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Overcoming the Photochemical Problem of Vitamin K in Topical Application

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Abstract

Topical application of vitamin K is beneficial in the treatment of various skin pathologies. However, its delivery to the skin is hampered by the photo-instability and phototoxicity of vitamin K (quinone form). Indeed, topical use of vitamin K is regulated in Europe owing to the photosensitive properties of this molecule. Here, we discuss the suitability of ester derivatives of vitamin K hydroquinone (VKH), the active form of vitamin K, for topical applications. Notably, VKH derivatives have the potential to overcome the photo-instability and phototoxicity problem of vitamin K and act as VKH prodrugs, as demonstrated in HaCaT human keratinocytes. Thus, VKH prodrug is a promising strategy for topical application of vitamin K without the need for special protection from light.

Keywords: vitamin K, photostability, phototoxicity, skin application, prodrug

1. Introduction

Skin application of vitamin K shows several beneficial effects, such as suppression of pigmentation and alleviation of bruising [1–3], prophylactically limiting the occurrence of acneiform side effects in patients receiving the monoclonal antibody cetuximab [4–6] and promoting wound healing [7].

Despite these potentially beneficial effects, vitamin K is unstable in the presence of light [8, 9]. Indeed, application of vitamin K on the skin can result in photodegradation without appropriate shielding of the application site, e.g., the face and hands, from light. Furthermore, in Europe, warnings have been issued regarding the use of vitamin K in cosmetics. The Scientific Committee on Consumer Safety has also reported phototoxicity of vitamin K in skin cells [10, 11]. As a result, the use of vitamin K as an external preparation for the skin is limited.

The molecules in the vitamin K family contain 2-methyl-1,4-naphthoquinone as the basic skeleton. Vitamin K molecules can be classified as phyloquinone (PK, vitamin K1) with a phytyl side chain at the 3-position, menaquinone (MK-n, vitamin K2) with an isoprenyl side chain consisting of n isoprenyl groups, and menadione (MD, vitamin K3)

with no side chain at the 3-position. PK and MK-4 are widely used as pharmaceutical treatments for vitamin K deficiency and osteoporosis.

Vitamin K (quinone form) delivered intracellularly is converted into vitamin K hydroquinone (VKH) by two-electron reduction. VKH functions as a cofactor for γ -glutamyl carboxylase (GGCX), which converts the glutamic acid (Glu) residue of vitamin K-dependent protein into the γ -carboxyglutamic acid (Gla) residue as a post-translational modification. Subsequently, VKH is oxidized to vitamin K epoxide (VKE). In addition, VKE is reduced to vitamin K (quinone form) to form the vitamin K cycle. Therefore, it is necessary to deliver sufficient VKH to the target site to achieve efficacy.

The active forms of PK and MK-4 are phylohydroquinone (PKH) and mena-hydroquinone-4 (MKH), respectively. However, these compounds cannot be used as preparations because they show extreme instability via oxidation. Therefore, PK and MK-4 that are stable against oxidation are used clinically. However, as described above, vitamin K (quinone form) is extremely unstable upon exposure to light. Thus, strict control of lighting is required during formulation, distribution, storage at medical institutions, and administration to patients. To achieve full efficacy of vitamin K, formulations with low photodegradation and phototoxicity are needed for effective topical delivery of VKH. The concepts underlying the delivery system of VKH using Vitamin K are shown in **Figure 1**.

Figure 1.
Schematic illustration of the vitamin K cycle.

2. Mechanisms of photodegradation and phototoxicity of vitamin K

Analysis of the PK photolysis reaction by Hangarter et al. showed that charge transfer from the β,γ -double bond of the isoprenyl side chain to the quinone moiety initiates intramolecular proton transfer from the side chain and yields a 1,3-quinone methide (meta-quinone methide) as a mixture of singlet and triplet species diradical in polar solvents, subsequently forming 1,2-quinone methide (ortho-quinone methide), which can be used to generate PK chromenol [9]. Chromenol levels tend to increase with irradiation time, and this compound is expected to be the final product of photodegradation of vitamin K [9, 12, 13].

Vitamin K is also expected to cause two types of phototoxicity during the above-mentioned series of photodegradation processes. After acquiring the excited state via light absorption, some chemicals cause oxidative damage to biological components, such DNA and proteins, through the generation of free radicals (type I reaction) by the electron rearrangement reaction and the generation of singlet oxygen from triplet oxygen (ground state) by the energy rearrangement reaction (type II reaction) [14, 15].

