Inhibitory Effects of a Novel Ascorbic Derivative, Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate on Melanogenesis

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We investigated the inhibitory effects of a novel amphiphilic ascorbic derivative, disodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-2Na), synthesized from a hydrophilic ascorbic derivative, sodium-2-*O*-L-ascorbyl phosphate (VCP-Na), on melanogenesis in cultured human melanoma cells, normal human melanocytes, and three-dimensional cultured human skin models. Melanin synthesis in melanoma cells treated with VCP-IS-2Na at 300 μM and melanocytes treated with VCP-IS-2Na at 100 μM decreased to 23% and 52% of that in non-treated cells, respectively, and the cell viability was not affected. VCP-IS-2Na also significantly suppressed the cellular tyrosinase activity of melanoma cells and melanocytes. Melanin synthesis in human skin models was evaluated by macro- and microscopic observations of its pigmentation and quantitative measurements of melanin. Treatment of the human skin models with 1.0% VCP-IS-2Na did not inhibit cell viability, while melanin synthesis was decreased to 21% of that in the control. In contrast, L-ascorbic acid (VC) and VCP-Na did not seem to inhibit melanin synthesis and cellular tyrosinase activity. These results indicate that VCP-IS-2Na may be an effective whitening agent for the skin, and we expect the application of VCP-IS-2Na in the cosmetic industry.

Key words disodium isostearyl 2-*O*-L-ascorbyl phosphate; amphiphilic ascorbate; melanogenesis; human tyrosinase; three-dimensional human skin model

Skin color is mainly determined by the amount of melanin present in the surface of the skin. Melanin is synthesized in melanocytes, which are normally found in the epidermal basal layer. Within melanocytes, melanin is bound to a protein matrix to form melanosomes. In the melanosomes, tyrosinase converts tyrosine to eumelanin or pheomelanin through the pathways of melanin biosynthesis. By blocking the pathways at various points, skin depigmentation agents can inhibit melanin biosynthesis, and can be used to treat local hyperpigmention or spots, which are caused by a local increase in melanin synthesis or uneven distribution. In the last decade, a number of depigmentation agents have been developed and utilized in cosmetics and pharmaceuticals for external use.

L-Ascorbic acid (VC) has various physiological and pharmacological functions in collagen synthesis,[1,2] anti-oxidation,[3,4] intestinal absorption of iron,[5] metabolism of some amino acids,[6] production of interferon,[7] antiviral[8] and antibacterial activity.[9] Because of its *in vivo* inhibitory action on melanin synthesis, VC is useful as a whitening agent in cosmetics. However, L-ascorbic acid is less resistant to oxidative conditions than other vitamins and is easily decomposed. To solve this problem, we have tried to develop an ascorbic derivative that is stable in cosmetics, can be converted to L-ascorbic acid in the skin, and has sustained L-ascorbic acid efficacy in the skin. Numerous stable derivatives of VC have been developed.[10] For example, magnesium-L-ascorbyl-2-phosphate (VC-PMG) is hydrolyzed to VC by phosphatase in the liver or skin.[11] Studies have shown that VC-PMG suppresses melanin synthesis in B16 melanoma cells and cultured human melanoma cells.[12] It is available as an additive in commercial cosmetics.

On the other hand, the rigid lipid lamellar structure of the stratum acts as a barrier, especially for the absorption of hydrophilic compounds.[13] Therefore, chemical modification of the hydroxyl group of VC a lipophilic molecule is necessary for the development of an efficient transdermal ascorbic derivative. A variety of lipophilic ascorbic derivatives has been developed,[14—16] but no available derivative is ideal in terms of stability, lipophilicity and biological activity.

Fatty acid esters of carbohydrates have been used as surfactants in cosmetics, detergents, and the food industry for a number of years.[17] These compounds are typically non-ionic, non-toxic and biodegradable.[18,19] Chemical alkylation of carbohydrates has been previously reported,[20,21] and can be accomplished efficiently and inexpensively using base-catalyzed transacylation. Therefore, the development of alkylated ascorbyl phosphate may be suitable for improved skin penetration of ascorbyl phosphate.

