Chapter

Photocatalysis in the Skin Related to UVA Photoaging

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Abstract

Skin aging is classified into chronological aging and photoaging, involving ultraviolet radiation (UV), visible light, and others. UVA and UVA-photosensitizers (involving photocatalysis) contribute to the production of chronically induced skin damage that results in photoaging, especially wrinkles that are associated with histopathological actinic elastosis in the dermis. Hydrogen peroxide produced by the photosensitization involving photocatalysis, such as flavin, has been proposed as a risk factor for photoaging. It was also revealed that hydrogen peroxide production by UVA is amplified through the following reactions. The photosensitization of type I and type II by riboflavin as an initiator oxidizes coexisted amino acids and vitamins. The oxidized amino acids and vitamins produce reactive oxygen species (ROS), including hydrogen peroxide, through secondary UVA-photosensitization. Finally, we proposed a screening method for detecting the effects of antioxidants on UVA-photosensitization. In our previous study, histidine and other antioxidants did not inhibit UVA-photosensitized by riboflavin, even though they have been reported to scavenge singlet oxygen and superoxide. In contrast, we demonstrated that ergothioneine suppressed the production of hydrogen peroxide by UVA-photosensitization. The purpose of this report is to provide new findings for the prevention of photoaging by discussing the characteristics of UVA-photocatalysts in the skin.

Keywords: photoaging, photosensitization, riboflavin, UVA, hydrogen peroxide

1. Introduction

In living organisms, photosensitizing reactions using photosensitizers (involving photocatalysts in this review) are used in a wide variety of ways. Beneficial uses include treatment of skin disease [1], elimination of cancer cells [2], and construction of tough collagen structures [3]. On the other hand, the photosensitizing reaction by amino acids and vitamin components in the living body through exposure to ultraviolet rays can cause skin aging. In this chapter, we reviewed the photosensitizing reactions in living organisms related to photoaging.

The important role of the skin is to protect the body from various external environmental factors. In other words, the skin has the role of preventing physical, chemical, and bacteriological invasion into the body and preventing water loss due to evaporation. The skin is composed of three layers: the outermost layer of the epidermis, the dermis, and the subcutaneous adipose tissue. Keratinocytes and melanocytes

are well-known cells that make up the epidermis. Keratinocytes contribute to the barrier function of the skin by differentiating, and melanocytes produce melanin pigment to protect the epidermis and dermis from ultraviolet rays. In the dermis, the extracellular components, produced by dermal fibroblasts, have collagen fibers, elastic fibers, hyaluronic acid, and proteoglycan as the main constituents. These extracellular components have a high water retention effect and contribute to the maintenance of the hydrophilic environment of the dermis. Subcutaneous adipose tissue is rich in mature adipocytes and has the role of reducing external pressure. In addition to those essential capabilities, the skin has also a role in thermoregulation, immune response, and social communication [4–8].

Like many other organs, the skin undergoes adverse changes over time in response to changes in lifestyle and hormonal balance. However, unlike most other organs, the skin receives major changes due to exposure to the environment, especially UV rays from the sun. Chronic exposure to UV rays causes an early aging phenotype (photoaging) that resembles the aging caused by the passage of time (chronological aging) [9]. As a result, areas of the body that are routinely exposed to the sun, such as the face, neck, and forearms, show the visible manifestation of aging (senile lentigo, wrinkles, sagging, etc.) faster than other areas of the body [10].

The effects of chronological aging and photoaging induce serious alteration in the dermis with detrimental changes to the extracellular matrix [11]. Collagen accounts for the majority of the dermal matrix. However, with age, normal collagen content decreases, and the ratio of collagen degenerated by oxidation, carbonyl modification, and glycation increases [12, 13]. In addition, the ability of fibroblasts to generate collagen is diminished by environmental factors in addition to chronological aging. In particular, the upper layers of the dermis on the face, neck, and back of the hands, which have been exposed to the sun's rays, are characterized by the accumulation of glycated elastic fibers (solar elastosis) [14]. Furthermore, photoaging is mainly induced by long-term UV exposure. UVA, a long-wavelength UV rays, causes serious damage to the skin due to ROS produced by the reaction with photosensitizers in the skin (**Figure 1**) [15]. However, due to the wide variety of *in vivo* photosensitizers associated with ROS production, understanding the mechanisms of ROS production and effective quenching methods is very complicated. In this chapter, we focused on the hydrogen peroxide generated by the photosensitization reaction by multiple photosensitizers and UVA in the living body. We also introduced a simple screening method for discovering active ingredients that are effective against the photosensitization reaction through UVA.

