Efficacy of fullerene capsule containing an amphipathic antioxidant vitamin

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INTRODUCTION

Ascorbyl 2-phosphate tocopherol (APT) is a free radical scavenger synthesized by connecting vitamin E with vitamin C (ascorbic acid) (AA) through a phosphate linkage. APT has been developed as an amphipathic antioxidant vitamin and is a water and oil soluble derivative of ascorbate and tocopherol, which is slightly soluble in oil but has a surface-active ability allowing the derivative to be used as an emulsifier and multi-layer liquid crystal nano capsule clathrate. Astaxanthin or other anti-oxidants as like fullerene were encapsulated in APT, giving a multi-layer liquid crystal emulsification nano capsule containing anti-oxidant, called "APT-capsule". Although the reaction rate constant of fullerene against superoxideanion radical seems to be lower than those of ascorbic acid (AA) (vitamin C) or tocopherol (vitamin E), the ability is known to be last longer than other anti-oxidants. For finding out the useful application of the unique anti-oxidation ability of fullerene, our laboratory showed that fullerene can suppress UV-B induced ascorbyl radicals generated from skin tissue treated with AA 1). Since fullerene was expected to be useful for stabilizing a vitamin C derivative capsule having multi-layer liquid crystal structure 2), fullerene was attempted to be encapsulated in the vitamin C derivative nano capsule, and the properties of this unique nano-capsule were evaluated. Fig. 1 shows the multi-layer liquid crystal structure of self emulsifying ascorbyl 2-phosphate tocopherol (APT) / lipid nano capsules with fullerene (APT-capsule). Since the liquid crystal structure of APT was constructed from water and lipid multiple layers (e.g. water-lipid-water-lipid, etc.), lipid-soluble anti-oxidants such as fullerene and astaxanthin were encapsulated stably in the lipid layers and lipid core. APT has been developed as an amphipathic antioxidant vitamin and is a water and oil soluble derivative of ascorbate and tocopherol, which is slightly soluble in oil but has a surface-active ability allowing the derivative to be used as an emulsifier and multi-layer liquid crystal nano capsule clathrate. Furthermore, this capsule is hydrolysed from the capsule surface by enzyme like a

phosphatase in tissue, and ascorbate, tocopherol and astaxanthin are released slowly from this capsule. The efficacy of this vitamin CE derivative nano capsule containing fullerene on UV-B irradiation was investigated. Furthermore, the stability of astaxanthin encapsulated nano-capsule was examined. When APT-capsule containing AA at a high concentration was irradiated by UV-B, the amount of AA radicals was observed. However, when APT-capsule containing anti-oxidant was irradiated, the generation of AA radicals was found to be reduced significantly, showing that anti-oxidant controlled the generation of AA radicals by ultraviolet rays with the presence of amphipathic vitamin C. APT-capsule was also found to inhibit the decomposition of astaxanthin, under a hyper oxidation condition of lipids. The experiments suggested that (1) APTF-capsule inhibited the oxidization of both water-soluble and lipid-soluble antioxidants, and (2) its anti-oxidation property was due to a possible synergistic effect of amphipathic vitamins and anti-oxidant. Being encapsulated in APT-capsule, unstable anti-oxidants such as astaxanthin or fullerene was preserved stably by the redox barrier of APT.

