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REVIEW

HYALURONIC ACID AND SKIN: WOUND HEALING AND AGING

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Hyaluronic acid (hyaluronan, hyaluronate, HA), isolated from the vitreous body of the bovine eye by Meyer and Palmer in 1934,[1] derives its name from the tissue in which it was found (*hyalos* = glassy) and one of its constituent sugars (uronic acid). Total HA content has been estimated to be about 15 g in an adult man; approximately one-third turns over daily.[2,3] Serum hyaluronic acid levels range from 10 to 100 μg per L, but can be elevated in cirrhosis, rheumatoid arthritis, and scleroderma, due either to impaired hepatic uptake or increased production. Its clearance from the systemic circulation is mainly via hepatic endothelial receptors and degradation to monosaccharides and their oxydation products. The half-life ($T_{1/2\beta}$) of hyaluronic acid in human plasma is 2.5 to 5.5 minutes. Urinary excretion of hyaluronic acid is less than 1% of total clearance.[4]

CHARACTERISTICS

Hyaluronic acid, a high-molecular weight polysaccharide, is a glycosaminoglycan (GAG), synthesized in the plasma membrane of fibroblasts and other cells, with a structure consisting of a 200 to 10,000 linear polyanionic polymer of repeating disaccharide units, [D-glycoronic acid (1-ß-3) N-acetyl-D-glycosamine (1-ß-4)]n,[4–6] giving a molecular weight between 1×10^5 and 5×10^6 Da, usually at the higher end of this range.[6,7] Regulation of its biosynthesis is not well understood; however, many inflammatory mediators and growth factors activate HA synthesis and the signal transduction seems to involve protein kinases.

HYALURONIC ACID AND SKIN

Hyaluronic acid occurs in all vertebrate tissue and body fluids in varying amounts; the highest concentrations have been observed in soft connective tissues and the lowest in blood (Table 1). In the skin, HA constitutes the primary reservoir of HA in the body, more than 50% of the total (Table 2).[8]

The synthesis of HA by keratinocytes could be inhibited by the addition of calcium chloride to the culture medium and was strongly stimulated by the addition of retinoic acid (RA).[9–13] Estradiol administration to mouse skin causes a considerable increase in HA.[14] Conversely, feeding of ascorbate[15] and administration of antiinflammatory corticoids[16–18] resulted in a reduction in hyaluronate synthesis by human skin fibroblasts in culture medium.

FUNCTIONS

The functions proposed for HA are usually presumed from the physicochemical properties of the polymer and its interaction with other macromolecular components.[3,6] As a predominant voluminous molecule of extracellular matrix (ECM), HA increases, whenever rapid tissue proliferation, regeneration, and repair occur. It is involved in structure and organization of ECM.[19] Bursts in HA deposition correlate with mitotic activity.[20,21] An elevated level promotes cell detachment and migration in proliferating tissues, whereas decreased levels coincide with the onset of differentiation.[22,23] Its water-attaining capacity suggests that HA may play a major role in the maintenance of the extracellular space, facilitate the transport of ion solutes and nutrients,[19,24] and preserves tissue hydration.[14,19,25,26]

Table 1. Concentration of Hyaluronic Acid in Human Tissues and Tissue Fluids*

Tissue Fluid	*Concentration (mg per L)*
Umbilical cord	4100
Synovial fluid	1420 – 3600
Vitreous body	140 – 338
Dermis	200
Thoracic lymph	8.5 –18
Urine	0.1 – 0.5
Serum	.01 – .1

*Data from reference 5: Laurent TC, Fraser JR. Hyaluronan. FASEB J 1992; 6:2397–2404.