Recently, we synthesized an amphiphilic ascorbic derivative, sodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-Na), which exhibited lipophilic and satisfactory thermal stability.[22] VCP-IS-Na has skin permeability superior to that of VC and sodium-2-*O*-L-ascorbyl phosphate (VCP-Na), and exhibits VC activity *in vitro* and *in vivo* after enzymatic hydrolysis to free VC by phosphatase and/or esterase.

In the present study, we examined the inhibitory effects of a novel amphiphilic ascorbic derivative, disodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-2Na), which is more stable than VCP-IS-Na, on melanogenesis in cultured human melanoma cells, normal human melanocytes, and three-dimensional human skin models.

Experimental

General Procedure ^{1}H- and ^{13}C-NMR spectra (δ, J in Hz) were recorded on a JEOL GSX-500 NMR spectrometer. Tetramethylsilane was used as the internal reference (δ 0.00) for ^{1}H-NMR spectra measured in chloroform-d_1. This solvent was also used for ^{13}C-NMR spectra. IR spectra were determined with a FT/IR-470 Pulse Fourier Transform Infrared Spec-

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trometer using a KBr disk. Fast atom bombardment high-resolution mass spectra (FAB-HR-MS) and FAB-MS were obtained on a JEOL JMS-HX 100 mass spectrometer. UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Optical rotations were measured with a JASCO DIP-1000.

Chemicals L-Ascorbic acid (VC) and synthetic melanin were obtained from Sigma-Aldrich Co., Ltd. (St. Louis, MO, U.S.A.). Sodium-2-*O*-L-ascorbyl phosphate (VCP-Na), L-DOPA and Advanced Protein Assay Reagent were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Synthesis of Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate (VCP-IS-2Na) Isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS) was synthesized from 5,6-*O*-isopropylidene-ascorbic acid and isostearyl dichlorophosphate by the methods from our previous report.[22] VCP-IS (1 mol) was reacted with sodium hydrate (2 mol). The reaction mixture was stirred at 0 °C for 3 h, ethanol was added, and it was then evaporated *in vacuo*. The residue was subjected to recrystallization in acetonitrile and ethanol to give VCP-IS-2Na (65% yield), as a white powder. For VCP-IS-2Na, ^{1}H-NMR ($CDCl_3$): δ 0.88 (24H, t, J=7.3 Hz, CH_3-11, 13, 15, 17), 1.00—1.05 (2H, m, 12-H), 1.1—1.19 (2H, m, 16-H), 1.21—1.25 (4H, m, 9, 10-H), 1.26—1.33 (1H, m, 11-H), 1.47—1.53 (1H, m, 15-H), 1.76—1.82 (1H, m, 8-H), 3.81 (2H, m, 7-H), 3.95 (2H, d, J=7.0 Hz, 6-H), 4.12 (1H, dd, J=1.8, 7.0 Hz, CH-5), 4.81 (1H, d, J=1.8 Hz, 4-H); ^{13}C-NMR δ: 171.18 (C-1), 167.63 (C-3), 110.58 (C-2), 77.41 (C-4), 69.89 (C-7), 69.04 (C-5), 63.25 (C-6), 51.23 (C-12), 48.56 (C-16), 45.98 (C-8), 38.03 (C-10), 31.21 (C-13), 31.05 (C-17), 30.90 (C-14), 30.06 (CH_3-13), 29.99 (CH_3-13), 29.93 (C-18), 29.87 (CH_3-17), 29.75 (CH_3-17), 29.56 (C-11), 28.67 (C-9), 22.62 (C-15), 18.76 (C-11), 18.50 (C-19); IR (KBr, cm^{-1}): 3384, 2955, 1731, 1592, 1230, 1047; FAB-HR-MS *m*/*z* (rel. int.): 533.2540 ([M$^+$], $C_{24}H_{43}O_9PNa_2$); UV λ_{max} (MeOH) nm (log ε): 241 (4.54), $[\alpha]_D^{24}$ +12.4° (c=0.10, MeOH). VCP-IS-2Na was identified as disodium isostearyl 2-*O*-L-ascorbyl phosphate [disodium 2-(1,3,3-trimethyl-*n*-butyl)-5,7,7-trimethyl-*n*-octyl-L-ascorbyl phosphate] (Fig. 1).