2. UVA-photosensitization reaction and photoaging

Sunlight is now considered to be one of the most harmful extrinsic factors that can induce ROS production [16]. Other well-known factors include tobacco smoke [17, 18], PM2.5, and air pollutants [19, 20]. The spectrum of sunlight includes infrared energy (greater than 760 nm), visible light (400–760 nm), and ultraviolet (UV) light (less than 400 nm). UVs are further classified into UVA (400–315 nm), UVB (315–280 nm), and UVC (280–100 nm) [21]. Photobiological reactions are primarily produced by exposure to UVB and UVA radiation. UV is a major cause of DNA damage in the epidermal skin cells [22, 23]. Furthermore, UV rays contribute to change in the stem cell niche, which can lead to photoaging [1, 24]. UVA accounts for about 95% of the UV rays that reach the surface of the ground and is likely to contribute to the risk of the initiation of human skin cancer [25].

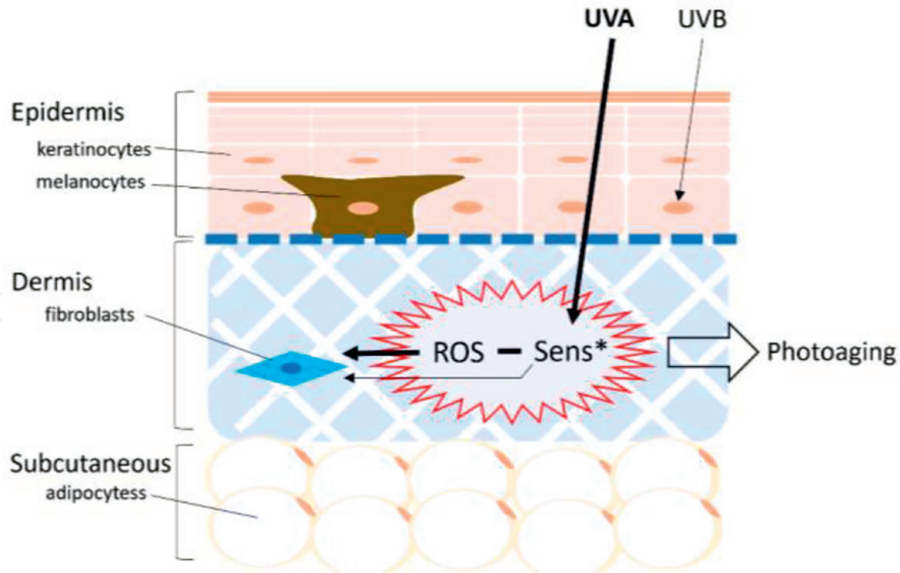

Figure 1.
Photoaging of the skin: Photoaging of the dermis is mainly induced by long-term UV exposure. UVA, a long-wavelength ultraviolet light, causes serious damage to the dermal skin due to ROS produced by the reaction with photosensitizers in the body. Sens: Activated photosensitizers.*

UVA indirectly damages DNA [26], in contrast to UVB, which is absorbed by DNA and causes direct cytotoxicity [27]. UVA-induced damage is mainly caused through interactions with the photosensitizers, which produce ROS [28]. UVA causes various changes in the dermis, which appear to be primarily involved in the initiation and progression of photoaging. These photosensitizers absorb photons/energy, resulting in a photosensitizer excited state called the singlet excited state [29, 30]. Two reactions can occur following this first reaction. One is a reaction that emits either heat or fluorescence and returns to the ground state, and the other is a triplet excited state due to intersystem crossing. This triplet excited state reacts with both DNA and molecular oxygen, resulting in DNA modification or the production of ROS, such as superoxide, hydroxyl radical, singlet oxygen, and hydrogen peroxide [31]. The term "photoaging" was coined to emphasize the importance of UV and the resulting ROS formation in the skin-aging process (**Figure 2**) [32].

