The physiological importance of hyaluronic acids and effects of topical applied formulations

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1. Introduction

The glycosaminoglycan **hyaluronic acid** (HA) is known as highly functional biopolymer, evolutionarily conserved from prokaryotes (bacteria) to eukaryotes (algae, yeast, mammals, birds molluscs etc.). However, it is not found in fungi, plants and insects.[1,2]
Although the HA structure seems to be "simple" by repeating disaccharide chains of N-acetyl-glucosamine and glucuronic acid, the corresponding biology is fascinating complex.
In the human body, the total content of HA is about 15g for a 70-kg adult.[3] HA is prevalently distributed around cells, where it forms a pericellular coating and is ubiquitously present in the extracellular matrix (ECM) of vertebrate tissues as one of the main components of the connective tissues.[4,5]. Approximately 50% of the total HA resides in the skin, both in the dermis and the epidermis.[6]

The diverse biological functions of HA include the regulation of cell adhesion, cell proliferation or the diffusion of nutrients and growth factors. Also it plays a significant role during tissue injury and modulation of inflammation due to its ability to interact with different receptors and other extracellular binding factors.[7]

Interestingly, depending on the context, HA has a differing or contradictory impact on such biological processes (e.g. be pro- or anti- -inflammatory, -migration or -proliferation processes). These disparate effects are explained in the literature by diverse mechanisms of function depending on certain properties related to HA:

- physicochemical parameters,
- size, distribution,
- functional modifications and binding partners in the ECM;
- synthesis and degradation, microenvironmental regulation and receptor engagement in the vicinity of the plasma membrane;
- intracellular signalling pathways.

The complex interaction of these mechanisms manifests as distinct biological effects at the cellular level but also on organs, and organism.[7]

One of these key properties is the size of the HA. The native form of hyaluronic acid has in most tissues a molecular mass of 1000 – 10000kDa. HA is synthetized by three HA synthase (HAS) proteins and can be degraded by hyaluronidases into smaller fragments or completely digested into disaccharide D-glucuronic acid-N-acetyl-D-glucosamine.[8] Smaller molecular weight fragments are observed in response to glycosidase activity upregulated by environmental cues, such as pH and reactive oxygen species.[9,10]
Predominantly fragments in the range of 200-2000 kDa are observed with different functionalities.[11,12]

2. Influence of HA on various physiological skin and wound repair processes

It is well known that HA takes a crucial role in the process of skin aging since levels of HA gradually decrease with age. In this context, the most visible effects observed are the loss of facial skin hydration, elasticity, and volume, which are responsible for wrinkles.[13,14] As HA provides a highly hydrated medium in the skin matrix, this influences the cell movement required in the early stages of injury, inflammation, and wound healing. HA-rich matrix can either facilitate cell migration by providing a hydrated and non-adhesive milieu to the migrating cells or inhibit such migration by increased binding of proteoglycans to the pericellular HA.[15,16]
The described ability of HA to facilitate tissue repair and wound healing is dependent on its molecular weight and tissue location, as well as the specific cell population HA interacts with. Therefore, HA rich matrix, both in the early inflammatory phase of wound repair and in the granulation tissue, facilitates cell migration into the provisional wound matrix by providing an open hydrated matrix and through direct interaction with cells via HA binding proteins.[16,17]

3. Implications for clinical and cosmetic applications

As HA size is one of the major determinants of its activity it is important to determine which species is used for a certain treatment. Aside to clinical usage like in ophthalmology as a drug delivery system[18], or in osteoarthritis for visco-supplementation,[19] HA has been implemented in a broad range of functional applications in minimally invasive aesthetic procedures, cosmetics or even dietary.[2,20-23]
Several studies have demonstrated that high molecular weight hyaluronic acid (>1 MDa) is anti-inflammatory and promotes epithelial cell homeostasis and survival. It was shown that HMW HA, at doses of 1 mg/ml or above are capable to inhibit inflammatory cell chemotaxis, phagocytosis, elastase release, and respiratory burst activity.(ref)

