Derivatives of Sugar Compounds Provide Anti-Aging Effects

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Abstract

The alpha-hydroxyacids (AHAs) have developed into a major ingredient technology for anti-aging and adjunctive therapeutic skincare over the past decade. Polyhydroxy acids (PHAs) evolved from AHA technology, offering similar anti-aging benefits as AHAs, with reduced sensory irritation and discomfort on sensitive skin. **Recent trends in skincare have embraced 'non-acid' technology, while still demanding the beneficial effects on skin that have been achieved with the use of AHAs.** One new approach in the development of novel ingredient technology utilizes sugar (saccharide) derivatives and naturally occurring aminosugar derivatives to provide anti-aging effects to skin. N-acetylglucosamine is one new compound that is being investigated for use in skin care. Clinical study results with this compound reveal significant anti-aging benefits to skin, including skin plumping and skin smoothing effects. In addition, it is well tolerated on skin. N-acetylglucosamine is a novel, naturally-derived, 'non-acid' ingredient with many potential future applications in cosmetic and therapeutic skin care.

Objective

This poster will review new clinical and safety data to support the use of N-acetylglucosamine for anti-aging skin care.

Introduction

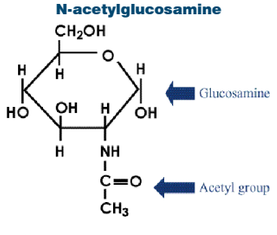
N-acetylglucosamine and glucosamine are constituents of naturally occurring glycoproteins, proteoglycans, and glycosaminoglycans (GAGs) in human connective tissue and skin. A GAG is a water-binding polysaccharide found extensively in the dermis, as well as the epidermis. These compounds are made up of repeating disaccharide units; one is always an aminosugar or N-acetyl aminosugar and the other is a polyhydroxy acid (PHA). N-Acetyl-D-glucosamine is an N-acetyl aminosugar found in the GAGs, keratan sulfate I and II, and **hyaluronic acid**, a lengthy GAG comprised of approximately 50,000 repeating disaccharide units.[1] Hyaluronic acid is well known in dermatologic skin care for its water binding properties and is frequently used in cosmetic preparations to provide moisturization. D-Glucuronic acid, a PHA, is the second part of the two repeating units in hyaluronic acid, as well as in other GAGs including chondroitin sulfate and heparan sulfate.[1]

The body routinely uses glucosamine as a substrate to build GAGs. Studies have shown that glucosamine can stimulate the synthesis of GAGs, as well as inhibit their degradation. In addition, glucosamine is thought to be involved in the repair process of connective tissue, such as damaged cartilage.[2] **In fact, oral glucosamine and acetylglucosamine are used widely in the treatment of arthritis as a result of their ability to promote the production of connective tissue pre-cursors, as well as inhibit their degradation.** N-acetylglucosamine is hydrolyzed to glucosamine[3] (which has a short half-life in blood), and is used to extend the duration of action of glucosamine for the treatment of arthritis[4-5].

Treatment of aging skin represents a new and exciting use for N-acetylglucosamine. Benefits to skin may be mediated through stimulatory effects on GAGs, which naturally decrease in concentration with age and exposure to UV from sunlight.[6] GAGs provide cushion to the skin and help to plump it, thereby keeping the skin looking healthy and youthful. The benefit of abundant GAGs in skin is easily visualized when one considers the natural plumpness of baby's skin, which is full of GAGs. **The potential to improve skin firmness and fullness through enhanced production of GAGs and/or diminished degradation of GAGs is an exciting opportunity in anti-aging skin care.**

N-acetylglucosamine – Chemical Attributes

N-acetylglucosamine



Non-Acid Anti-Aging Technology

- **INCI Name**
  - Acetyl glucosamine
- **Chemical Composition**
  - $C_8H_{15}NO_6$

Safety Profile of N-acetylglucosamine

Naturally occurring polymers of N-acetylglucosamine exist abundantly in nature as chitin, the primary structural bio-polymer found in the shells of crabs, shrimp and lobsters. This polymeric compound is considered to be both nontoxic and biodegradable, with lethal doses in mice being comparable to sucrose and sodium chloride.[7] Dietary supplementation of 1g/day N-acetylglucosamine in humans does not increase blood glucose levels.[8]

The following studies further support the safety of this compound.[9]