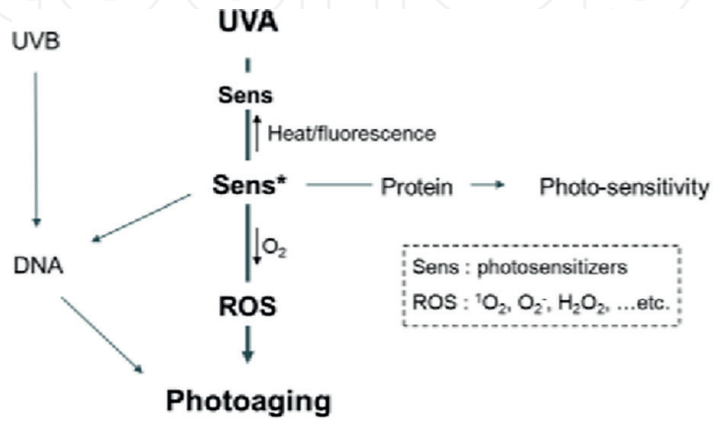

Figure 2.
Scheme of photosensitization and Photoaging: UVA that penetrates the epidermis and reaches the dermis is absorbed by the photosensitizers in the skin tissue and produces ROS under existing O_2 molecules.

3. Photosensitizing reactions and photosensitizing components that occur in the presence of oxygen molecules

Under certain circumstances, endogenous photosensitizers, such as porphyrins, melanin, urocanic acid, bilirubin, flavins, pterins, and amino acid, such as tryptophan, act as photosensitizers [31–33]. Photosensitization, such as melanin and bilirubin, out of the major pigments of the skin, are known as the major absorbers of visible regions of the spectrum over 300 to 600 nm. On the other hand, other photosensitizers, such as urocanic acid (250 to 300 nm), riboflavin (355 nm), and pterin (345 to 375 nm), show maximum absorption in the UV range and are hardly absorbed in the visible region of the spectrum [34, 35]. Photosensitized reactions involving oxygen molecules are reported as either type I or type II. Previously, the definition of type II reaction involved the formation of singlet oxygen (major reaction) and superoxide (minor reaction) [36, 37]. Currently, it was revised, and the definition of type I reaction now involves the formation of superoxide because we define type I on the basis of the formation of radicals. Type II is now established as the sensitized formation of singlet oxygen. This review followed the definition of guidelines in Baptista et al. [38].

Dermal fibroblasts are often used as a research target for skin aging [39]. The senescence of dermal fibroblasts is thought to have a significant effect on dermal matrix metabolism and degenerate dermal structure [40]. It is well-known that matrix degradation by activation of matrix metalloproteinases (MMPs) contributes to the formation of wrinkles and sagging skin [41]. In addition, it has been reported that aging fibroblasts, which showed an increase of SA-β-galactosidase [42], intercellular ROS, p16 expression [43], DNA damage, and other typical cellular senescence phenotypes, are present in the dermis at the photoaging site. *In vitro*, UVA and UVA-photocatalysts are often used to induce cellular senescence (**Figure 3**). Thus, the ROS generated by photosensitization reactions are considered to be important targets for the development of anti-photoaging agents.