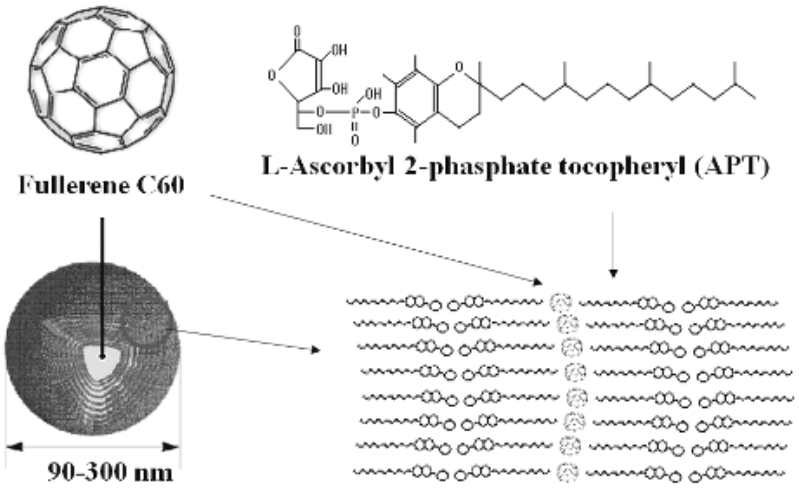

Figure 1. Multi-layer liquid crystal structure consisting of self-emulsifying ascorbyl 2-phosphate tocopheryl (APT) / lipid nano-capsules. APT liquid crystal structure is constructed from water and lipid multiple layers of (water-lipid-water-lipid, etc.). Lipid-soluble anti-oxidants such as fullerene and astaxanthin can be mixed in the lipid layers and lipid core. When unstable anti-oxidants such as astaxanthin are encapsulated in the capsule, the anti-oxidants are allowed to stay stably in the structure by its redox barrier. Fullerene can inhibit pro-oxidant (ascorbyl radical) of ascorbate strongly.

ESR analysis

The ESR peak intensities ratio (EPI) of free radical on skin tissue samples after UV-B irradiation shown in Fig. 2. ESR is an abbreviated designation of “electron spin resonance”. ESR is the one of the convenient analysis method which can detect a kind of each free radical species easily and directly from 3D skin tissu in a short time. The relative EPI of HO• (hydroxyl radical), H• (hydrogen radical), O_2• (superoxide anion radical) (0h.) and ascorbyl

radical (24h.) detected in the skin tissue after UV-B irradiation. HO•, H• and O_2• were significantly increased by UV-B irradiation, compared to the control (non-UV-B-irradiated specimens) at 0 h (Fig. 2-A). The application of AA (100 mmol/L), APT (100 mmol/L), APT(100 mmol/L) with fullerene (30μmol/L) significantly decreased H•, O_2•, and HO•, compared to the negative control ($P < 0.001$, Fig. 2-A). However, AA• (ascorbyl radical) increased with the application of AA (100mmol/L, $P < 0.01$, against the positive control) at 24 h after the irradiation (Fig. 2-B). AA• inhibited with the application of 100 mmol/L APT and 30μmol/L fullerene in Fig.2-B (against the positive control) at 48 h after the irradiation. Especially, the nano capsule of 100 mmol/L APT with 30 μmol/L fullerene strongly inhibited AA• (Fig. 2-B), compared to the positive control ($P < 0.01$) at 48 h.

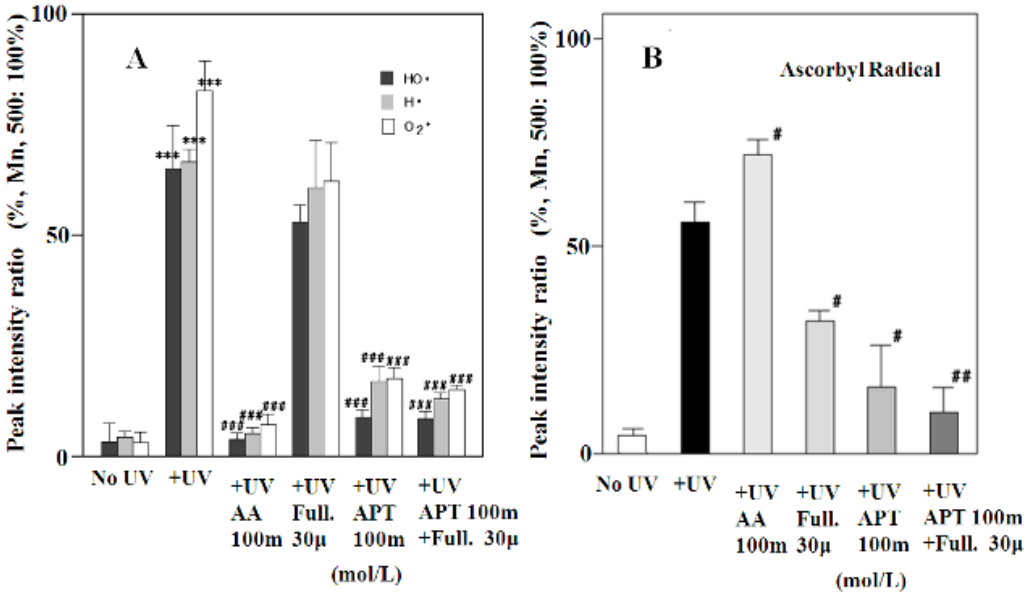

Figure 2. ESR peak intensities ratio (EPI) of HO・, H・, O2・, and Ascorbyl radical after the application of ascorbic acid (AA), fullerene, APT, or APT with fullerene to skin tissue irradiated by UV-B. The bars and lines represent the mean count and SEM of the peak intensity ratio (n = 8), respectively. *$P < 0.05$, ***$P < 0.001$ against the negative control (non UV-B control). #$P < 0.05$, ##$P < 0.01$ and ###$P < 0.001$ against the positive control (UV-B irradiation control) at the same time.

