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Ozonized glycerin (OG)-based cosmetic products lighten age spots on human facial skin

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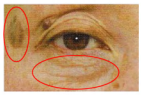
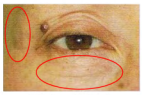
Introduction:

Ozone is known as an excellent disinfectant because its disinfecting action is powerful and instantaneous, it does not cause environmental pollution, it is extremely safe because it becomes oxygen when reduced, and it does not remain in living organisms or the environment. In addition, ozone has been reported to exhibit various biological activities in addition to disinfection.
However, ozone has a short half-life, about 30 min even in solubilized ozone water, and its lack of sustained action is a major disadvantage.To compensate for this disadvantage, Niinomi et al. developed a cream formulation of ozonized glycerin (OG) by solubilizing ozone in glycerin in 2004.

Glycerin from RSPO

Ozonized glycerin has various physiological effects such as anti-inflammatory, cytoprotective, wound healing, antibacterial, antiviral, and hemostatic effects in cell culture systems and various animal models.For the skin, it has been reported to promote granulation and epithelial formation, synthesis of extracellular matrixes such as collagen fibers and hyaluronic acid, and suppression of inflammation around ulcers in animal models of wound healing.Regarding safety for the application of OG to the skin, none of toxicities was found in animal studies so far.In the meantime, OG has been developed for cosmetics by taking advantage of its ability to maintain high ozone concentrations for a long period, but the effects of OG on normal skin tissue have not yet been fully clarified.
One of the current focus of attention in cosmetics development is facial age spots caused by UV exposure, other inflammatory stimuli, and aging. Melanin synthesis, secretion, and deposition in epidermal cells are major factors in the formation of dark spots in face. Although many cosmetic components have been found to inhibit melanin synthesis, no cosmetic components are shown to be able to safely decompose or lighten dark spots once they have formed. OG can be expected to chemically eliminate aging spots due to its inherent oxidative
bleaching properties and also expected to inhibit melanin synthesis and enhance skin metabolism by inducing antioxidant factors and suppressing inflammatory responses. In this study, we clarified the effects of OG on the skin, especially on age spots in the face.


before


After(6month later)

Materials & Methods:

***In vitro* study**

High-concentration formulation (water, pentylene glycol (Symrise), and xanthan gum (DSP Gokyo Food & Chemical)) containing 800 ppm ozonized glycerin (OG, 50% of final concentration of glycerin) and its control formulation without OG, and low-concentration formulation (water, pentylene glycol, xanthan gum, and sodium hyaluronate (Spera Nexus)) containing 80 ppm OG (40% of final concentration of glycerin) and its control formulation were used for melanin degradation assay. The oxidizing power of OG was measured by iodometric titration method, and then, activity/concentration was expressed in ppm. Hydroxide peroxide solution (10 mM, FUJIFILM Wako Chemicals) was used as a positive control in the assay.For measurements of mRNA expression of skin cells, OG samples, 1% (20), 2% (40) and 4% (80 ppm), were prepared by diluting 2000 ppm of OG with culture medium. Glycerin was also prepared by dilution as a control to match the final concentration of OG.

Measurement of melanin degradation activity

Synthetic melanin (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (FUJIFILM Wako Chemicals) and diluted 2-fold with ultrapure water to make a melanin solution. Then, mix this solution and the test product in equal amounts, and dispense it into 96-well plates at 100 µl/well and incubated at 37ºC. After 6 and 24 h, and 2 and 4 weeks, melanin content in each well of the plate was measured at a 490 nm by spectrophotometer (ImmunoMini, NJ-2300, Biotec).

Measurement of mRNA expression of differentiation markers in human normal epidermal keratinocyte (HNEK)

NHEK cells were seeded in 96-well plates (IWAKI) at a density of 2.0 × 104 cells/100 µL/well using HuMedia-KG2 (Kurabo) and incu- bated with 0, 1% (20), 2% (40) and 4% (80 ppm) of OG for 24 h at 37ºC and 5% CO2. After incubation, the cells were washed with PBS(–), total RNA was extracted using the Ambion Cells-to-CT kit (Thermo Fisher Scientific), and cDNA was synthesized by reverse transcrip- tion reaction (37ºC, 60 min–95ºC, 5 min) using the StepOne Real- Time PCR system (Applied Biosystems, Thermo Fisher Scientific). The mRNAs of involucrin and serine palmytoltransferase, differen- tiation markers of NHEK, were amplified using the primer (Takara) below, and relative quantification was performed using the ΔΔCt method. The relative expression levels of each gene were normal- ized to the house keeping gene, GAPDH.