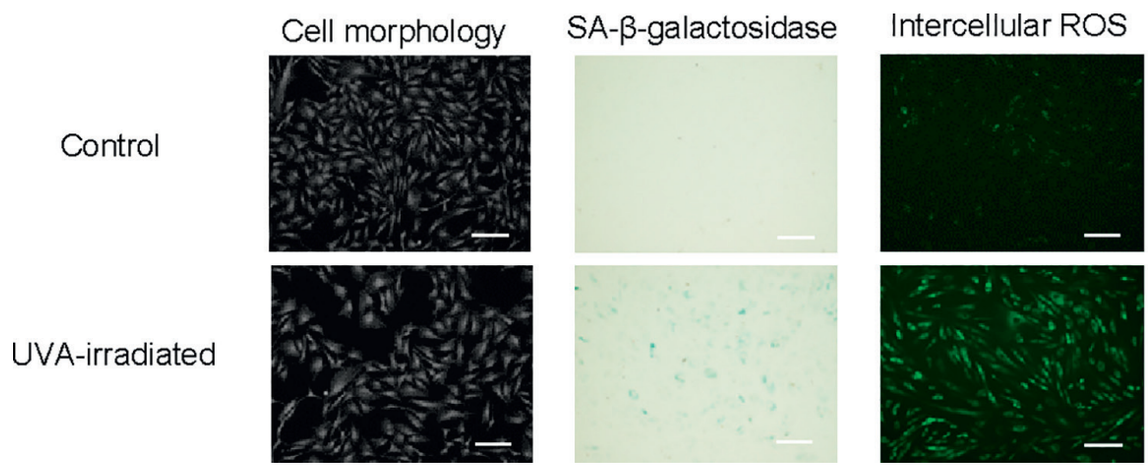

Figure 3.
Senescence phenotype of fibroblast induced by UVA irradiation in vitro: Repeated UVA irradiation of human dermal fibroblasts at a dose of 36 J/cm²/10 days in the condition of riboflavin coexisted to amino acids and vitamins induced typical phenotype of cellular senescence. Left: Flattened and larger cells have a greater diameter ratio compared to nonirradiated control cells (calcein-AM staining). Center: Increased of blue coloration cells (senescence-associated β-galactosidase staining) compared to nonirradiated control cells. Right: Increased higher level of green fluorescence intensity (dihydrorhodamine 123 staining) compared to nonirradiated control cells. Scale bar; 200 μm.

4. Study of UVA-photosensitization *in vitro*

Many researchers have investigated photosensitization reactions *in vitro* system using a single photocatalyst [44–50]. On the other hand, some research groups have shown that the coexistence of multiple components induces stronger cytotoxicity than a single photosensitizer condition [44, 51]. It has been reported that a low concentration of photocatalyst, about 0.4 μg/mL riboflavin, can induce cell damage in coexisting conditions with amino acids and vitamins [52]. On the other hand, in single riboflavin conditions, it takes about 100 μg/mL of riboflavin to induce cell toxicity *in vitro* [53]. In the condition that photocatalysts and multiple components coexist, the type I mechanism and the type II mechanism may be amplified at the same time in photosensitization. Therefore, it is necessary to consider which of the type I and type II reactions is the main reaction under the coexistence conditions of photocatalysis and multiple components.

4.1 Combination of components with type I as the main reaction (riboflavin and folic acid)

One type of photosensitization occurs in aqueous solution containing riboflavin and folic acid. Folic acid coexisting riboflavin in aqueous solution easily undergoes oxidative degradation upon UVA exposure to produce a pterin derivative [54]. Pterin derivatives are reported to accumulate in vitiligo skin [55], and to induce UV stress in melanocytes. Pterin derivatives are known to produce superoxide, hydrogen peroxide, and other types of ROS through UVA-photosensitizing [56]. In our previous study, when aqueous solution containing both folic acid and riboflavin was exposed to UVA, blue fluorescence derived from pterin derivatives appeared earlier than in aqueous solution containing folic acid alone. Those results indicate that the oxidative degradation of folic acid proceeds only very slowly in HBSS containing folic acid alone, but occurs rapidly in the presence of the photosensitizer riboflavin. Since this reaction was not inhibited by NaN_3, a singlet oxygen scavenger, it was thought that the oxidative degradation of folic acid was possibly promoted via photosensitization of type I generated by riboflavin photosensitization [52]. Therefore, it is considered that the superoxide quencher is effective for these types of reactions.

4.2 Combination of components with type II as the main reaction (riboflavin and tryptophan)

The other type of photosensitization occurs between riboflavin and tryptophan. Since tryptophan has a maximum absorption wavelength in the UVB region (especially at 280 nm), exposure to UVB is known to produce tryptophan oxides, such as FICZ and kynurenine derivatives. These tryptophan oxides have absorption wavelengths in the UVA region, and it has been reported that UVA exposure produces superoxide, H_2O_2, and other types of ROS [57–59]. In our previous study, since tryptophan does not have a UV absorption region, UV exposure to tryptophan alone did not cause the oxidative degradation of tryptophan and did not produce kynurenine. However, exposure to UVA in the presence of riboflavin decreased the 280 nm absorption by tryptophan and increased the 360 nm absorption by kynurenine. Those results indicate that the oxidative degradation of tryptophan, which does not occur in aqueous solution with tryptophan alone, may be initiated by the photosensitization of riboflavin. This phenomenon

was markedly suppressed by the addition of NaN_3, which suggests that the oxidative degradation of tryptophan may be promoted by a singlet oxygen generated at an earlier time by the photosensitization of riboflavin [52]. Therefore, the singlet oxygen quencher is considered to be effective for these types of reactions.

