

Confidential

Technical Information
on
TPNaTM
INCI name: Sodium Tocopheryl phosphate

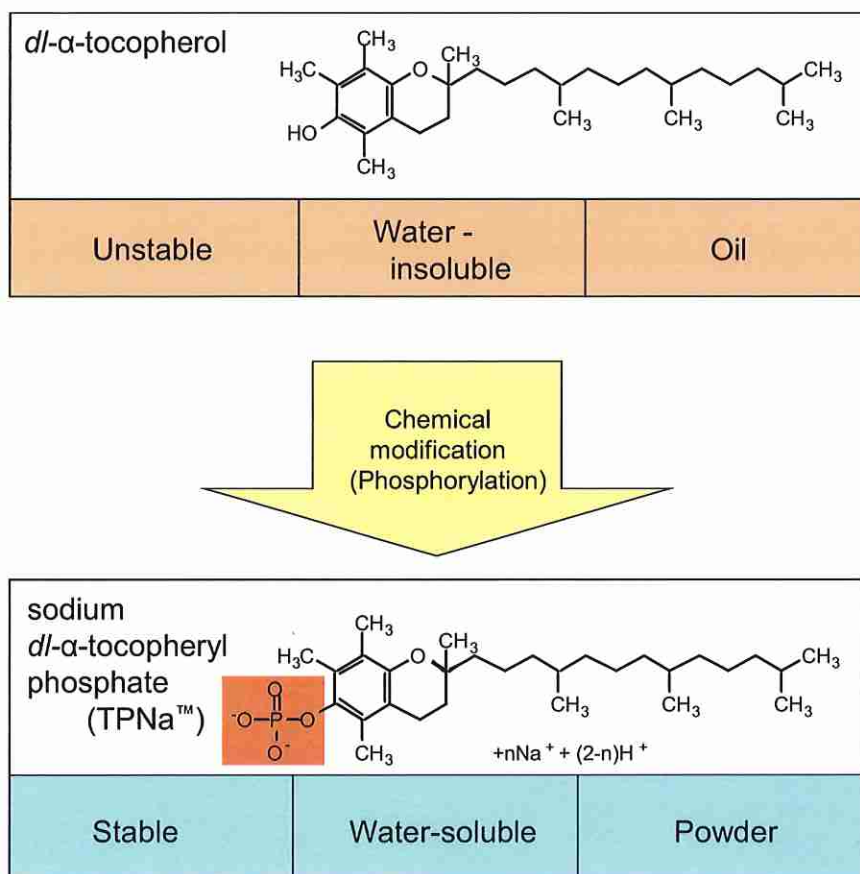


Showa Denko K.K.
Functional Chemicals Division

Ver. 1412NA

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Vitamin E Modification



TPNa™ is a stable, water-soluble powdery vitamin E.

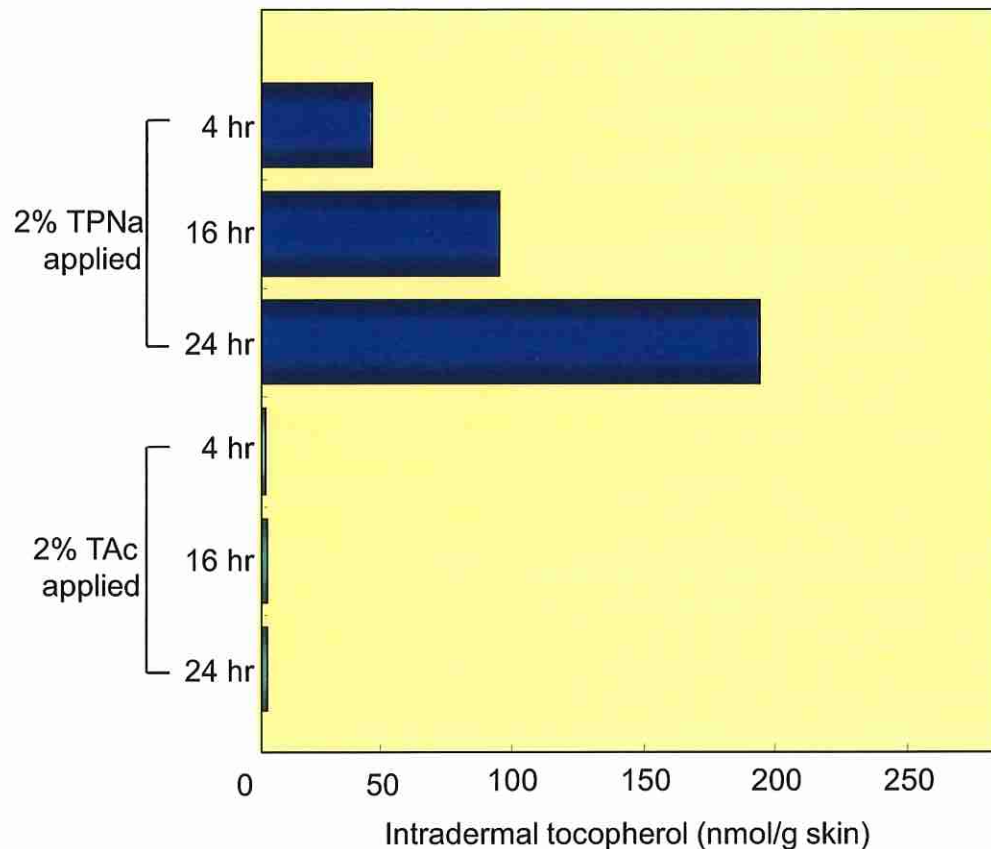
Alpha-Tocopherol (vitamin E) is one of the most important vitamins for cosmetics. Acting in harmony with ascorbate (vitamin C) vitamin E protects skin cells from oxidative attacks of radicals and peroxides.

As tocopherol is easily oxidized and quickly loses the reductive activity, tocopheryl acetate, a stable derivative of tocopherol, is popularly formulated. However, its use for aqueous formulations is restricted due to its oily form and poor solubility in water.

Our new product, Vitamin E Phosphate (sodium *dl*-α-tocopheryl phosphate, TPNa™) is a water-soluble powdery vitamin E. The oxygen-sensitive hydroxyl group of tocopherol is chemically modified and protected with a phosphoryl group. Once absorbed in skin, TPNa™ is readily converted to the active tocopherol via hydrolysis catalyzed by phosphatase, a ubiquitous enzyme in skin.