Physiologic responses to HA are, in part, mediated through the immune system, resulting in either acute or chronic inflammation,[24] manifested by the production of specific inflammatory mediators. Low molecular weight HA promotes the production of inflammatory mediators.[25,26] Similarly, high molecular weight HA inhibits production of pro-inflammatory mediators,[27] suggesting differential macrophage activation by different molecular weight HAs.[26,28,29]

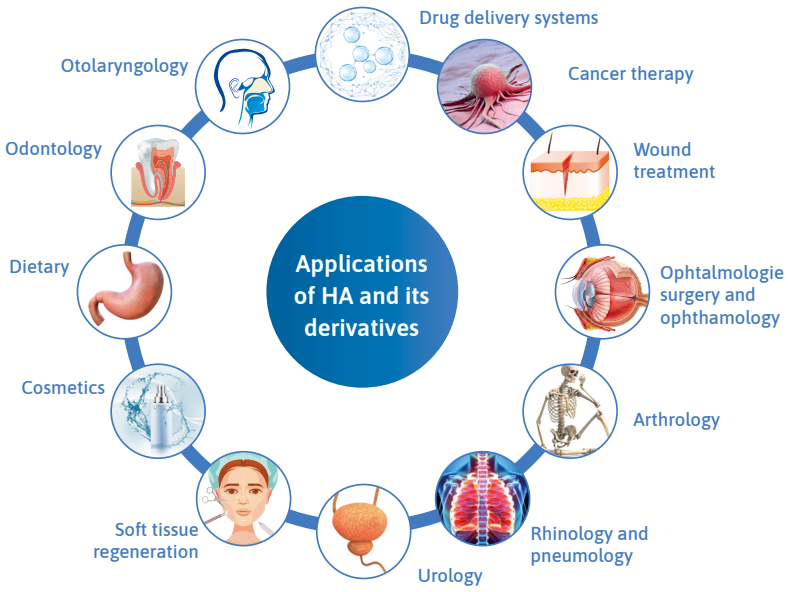

Fig. 1: Medical, pharmaceutical, cosmetic and dietary applications of HA and its derivatives. *(modified from Fallacara et al 2018, Hyaluronic Acid in the Third Millennium)* [2]

In cosmetics, HA is widely used as a moisturizing ingredient in various formulations (gels, emulsions, or serums). Main goal is to improve or maintain the physiological microenvironment of healthy young skin. Consequently, most of these HA containing products aim for a restoration of skin hydration and elasticity. Dependent on the product, an anti-wrinkling effect might be achieved.

As described, the hydrating effect of HA largely depends on its molecular weight (MW), which is also related to the HA stability to hyaluronidases in the microenvironment of the tissue like the skin. High-MW HA mainly acts as a film-forming polymer which ablates water evaporation from the skin surface. In contrast, medium-MW and low-MW HA molecules bind moisture from the environment, due to their high hygroscopicity.[2] At a high concentration of such HA molecules, this water binding capacity lead to the point that HA even extract humidity from the skin reverting the expected hydrating effect.[2] In addition, it is reported that hyaluronic acid has also protecting properties against the harmful effects of ultraviolet irradiation which is based on its free radical scavenging effects.[2,30 31]

4. Preclinical In Vivo Results of HA- containing formulations

In-vivo studies have shown that topically applied HA on the skin reduces signs of skin aging. HA, especially of high molecular weight, hydrate the skin by forming a film on the skin surface and preventing water loss. Whereas, low molecular weight of HA can penetrate skin to protect and support the epidermal hydration, to moisturize the stratum corneum continuously to assure high quality of the epidermal texture. [3, 7, 9]

Essendoubi and colleagues investigated in an in-vitro study the penetration of different sized HA molecules into skin by Raman spectroscopy. He reported that HA with low molecular weight (20–300 kDa) passes through the stratum corneum in contrast of the impermeability of high molecular weight HA (1000–1400 kDa). Recently, Mazzucco evaluated in another study how molecular weight influences the activity of HA and its possible anti-wrinkle effect.[5] He tested two solutions containing either HA with molecular weight from 1000 to 1800 kDa (Standard, Sol. A) or with 50 to 2500 kDa (Second Generation, Sol. B). Both type of formulations revealed to be similar with regards to its safety and high stability, showing no signs of cytotoxicity. Interestingly, Sol. B showed in a quantitative protein analysis that the amount of HA binding receptor CD44 was significantly increased. The authors discuss that this correlates to the fact that low molecular weight HA can penetrate deeply ensuring interaction with specific receptors and in particular with CD44 located on cell membranes. This interaction induces a reactivation of cellular metabolism to help fight environmental stress through mitosis and cell proliferation.