As a point to be noted, it has been reported that HEPES and phenol red can enhance the cytotoxicity and the production of ROS by photosensitization reaction under the coexistence condition with riboflavin [60, 61]. When considering a photosensitizing reaction with multiple components, it is necessary to consider the possibility that a component other than the object to be evaluated may become noise.

5. Antioxidants and inhibition of the UVA-photosensitization reaction

The following is a summary of typical antioxidants (**Table 1**). Oxygen radicals scavenger (trolox [62], lutein [63], allicin [64], resveratrol [65], isoflavones [66], quercetin [67], catechin [68], theaflavin [69], curcumin [70], chlorogenic acid [71], and superoxide dismutase [72]), singlet oxygen quencher (astaxanthin, histidine [73], and lycopene [74]), hydrogen peroxide scavengers (catalase and glutathione peroxidase), and scavengers for all type of ROS (ascorbic acid [75], ergothioneine [76], L-cysteine, glutathione [77], and tocopherol [78]) are suggested to be effective in suppressing UVA-photosensitization.

However, these antioxidants have not been investigated for the effects of the photosensitizing reaction. Therefore, it is necessary to know if these antioxidants can promote the photosensitizing reaction when coexisting with a photocatalyst, such as riboflavin.

6. Screening method for antioxidants that suppress the photosensitizer reaction *in vitro*

We have proposed an *in vitro* assay using cytotoxicity and hydrogen peroxide as detection indicators in a screening method for compounds that suppress cell

Target	Antioxidants		
Oxygen radicals	Allicin	Resveratrol	
	Catechin	Quercetin	
	Chlorogenic acid	Superoxide dismutase	
	Curcumin	Theaflavin	Ascorbic acid
	Isoflavones	Trolox	L-cysteine
	Lutein		Ergothioneine
Singlet oxygen	Astaxanthin		Glutathione
	Histidine	Lycopene	Tocopherol
Hydrogen peroxide	Ascorbate peroxidase	Glutathione peroxidase	
	Catalase		

Table 1.
The classification of typical antioxidants.

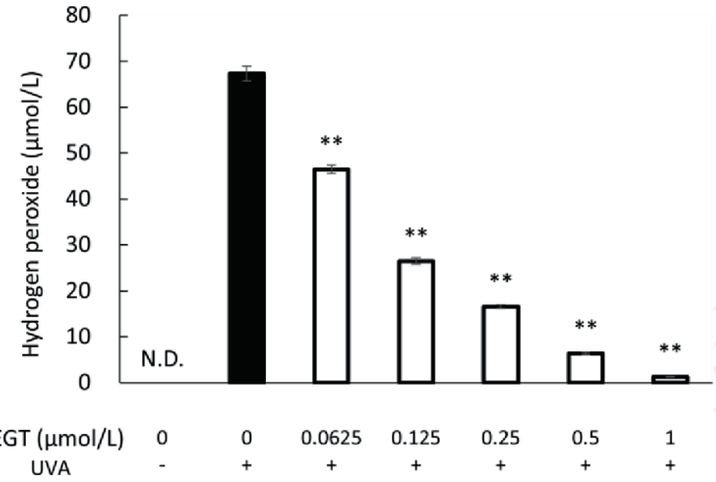

Figure 4.
*Efficacy of ergothioneine against the production of hydrogen peroxide through UVA-photosensitization: Samples were added to HBSS containing 1 μmol/L riboflavin, 30 μmol/L folic acid, and 100 μmol/L tryptophan. Hydrogen peroxide is detected in UVA irradiation control in this assay. For samples, each dose of ergothioneine (EGT) was used. A lamp (Toshiba Lighting & Technology Corporation, Yokosuka, Japan) emitting a UVA spectrum (340–410 nm) was used as the UVA source and was adjusted to an intensity of 1.0 mW/cm². Each sample solution was irradiated for 1 hour in an ice box for temperature control (3.6 J/cm²). The amount of hydrogen peroxide generated in each solution after UVA irradiation with 3.6 J/cm² was measured by ADHP/HRP methods. Data are expressed as means ± S.D. statistical analysis was performed by Student's t-test with p-value <0.010 (**p < 0.010) considered statistically significant differences.*

damage caused by UVA-photosensitization. This is because past studies have suggested that hydrogen peroxide generated in the solvent by the UVA photosensitization reaction is the main cause of cell damage [52, 79]. Furthermore, by coexisting with the target compound during UVA exposure, it is possible to evaluate the photosensitization reaction between the target active ingredients and other components.

