

Review Article

Induction of Hyalurosomes by Topical Hyaluronate Fragments Results in Superficial Filling of the Skin Complementary to Hyaluronate Filler Injections

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Keywords

Hyaluronate · Hyalurosomes · Dermatoporosis · Epicutaneous filling · Retinoids

Abstract

Hyaluronate (HA) plays a major role in the process of skin aging. The main use of HA has been for hydration and dermal fillers. Another approach, based on the discovery of the signaling effects of topically applied hyaluronate fragments (HAF), has subsequently been developed. It has been thoroughly demonstrated that topical applications of HAF of a very specific size induce HA filling of the epidermis and the upper dermis. These effects are particularly visible in dermatoporotic patients. Moreover, the combination of HA-based filler injections with topical applications of HAFs/retinoids showed an optimization of the effects of HA. Thus, a new classification of the different effects of HA is proposed here.

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Hyaluronate (HA, hyaluronan, hyaluronic acid) was discovered in 1934 by Meyer and Palmer. It can be found in skin, joints, eyes, and most other organs and tissues. HA is a major component of the extracellular matrix, of which the skin is the main reservoir [1]. It is synthesized at the cell membrane of many cells, in particular in fibroblasts and keratinocytes [2]. It is composed of repeating units of the disaccharide $\text{GlcNAc}\beta(1\rightarrow4)\text{-GlcUA}\beta(1\rightarrow3)$ throughout a molecule that can have a molecular weight of up to 10^6 Da. HA belongs to the glycosaminoglycan family with the particularity that it is not sulfated in comparison with other glycosaminoglycans. HA acts through a large number of proteins with the ability to bind to it, called hyaladherins [3]. The main cell surface receptor of HA is CD44, which can exist in numerous isoforms through alternative splicing of 10 variant exons in different combinations [4].

Hyaluronate: Up to Now

In the skin, HA provides a highly hydrated medium facilitating the cell movement that occurs in the early stages of injury, inflammation, and wound healing [5, 6]. It may also contribute to the elastic properties of the dermis by forming a network of helicoidal structures, to epidermal differentiation, and also to lipid synthesis/secretion [7–9].

HA is a crucial molecule in the process of skin aging since levels of HA gradually decrease with age [10]. Cosmetics often propose HA as an active ingredient, but topical HA applications do not lead to an increase in HA-specific staining intensity, suggesting that the large molecule is poorly able to permeate the stratum corneum of viable human epidermis, both *ex vivo* and *in vitro* [11, 12]. Another solution to correct wrinkles and folds is the use of HA injections [13]. HA fillers provide structural support for the collagen fibers. Treatment with HA injections is not permanent, and visible results last about 6 months. Several fillers received approval by the US Food and Drug Administration. HA dermal fillers are considered to be safe and effective [14–16]. Their advantage is that they offer longer-lasting correction than collagen fillers [17, 18].

Hyaluronate Fragments: From Now On

In addition to its viscoelastic properties, HA may be cleaved into fragments of smaller molecular weight, called hyaluronate fragments (HAF), which are the degradation products derived from the action of hyaluronidases, β -glucuronidase, or hexosaminidase on HA. HAF have distinct physiological properties, such as stimulation of cell turnover, angiogenesis, tissue remodeling, or activation of the innate immune defense [19–21].

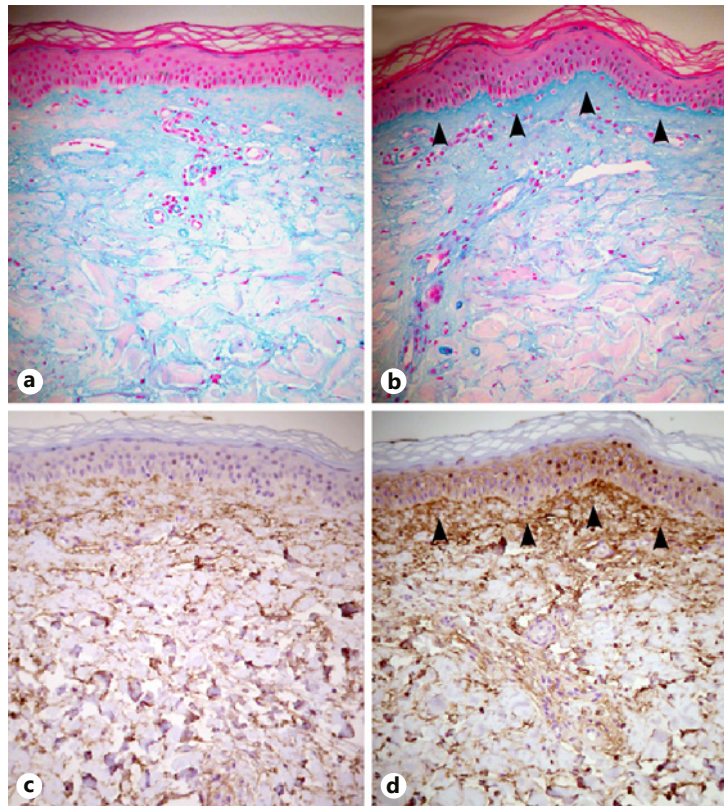
Many studies suggest that cell responses after HA treatment depend on the size of the fragments as well as on the cell type. Thus, HAF may exert differential regulation on the wound-healing process [22]. They also induce epithelial cell proliferation and HA synthase expression, thereby stimulating endogenous HA production [12]. However, we have demonstrated that only HAF between 50 and 400 kDa (intermediate size, HAFi) were able to induce an epidermal hyperplasia, to increase the density of the dermis and HA content through a CD44-dependent pathway [12]. In a recent study that we conducted on 10 subjects, we showed that daily topical application of HAFi for 5 days increased the HA content in the superficial dermis of the skin already injected by a noncross-linked HA (Fig. 1). Therefore, HAFi act as an epicutaneous (or topical) HA filler (Fig. 2).

