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Mediplus Pharma, Inc.

Novel glycerin compounds improve skin health

Poster ID408

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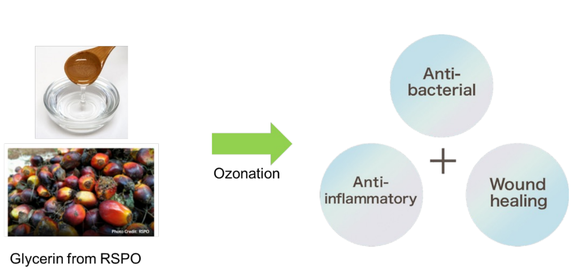
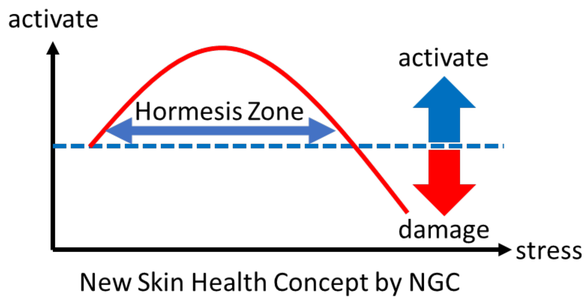
Introduction:

Novel glycerin compounds (NGC) were developed which generated new reaction products, endoperoxide derivatives. NGC was made by solubilizing ozone in high-purity glycerin (>99 %) using an ozone generator, and has been reported to have antiviral and antimicrobial effects due to its oxidizing action. 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 also NGC showed therapeutic efficacy for skin ulcers and dermatitis. Regarding safety for application of NGC to the skin, none of toxicities was found in human clinical study so far. Meanwhile, clinical trials were conducted in order to develop NGC as a cosmetic raw material and demonstrated that NGC directly decomposed synthetic melanin pigments and lightened facial age spots. However, the detailed mechanism of action of NGC on normal human skin is not well understood. In the present study, we investigated the effects of NGC using culture system of normal human epidermal keratinocytes (NHEK).





New Skin Health Concept by NGC

For many years, oxidative stress has been the focus of toxicity research.Antioxidants alone have limited ability to activate biological functions.On the other hand, as shown in Nrf2 and redox studies, mild oxidative stress contributes to body homeostasis.
The hormesis effect of mild oxidative stress is very important for skin health.We thought that mild oxidative stress may contribute to the strengthening of the skin itself.

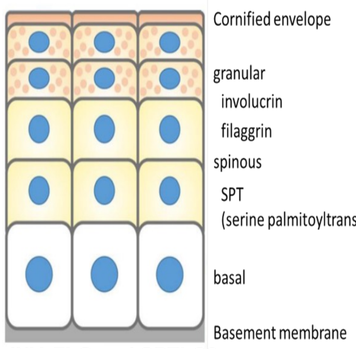
Materials & Methods:

Glutathione (GSH) assay for cultured human normal epidermal keratinocyte (HNEK)

GSH and GSSG contents were measured in a recycling assay using 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB). HNEK were plated onto 96-well culture plates at 2 x10^4 cells/well and maintained in a 100 µL culture medium (HuMedia-KG2, Kurabo, Japan) for 24 h. The cells were treated with vehicle or NGC and cultured in the absence for further 24 and 48 h. Cells were extracted using 0.5% Triton X-100/PBS (100 µL per well) . The extracted solution (25 µL) was mixed with reaction solution (2 mM NADPH, 0.12 units/mL glutathione reductase, 0.1 mM PBS containing 0.5mM EDTA, 175 µL). After incubation at 37 C for 10 min, 10 mM DNTB (25 µL) was added and then measured for absorbance at 405 nm.

Measurement of mRNA expression of antioxidant and differentiation markers in HNEK

NHEK cells were seeded in 96-well plates (IWAKI, Japan) at a density of 2.0x10^4 cells/100 µL/well using HuMedia-KG2 and incubated with 0, 1 % , 2 % and 4 % of NGC for 24 hours at 37° C and 5% CO_2. After incubation, the cells were washed with PBS(-), total RNA was extracted using the Ambion Cells-to-CT kit (Thermo Fisher Scientific, USA), and cDNA was synthesized by reverse transcription reaction (37° C, 60 min→95° C, 5 min) using the StepOne Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific, USA). The mRNAs of heme oxygenase-1 (HO-1), DAD(P)H quinone dehydrogenase 1 (NQO1), involucrin (INV), filaggrin (FLG) and serine palmytoiltransferase (SPTLC2), differentiation markers of NHEK, were amplified using the primer (Takara, Japan) below and relative quantification was performed using the ΔΔCt method. The relative expression levels of each gene were normalized to the house keeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).