As an interesting example, histidine, which is used as a singlet oxygen quencher, has not been shown as an effective compound in this *in vitro* assay. This was because histidine enhances the photosensitizing response to riboflavin during UVA exposure [51]. These indicate that they may not be useful depending on the conditions of amino acids and vitamins in which existing antioxidants that are expected to have a photosensitizing effect coexist. Ergothioneine is a powerful antioxidant that has been reported to eliminate singlet oxygen, superoxide, and hydrogen peroxide. A previous study reported that ergothioneine has a protective ability against hydrogen peroxide and other ROS [76, 80]. It was indicated that ergothioneine has an anti-photosensitization efficacy because ergothioneine was treated only during UVA irradiation [81]. In our *in vitro* assay, using hydrogen peroxide as an index, the amount of hydrogen peroxide produced was suppressed in a concentration-dependent manner, without increasing hydrogen peroxide production at any concentration (**Figure 4**). These findings suggest that ergothioneine may prevents the progression of photoaging in the skin.

7. Discussions

In this paper, we discussed the possibility that ROS production through photosensitization reaction in the living body may be important factor for photoaging of the skin, especially damage to dermal fibroblasts caused by UVA.

In addition, the concentration of amino acids and vitamins in the human skin, especially in the dermis, must be clarified to discuss the role of sunlight on skin photoaging. There are some reports of the concentration of vitamins in the blood. It has been reported that riboflavin may exist at approximately 1–300 ng/mL [82, 83], folic acid may exist at approximately 13–57 ng/mL [84], and tryptophan may exist at approximately 12 μg/mL [85]. However, as far as we have investigated, there have been no reports of detailed verification of the concentrations of amino acids and vitamins in the dermis. In the future, we hope that the concentrations of amino acids and vitamins in the skin must be clarified by detailed studies on the mechanism of production of ROS by the UVA-photosensitization reaction in the skin.

Photosensitivity, unlike photoaging, is an acute response to light. Among photosensitivity research, there are also reports on the reduction of phototoxicity by studying combinations of ketoprofen with several antioxidants. The report investigates the effects of eight known radical scavengers on UV-induced photodegradation of ketoprofen and the production of ROS. Interestingly, quercetin was the only one that simultaneously suppressed the photolysis of ketoprofen and the production of ROS. Tocopherols eliminated ROS but did not suppress the photolysis of ketoprofen [86]. It can be inferred that quercetin directly quenched the photosensitizing reaction of ketoprofen.

It should be noted that it is important to look for antioxidants that suppress the reaction of the photosensitizer, which is effective not only for photoaging caused by ROS production through photosensitization but also for the prevention of photosensitivity. We concluded that understanding of the photosensitizing mechanism of environmental components, such as amino acids and vitamins in the skin, will be effective in reducing or preventing harmful skin symptoms induced by phototoxicity, which is caused by UVA.

In addition, in recent years, much attention has been paid to treatments targeting senescent cells, such as senolytics [87]. In 2018, Yoon reported that the elimination of nearby aging fibroblasts was effective in improving senile pigmented spots [88]. This strongly suggests that the phenomenon of skin aging may be caused by cell aging of fibroblasts. Therefore, the prevention of cellular senescence, especially the prevention of photoaging and photodamage caused by ultraviolet rays, is considered to be an even more important issue than before. For anti-photoaging to be effectively implemented, we need studies to elucidate the photosensitizing-reaction mechanism considering various components in the skin.

8. Conclusion

Until now, the role of photosensitizers involving photocatalysis in the skin components is poorly understood in photoaging. In this review, we focused on the hydrogen peroxide generated by the UVA-photosensitization reaction by multiple photosensitizers, such as riboflavin, amino acids and vitamins. We also introduced a simple screening method for discovering compounds that are effective for UVA-photosensitization, using ergothioneine as an example.

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