Cell Viability ratio

Fig. 3 shows NIH-3T3 cell viability ratio after UV-B irradiation (100 J/m^2) for 48 h. The cell viability ratio of the positive control with UV-B irradiation (+UV) showed a significant decrease to 46%, compared to that of the negative control without UV irradiation (no UV) ($P < 0.001$). Although 30 μmol/L fullerene +UV group increased its cell viability ratio significantly (69%), 100 μmol/L APT +UV group increased the ratio significantly (80%), and 100 mmol/L APT with 30 μmol/L fullerene + UV group showed an increase significantly (91%), compared to the vehicle + UV control.

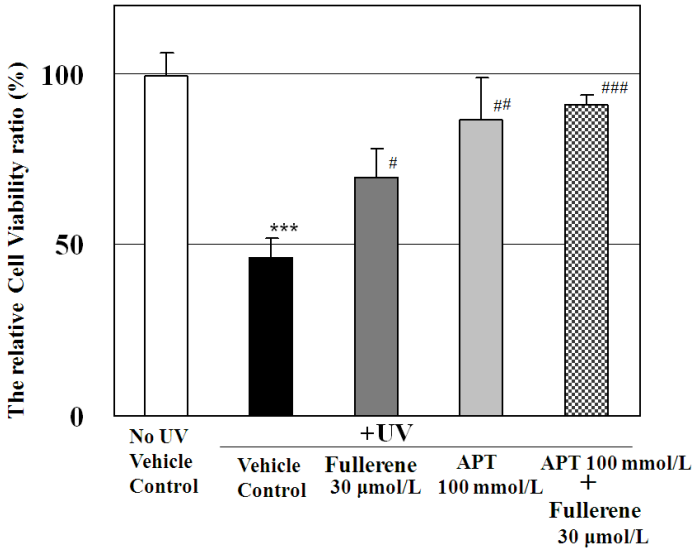

Figure 3. NIH-3T3 cell viability ratio at 48 h after UV-B irradiation. UV-B irradiation induced NIH-3T3 cell viability in vitro was detected by a cell viability analyzer. Each bar represents the mean count with SEM of the relative erythema index (n = 8). *** : $P < 0.01$ against no UV-B irradiation group. # : $P < 0.05$ against UVB irradiation group. ## : $P < 0.01$ against UVB irradiation group. ### : $P < 0.001$ against UV-B irradiation group.

Apoptosis index

Fig. 4 shows relative Yo-Pro-1 (apoptosis index) fluorescence intensity ratio (RYI) as an apoptosis index of NIH-3T3 cell after UV-B irradiation for 48 h. Apoptosis is a cell damage index by UV-B. The vehicle control with UV irradiation (+ UV) showed a significant increase in apoptosis index to 64% (mean RYI), compared to the negative control ($P < 0.01$). Fullerene (30 µmol/L) group showed a decrease to 178% (non-significant), compared to the vehicle control + UV. The mean RYI of the vehicle control + UV decreased significantly to 132% with the application of 100 µmol/L APT. Nano-capsule containing 100 mmol/L APT and 30 µmol/L fullerene significantly decreased the mean RYI to 119% ($P < 0.05$).