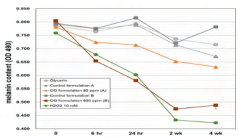
Clinical Study

High-concentration formulation (OG formulation 800 ppm) and its control formulation were applied to half the face of the same subject. It was randomly determined which half of the face they would be applied to. In the same way, low-concentration formulation (OG formulation 80 ppm) and its control formulation were applied to half the face of the same subject. After cleansing face twice a day (morning and night), 2 pumps of each formulation bottle (push type, 30 ml) were dispensed into the palm of hand and applied to the designated half of the face. Since the oxidizing power of OG is thought to be mediated by the action of free radicals, its duration of action was set to be short and in accordance with the existing dosage for general cosmetics (twice a day, morning and evening).

Evaluation

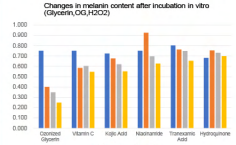
On first visit, skin diseases, scars and damages on the test site were checked. After washing the face with make-up remover (if make-up is worn) and facial cleanser that the subject normally uses, acclimatization was performed in a thermo-hydrostatic chamber (temperature: 21 ± 1ºC, humidity: 50 ± 5%) for 20 min, and then, one aging spot site was selected on each facial side. Then, its size was selected should be less than 5 mm. Thereafter, melanin content of the spots was analyzed. Melanin content was measured at aging spot areas on the right and left cheeks of subjects by a Mexameter MX18 (Courage + Khazaka electronic GmbH). Measurements were performed 5 times. Measured value was expressed as melanin index. The maximum and minimum values of 5 data are excluded, and the average value is calculated. The Δmelanin index was calculated by subtracting the baseline melanin index from those at 4 or 8 weeks. On Weeks 4 and 8, after adverse events on the applied facial sites were assessed and melanin contents measured in the same manner.

Results & Discussion:

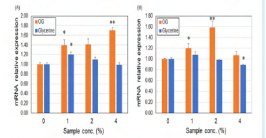


	untreated	2weeks later	4weeks later
Ozonized Glycerin 1%			
Vitamin C 1%			
Kojic Acid 1%			
Niacinamide 1%			
Tranexamic Acid 1%			
Hydroquinone 1%			

Changes in melanin content after incubation in vitro (Glycerin,OG,H2O2)

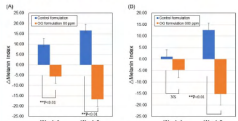


Changes in melanin content after incubation in vitro (OG,Vitamin C,Kojic Acid,Niacinamide,TranexamicAcid,HQ)

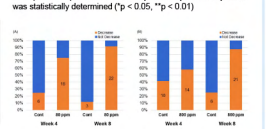


Compared to five types (vitamin C, hydroquinone, kojic acid, niacinamide, tranexamic acid), which are mainly famous as blighting active ingredients, The ozonized glycerin has the effectiveness of Melanin index dissolution was confirmed . It was confirmed that the concentration (depth) of melanin by 66% was reduced in 4 weeks.

Effects of OG on differentiation markers of human normal epidermal keratinocytes (NHEKs). NHEKs were treated with 0, 1, 2, and 4% of vehicle (glycerine) or ozonized glycerine (OG) for 24 h. Relative involucrin (A) and serine palmitoyltransferase (B) mRNA levels were analyzed by real-time PCR. Data are presented as mean ± standard error (n = 3 or 4) in one out of two repeated experiments. p value was statistically determined (*p < 0.05, **p < 0.01)



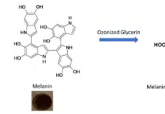
Comparison of Δmelanin index between control and OG formulations 80 (A) or 800 ppm (B) at 4 and 8 weeks. Data are presented as mean ± standard error (n = 24). p value was statistically determined (**p < 0.01)



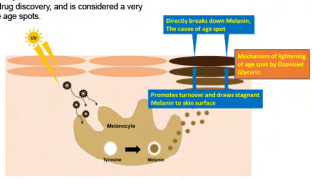
Number of subjects with a decrease in melanin index from baseline to 4 or 8 weeks in control formulation (Cont), OG formulations 80 (A), and 800 ppm (B) (n = 24)

Conclusions:

1.Ozonized Glycerin can directly decompose melanin in vitro.
2.Ozonized Glycerin can express of differential markers ofInvolucrin,serine palmitoyltransferase in NHEK.
3.By conituing to use cosmetics containing Ozonized Glycerin in clincal study,Confirmed the effect of reducing age spots
On the facial skin.
These data suggest that new mechamism of lightening of Age spot by Ozonized Glycerin.
A.Promote turnover and draws stagnant melanin to the skin Surface.
B.Directly breaks down melanin,the cause of ages spot.
This mechanism does not inhibit melanin synthesis, but rather normalizes turnover and pushes melanin that has been stagnant to the surface layer.
The melanin pushed up to the surface is decomposed by ozonated glycerin in a simple way.This is why it isconsidered a hypoallergenic melanin decomposition method discovered in the process of atopic disease drug discovery, and is considered a very safe method to decompose age spots.



Ozonized glycerin can directly decompose melanin *in vitro*.



Mechanism of lightening of age spot by Ozonized Glycerin

Acknowledgements:

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