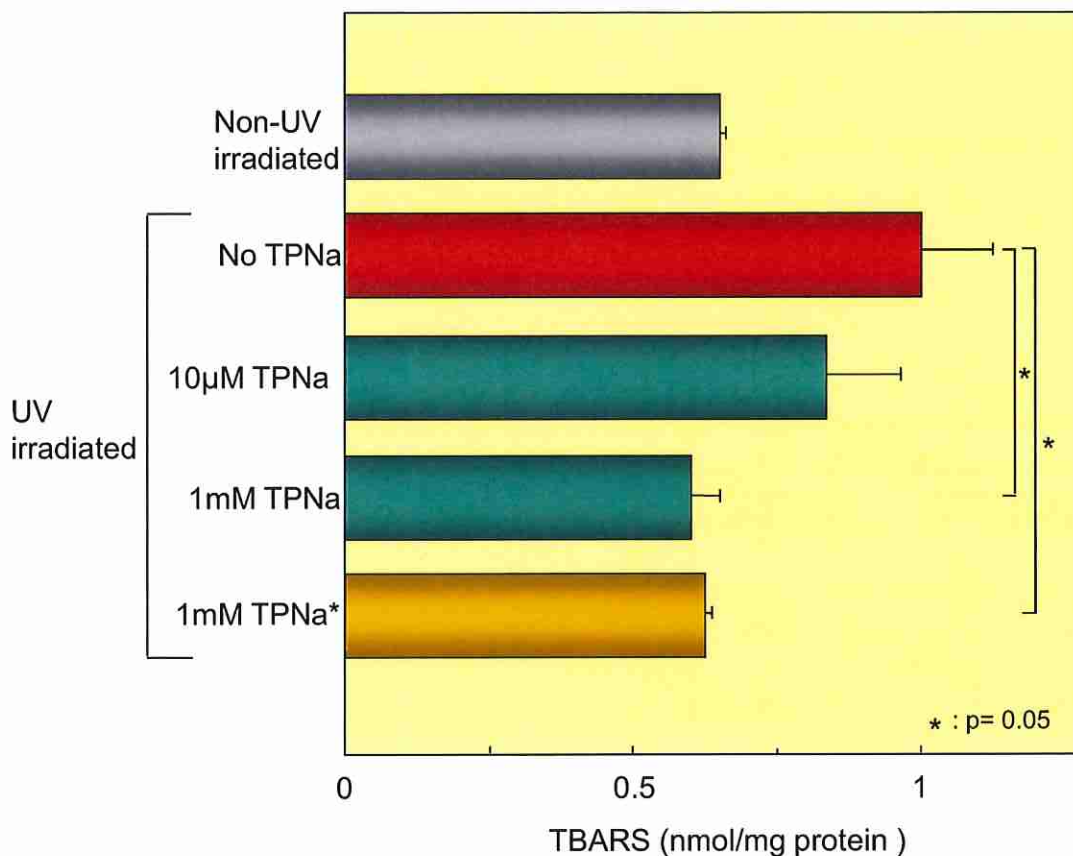
V0010-1.3.1E

Permeation and Conversion in Skin Model



The permeation of sodium α -tocopheryl phosphate (TPNa™) and its conversion to α -tocopherol (Toc) was examined using a three dimensional restructured human skin model (TESTSKIN™ LSE-high, TOYOBO, Japan). TPNa™ or α -tocopheryl acetate (TAc) was dispersed in HEPES buffer (pH 7.2) containing 5% ethanol at the final concentration of 20 mg/ml (2%). 100 μ l of each solution was applied onto the skin model's surface and the skin model was incubated at 37°C. At 8, 16, 24 hours of incubation the skin model was homogenized and intradermal concentrations of released Toc was determined by HPLC. In these experiments TPNa™ showed a quick release of Toc, while there was almost no Toc release observed by TAc application.

V0040-2.1.1E

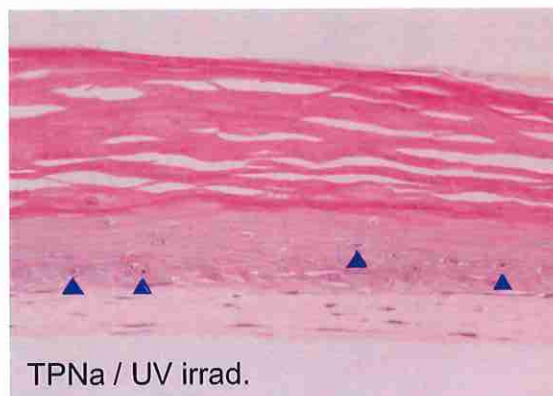
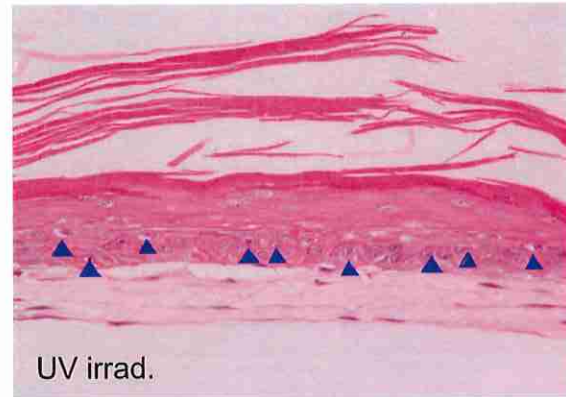


Suppression of lipid peroxidation caused by UV irradiation.

Anti-oxidative effects of sodium *d*- α -tocopheryl phosphate (TPNa™) were examined using cultivated human fibroblast (NB1RGB).

Cells were precultivated in MEM containing 10% FBS prior to the TPNa™ treatment (at 10 μ M or 1mM for one hour) in PBS replaced from the medium. At the end of treatment UVA was irradiated at 5.0 J/cm². Cells were then collected, washed, and disrupted by ultrasonic treatment in ice bath before the measurement of thiobarbituric acid-reacting substance (TBARS) content as an indicator of lipid peroxidation.

