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 activities. 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 photoaging 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 hyaluronic acid'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 hyaluronidase activity in an *in vitro* and cell-free assay.

Results: The antioxidant glycine saponin induced endogenous HA synthesis in fibroblasts, while the antioxidant 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 cryosectioning at 6 µm. Cryosections were stained for hyaluronic acid and visualized by immunofluorescence. Additionally, fibroblasts were incubated for 72 hours with 100 µg/ml Glycine Saponin (Glycine Soja) and compared to untreated control cells. HA level in the supernatants were quantified via HA ELISA (n=9). Cells were immunostained with biotinylated HA binding protein and cell nuclei stain DAPI.

Hyaluronidase inhibition assay

The enzymatic kinetics of HYAL1 was evaluated with and without the presence of glycyrrhetinic acid. HA (0-2 mg/ml) was incubated with 0.035 mg/ml hyaluronidase 1 +/- 0.0125% glycyrrhetinic acid at 37ºC for 24 hours (n=6). Enzymatic degradation was determined via quantitative measurement of N-acetylglucosamine (NAG) release using a specific colorimetric reaction and analysis of the data based on Michaelis-Menten kinetics.

Results

FIGURE 1. Bio-active Glycine Saponin (Glycine Soja) stimulates endogenous hyaluronic acid synthesis

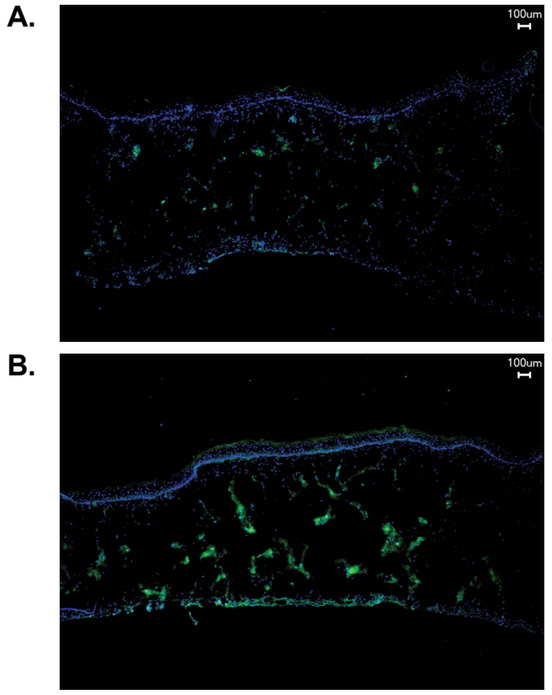

FIGURE 1. Bio-active Glycine Saponin (Glycine Soja) stimulates endogenous hyaluronic acid synthesis. Bioactive Glycine Saponin [100 µg/ml] was applied on the surface of an *in vitro* reconstructed skin model and incubated for 24 hours, followed by cryosectioning at 6 µm. Cryosections were stained for hyaluronic acid and visualized by immunofluorescence (HA in green, cell nuclei in blue [DAPI]). A. Untreated control. B. Topical treatment with Glycine Saponin, 0.01%.

FIGURE 2. Quantification and visualization of Bio-active Glycine Saponin (Glycine Soja) stimulated endogenous hyaluronic acid synthesis

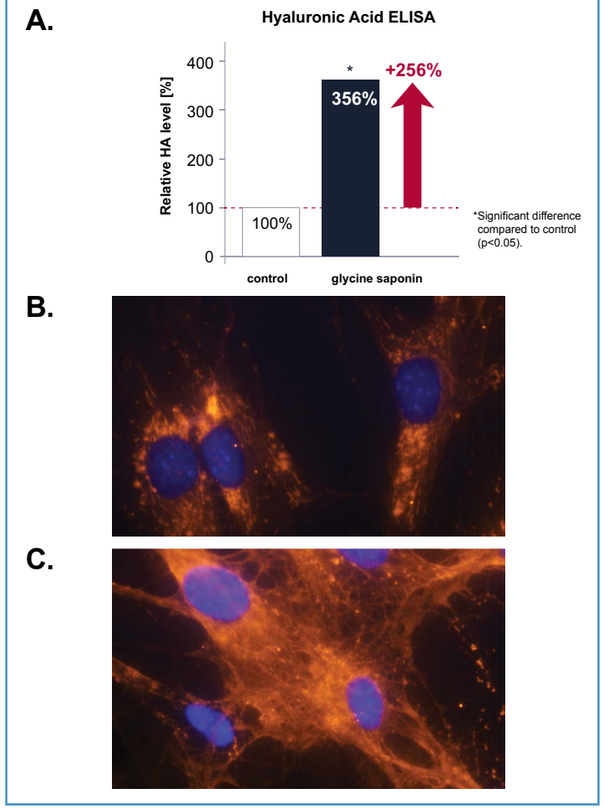

FIGURE 2. Quantification and visualization of Bio-active Glycine Saponin (Glycine Soja) stimulated endogenous hyaluronic acid synthesis. Fibroblasts were incubated for 72 hours with 100 µg/ml Glycine Saponin (Glycine Soja) and compared to untreated control cells. Cells were immunostained with biotinylated HA binding protein (orange) and cell nuclei stain DAPI (blue). A. Hyaluronic Acid ELISA. B. Untreated control. C. Glycine Saponin treated cells.

