadmium-induced metallothionein [83]. Metallothionein plays important roles in the detoxication of heavy metals and regulation of the metabolism of essential trace metals. However, there is increasing evidence that it can act as a free radical scavenger [84]. Metallothionein in the epidermis may contribute to the protection against phototoxic injury in association with enzymatic and non-enzymatic antioxidants [85]. This approach to induce endogenous antioxidant levels seems to be beneficial because a well-balanced elevation of antioxidant network can be achieved in a reasonable way if a safe and practical agent is found. In any case, the possible use of an antioxi-

dant that attenuates photo-oxidative toxicity is one of the important issues in photoaging.

5. Concluding remarks

A growing body of evidence suggests that cumulative photo-oxidative damage should contribute to the photoaging process, and thus antioxidant therapy that modulates photo-oxidative stress is believed to be an important strategy against photoaging. Several antioxidants have readily been proven to work in experimental conditions and, in the near future, it is hopefully possible that antioxidants become routine in clinical use.

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