Evaluation of Antioxidants’ Ability to Enhance Hyaluronic Acid-Based Topical Moisturizers

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Abstract

Introduction: Hyaluronic acid (HA) is a unique molecule of the extracellular matrix with multiple biological actions. In skin, HA plays an essential role as a humectant, capable of binding up to 1000 times its mass with water, providing skin with moisture and viscoelastic properties. HA concentration and synthesis decrease significantly in aging skin, due to exogenous and endogenous factors, including photaging and HA metabolism, respectively. A key driver for HA degradation and decrease in concentration is mediated via the induction of reactive oxygen species (ROS) and other free radicals. In this study, we evaluate antioxidant ingredients essential in the development of next-generation multi-weight HA-based topical formulations aimed at leveraging HA’s ability to maximize anti-aging properties.

Methods: Two antioxidants, glycine saponin and glycyrrhetinic acid, were evaluated for stimulation of endogenous HA production and inhibition of endogenous hyaluronic acid activity in an in vitro and cell-free assay.

Results: The antioxidant glycine saponin induced endogenous HA synthesis in fibroblasts, while the significant glycyrrhetinic acid decreased the degradation rate of HA by hyaluronidase 1 (HYAL 1) by up to 54%.

Conclusion: This study demonstrates the ability of two antioxidants (glycine saponin and glycyrrhetinic acid, essential in the development of the next generation of multi-weight HA plus antioxidant complex-based topical skin formulations) to limit the signs of aging skin, addressing limitations of many currently available topical HA-based formulations.

Materials and Methods

Endogenous HA stimulation assay (via Glycine Saponin)

Bioactive Glycine Saponin [100 µg/ml] was applied on the surface of an in vitro reconstructed skin model and incubated for 24 hours, followed by crossectioning at 6 µm. CROSsections were stained for hyaluronic acid and visualized by immunofluorescence. Additionally, fibroblasts were incubated for 72 hours with 100 µg/HA Glycine Saponin (Glycine Soja) and compared to untreated control cells. HA level in the supernatant were quantified via HA ELISA (n=9). Cells were immunostained with biotinylated HA binding protein and cell nuclei stained DAPI.

Hyaluronidase inhibition assay

The enzymic kinetics of HYAL1 was evaluated with and without the presence of glycine saponin. HA (0.02 mg/ml) was incubated with 0.035 mg/ml Hyaluronidase 1 in 0.0125% glycine saponin at 37°C for 24 hours. Hyaluronidase degradation was determined via quantitative measurement of NAG release using a specific colorimetric reaction and analysis of the data based on Michaelis-Menten kinetics.

Summary and Conclusions

- Advances in aging and photodamaged skin have shown the process is a result of extrinsic and intrinsic properties.
- One of the major biomoerires involved in skin moisture and extracellular matrix structure is hyaluronic acid (HA), which has been studied since the 1970s. The glycaminoglycan, HA, is a unique molecule that plays a crucial role in skin hydration, with the ability to hold up to 1000 times its mass of water, a key component of its functional role of maintaining softness with elasticity and structure in skin.

This study describes the evaluation of two antioxidants (glycine saponin and glycyrrhetinic acid) essential for the development of the next generation of multi-weight HA and antioxidant complex-based topical skin formulations to limit the signs of aging skin, addressing limitations of many currently available topical HA-based formulations.

- The antioxidant glycine saponin induced endogenous HA synthesis in fibroblasts.

- Glycyrrhetinic acid decreased the degradation rate of HA by hyaluronidase 1 (HYAL 1) by up to 54%.

- While HA has been included in numerous topical skin products, critical aspects of HA metabolism, especially in aging skin, have often been overlooked including decreases in HA synthesis in increasing age, and increases in HA degradation mediated by excessively induced reactive oxygen species and free radicals and increased enzymatic degradation by exogenous factors. Here, we describe a unique approach to inclusion of two antioxidants essential for the development of the next generation of anti-aging HA-based formulations.

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