Cell Culture Human melanoma cells (HMV-II) were provided by the Cell Resource Center for Biomedical Research, Tohoku University. HMV-II cells were maintained in F12/Dulbecco's modified Eagle's Medium (DMEM) (GIBCO) supplemented with 10% fetal bovine serum. Normal human epidermal melanocytes (NHEM) from neonatal Asian foreskins and Medium 254 were obtained from Kurabo Industry Co., Ltd. (Osaka, Japan). These cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. The three-dimensional cultured human skin model, MelanoDerm (MEL300A; a co-culture of normal human melanocytes from Asian donors and normal human keratinocytes), was purchased from Kurabo Co., Ltd. (Osaka, Japan). The human skin model was maintained according to the manufacturer's instructions.

Inhibitory Effect on Melanogenesis Using HMV-II Cells HMV-II cells (4.0×10^5 cells) were planted with 5 ml of medium in a 25-cm^2 flask and cultured for 10 d. On the 1st, 3rd, and 7th day, the medium was changed to fresh medium containing various concentrations of VC, VCP-Na, and VCP-IS-2Na.

Inhibitory Effect on Melanogenesis Using NHEM NHEM (4.0×10^5 cells) were planted with 5 ml of Medium 254 in a 25-cm^2 flask and cultured for 7 d. On the 1st and 3rd day, the medium was changed to fresh medium containing various concentrations of VC, VCP-Na, and VCP-IS-2Na.

Determination of Cell Proliferation and Melanin Content in HMV-II Cells and NHEM HMV-II cells and NHEM were treated with 0.25% trypsin and the cell number was counted with a hemocytometer. Melanin content was measured using a modified version of Oikawa and Nakayasu[23] and Hosoi *et al.*[24] The cells were pelleted by centrifugation at 1000×***g*** for 10 min and sonicated in 250 μl of 0.1% Triton X-100/phospate buffer saline (PBS)(−). The lysate was solubilized by treatment with 500 μl of 10% dimethyl sulfoxide in 1 N NaOH aqueous solution at 80 °C for 2 h. The absorbance of solutions was measured at 470 nm. Synthetic melanin was used as a standard.

Assay of Tyrosinase Activity by HMV-II Cells and NHEM HMV-II cells and NHEM were planted at a density of 2.0×10^4 cells/well in 100 μl of medium in 96-well plates. After 1 d of culture, the medium was changed to fresh medium containing various concentrations of VC, VCP-Na, and VCP-IS-2Na. HMV-II cells and NHEM were incubated for 10 d and 7 d, respectively, and then washed with PBS(−) and lysed with 100 μl/well of 0.1% Triton X-100/PBS(−). After mixing the lysates by vibration, 50 μl of 10 mM L-DOPA was added to each well. After incubation at 37 °C for 3 h, the absorbance was measured at 475 nm.

Inhibitory Effect on Melanogenesis Using Cultured Human Skin Models The human skin model was placed in 6-well plates and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 1 h of culture, 50 μl of each sample solution at concentrations of 0.5% and 1.0% was applied to the surface of the tissue. The tissue was incubated for 3 weeks, and fed with 5 ml of fresh medium every other day.

Determination of Melanin Content in Cultured Human Skin Models The human skin model was homogenized in 450 μl of 1.0% sodium dodecylsulfate (SDS) containing 0.05 mM ethylenediaminetetraacetate (EDTA), and 10 mM Tris HCl, pH 6.8. To each homogenate, 20 μl of 5 mg/ml proteinase K was added. After incubation at 45 °C for 18 h, the homogenate was made basic by adding 50 μl of 500 mM sodium carbonate, and 10 μl of 30% hydrogen peroxide was added. Samples were maintained at 80 °C for 30 min. The mixture was extracted with 100 μl of chloroform : methanol (2 : 1) mixture. After centrifugation at 10000×***g*** for 10 min, the optical density at 405 nm was determined. Synthetic melanin was used as a standard.