UVA irradiation increased the TBARS content by 50%, which was significantly reduced by the pretreatment with TPNa™. 1mM TPNa™ completely canceled the increase of TBARS formation. There was no difference observed in TBARS suppression in the cells thoroughly washed before the UVA irradiation (bar with asterisk*), which suggested that TPNa™'s UV-blocking effect was negligible in this experimental system.



TPNa™ protects skins from UV irradiation.

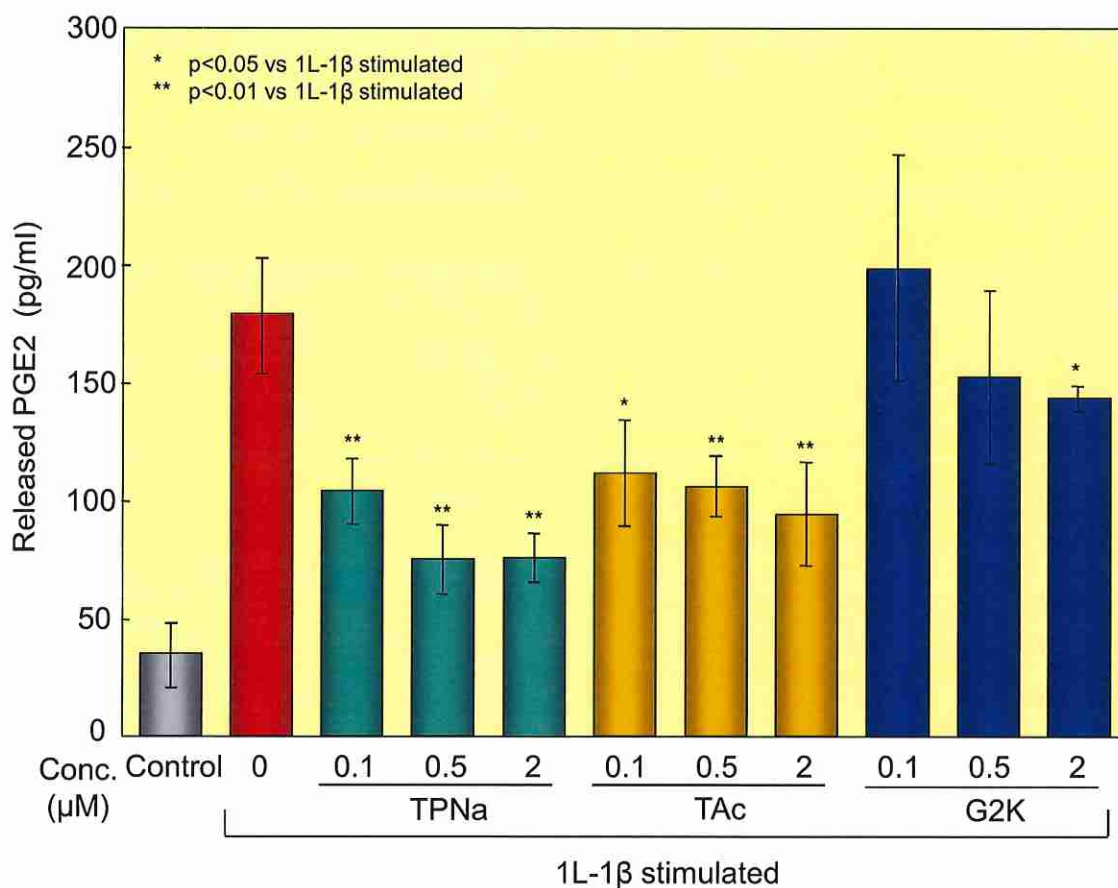
The protective effect of sodium *dl*- α -tocopheryl phosphate (TPNa™) against UV irradiation was examined using a three dimensional restructured human skin model (TESTSKIN™ LSE-high, TOYOBO, Japan).

After irradiation by UVB at an energy of 80 mJ/m², the skin models were cultivated with 2% solution of TPNa™ on its surface. After 2 hours, TPNa™ solution was removed and the skin models were cultivated for another 22 hours.

After 24 hours cultivation, the skin models were fixed and stained with 1% hematoxylin and eosin by the ordinary method.

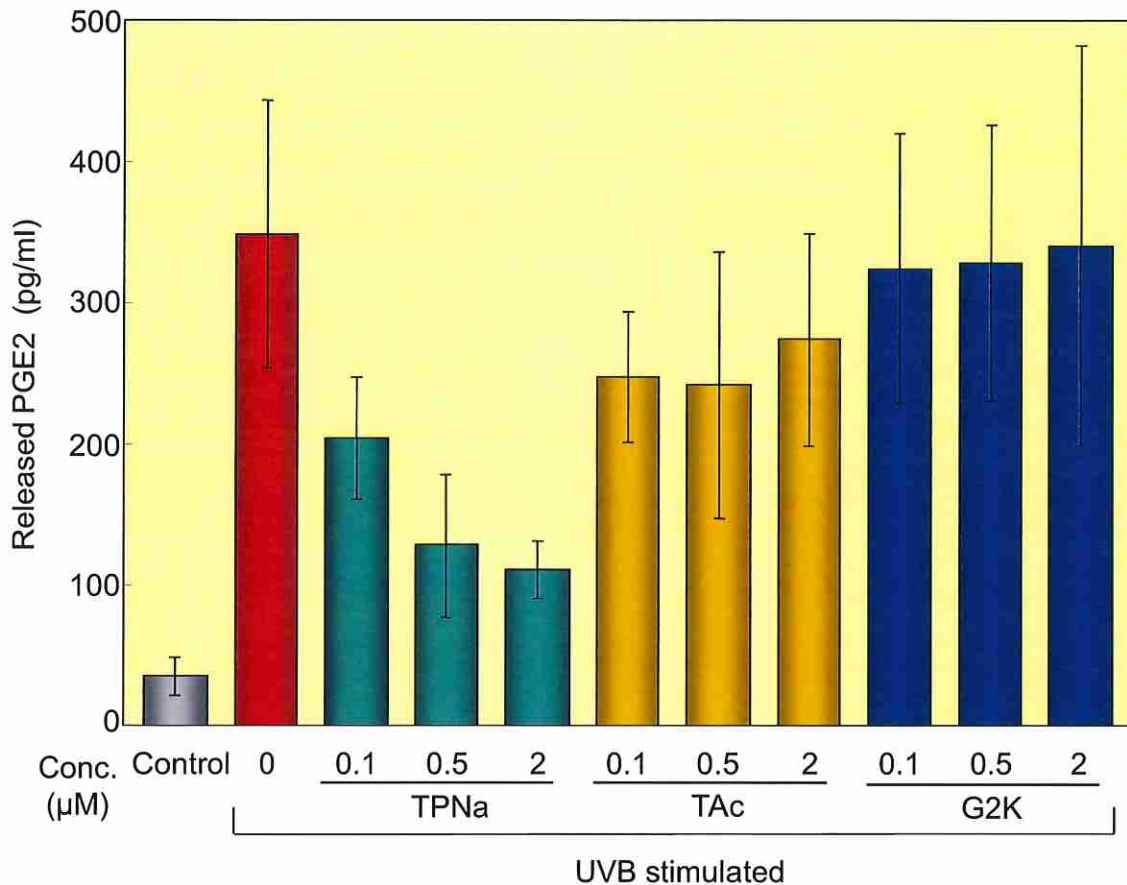
The UV-damaged cells were stained darker (sunburn cells, typically indicated by blue arrows).