- **Ames II Assay:** This mixed strain assay measures frame shift and base pair substitution mutations in six different strains of Salmonella typhimurium in the presence of S9 fraction. Cultures with S9 fraction simulate metabolic conditions in the human body in which a compound is metabolized by the liver. Aqueous solutions of N-acetylglucosamine with a maximum concentration of 10% were tested in this assay in comparison to a negative control, sterile deionized water.
  - **Result: Non-mutagenic:** the test material did not cause base pair substitution or frame shift mutations.
- **Epiderm Skin Model (EPI-100) – PGE_2, LDH, IL-1a endpoints** (MatTek Corp.):
  The EPI-100 assay utilizes a living skin equivalent consisting of cultured keratinocytes that ultrastructurally resemble human epidermis. This *in vitro* method is used to assess skin irritation via quantitation of cytotoxicity following exposure to test materials. Cell viability is measured through incorporation of a purple dye into living cell mitochondria. Following extraction from the cells, the intensity of purple color is directly proportional to cell viability and inversely proportional to toxicity from the test material. **The following assays were conducted following application for one, four, and twenty-four hours of a cream formulation containing 8% N-acetylglucosamine (pH 5.4). Deionized water served as a negative control and Triton-X 100 (1%), a mild irritant, was used as the positive control.**
  - **Overall cell viability:** N-acetylgluosamine (8% cream) is classified as a **non-irritant.**
  - **PGE_2 assay:** Quantitation of prostaglandin release correlates to inflammation. N-acetylgluosamine (8% cream) scored **lower than the negative control.**
  - **Lactate Dehydrogenase (LDH):** Quantitation of cytosolic enzyme release from cells resulting from cell lysis. N-acetylgluosamine (8% cream) scored **lower than the negative control.**
  - **Interleukin-1a:** Assessment of cytokine release from keratinocytes in response to injury. This is a measure of irritation resulting from application of the test material. N-acetylgluosamine (8% cream) scored **lower than the negative control.**
  - **Summary Result: N-acetylgluosamine (8% cream) is non-irritating to the living skin equivalent.**
- **Irritancy and Allergenicity Testing**
  - A cream containing **10% N-acetylglucosamine** was tested in a human **Repeat Insult Patch Test** (RIPT) on 109 healthy volunteers under semi-occlusive conditions. **N-acetylgluosamine (10% cream, pH 6.1) did not indicate a potential for dermal irritation or allergic contact sensitization.**

Effects of N-acetylglucosamine on Skin

Preliminary observations by dermatologist, Eugene J. Van Scott, MD, and dermatopharmacologist, Ruey J. Yu, PhD, OMD indicate that N-acetylglucosamine can increase skin thickness and improve wrinkles and photoaged skin.[10] Other work has shown that N-acetylglucosamine has a moderate effect in enhancing cell turnover using the dansyl chloride model.[9] In addition, this compound has been found to alleviate itching in nummular eczema, atopic dermatitis and senile pruritis.[10] New clinical data supporting the use of N-acetylglucosamine in anti-aging skin care is described.

Anti-Aging Clinical Study of N-Acetylglucosamine[9]

Method

- This was a 12-week, controlled-use anti-aging study of a single test product. Visits were conducted at 0, 6 and 12 weeks. Statistical comparisons were made to baseline conditions for the face and versus an untreated control for the forearm.
- **Population:** 37 women (Fitzpatrick types I, II, and III) with clinically graded mild to moderate photodamage on the face.
- **Conditioning phase:** All participants discontinued use of topical products, with the exception of make-up (glamour products) and cleanser, for 3 to 5 days before the baseline visit.
- **Test Product:** 8% N-acetylglucosamine cream formulation at native pH=4.25
- **Test Product Application:**
  - **Face:** the test cream was applied twice daily after cleansing
  - One **forearm**: product was applied to one forearm three times daily; the untreated forearm served as a control for skin thickness measurements.
- **Clinical Evaluations (baseline, week 6, week 12):**
  - **Performance parameters:** the face was evaluated by a trained visual grader using a 10-cm scale with 0.25cm increments (where 0 = none and 10 = severe) for the parameters: fine lines, coarse wrinkles, pore size, roughness, firmness (laxity), mottled pigmentation, sallowness (dullness), and clarity.
  - **Irritation parameters:** facial irritation was graded *objectively* for the parameters: erythema, edema and dryness, and *subjectively* for the parameters: burning, stinging, itching, tightness, and tingling, using a 0-3 scale.
  - **Pinch recoil:** measurements were taken of the left under eye area to assess skin elasticity by pinching the skin and recording time with a stopwatch (in 0.01 second increments) to full recovery of the skin. The measurements were performed in triplicate, and the average score was reported. Pinch recoil is a recognized indicator of skin resiliency and firmness.[11]
  - **Total skin thickness:** measurements were collected on the outer forearms using a hinged pinching device and digital calipers as previously described.[12] Duplicate measurements representing a two-fold thickness of skin were taken and averaged at baseline and endpoint for both the treated and untreated control arms.
  - **Self-assessment:** questionnaires were completed by the panelists at each study visit.