We have previously confirmed that irradiation of PK and MK-4 with UVA increases singlet oxygen generation, intracellular reactive oxygen species (ROS) generation, and cytotoxicity in HaCaT human keratinocytes. Thus, vitamin K has phototoxic properties [12, 13]. Moreover, 1,3-quinone methide diradical and 1,2-quinone methide, which are produced during the photodegradation of vitamin K, are highly reactive and show phototoxicity via type I reactions. Additionally, singlet oxygen generation through a type II reaction is an early-stage phototoxic reaction that

Figure 2.
Schematic diagram of vitamin K photodegradation and phototoxicity.

generates additional secondary ROS [16]. Singlet oxygen is also known to promote the peroxidation of skin surface lipids, resulting in the induction of skin inflammation [17]. Because no enzymes are known to scavenge singlet oxygen in the body, singlet oxygen is thought to exhibit extremely strong cytotoxicity. **Figure 2** shows a schematic diagram of the processes of vitamin K photodegradation and phototoxicity.

3. Application of VKH derivatives to overcome vitamin K photodegradation and phototoxicity

3.1 VKH derivatives strategy for applying vitamin K to the skin

Vitamin K photodegradation and phototoxicity are derived from its quinone structure [9]. Therefore, as far as it has a quinone structure, the photoreaction is unavoidable. We have previously synthesized VKH derivatives without a quinone structure in which the two hydroxyl groups of the VKH are protected by ester bonds. In addition, we have previously reported cationic VKH derivatives which have *N,N*-dimethylglycine (DMG) and anionic VKH derivatives which have succinic acid (SUC) as promoieties (**Figure 3**) can deliver VKH to the liver and to hepatocellular carcinoma cells by being hydrolyzed without a reductive activation process and exhibit strong antitumor effects compared with Vitamin K [18–20]. Accordingly, these VKH derivatives may act as delivery systems for VKH to avoid photodegradation and phototoxicity. Also, Vitamin K (quinone-type) is the difficulty in dose adjustment owing to its high lipid solubility and insolubility in aqueous media. Since VKH derivatives are designed in the form of powders, they are comparatively easy to prepare. Moreover, VKH derivatives are hydrophilic and can be dispersed in aqueous media. These aqueous formulations can be used in *in vitro* experiments without using solubilizing agents such as surfactants.

Here, we assessed their photostability and phototoxicity in order to further development of a vitamin K skin application.

3.2 Evaluation of the photostability of VKH derivatives

The ethanol solutions of PK, MK-4, phyllohydroquinone derivatives (PKH-DMG and PKH-SUC), and menahydroquinone-4 derivatives (MKH-DMG and MKH-SUC) in quartz cells were exposed to artificial sunlight (12000 lx) from the vertical

Figure 3.
Structure of the VKH derivatives and hydrolytic of VKH derivatives to VKH.

direction at 25°C with and without shading. The residual concentrations were determined by liquid chromatography tandem mass spectrometry [12, 13].

All samples were photodegraded according to the apparent first-order rate equation by artificial sunlight irradiation, and their apparent first order rate constants (*k*) and half-lives (*t*_{1/2}) of degradation are shown in **Table 1**. The concentrations of vitamin K (quinone form) and DMG ester derivatives were unchanged under shading, whereas the concentrations of SUC ester derivatives decreased both with and without shading. The decreased concentration of with shading was related to hydrolysis of the bis-ester to monoesters. The difference in the degree of hydrolysis for DMG and SUC ester derivatives are probably due to the stability of the ester bonds.

The half-lives of PK and MK-4 irradiated with artificial sunlight were 0.125 and 0.08 h, respectively. Moreover, the half-lives of PKH-SUC and MKH-SUC were approximately 5- and 3-fold more stable than those of PK and MK-4, respectively, although the stability was not greatly improved. In contrast, the half-life of PKH-DMG was approximately 40-fold greater than that of PK, and the half-life of MKH-DMG was approximately 50-fold greater than that of MK-4, supporting that high light stability could be ensured against artificial sunlight. Note that no formation of chromenol was observed from irradiating the VKH derivatives.