INV, FLG and SPTLC2 were representative differentiation markers of epidermal cells

Results & Discussion:

NGC increased expression of HO-1 and NQO1 in concentration-dependent manner

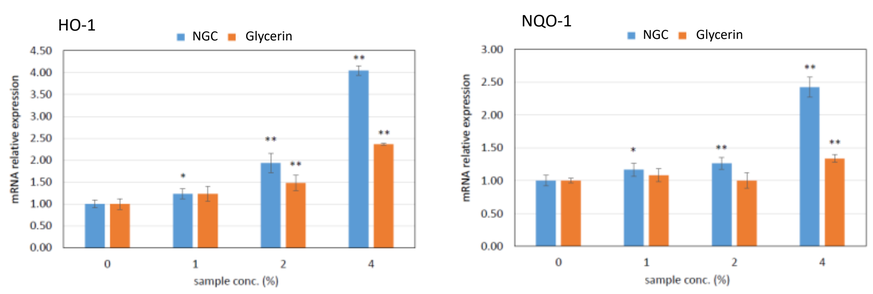


Figure 1.Effects of NGC on differentiation markers of human normal epidermal keratinocytes (NHEKs).
NHEKs were treated with 0, 1, 2 and 4 % of vehicle (glycerine) or Novel Glycerine compound (NGC) for 24 hours.
Relative HO-1,NQO-1 mRNA levels were analyzed by real-time PCR. Data are presented as mean ± standard error (n=3 or 4) in one out of two repeated experiments.P value was statistically determined (*P<0.05, **P<0.01).

NGC increased expression of GSH in concentration-dependent manner

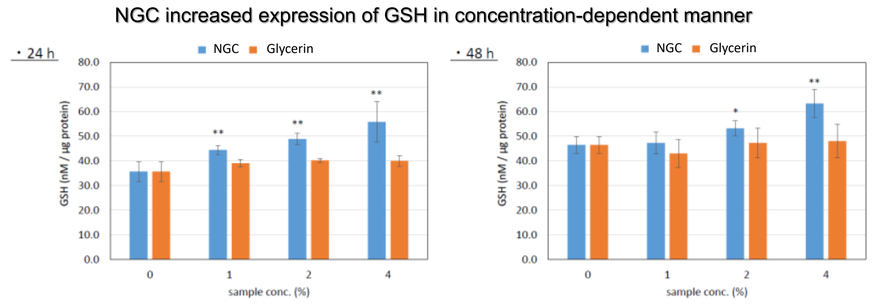


Figure 2.Effects of NGC on differentiation markers of human normal epidermal keratinocytes (NHEKs).
NHEKs were treated with 0, 1, 2 and 4 % of vehicle (glycerine) or Novel Glycerine compound (NGC) for 24hours,48hours.
Relative GSH mRNA levels were analyzed by real-time PCR.
Data are presented as mean ± standard error (n=3 or 4) in one out of two repeated experiments. P value was statistically determined (*P<0.05, **P<0.01).

Results & Discussion:

NGC increased expression of INV,FLG and SPTLC2 in concentration-dependent **manner**

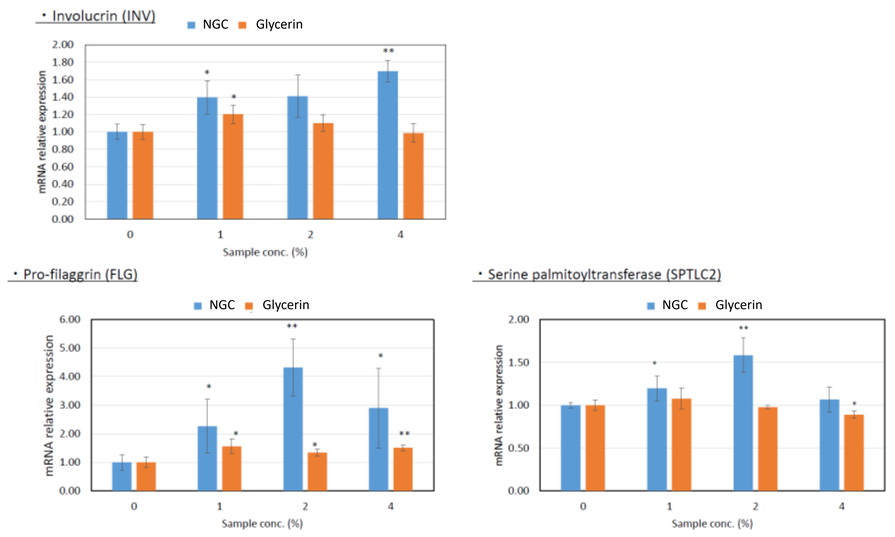


Figure 3.Effects of NGC on differentiation markers of human normal epidermal keratinocytes (NHEKs).
NHEKs were treated with 0, 1, 2 and 4 % of vehicle (glycerin) or Novel Glycerin compound (NGC) for 24 hours. Relative Involucrin,FLG and SPTLC2 mRNA levels were analyzed by real-time PCR. Data are presented 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).