The observations suggested that the damage of corneous layer and the sunburn cells formation was effectively suppressed by the treatment with TPNa™.



TPNa™ suppresses prostaglandin E2 generation.

The suppressive effects of TPNa™ on inflammation was examined using cell cultivation system. Human keratinocyte SVHK was cultivated until confluent and pretreated with 0.1 to 2 μM TPNa™ for twenty four hours. The cells were stimulated by addition of 10 ng/ml of recombinant human interleukin 1-beta (IL-1β) to trigger the simulated chain reaction of inflammation. After the cultivation in this medium for twenty four hours, the medium was sampled for the measurement of released prostaglandin E2 (PGE2), an inflammation marker, by the ELISA method. The cell amount was spectrophotometrically determined as absorbance at the wavelength of 570 nm (A570) by Alamar Blue method after the medium sampling. The PGE2 generation was significantly enhanced by addition of IL-1β, while it was suppressed almost completely by the pretreatment with TPNa™. The weaker suppression was observed by the pretreatment with tocopheryl acetate (TAc) or dipotassium glycyrrhizate (G2K) at the same concentration.



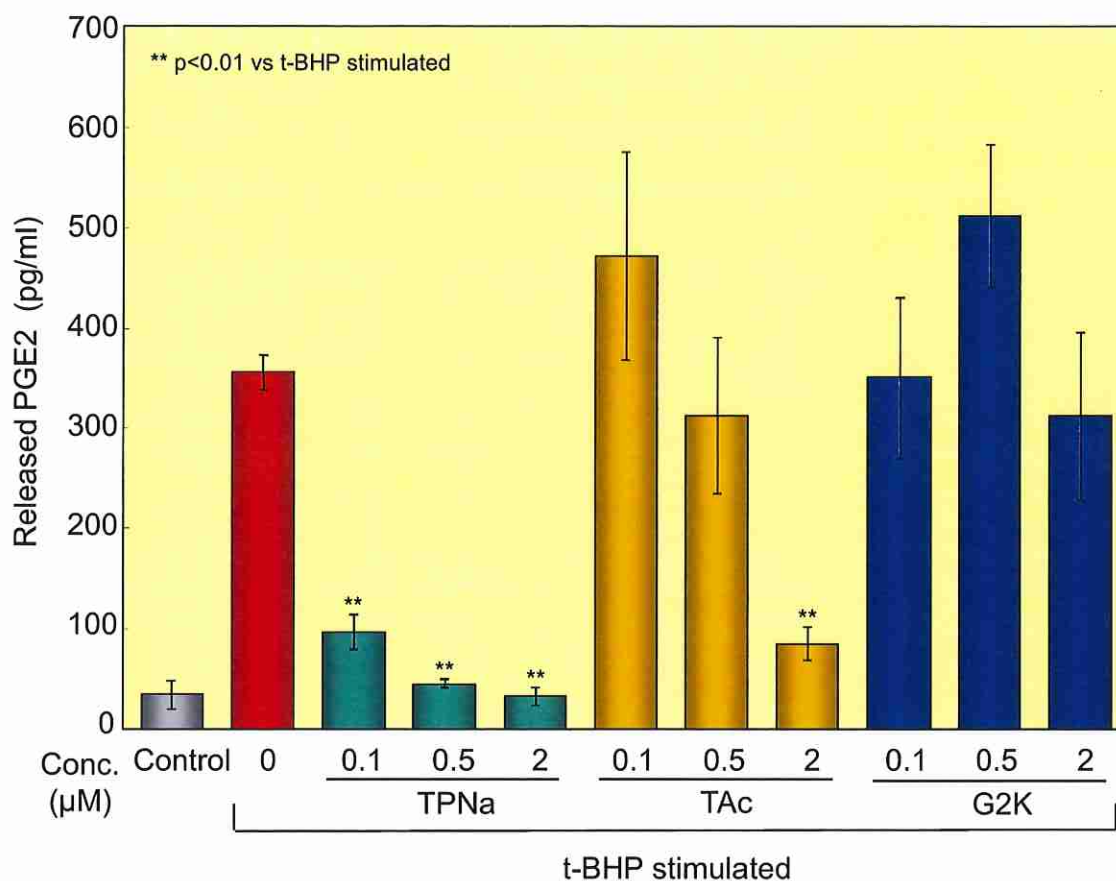
Suppression of inflammation caused by UV irradiation.

Sodium *dl*- α -tocopheryl phosphate (TPNa™) suppresses the inflammation caused by strong UV-B irradiation.

0 to 2 μ M TPNa™ was added to the medium of human keratinocyte grown confluent, and the cells were cultivated for twenty four hours. After the medium was replaced with the one containing no TPNa™, the cells were irradiated by UVB at an energy of 60 mJ/cm². The amount of secreted prostaglandin E2 (PGE2) was determined by ELISA method after another twenty four hours post-cultivation.