The wavelength distribution of sunlight is wide from ultraviolet to infrared. To examine the wavelength characteristics of photodegradation, the photostability of quinone-type vitamin K and VKH derivatives was evaluated after irradiation with monochromatic light at 279, 341, 373, 404, or 435 nm. **Table 2** shows the photodegradation rate (*k*) and the irradiation energy of each wavelength at which the residual concentration reaches half (*E*_{1/2}). Photodegradation of PK and MK-4 occurred at all measured wavelengths (279–435 nm), and the decomposition rate accelerated with

Compound ^a	Irradiation conditions	<i>k</i> (h ⁻¹)	<i>t</i> _{1/2} (h)
PK	Sunlight	5.532	0.125
	Shading ^b	- ^c	- ^c
PKH-DMG	Sunlight	0.140	4.950
	Shading ^b	- ^c	- ^c
PKH-SUC	Sunlight	1.219	0.569
	Shading ^b	0.577	1.201
MK-4	Sunlight	8.239	0.084
	Shading ^b	- ^c	- ^c
MKH-DMG	Sunlight	0.167	4.150
	Shading ^b	- ^c	- ^c
MKH-SUC	Sunlight	2.796	0.248
	Shading ^b	0.883	0.785

^aThe initial concentration was 1 μM in ethanol.

^bDuring irradiation, the compound was covered with aluminum foil to provide shade.

^cNo decomposition.

Table 1.
Apparent first order rate constants (*k*) and half-lives (*t*_{1/2}) of degradation of vitamin K and VKH derivatives in ethanol under irradiation using artificial sunlight (12000 lx) at 25°C.

Compound ^a	Wavelength (nm)	k ($\text{J}^{-1} \times \text{cm}^2$)	$E_{1/2}$ ($\text{J} \times \text{cm}^{-2}$)
PK	279	0.549	1.262
	341	0.359	1.933
	373	0.094	7.390
	404	0.026	26.260
	435	0.021	32.459
PKH-DMG	279	0.146	4.750
	341	_b	_b
	373	_b	_b
	404	_b	_b
	435	_b	_b
PKH-SUC	279	0.137	5.047
	341	0.070	9.889
	373	0.076	9.169
	404	0.078	8.860
	435	0.079	8.828
MK-4	279	0.533	1.301
	341	0.422	1.643
	373	0.151	4.583
	404	0.049	15.800
	435	0.035	19.738
MKH-DMG	279	0.146	4.750
	341	_b	_b
	373	_b	_b
	404	_b	_b
	435	_b	_b
MKH-SUC	279	0.110	6.323
	341	0.069	10.036
	373	0.059	11.792
	404	0.061	11.296
	435	0.068	10.253

^aThe initial concentration was 1 μM in ethanol.

^bNo decomposition.

Table 2.
The rate constants (k) and half-lives ($E_{1/2}$) of degradation of vitamin K and VKH derivatives in ethanol under different irradiation intensities of monochromatic light at 25°C.

shorter wavelengths. By contrast, the photodegradation of PKH-DMG and MKH-DMG accelerated at a wavelength of 279 nm. In addition, the degradation of PKH-SUC and MKH-SUC occurred at all wavelengths, and the photodegradation rates were almost the same at wavelengths above 341 nm. Therefore, these findings clarified that

decomposition at wavelengths above 341 nm involved hydrolysis to the monoester, but not photodegradation, and photodegradation of PKH-SUC and MKH-SUC was accelerated at a wavelength of 279 nm.

The above results clearly confirmed that the VKH derivative is more stable to sunlight than vitamin K (quinone form) and has a narrow wavelength range for photodegradation.

3.3 Evaluation of phototoxicity

To confirm whether the VKH derivatives had phototoxic properties, singlet oxygen generation, intracellular ROS generation, and cytotoxicity after irradiation with UVA were evaluated in HaCaT cells [12, 13].

Figure 4 shows the amounts of singlet oxygen produced by each compound (200 μ M) irradiated with UVA (15 J/cm²) in phosphate-buffered saline (PBS). Ketoprofen was used as a positive control, and sulisobenzene was used as a negative control. Vitamin K (quinone form) showed singlet oxygen generation depending on UVA irradiation energy, whereas VKH derivatives showed almost no singlet oxygen generation.

Analysis of intracellular ROS generation and cell viability following UVA irradiation at the time of MK-4, MKH-DMG, or MKH-SUC addition (50 μ M) in HaCaT cells is shown in **Table 3**. MK-4 irradiated with UVA (5 J/cm²) increased intracellular ROS generation and decreased cell viability, whereas the MKH derivative did not. Similar trends were observed with PK and PKH derivatives [12].