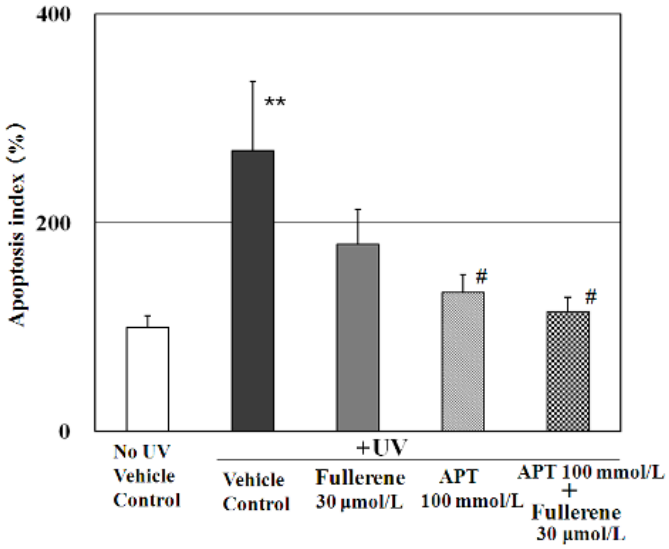

Figure 4. Yo-Pro-1/PI assay (an apoptosis index) at 48 h after UV-B irradiation. Irradiation induced NIH-3T3 apoptosis in vitro was detected by Yo-Pro-1/PI assay. Each bar represents the mean count with SEM of the relative apoptosis index (n = 8). * : $P < 0.05$ against non UVB irradiation group. # : $P < 0.05$ against UVB irradiation group.

Lightness value (L-value of Lab color analysis)

Fig. 5 shows the L-value changes of 1% astaxanthin stored at 40 °C for 80 days. L-value is an index to express whiteness of skin, when astaxanthin disintegrates, this numerical value rises. The L-value of 1% astaxanthin in APT-F emulsion (no nano-capsule) was found to increase significantly (L value: 213, value in APT-F multi-layer liquid-crystal decreased significantly (L value: 198, $P < 0.001$), compared to the vehicle control ((L value: 239) at 80 days.

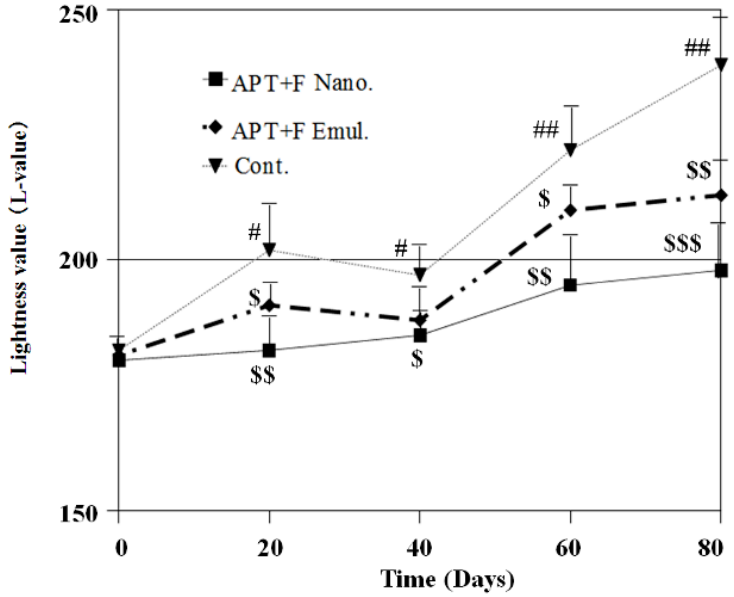

Figure 5. L-value changes of 1% astaxanthin stored at 40 ℃ for 80 days. Each bar represents the mean value with SEM of the L-value (n = 8). # : $P < 0.05$ against 0 day. ## : $P < 0.01$ against 0 day. $: $P < 0.05$ against the control at the same day. $$: $P < 0.01$ against the control at the same day, $$$: $P < 0.001$ against the control at the same day.

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CONCLUSIONS

The results suggested that fullerene in APT nano-capsule (APTF-capsule) inhibited ascorbyl radicals in skin tissue that was irradiated by UV-B. And fullerene in the capsule more significantly inhibited apoptosis and cell death induced by UV-B irradiation than the solo application of individual components. Additionally, APTF-capsule more significantly inhibited the color change of encapsulated astaxanthin stored at 40 °C for 80 days than mono-layer emulsification capsule. This multi-layer APT nano-capsule containing fullerene is a promising basic tool for developing anti-aging products including cosmetics and medicines, because this tool has an effective redox balance, which allows water-soluble and lipid-soluble redox molecules to synergistically work for expressing its anti-oxidant ability. In addition, kind and level of anti-oxidant in this capsule were able to change by user opption. Please contact to the writer, if a reader is interested in this sample.

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