Unlike the effects against the IL-1 β induction, very low concentrations of TPNa™ pretreatment gave the significant suppression of inflammation. The weaker or no suppression were observed by the pretreatment with tocopheryl acetate (TAc) or dipotassium glycyrrhizate (G2K) at the same concentration.

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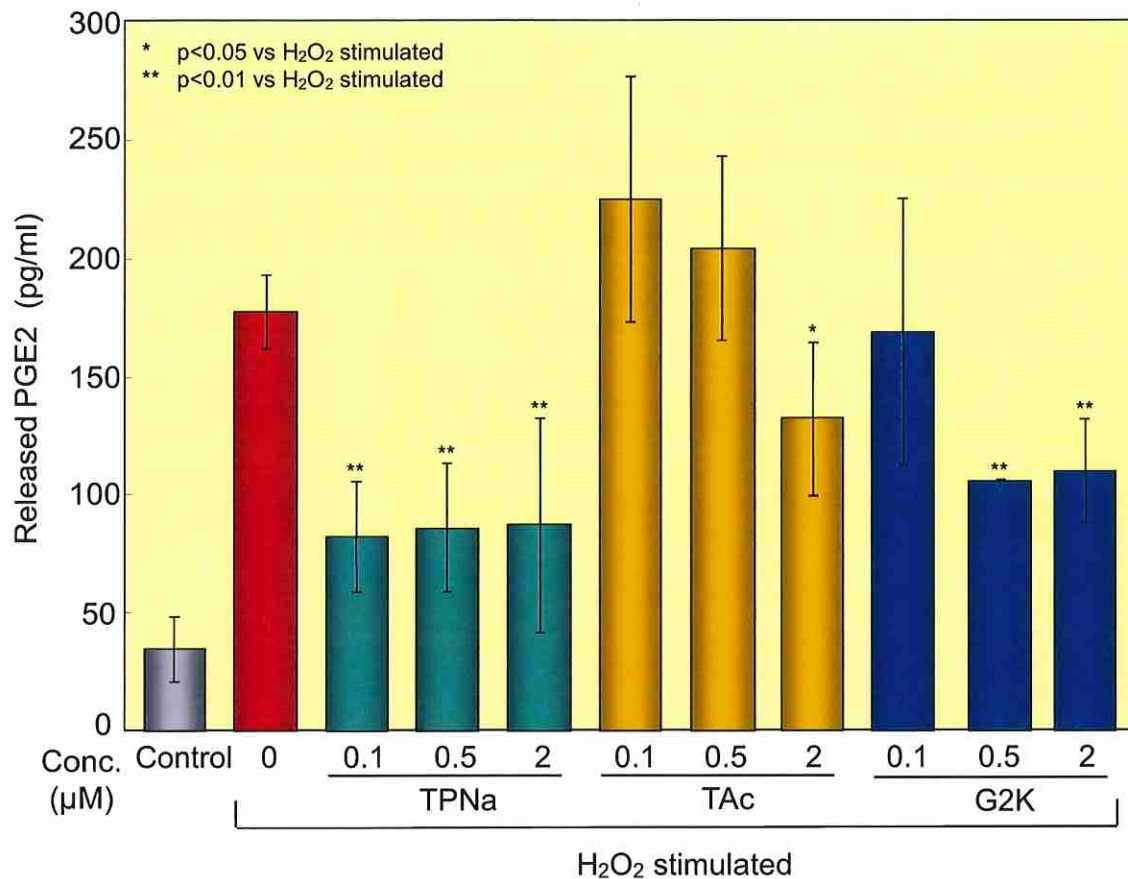


Suppression of inflammation caused by hydrophobic oxidant.

Sodium *d*/ α -tocopheryl phosphate (TPNa™) strongly suppresses the inflammation caused by tert-butyl hydroperoxide (t-BHP).

0 to 2 μM TPNa™ was added to the medium of human keratinocyte grown confluent, and the cells were cultivated for 24 hours. The medium was replaced with the one containing 0.5 mM t-BHP for 30 minutes, and the amount of secreted prostaglandin E2 (PGE2) was determined by ELISA method after another 24 hours post-cultivation.

TPNa™ suppressed the PGE2 generation almost completely, while tocopheryl acetate (TAc) or dipotassium glycyrrhizate (G2K) showed weaker or no suppressive activity.



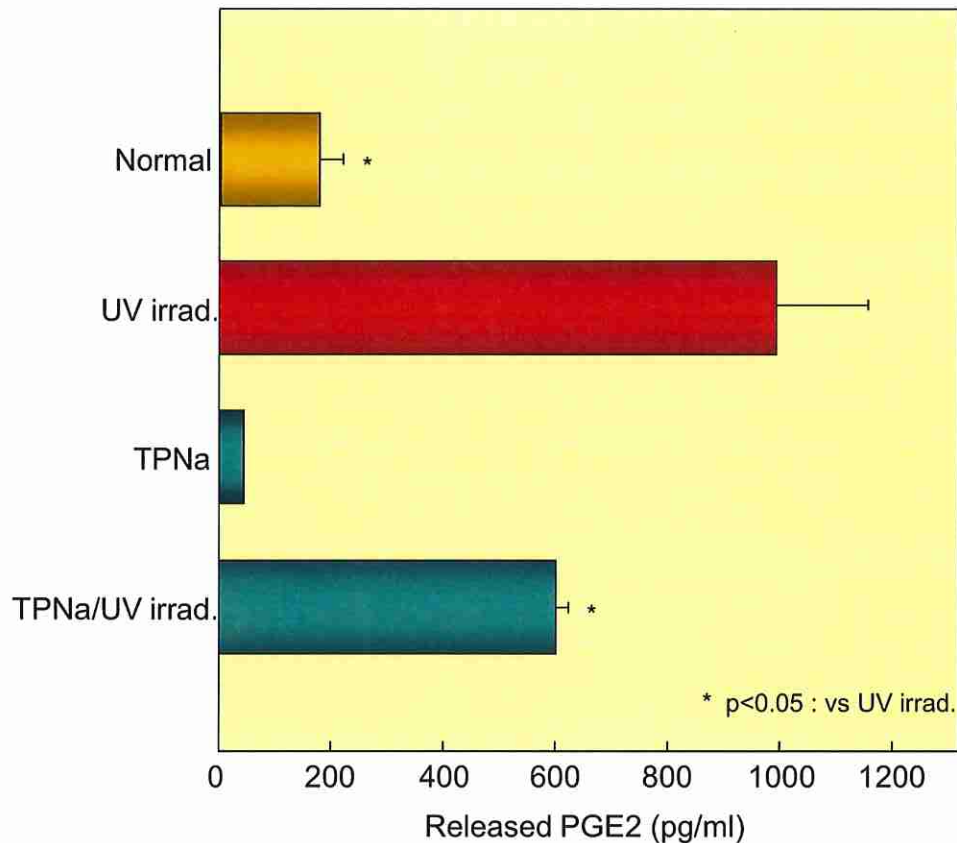
Suppression of inflammation caused by hydrophilic oxidant.