As mentioned above, the photodegradation and phototoxicity of vitamin K (quinone form) is charge transfer from the β , γ -double bond of the isoprenyl side chain to the quinone moiety initiates intramolecular proton transfer from the side chain. Since

Figure 4. Singlet oxygen generation in aqueous solutions of various compounds (200 μ M) exposed to UVA (15 J/cm²). Data represent means $\pm$ standard deviations ($n = 3$).

Compound ^a	UVA irradiation intensity (J/cm ²)	ROS generation (%control without UVA)	Cell viability ^b (%control without UVA)
Control	0	100 ± 6.19	100 ± 6.04
	5	275 ± 22.0	86.6 ± 5.44
MK-4	0	99.2 ± 6.87	104 ± 2.40
	5	1150 ± 131	11.3 ± 2.17
MKH-DMG	0	126 ± 6.63	104 ± 1.93
	5	282 ± 4.40	101 ± 2.00
MKH-SUC	0	125 ± 4.54	93.9 ± 4.79
	5	271 ± 15.5	88.8 ± 6.07

^aCells were treated with PBS containing 50 µM MK-4, MKH-DMG, or MKH-SUC. Data represent means ± standard deviations (n = 3).

^bCell viability was measured at 24 h after UVA irradiation.

Table 3.
Percent of intracellular ROS generation and cell viability in HaCaT cells in aqueous solutions of vitamin K and VKH derivatives with or without UVA irradiation (5 J/cm²).

VKH derivatives in which the two hydroxyl groups were protected by ester bond, it is considered that the charge transfer from the isoprenyl side chain that triggers a photochemical reaction was suppressed. These results strongly supported that VKH derivatives without a quinone structure did not show the same photodegradation and phototoxicity as vitamin K (quinone form) and may therefore be applied topically to the skin.

4. VKH delivery into HaCaT cells with vitamin K and VKH derivatives

To confirm whether VKH derivatives function as VKH prodrugs in skin-derived cells, the delivery properties of VKH to HaCaT cells were evaluated [12, 13]. **Table 4** shows the area under the curve (AUC) of intracellular VKO up to 72 h after the administration of MK-4 and VKH derivatives to HaCaT cells. VKO was used as an index of VKH because it is stoichiometrically produced from VKH after functioning as a cofactor for GGCX.

Compound ^a	AUC _{VKO(0-72h)} (nmol × h/mg of protein)
PK	1.176 ± 0.056
PKH-DMG	0.872 ± 0.138
PKH-SUC	26.967 ± 2.030
MK-4	10.543 ± 0.795
MKH-DMG	10.786 ± 1.696
MKH-SUC	17.304 ± 1.068

^aCells were treated with medium containing 5 µM compounds. Data represent means ± standard deviations (n = 3).

Table 4.
Area under the curve over 72 h of VKO treated with PK, PKH derivatives, MK-4 or MKH derivatives in HaCaT cells.

The $AUC_{VKO(0-72h)}$ values of PKH-DMG and PKH-SUC were 0.741- and 22.9-fold higher than that of PK, respectively. Additionally, the $AUC_{VKO(0-72h)}$ values of MKH-DMG and MKH-SUC were 1.02- and 1.64-fold higher than that of MK-4, respectively.

Based on these findings, vitamin K (quinone form) and VKH derivatives are converted to VKH in HaCaT cells and function as cofactors for GGCX. Thus, VKH derivatives can function as prodrugs of VKH. Furthermore, VKH derivatives could deliver VKH at concentrations equal to or higher than vitamin K (quinone form).

5. Conclusion

Although many studies have supported the application of vitamin K for the treatment of skin pathologies, this compound is difficult to use as an external preparation to the skin owing to its photo-instability and phototoxic properties. The photodegradation and phototoxicity of vitamin K are derived from its quinone structure. Avoiding chromenol formation may suppress photodegradation via singlet oxygen and radical formation. Moreover, VKH derivatives in which the quinone structure is protected by ester bonds do not show chromenol formation and can be used to overcome the photo-instability and phototoxicity associated with vitamin K while promoting VKH delivery to skin cells. Thus, VKH derivatives may be used for application to sites where shading may be difficult, as alternatives to vitamin K (quinone form).

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Conflict of interest

The authors declare no conflict of interest.

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