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Hyaluronan exists in free and tissue-bound form.[19,27,28] The HA-binding proteins (HABPs), hyaladherens or hyaladherins, are a class of molecules that are only now gaining recognition.[29] It was believed that effects of HA upon cell behavior depend on its physiochemical properties and its interactions with HABPs. Toole[6] previously classified HABPs into two groups: structural HABPs, such as HA-binding proteoglycans and link proteins, and cell-surface-associated HABPs that he thought had HA-receptor properties. Currently, it is believed that these two groups of HABPs have homologous HA-binding domains, akin to a single family of proteins.[6] Among hyaladherens are fibrinogen,[30] collagen,[31–34] CD44,[35–38] Receptor for Hyaluronan-Mediated Motility (RHAMM),[39–41] albumin, and hyaluronidase itself, as well as proteins the functions of which are unknown. It is likely that other hyaladherens will be identified.

Dense hyaluronan exists in human epidermis and dermis. Compartmentalization in different layers of the epidermis was detected; hyaluronan intensity is highest in the middle spinous layer and is lower in the basal layer, whereas it is completely absent in the granular and keratin layers.[24] In the dermal layer, HA appears spatially related to the collagen microfibrils and lies between collagen and elastic fibers.[26] The patterns of HA in epidermal and dermal layers change as a function of age (Table 3).

Diffusion of water through the skin is blocked by extracellular lipids originating from keratinosomes in the stratum granulosum; this boundary corresponds to the site where hyaluronan-staining ended. Hyaluronan-rich extracellular spaces in the vital epidermis may drag water from the dermis, whereas the lipid-rich extracellular water barrier prevents its leakage beyond the granular cell layer; both processes are crucial for the maintenance of skin hydration.[24]

ROLE IN WOUND HEALING

Effect in Wounds

Wounding results in discontinuity in tissue integrity and healing is the process of reconstituting that integrity. Scarring occurs when there is a failure in this healing process to allow the complete degree of organization that occurs in uninjured tissue. The recognition that surgically repaired fetal wounds, rich in HA, healed without a scar is an exciting finding. Therefore, studies of fetal wound healing may provide insights into the initiation and regulation of scarless repair processes akin to regeneration. Fetal extracellular matrix contains abundant HA rather than collagen, distinctly different from that of adults.[42,43] In comparison to adult fibroblasts, fetal fibroblasts have an increased density of cell-surface HA-receptors.[44] Unlike adult wounds, in which hyaluronic acid is present only transiently, a high level of HA in fetal wounds persists in the fetal matrix until repair is completed.

Table 2. Distribution of Hyaluronic Acid Between Different Organs of the Rat*

Organ	*Total Hyaluronan (mg)*	*Percent of Total*
Skin	33.8	55.9
Muscle	4.69	7.8
Skeleton and supporting structures	16.2	26.8
Intestine and stomach	0.50	0.8
Remaining internal organs	5.25	8.7
Whole rat	60.5	100

*Data from reference 5: Laurent TC, Fraser JR. Hyaluronan. FASEB J 1992; 6:2397–2404.

The reasons for this persistent enrichment with HA in fetal wounds may be the presence of hyaluronic acid-stimulating activity (HASA) found in fetal serum, wound fluid, and amniotic fluid, or could be the result of a decrease in degradation of HA.[45] Mast et al.[46] observed that HA inhibited fetal fibroblast proliferation, but stimulated collagen and noncollagen protein synthesis. This study in conjunction with previous findings in utero[47] suggested that HA may have a regulatory effect on scarless fetal wound healing. *In vivo* degradation of hyaluronic acid in fetal wounds by addition of hyaluronidase leads to increased fibrosis, inflammation, collagen deposition, and angiogenesis.[47] Reduced scar formation is found in postnatal wounds treated with HA extracted from the topical tissue.[48] The ability to modulate the repair of adult tissue to make it more "fetus-like" is an ideal approach to reduce scar formation in wound healing.