MTT Assay of Cultured Human Skin Models The human skin model was placed in a 24-well plate, 300 μl of 1 mg/ml MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) solution was added to each well, and the tissue was incubated for 3 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. After incubation, the tissues were washed with PBS(−), and 2 ml of isopropanol containing 0.04 M HCl was added to each well. The plate was shaken gently at room temperature for 2 h. The absorbance of extracts was measured at 570—660 nm.

Statistical Analysis The proliferation, melanin content and tyrosinase activity data were expressed as the mean±standard error (S.E.), and subsequent inspection of means was evaluated by Student's t-test between two groups at a significance level of $p<0.05$.

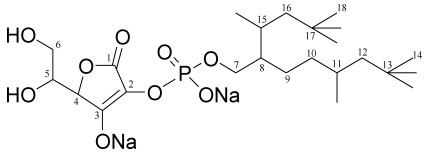

Fig. 1. Structure of Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate (VCP-IS-2Na)

Results

Effect of VCP-IS-2Na on Melanogenesis in HMV-II Cells To investigate the effects of VCP-IS-2Na on melanogenesis in human melanoma cells, HMV-II cells were cultured in medium containing various concentrations of VCP-IS-2Na. The melanin content of HMV-II cells was decreased by the addition of VCP-IS-2Na to the medium at concentrations of 100—300 μM (Fig. 2A). Melanin content was significantly inhibited at 300 μM, and the melanin content was reduced to 23% of that in non-treated cells. In contrast, VC and VCP-Na did not seem to inhibit melanin content at concentrations below 300 μM.

To exclude the possibility that the above inhibitory effects of VCP-IS-2Na on melanin synthesis might be caused by the inhibition of cell growth in human melanoma cells, we determined the number of cell grown of HMV-II cells in the presence and absence of treated with VCP-IS-2Na (Fig. 2B). VCP-IS-2Na did not seem to inhibit the cell viability at concentrations below 500 μM. These results indicate that VCP-IS-2Na had an inhibitory effect on melanogenesis at non-cytotoxic concentrations below 500 μM.

Effect of VCP-IS-2Na on Melanogenesis in NHEM To investigate the effects of VCP-IS-2Na on melanogenesis in melanocytes, NHEM were cultured in medium containing various concentrations of VCP-IS-2Na. The melanin content of NHEM was decreased in a dose-dependent manner by the addition of VCP-IS-2Na at concentrations of 50—100 μM (Fig. 3A). Melanin content was significantly inhibited at 100 μM, and the melanin content was reduced to 52% of that in non-treated cells. In contrast, VCP-Na did not seem to inhibit melanin content at concentrations below 100 μM, while