Sodium dl- α -tocopheryl phosphate (TPNaTM) also suppresses the inflammation caused by hydrogen peroxide (H₂O₂).

0 to 2 μ M TPNaTM was added to the medium of human keratinocyte grown confluent, and the cells were cultivated for 24 hours. The cells were treated with the medium containing 1 mM H₂O₂ for 30 minutes, and the amount of secreted prostaglandin E2 (PGE2) was determined by ELISA method after another 24 hours post-cultivation.

TPNaTM suppressed the PGE2 generation, while tocopheryl acetate (TAc) or dipotassium glycyrrhizate (G2K) showed weaker or no suppressive activity.

TPNaTM shows the suppressive effect on the inflammation caused by both of the hydrophilic and hydrophobic oxidant, which suggests to come from its amphipathic property.



Suppression of inflammation caused by UV irradiation in skin model

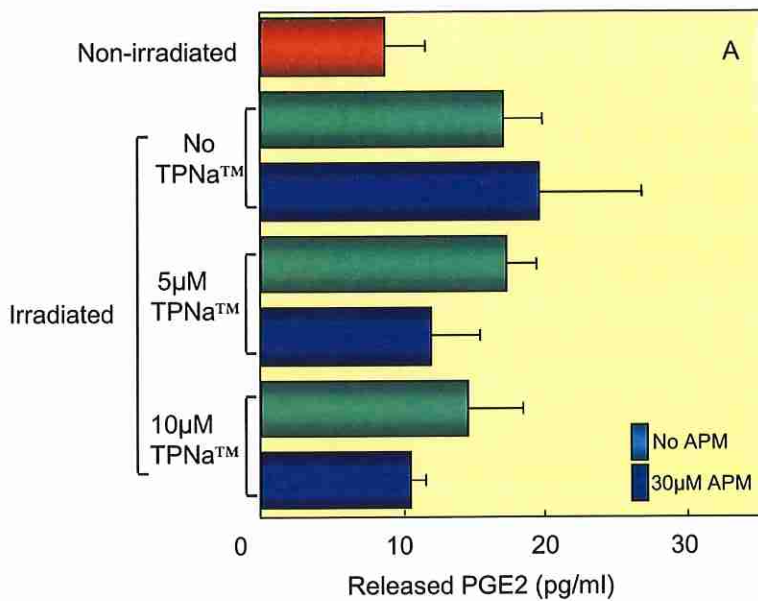
The suppressive effect of sodium *d*- α -tocopheryl phosphate (TPNa™) on inflammation in skin was examined using a three dimensional restructured human skin model (TESTSKIN™ LSE-high, TOYOBO, Japan).

After irradiation by UVB at an energy of 80 mJ/m², the skin models were cultivated with 2% solution of TPNa™ on its surface. After 2 hours, TPNa™ solution was removed and the skin models were cultivated for another 22 hours.

After 24 hours cultivation, the amount of secreted prostaglandin E2 (PGE2) in the medium was determined by ELISA method.

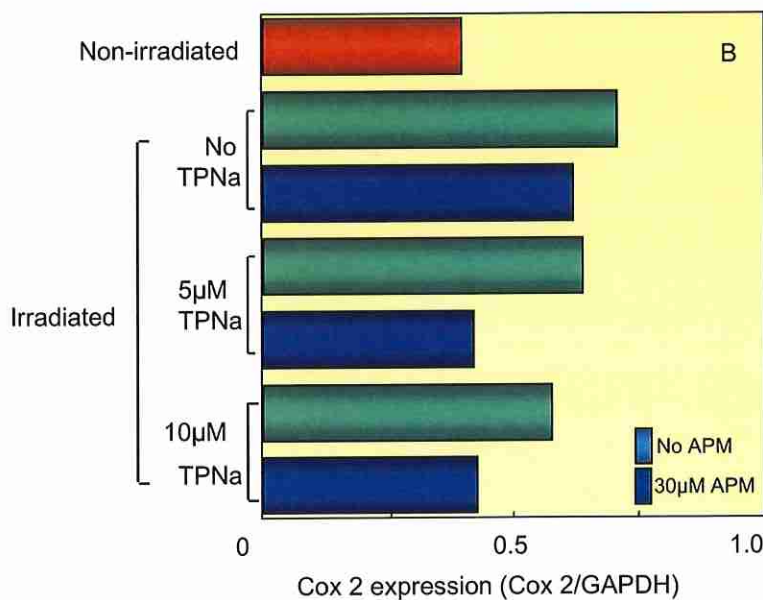
The PGE2 generation was significantly enhanced by UVB irradiation, while it was suppressed by the post-treatment with TPNa™.

Synergy with Vitamin C



The synergistic effects of sodium vitamin E phosphate (sodium α -tocopheryl phosphate, TPNa™) with vitamin C phosphate (Magnesium ascorbyl phosphate, APM) on inflammation was examined in a cell cultivation system.

Human keratinocyte (SVHK) was cultivated in the medium containing 0, 5, or 10 μ M TPNa™ for 24 hours. After the cultivation the medium was replaced with phosphate buffer, and the cells were irradiated by UVB (30 mJ/cm²). The concentration of released prostaglandin E2 (PGE2) in the buffer was determined by ELISA method after another 24 hour-incubation with or without 30 μ M APM.



Supplementation of APM significantly enhanced the suppressive effects of TPNa™ on PGE2 generation. The PGE2 level of the cells treated with 10 μ M TPNa™ and 30 μ M APM remained almost the same as that of non-UVB irradiated cells (A).