Scavenge effect of superoxide anion radical by NGC.

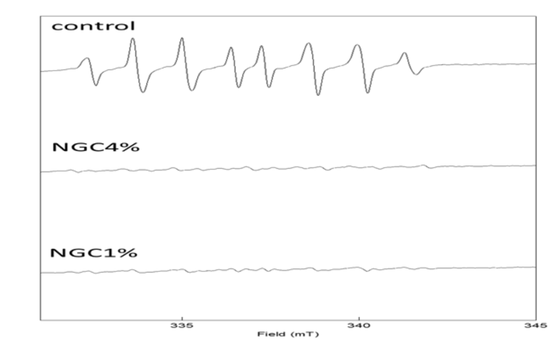
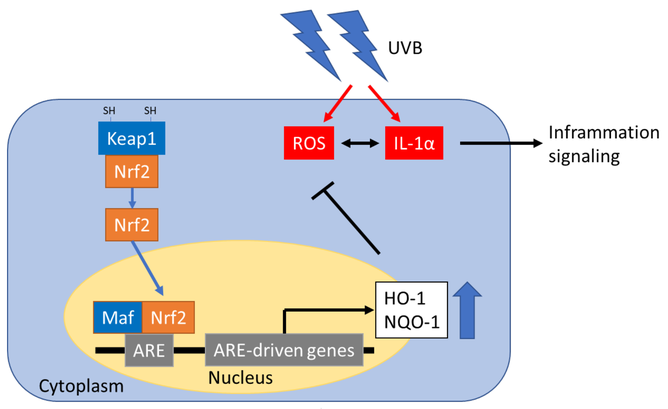


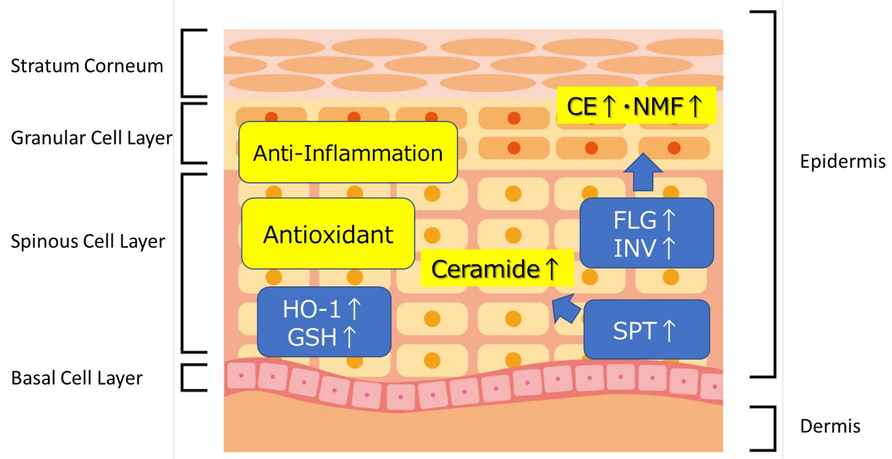
Figure 4. Scavenge effect of superoxide anion radical by NGC. Superoxide anion radical generated by HPX-XOD reaction system.Detection of radical with DMPO(spin trap agent) by ESR.
NGC almost scavenged superoxide anion radicals at 1% and 4%, and the scavenging ability was not significantly different between 1% and 4%.

Conclusions:

These results suggest that the oxidative property of NGC activates Nrf2 transcription factor, which is a biological defense sensor, and induces the expression of antioxidant factors. Since their expression was caused by oxidative stimulation at low concentrations of NGC, it was strongly suggested that their hormesis effect would be working protectively for the skin. Consistent with this, the expression of INV, FLG, and SPT genes also increased at low concentrations of NGC: FLG showed a threefold increase compared to the control group. These results suggest that NGC promote epidermal cell differentiation and have positive effects on skin barrier functions. We are currently conducting detailed analysis of the effects of NGC using three-dimensional cultures of human epidermis. Mild oxidative stimulation of NGC can lead to healthy skin through hormesis effect.



Expression of HO-1,NQO-1 and GSH by Nrf2-Keap1 signal



Multi effect of NGC to improve skin health

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