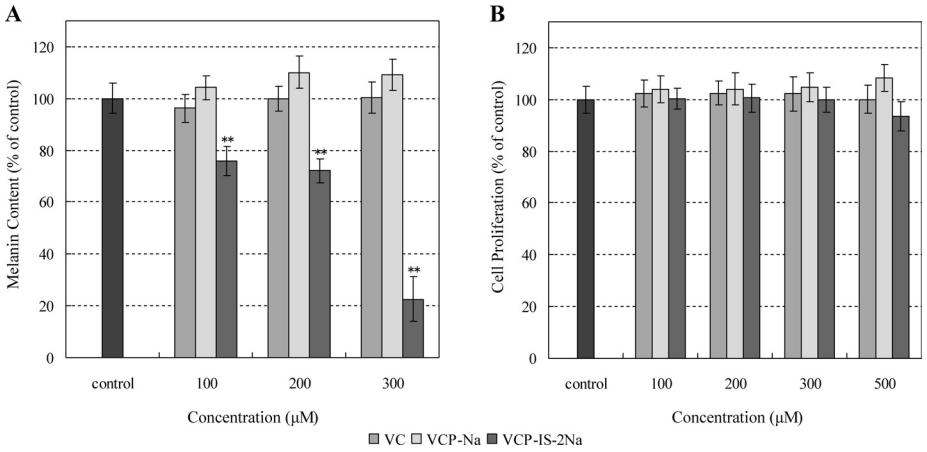

Fig. 2. Effects of VCP-IS-2Na on Melanogenesis and Cell Growth in HMV-II Cells

HMV-II cells (4.0×10^5 cells) were incubated in 5 ml of medium containing various concentrations of VC, VCP-Na, and VCP-IS-2Na for 10 d. Quantification of melanin synthesis (A) and measurement of cell proliferation (B) were performed as described in Experimental. Data are expressed as a percentage of control. Bars represent mean±S.E. of three independent experiments. ** Significantly different from the non-treated control group at $p<0.01$.

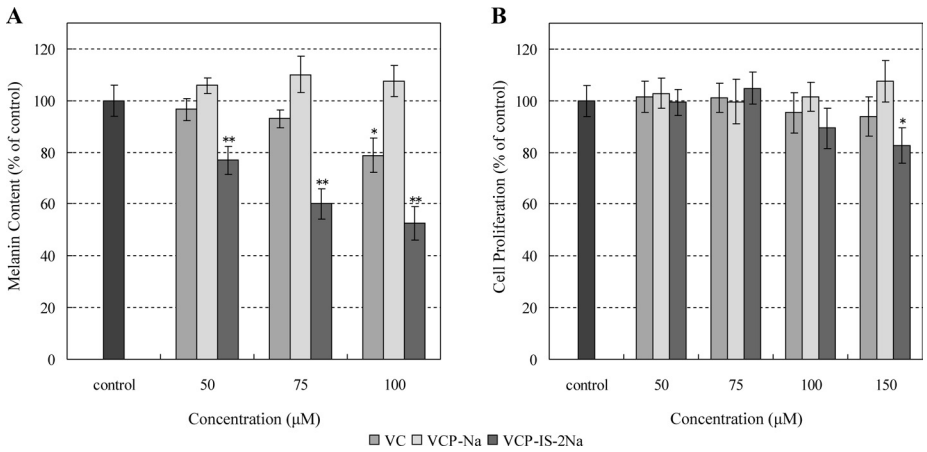

Fig. 3. Effects of VCP-IS-2Na on Melanogenesis and Cell Growth in NHEM

NHEM (4.0×10^5 cells) were incubated in 5 ml of medium containing various concentrations of VC, VCP-Na, and VCP-IS-2Na for 7 d. Quantification of melanin synthesis (A) and measurement of cell proliferation (B) were performed as described in Experimental. Data are expressed as a percentage of control. Bars represent mean±S.E. of three independent experiments. ** Significantly different from the non-treated control group at $p<0.01$. * Significantly different from the non-treated control group at $p<0.05$.

VC reduced melanin synthesis to 79% of that in non-treated cells at a concentration of 100 μM.

To exclude the possibility that the above inhibitory effects of VCP-IS-2Na on melanin synthesis might be caused by the inhibition of cell growth, we compared the number of NHEM grown in the presence and absence of VCP-IS-2Na (Fig. 3B). VCP-IS-2Na did not seem to inhibit the cell viability at concentrations below 100 μM. These results indicate that VCP-IS-2Na had an inhibitory effect on melanogenesis at non-cytotoxic concentrations below 100 μM.