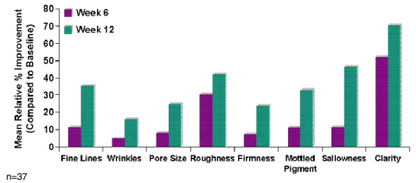
Data Analysis

- Mean scores of clinical grading parameters and pinch recoil measurements were statistically analyzed compared to baseline scores using a paired t-test at the $p<0.05$ significance level. Mean percent changes from baseline were calculated.
- Mean skin thickness measurements for the treatment group and the untreated control were statistically analyzed compared to baseline scores and compared to each other using a paired t-test at the $p<0.05$ significance level.
- Self-assessment questionnaires were tabulated and graphed.

Results

- Clinical grading revealed significant anti-aging effects.
- Elasticity was significantly improved (>12%).
- Total skin thickness was increased (>16%).
- The test product was well tolerated by users.
- Improvements were self-assessed for fine lines and wrinkles, and no acnegenic effect was noted.
- Over 50% of panelists rated product ***BETTER THAN*** their usual moisturizer.

Visually Assessed Anti-Aging Effects on the Face



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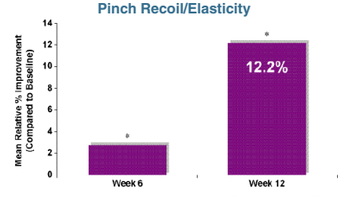
All clinically-graded aging attributes were significantly improved at Week 6 & Week 12 compared to baseline conditions, $p<0.05$.

Pinch Recoil/Elasticity



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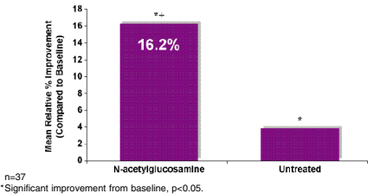
*Significant improvements in skin elasticity were observed at Week 6 and Week 12 compared to baseline, $p<0.05$.
Pinch recoil/elasticity was improved 12.2% at endpoint, compared to baseline.

Total Skin Thickness

Digital Caliper Measurements – Percent Change



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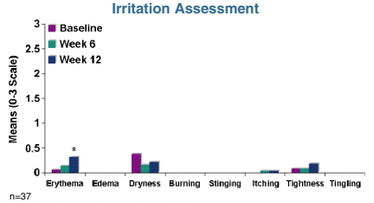
*Significant improvement from baseline, $p<0.05$.
+N-acetylglucosamine 8% improved skin thickness significantly better than untreated at endpoint, $p<0.001$.
N-acetylglucosamine 8% provided a 16.2% increase in total skin thickness.

Irritation Assessment



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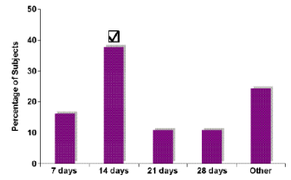
*Significantly worse than baseline, $p<0.05$.
N-acetylglucosamine 8% was well tolerated by the study participants.

Self-Assessment

Number of Days Until Skin Looked and Felt Younger



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☑ Over 50% of the panelists noticed an improvement in their skin within 2 weeks.

Conclusion

Results of the clinical study indicate that N-acetylglucosamine 8% cream is well-tolerated on facial skin. Furthermore, statistically significant improvements in the visual signs of photoaging were achieved at both the 6 and 12-week timepoints. Elasticity, as measured by pinch recoil, improved by 12.2% at 12 weeks. Total skin thickness (skin plumping) increased by 16.2% compared to 3.8% on the untreated control. This is the strongest skin plumping effect observed to date among other anti-aging technologies evaluated by the sponsor company using this model.[9] This finding is not unexpected considering the biological role of glucosamine in the development and structure of some epidermal and dermal GAGs. Consumer perceivable improvements to skin were noted for wrinkles and fine lines, with no evidence of an acnegenic effect. Fifty percent of the participants rated the N-acetylglucosamine-containing product better than their currently used moisturizer. Fifty-four percent of the participants felt their skin looked and felt younger within 2 weeks, and two-thirds felt their skin was noticeably improved within 3 weeks of product use.
N-acetylglucosamine is a natural sugar derivative that is safe and effective for use as a novel, non-acid anti-aging ingredient.

References

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