FIGURE 3. Glycyrrhetinic acid inhibition of Hyaluronidase 1 (HYAL1) enzymatic kinetics

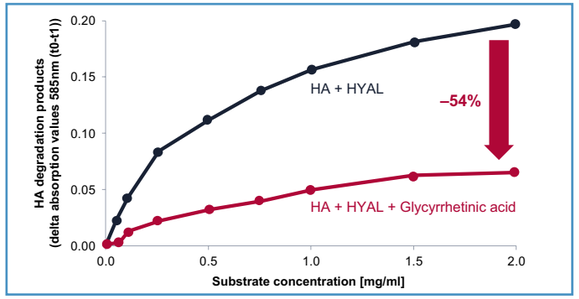

FIGURE 3. Quantification of HA degradation products (NAGs) with increasing substrate (low molecular weight HA) concentration and Hyaluronidase 1 (HYAL1) +/- GA. The enzymatic activity of HYAL1 was evaluated with and without the presence of glycyrrhetinic acid. HA (0-2 mg/ml) was incubated with 0.035 mg/ml hyaluronidase 1 +/- 0.0125% glycyrrhetinic acid at 37ºC for 24 hours (n=6). Enzymatic 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 that the process is a result of extrinsic and intrinsic properties
- One of the main biomolecules involved in skin moisture and extracellular matrix structure is hyaluronic acid (HA), which has been studied since the 1970s. The glycosaminoglycan, 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 exogenously induced reactive oxygen species and free radicals and increased enzymatic degradation by endogenous hyaluronidases. Here we describe a unique approach to inclusion of two antioxidants 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

REFERENCES: 1. Bravo B, Correia P, Gonçalves Junior JE, et al. *Dermatol Ther.* 2022;35(12):e15903. **2.** Kang HY, Lee JW, Papaccio F, et al. *Pigment Cell Melanoma Res.* 2021;34(4):800-813. **3.** Widgerow AD, Ziegler ME, Garruto JA, et al. *J Cosmet Dermatol.* 2022;21(7):2865-2870. **4.** Makino ET, Huang PC, Emmerich T, et al. *Clin Cosmet Investig Dermatol.* 2023;16:1123-1134. **5.** Galvez-Martin P, Soto-Fernandez C, Romero-Rueda J, et al. *Int J Mol Sci.* 2023;24(5):4774. **6.** Juncan AM, Moisă DG, Santini A, et al. *Molecules.* 2021;26(15):4429. **7.** Gupta RC, Lall R, Srivastava A, et al. *Front Vet Sci.* 2019;6:192. **8.** Essendoubi M, Gobinet C, Reynaud R, et al. *Skin Res Technol.* 2016;22(1):55-62. **9.** Marinho A, Nunes C, Reis S. *Biomolecules.* 2021;11(10):1518. **10.** Draelos ZD, Diaz I, Namkoong J, et al. *Dermatol Ther (Heidelb).* 2021;11(4):1385-1394. **11.** Borchers S, Pirrung M. ChemRxiv. *Cambridge: Cambridge Open Engage;* 2023. **12.** Oftadeh R, Azadi M, Donovan M, et al. *PNAS Nexus.* 2023;2(8):pgad240. **13.** Humzah D, Romagnoli M, Tateo A, et al. *Esperienze Dermatol.* 2020;22:36-41. **14.** Draelos ZD. *J Drugs Dermatol.* 2021;20(6):695-699. **15.** Bukhari SNA, Roswandi NL, Waqas M, et al. *Int J Biol Macromol.* 2018;120(Pt B):1682-1695. **16.** Nakab L, Hee CK, Guetta O. *Plast Reconstr Surg Glob Open.* 2020;8(3):e2723. **17.** Mazzucco A. *Int J Chem Res.* 2018;1(1): 13-18. **18.** Ballance WC, Qin EC, Chung HJ, et al. *Biomaterials.* 2019;217:119292. **19.** Anderegg U, Simon JC, Averbeck M. *Exp Dermatol.* 2014;23(5):295-303. **20.** Lianjie Wang, Yves Queneau. Green Chemistry and Chemical Engineering, Springer New York, pp.349-383, 2019. **21.** Nichifor M. *Polymers (Basel).* 2023;15(5):1065. **22.** Lin C-A, Ho H-M, Venkatesan P, et al. *Pharmaceutics.* 2021;13(10):1569. **23.** Baldi M, Reynaud R, Lefevre F, Fleury M, Scandolera A, Maramaldi G. *Eur Rev Med Pharmacol Sci.* 2023;27(4):1450-1466. **24.** Südel KM, Venzke K, Mielke H, et al. *Photochem Photobiol.* 2005;81(3):581-587.

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