No suppressive effect was observed in APM-alone treated cells, which suggested that vitamin C reacted on vitamin E but not on the irradiation cascade directly.

The expression of cyclooxygenase (Cox2) gene, which was responsible for the inflammation, was also examined in the same experimental system. The expression level showed a good correlation with PGE2 concentration in each tested condition (B).

V0130-1.2.3E

Comparison of Anti-inflammatory Agents



Properties	TPNa™	TAc	G2K
Conversion to active tocopherol	+++	+	n.t.
Antioxidative activity	+++	+	n.t.
Anti-irradiation activity	+++	+	
Anti-inflammatory activity	+++	++	+

n.t. ; not tested

TPNa™ shows better activities than the other anti-inflammatory agents.

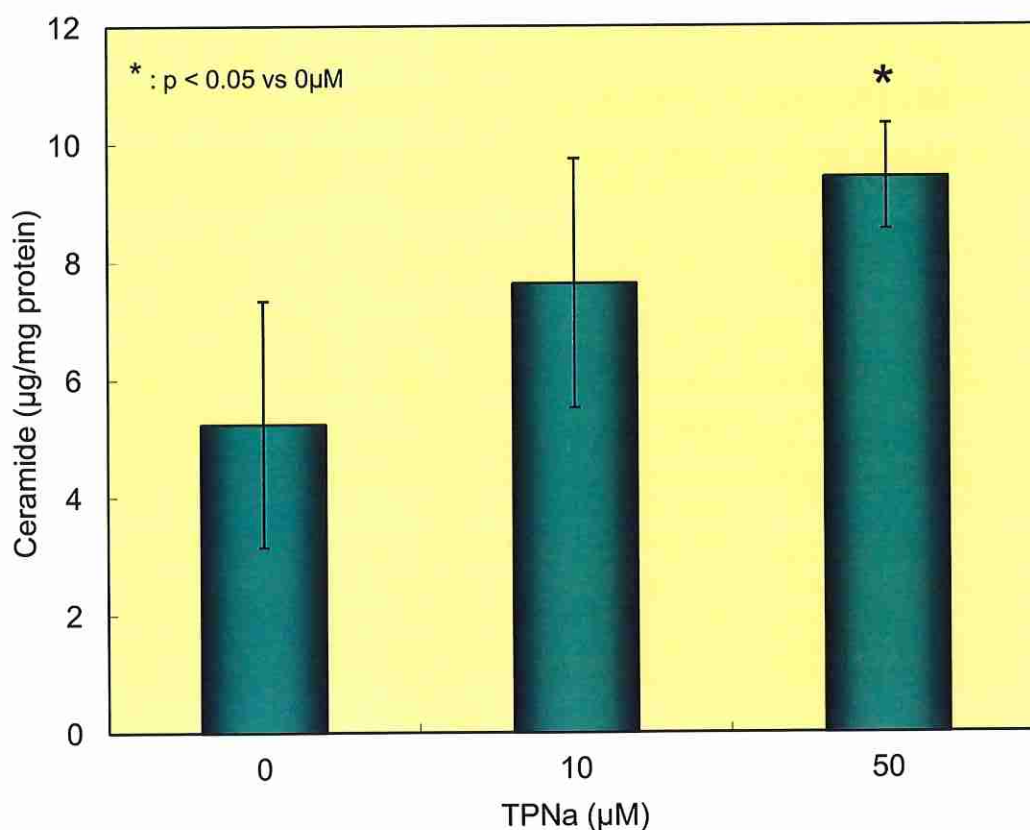
The conversion efficiency of TPNa™ to active tocopherol is much higher than that of tocopheryl acetate (TAc), so the efficacy of TPNa™ as a provitamin E is higher than TAc.

Dipotassium glycyrrhizate (G2K) is a famous anti-inflammatory agent and used in cosmetics widely. The anti-inflammatory activity of G2K is much lower than TPNa™, and it has no antioxidative activity, such as radical-scavenging.

TPNa™ is the best agent to prevent skin from damages caused by UV irradiation or oxidative attacks.

V0190-1.1.1E

Enhancement of Ceramide Synthesis



TPNa™ enhances the ceramide synthesis in skin.

Ceramides play an essential role in structuring and maintaining the water-retention and water permeability barrier function of the skin, a key substance for the skin moisture. Its amount is decreased by aging or skin diseases, such as atopic dry skin.

The effects of TPNa™ on ceramide synthesis was examined using cell cultivation system. Human keratinocyte SVHK was cultivated until semi-confluent and treated with the medium containing 0, 10 or 50µM TPNa™ for another 24 hours. The sphingolipid in the cell was extracted by Bligh & Dyer method and hydrolyzed to the ceramide. The ceramide was then determined after the fluorescent derivatization.

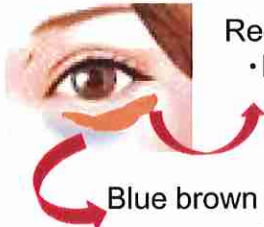
TPNa™ showed a significant enhancement of ceramide synthesis depend on its concentration.