Effects of VCP-IS-2Na on Cellular Tyrosinase Activity To identify how melanin synthesis was inhibited, we examined the inhibitory action of VCP-IS-2Na on cellular tyrosinase activity in HMV-II cells and NHEM. As shown in Fig. 4A, treatment of HMV-II cells with VCP-IS-2Na at concentrations of 100—500 μM suppressed tyrosinase activity in a dose-dependent manner. VCP-IS-2Na inhibited 85% of tyrosinase activity at concentration of 500 μM in HMV-II cells. The 50% inhibition concentration (IC_{50}) value of VCP-IS-2Na was 314 μM in HMV-II cells. In contrast, VC and VCP-Na did not seem to inhibit tyrosinase activity at concentrations below 500 μM. Tyrosinase activity in NHEM was suppressed by the addition of VCP-IS-2Na at concentrations of 50—150 μM (Fig. 4B). VCP-IS-2Na inhibited 67% of tyrosinase activity at a concentration of 150 μM in NHEM. The IC_{50} value of VCP-IS-2Na was 125 μM in NHEM. VCP-Na inhibited 43% of tyrosinase activity at a concentration of 150 μM, and VC slightly inhibited.

Effects of VCP-IS-2Na on Melanogenesis in Cultured Human Skin Models To identify the effects of VCP-IS-2Na on natural skin pigmentation, we used three-dimensional cultured human skin models. Figure 5 shows macroscopic darkening of a human skin model grown in the presence of different amounts of VCP-IS-2Na. Darkening of the model was clearly inhibited by the addition of 0.5% VCP-IS-2Na. When the cultured tissue was observed under an inverted microscope, there appeared to be fewer darkened melanocytes in VCP-IS-2Na-treated tissue than in non-treated tissue (Fig. 6). In contrast, macro- and microscopic observations of tissues treated with VC and VCP-Na did not appear to show fewer darkened melanocytes.

To quantitatively evaluate the inhibitory effect of VCP-IS-

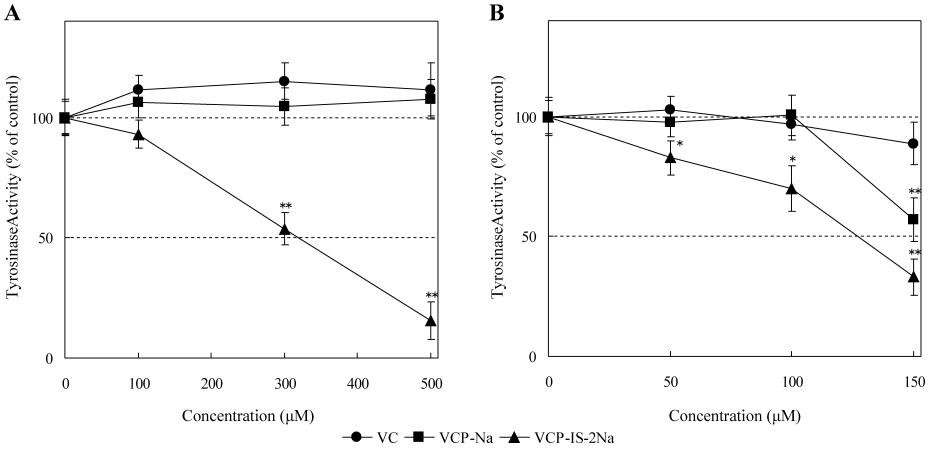

Fig. 4. Effects of VCP-IS-2Na on Cellular Tyrosinase Activity

HMV-II cells (A) and NHEM (B) were cultured for 10 d and 7 d, respectively, as described in Experimental. Bars represent mean±S.E. of three independent experiments. ** Significantly different from the non-treated control group at $p<0.01$. * Significantly different from the non-treated control group at $p<0.05$.