The distribution and amount of HA are different in various scar tissues. In a mature scar, the distribution resembles that of normal uninjured tissue; however, the layer of HA is thinner. In hypertrophic scar tissue, HA occurred mainly as a narrow strip in the papillary dermis. Keloid revealed the least HA-staining of the papillary layer and resembled that of the bulging reticular dermis. In contrast, the thicker granular and spinous layer of the keloid epidermis showed an intense HA-staining. The variations in HA of different scar tissue may contribute to their different clinical appearance.[49]

The facilitating effect of HA exogenously applied was a surprising observation in wound healing. Beneficial effects of topical HA on the healing of cutaneous ulcers in clinical and histologic studies have been documented.[50–54] The results were claimed to be better than with conventional treatments.[50,53,55] Topical application of hyaluronic acid to wounds in rats leads to enhanced epithelial migration and differentiation[55] and accelerates wound healing with reduced tissue fibrosis.[56] King et al. found that artificial enrichment of the wound tissue with HA improved the microcirculatory

perfusion of repairing tissue in the hamster cheek pouch, reduced wound-induced extravasation, and accelerated wound closure.[57]

The roles of HA in wound healing have been investigated intensively. Doillon et al.[58,59] demonstrated, both in *in vitro* and *in vivo* models, that HA and fibronectin are effective in enhancing fibroblast movement into a collagen sponge and in depositing collagen fibers during the early phases of wound healing. Burd et al.[60] proposed that the collagen protein complex associated with HA contributes to the reorganization of the wound matrix. Alternatively, HA might be part of a feedback loop that promotes cell proliferation and migration in actively growing tissues. Additionally, the action of HA in water homeostasis could favor tissue hydration, which has a positive effect on healing.[57]

Ear surgeons observed in the rat that perforations in the tympanic membrane healed more quickly with hyaluronan applied to the middle ear.[48,61] Some authors noted that exogenous hyaluronan may be beneficial in wound healing. The mechanism is unknown, but it has been hypothesized that hyaluronan promotes epithelial migration, as it seems to surround proliferating and migrating cells in regenerating, remodeling, or healing tissues.[48,57,62]

Glucocorticoid treatment reduces the quantity of glycosaminoglycans, especially of HA, and, moreover, changes the distribution, relative proportion, and structure of connective tissue proteoglycans. These effects probably underlie the development of profound skin changes such as skin atrophy, striae, or impaired wound healing, induced by long-term use of potent glucocorticoids.[63]

IMPACT ON CUTANEOUS AGING

With advancing age, there is a decline in the quality of human connective tissue and its repair processes. Nowhere is this deterioration more obvious than in the skin. Glucosaminoglycans are a major component of the extracellular matrix of the skin primarily composed of hyaluronic acid and dermatan sulfate, with smaller amounts of chondroitin sulfate and heparan sulfate. The water-attracting property of HA produces a swelling pressure in extracellular matrix allowing the rapid diffusion of water-soluble molecules. Decreasing levels of HA during aging imply a shrinkage of the ground substance and a reduction in its viscosity, altering the rate of diffusion of ions and macromolecules from the blood to the tissues and vice versa, probably accounting for the dried and wrinkled appearance of aged skin.[64] The voluminous water of hydration associated with hyaluronan may be a mechanism for maintaining the normal hydration of the skin.

An age-dependent decrease in the content of GAGs, especially HA, has been postulated.[64–69] To verify this hypothesis, several studies to localize and characterize HA in the skin have been conducted. Nevertheless, the correlation of HA with age remains inconclusive.

In vivo studies documented that the amount of HA in skin decreases sharply during maturation,[66,70] as well as during cellular aging *in vitro*.[67,68] Ghersetich et al.[26] demonstrated an age-related decrease in the number of electron-dense granules of HA and of their filament in the dermis by using the cationic dye, alcian-blue, staining method. They proposed that the remarkable alterations of aged skin, including the decrease in turgidity, less support for microvessels, wrinkling, and altered elasticity could be the result of variations in the levels of HA in the dermis. A significant increase of HA staining, located in the elastic fibers of collagen and in the soluble matrix was observed after treatment of wrinkles with electrorydesis.[71] These observations suggested that the swelling and disappearance of wrinkles after treatment may be the result of the increased levels of GAGs and the subsequent edema of the dermis due to the water-retaining property of HA.