V0170-1.1.1E

Eliminate Effect on Blood Origin Pigments For Eye Care



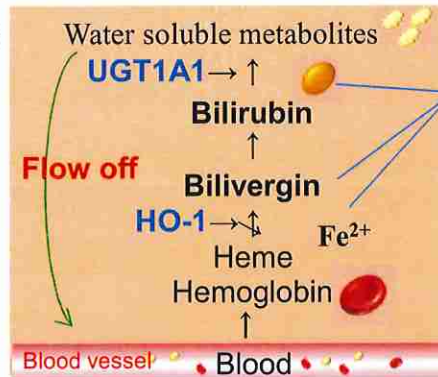
Dark Circles around Eyes



Reddish under lid and lid
• Inflammation

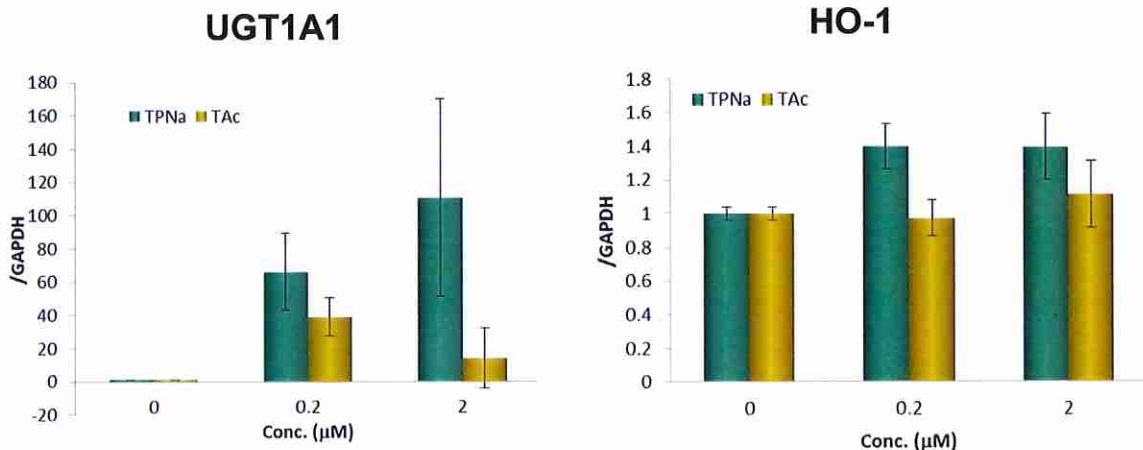
Blue brown dark circles

- Low microcirculations
- Accumulate blood origin pigments (Bilirubin, Bilivergin, Fe²⁺)



Cause of blue brown dark circle

Digest and remove pigments like bilirubin by enzymes (UGT1A1, HO-1) makes your eyes brighter.



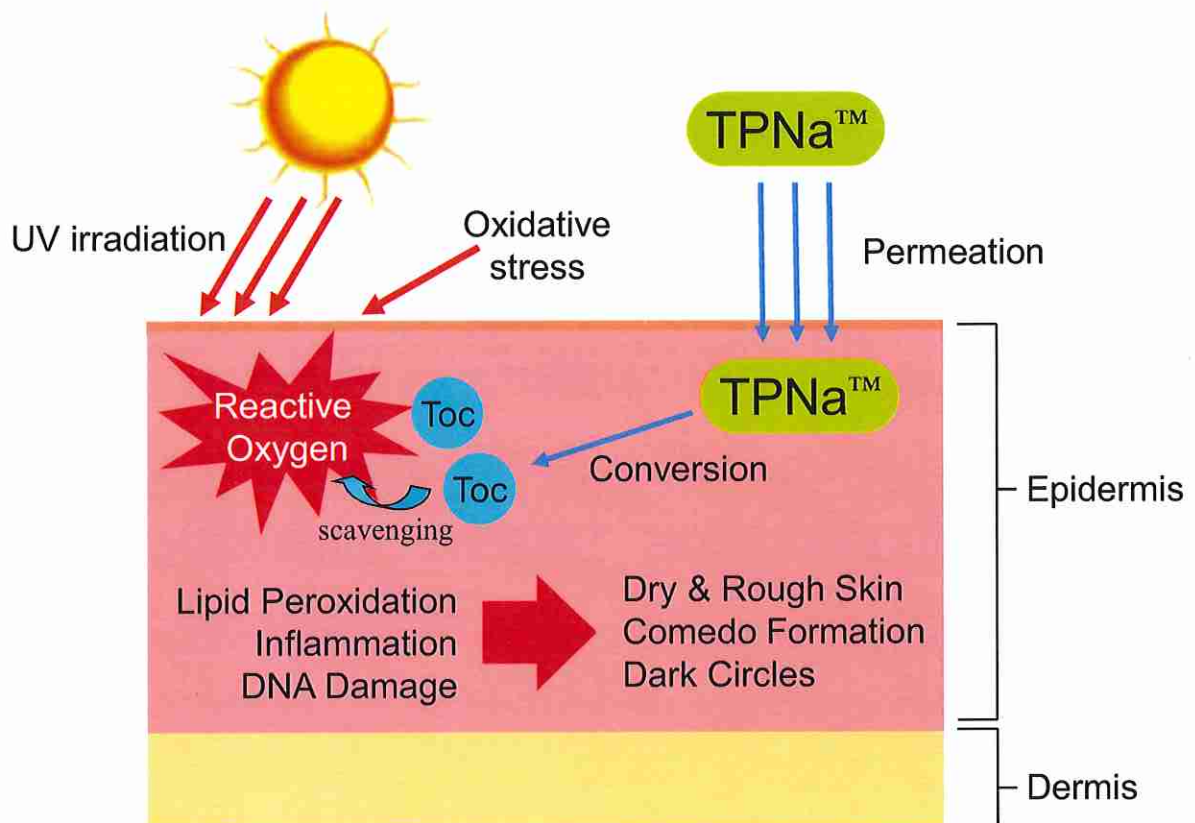
Promotion of gene expression for digesting pigments originated from blood.

Gene expression of enzymes working on digestion of pigments like bilivergin and bilirubin were assessed by quantitative RT-PCR, when *d*- α -tocopheryl phosphate (TPNa™) and tocopherol acetate (TAc) were applied on human dermal fibroblasts (NRGB1) for 48 hrs. The graphs above are shown relative levels of gene expression against GAPDH, a house keeping gene.

TPNa™ can clearly promotes gene expression of uridine diphosphate (UDP)-glucuronosyl transferase 1A1 (UGT1A1) and hemeoxygenase-1 (HO-1) and shows higher efficacy than TAc. As described before, TPNa™ has high anti-inflammatory effect, which might be effective on reddish skin color in eye zone caused from weak inflammation.

These results suggest that TPNa™ is promising for prevention of dark circles and uneven skin tone around eyes.

Mechanism of TPNa™ Function



TPNa™ is easily converted to α -tocopherol and protects the skin from the reactive oxygen.

UV irradiation or the oxidative stress causes reactive oxygen in the skin, ultimately causes comedo formation, rough and dry skin. TPNa™ easily permeates into the skin, and is converted continuously to α -tocopherol (Toc, vitamin E). α -Tocopherol scavenges the reactive oxygen, which prevents the lipid peroxidation, inflammation or DNA damage in the skin.

V0180-1.1.1E