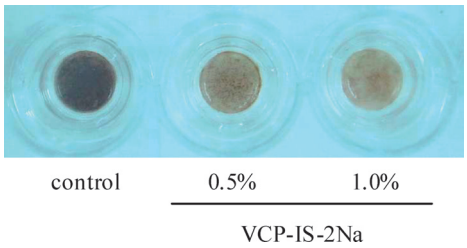

Fig. 5. Macroscopic Views of Cultured Human Skin Models Treated with VCP-IS-2Na

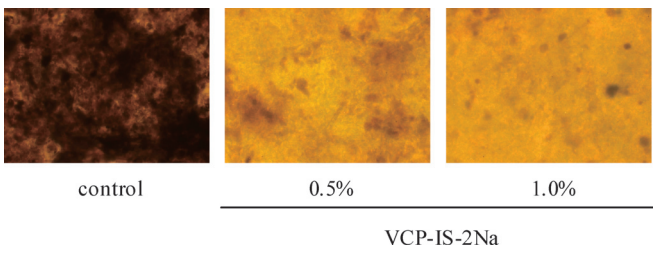

Fig. 6. Microscopic Views of Cultured Human Skin Models Treated with VCP-IS-2Na (100×)

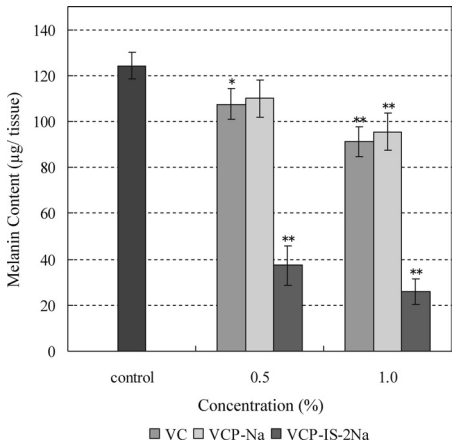

Fig. 7. Effects of VCP-IS-2Na on Melanin Synthesis in Cultured Human Skin Models

The human skin model was incubated for 3 weeks, as described in Experimental. Bars represent mean±S.E. of three independent experiments. ** Significantly different from the non-treated control group at $p<0.01$. * Significantly different from the non-treated control group at $p<0.05$.

2Na on cellular melanogenesis, the amount of melanin in the human skin models was measured (Fig. 7). With VCP-IS-2Na treatment at concentrations of 0.5% and 1.0% for 3 weeks, melanin production was significantly reduced to 30% and 21% of that in non-treated cells, respectively. The inhibitory effects of VC and VCP-Na on melanogenesis were significantly lower than that of VCP-IS-2Na.

Further, we evaluated cell viability in human skin models, which had been topically treated with different concentrations of VCP-IS-2Na, by MTT assay. No significant decrease in cell viability was observed when the tissue was treated with up to 1.0% of each sample (Fig. 8). These results suggest that topical treatment of tissue with VCP-IS-2Na decreased melanin synthesis without affecting cell viability.

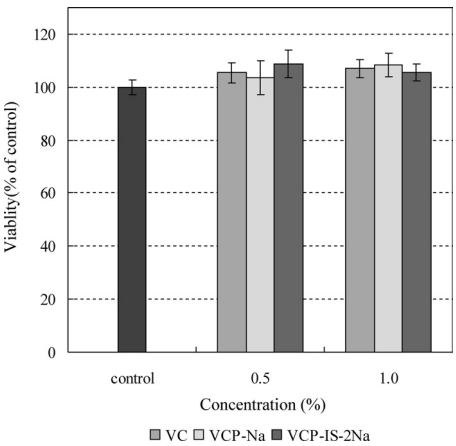

Fig. 8. Effects of VCP-IS-2Na on Cytotoxicity in Cultured Human Skin Models

The human skin model was incubated for 3 weeks. The viability of the human skin model was evaluated by MTT assay, as described in Experimental. Bars represent mean±S.E. of three independent experiments.