Therapeutic Applications of HAF

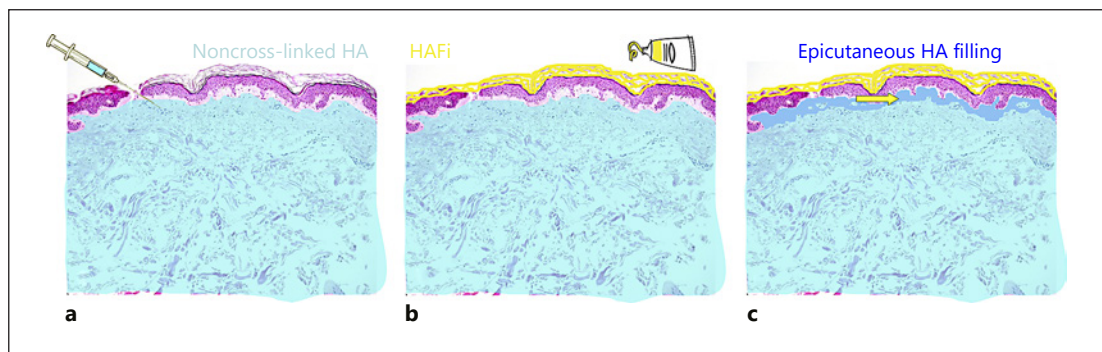
Dermatoporosis

The term “dermatoporosis” was proposed to cover the different manifestations and implications of the chronic cutaneous insufficiency/fragility syndrome in the elderly and to facilitate the understanding that, as osteoporosis, dermatoporosis should be treated to prevent complications [23, 24]. We have shown that dermatoporosis is due to the dysfunction of hyalurosomes, which is a putative multimeric macromolecule complex composed of molecules involved in HA metabolism and cell signaling in keratinocytes, such as CD44, heparin-binding epidermal growth factor (HB-EGF), and its receptor erbB1 [25]. Epidermal Lrig1+ progenitor cells, Wnt/ β -catenin pathway, calcium signaling, and p16^{Ink4a} pathway also play a role in the pathogenesis of dermatoporosis [24, 26, 27]. HAFi might constitute a target molecule for the prevention of cutaneous aging and the reversal of the skin atrophy observed in dermatoporotic patients [12]. Inhibition of a putative hyalurosomes complex in keratino-

Fig. 1. HAFi increase the HA in the superficial dermis of HA-injected human skin. Colloidal iron (a, b) and HABP (c, d) staining of human skin injected with a non-cross-linked HA (a–d) and subjected to a daily topical application of HAFi for 5 days (b, d). Note the diffuse dermal staining of HA in blue (a, b) and in brown (c, d) and the accumulation of HA in the superficial dermis just beneath the dermal-epidermal junction in HAFi-applied skin (b, d, arrowheads). HABP, hyaluronan binding protein.



Color version available online



Color version available online

Fig. 2. Schematic representation of the epicutaneous filling effect of HAFi. Noncross-linked HA shows its filling effect (light blue color) in the mid- and deep dermis (a). Topically applied HAFi (b) act as an epicutaneous filler by filling the superficial dermis with HA (dark blue color, yellow arrow in c).

cytes seems to be the molecular mechanism for corticosteroid-induced dermatoporosis [28]. We have recently demonstrated that HAFi induced hyalurosomes complex resulting in EGFR activation via CD44v3 [12, 28]. The resulting effect was the stimulation of cell proliferation and epidermal hyperplasia in dermatoporotic patients [12]. Topical HAFi induced the expression of hyalurosomes components in dermatoporotic skin [29].

HAFi in Association with Retinoids

Retinoids, including retinaldehyde (RAL), are well known in the treatment of skin aging [30]. RAL induces keratinocyte proliferation through a CD44-dependent mechanism and

Table 1. The different types of biological activity of hyaluronic acid

Administration	Type of HA	Method of action	Type	Effect
Intradermal	Native molecule	Increases dermal mass	1	Filling and viscoelastic effects in the dermis
Topical (epicutaneous)	Fragments (HAFi)	Induces HA production in the epidermis and upper dermis	2	Filling and viscoelastic effects in the epidermis and upper dermis
Topical (epicutaneous)	Native molecule	Traps water	3	Water retention

upregulates the HA synthesizing enzymes in mouse skin [25, 31]. We have recently demonstrated that topical applications of RAL and/or HAFi also induce CD44 expression in the epidermis, as well as increasing the dermal and epidermal HA content. These effects are more significant than treatment with RAL or HAFi alone, suggesting that these 2 components have a synergistic action [25, 29].

A recent clinical study showed that applications of a topical preparation containing a combination of RAL and HAFi optimized the results obtained with injectable HA for both parameters measured, skin texture and dermal thickness [32]. These results suggest that topical HAFi and HA fillers have complementary biological activities.

Proposal of a New Classification of the Biological Activities of HA

A new classification of the biological activities of hyaluronic acid may be proposed based on size and method of administration (Table 1). The 2 biological activities of HA involving (1) viscoelastic filling (type 1) and (2) its role as an intercellular messenger, HAFi (type 2), could be used with a view to prevent and reverse cutaneous aging and dermatoporosis. It follows from this reasoning that HA skin treatment should combine type 1 and type 2 activities, with a variable type 1/type 2 ratio.

Type 3 activity corresponds to HA's function in water retention and its ability to combine with water to form a complex in the intercellular environment and in connective tissue in particular. In this way, the space it occupies enables the retention of smaller molecules, such as growth factors or electrolytes.

Disclosure Statement

The authors declare no conflicts of interest.

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