By using an enzyme-linked immunosorbent assay (ELISA)-like technique, Meyer and Stern[19] also found that with increasing age a steady decline of HA occurs in the upper epidermis, with a concomitant increase in the basal layer of the epidermis and the upper portions of the papillary dermis. In senile skin, HA was still present in the upper dermis, but entirely absent in the epidermis. Moreover, the overall staining intensity continued to decrease with age. In contrast to most previous observations, they noted that neither the total HA concentration in the skin nor the polymer size changes with increasing age; however, differences in extractibility were detected with increasing tissue binding as a function of age which may underlie some of the changes in human skin that occur with aging. As previously mentioned, it is believed that hyaluronan exists both in free and in tissue-bound forms. Higher levels of bound HA are observed with increasing age suggesting that the

Table 3. Localization of Epidermal and Dermal Hyaluronan in Adult Humans*

Layer	*Positive Staining Location*† *Location of Positive Staining*	*Change with Age*†
Epidermis	Middle stratum spinosum§	⇓
	Stratum basalis	⇓
Dermis	Papillary dermis	⇑
	Periphery of collagen microfibrils	⇓
	Between collagen and elastic fibers	⇓

*Data from references: 19, 24, 26.
† Staining intensity: epidermal intercellular space > papillary dermis > reticular dermis; ‡ Overall staining intensity decreasing with age; § highest intensity; ⇓ decreased staining; ⇑ increased staining.

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patterns of HABPs undergo major age-related changes, concurrently with increasing concentrations of such proteins. Miyamoto and Nagase[72] found an age-related change in the molecular weight of HA in rat skins. This might be the basis for the alteration of HA in the aging process of human skin. Additional experiments are required to address this point more fully. Age-dependent changes in the profiles of skin hyaladheren may occur and play a significant role in aging.

EFFECTS OF RETINOIC ACID

As shown in many *in vitro* studies, retinoic acid (RA) induces the synthesis of HA.[9–13] This is supported by the recent *in vivo* findings of Lundin et al.[73] that 6 months after treatment of photodamaged skin with topical RA, the thickness of the vital epidermis had increased by 23%. With a specific immunohistochemical method using hyaluronan-binding protein, they showed a 31% increase in the thickness of the HA-staining meshwork in the upper stratum spinosum compared with pretreatment skin. The mean concentration of HA in blister fluid had also increased significantly after 2 weeks of treatment of the treated areas. The increase in the thickness of epidermal HA meshwork and the blister fluid suggests that HA is involved in the epidermal change induced by topical RA therapy. Tammi et al.[10] demonstrated that RA increases the accumulation of epidermal HA by stimulating its synthesis in keratinocytes. The property of RA shown earlier to enhance keratinocyte mobility,[74,75] and to modulate the normal maturation of epidermal keratinocytes[11,12,76–82] raises the question of whether these are primary or secondary responses to retinoids.

CONCLUSIONS

All functions of HA on the skin are not known at present, but it has been suggested to be essential for cell proliferation and migration. At present attention focuses on the beneficial actions of HA in wound healing. The water-binding property associated with HA may serve as a means for water homeostasis in the skin. The altered pattern and level of HA with age may account for the deterioration of the aging skin.

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Eczema in 1837

Eczema on the forehead, face, neck and shoulders, as well as in other parts in older children, was generally found connected with errors in diet, or more or less gastric or intestinal derangement, and yielded to means for correcting these, with the frequent application of warm water, or the use of the warm bath; with particular attention to diet, restricting the patients, as far as possible, to farinaceous articles and plainly cooked fresh meat. These varieties frequently require some of the ointments before alluded to, especially when the disease, either from its duration or from neglect and irritation, has given rise to more or less ulceration of the outer surface of the dermis. *From Bulkley HD. Bulkley on diseases of the skin. New York J Med & Surg 1840; 2:124.*