Discussion

In the present study, the novel amphiphilic ascorbic derivative VCP-IS-2Na significantly inhibited the synthesized melanin content in cultured human melanoma cells, normal

human melanocytes, and three-dimensional cultured human skin models. VCP-IS-2Na also significantly suppressed the cellular tyrosinase activity of cultured human melanoma cells and melanocytes. This decrease in the synthesized melanin content was well correlated with the inhibition of cellular tyrosinase activity in VCP-IS-2Na-treated melanoma cells and melanocytes. It has been suggested that the influence of VC on the monopherase activity of tyrosinase results from its ability to reduce enzymatically generated *O*-quinones. Therefore, we conclude that VCP-IS-2Na directly or indirectly suppresses melanin synthesis catalyzed by human tyrosinase. Tyrosinase Related Protein-1 (TRP1) has been suggested to have a variety of catalytic functions including low levels of the enzymatic activities ascribed to tyrosinase.[25,26] It has been reported that TRP1 possesses 5,6-dihydroxyindole-2-carboxylic acid oxidase activity, which can be blocked by VC. Moreover, VC may suppress melanin synthesis at various oxidative steps, such as 5,6-dihydroxyindole oxidation. Therefore, the inhibitory effects of VCP-IS-2Na on cellular tyrosinase activity may be due to the suppression of TRP1 activity with VC, which is released from VCP-IS-2Na.

We also evaluated the effect of VCP-IS-2Na on three-dimensional human skin models. Its inhibitory effect on melanogenesis was confirmed by quantitative measurements of melanin as well as macro- and microscopic observations of tissue pigmentation. Several researchers have indicated that human epidermal equivalents containing melanocytes and keratinocytes provide a convenient and reliable alternative to animal testing for evaluating the regulation of mammalian pigmentation.[27,28] Our results indicated that VCP-IS-2Na affects melanin synthesis in these three-dimensional human skin models. Furthermore, this effect was observed without any detectable cytotoxicity to the human skin models.

In this study, it was difficult to compare the inhibitory effect of VCP-IS-2Na on the three-dimensional human skin models with that on human cells. When 500 μg of VCP-IS-2Na (50 μl of 1.0%) was applied to the tissue, the original concentration was approximately calculated as 19 mM, which is much higher than that applied to cells. However, the final concentrations of VCP-IS-2Na that permeated through the tissue into the medium were estimated to be much less than those of the applied VCP-IS-2Na. This may partly explain the difference in sensitivity between the three assay systems that we used. Further we are trying to characterize the inhibitory effects of VCP-IS-2Na on the expression of the tyrosinase gene and pigmentation in human skin.

VCP-IS-2Na inhibited melanin synthesis and tyrosinase activity in cultured human melanoma cells, normal human melanocytes, and three-dimensional human skin models to a greater degree than VC and VCP-Na. We have previously reported that VCP-IS-Na has superior skin permeability and releases more free VC by phosphatase and/or esterase hydrolysis than VC and VCP-Na in the cells. In this study, no significant difference was observed in melanin synthesis in cultured human melanoma cells, normal human melanocytes, and three-dimensional human skin models, and skin permeability in three-dimensional human skin models, between VCP-IS-2Na and VCP-IS-Na (data not shown). The rigid lipid lamella structure of the stratum works as a barrier, especially for the adsorption of hydrophilic compounds.[13] These results suggest that the difference in the inhibitory effects of VC, VCP-Na, and VCP-IS-2Na on melanogenesis was due to skin permeability and the releasing activity of VC in human skin cells.

We found that the melanin content in normal human melanocytes was slightly inhibited by the addition of VC to the medium. It is well known that VC reduces melanin. Therefore, these results indicate that when the medium was changed to fresh medium containing various concentrations of VC on the 3rd day, there was a reduction in the melanin content.

In conclusion, VCP-IS-2Na inhibits melanogenesis in cultured human melanoma cells, normal human melanocytes, and three-dimensional human skin models to a greater degree than VC and VCP-Na. VCP-IS-2Na also significantly suppresses the cellular tyrosinase activity of cultured human melanoma cells and normal human melanocytes. Therefore, the inhibitory effect of VCP-IS-2Na on melanin synthesis is due to an inhibitory effect on cellular tyrosinase activity in human melanoma cells and normal human melanocytes. These results indicate that VCP-IS-2Na may be an effective whitening agent for the skin, and is metabolized from VCP-IS-2Na by phosphatase and/or esterase in the skin. Moreover, we expect the utilization of VCP-IS-2Na in the cosmetic industry.

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