Medium molecular weight HAs that penetrates through the skin and provides the water necessary to preserve turgidity and firmness of the skin. C) HA with high molecular weight which do not penetrate the skin, but form a film on the skin surface blocking the evaporation of water, thus counteracting dehydration.

5. Clinical Observations

Several clinical studies were published aiming to demonstrate the safety and efficacy of dermo-cosmetic HA formulations on different skin aging phenomena. Evidence-based analysis revealed that the anti-wrinkle efficacy of HA is molecular weight dependent which is expected to be due to differences in percutaneous absorption of different molecular weight HA across the stratum corneum.[32]

In a study performed by Pavicic and coworkers 33 they analyzed the efficacy of topical application of 0.1% hyaluronan formulations of different molecular weights (MW) (50, 130, 300, 800 and 2000 kDa, respectively) in the periocular area as anti-wrinkle treatment. Authors performed a clinical trial by including 76 females aged between 30 and 60 years having periocular wrinkles. The different formulations were applied twice daily for a period of 60 days. As result they found greater improvements in the skin hydration level, skin elasticity, and reduction in peri-ocular wrinkles where a formulation containing low molecular weight HA was used.[33]

A further anti-wrinkle efficacy study of HA based topical cream formulation have been summarized by Poetschke and colleagues.[34] In this study, authors have tested four topical cream formulations (Balea, Nivea, Lancome, Chanel) containing HA on 20 women with periorbital wrinkles for 3 months. The creams were applied daily. After treatment period, researchers observed significant improvement in skin elasticity and tightness (13–30%), significant reduction in wrinkle depth (10–20%), and improved hydration level in all treatment subjects.[34]

Because of molecular weight dependent percutaneous absorption, also ultra-small sized HA (nano-HA) containing topical formulations (lotion, serum, and cream) were tested.[35] In this study 33 women with periorbital wrinkles were treated for eight weeks. Results revealed a significant improvement in fines of skin within 2 weeks and improved skin elasticity in 2 to 8 weeks of treatment. The fast anti-wrinkle and skin rejuvenating effects of nano-HA containing topical formulations were addressed to the superior percutaneous absorption of ultra-small HA molecules.[35 36]

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5. amilera® as a second generation HA containing solution

As described before, the biological activity of HA is based on two basic mechanisms, either acting as a passive structural molecule or functioning as a signaling molecule. Both of the mechanisms have been shown to be size-dependent.[2,3,37]
Recent in-vitro data clearly indicate that cosmetic formulations which contain a mixture of small (<500kDa), medium (>500 and <1800) and high molecular weight (>1800 kDa) species of hyaluronic acid have not only a superficial hydration effect but additional positive physiological functionalities e.g. by interacting with the CD44 receptor or other downstream molecules.[38]

Based on the described very promising in-vitro and clinical data, a state of the art formulation for the topical application of HA, like in products as amilera®, should contain a mixture of different molecular weight HA species between 100 and 2500 kDa. Formulations like amilera® products show a high stability and excellent dermatological safety profile, and one may expect that these products should ensure the broad range of HA based effects like improvement in skin hydration and elasticity as well as significant reduction of wrinkle depth.

If additional skin active ingredients are implemented in such HA formulation as e.g. amilera® or other products, the broad range of HA attributed effects may support additional physiological effects within the microenvironment provided by the complementary ingredients. Here are further in vivo studies required to elucidate the full range of beneficial properties of such products.

6. Conclusion

The topical application of mixtures of different molecular weight species of HA in formulations likewise used in amilera® products should ensure a broad range of beneficial effects on aged skin to improve skin hydration and elasticity as well as reduction of wrinkle depth. In addition, it might provide some UV protective effects due to certain radical scavenging properties.

7. References

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