

NEW THERAPEUTIC TARGETS IN DERMATOPOROSIS

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Abstract: Dermatoporosis is a novel term proposed to describe the chronic cutaneous insufficiency/fragility syndrome characterized by an extreme skin atrophy. Dermatoporosis is principally due to chronological aging and long-term and unprotected sun exposure, but it may also result from the chronic use of topical and systemic corticosteroids. We have recently proposed a membrane organelle, hyalurososome, composed of molecules involved in hyaluronate (HA) metabolism and cell signaling in the keratinocytes, such as principal HA receptor CD44, heparin-binding epidermal growth factor (HB-EGF), HB-EGF receptor erbB1 and HA synthase 3 (HAS3), which is functionally defective in dermatoporosis and may be a target for intervention. Several lines of evidence suggest that hyalurososome is located in keratinocyte filopodia, thin, actin-rich plasma membrane protrusions implicated in cell motility. We have recently shown that keratinocyte filopodia are downregulated by corticosteroids in vitro. Intermediate size HA fragments (HAFi) inhibited the downregulation of filopodia induced by corticosteroids. Topical HAFi prevented the skin atrophy induced by topical corticosteroids in mice without interfering with their anti-inflammatory effect. Topical treatment with HAFi 1% of atrophic forearm skin of dermatoporosis patients for 1 month resulted in a significant clinical improvement and induced the expression of hyalurososome molecules. Topical retinaldehyde (RAL) and HAFi showed a synergy in HA production and pro-HB-EGF expression in mouse skin and in the correction of skin atrophy in dermatoporosis patients. Uncovering the molecular mechanisms implicating hyalurososome seems to be crucial to better understand the pathogenesis of dermatoporosis and to develop new therapeutic strategies.

Key words: Dermatoporosis, CD44, hyaluronate, hyalurososome, filopodia, HAFi, RAL, corticosteroids.

Introduction

Dermatoporosis is a novel term proposed to describe the chronic cutaneous insufficiency/fragility syndrome (1-3). As in osteoporosis where the bone mass is progressively decreased, the dermatoporosis is caused by the progressive decrease of the main structural elements of the skin (Figure 1). Dermatoporosis is considered to be the functional face of skin aging beyond cosmetics and appearance. As of 40-60 years-old, the trivial signs of skin aging such as wrinkles and appearance changes occur. At 70-80 years-old, morphological markers of dermatoporosis start to appear. As from 80 years-old, functional expression of skin fragility with the complications of dermatoporosis begins. This syndrome needs to be better studied in order to find preventive solutions. With increasing numbers of elderly patients over 80 years-old, we start talking about the epidemics of dermatoporosis. Chronic systemic or topical steroid therapy and chronic exposure to ultraviolet irradiation seem to be the major causes of dermatoporosis (2).

Morphological markers of dermatoporosis

Skin atrophy

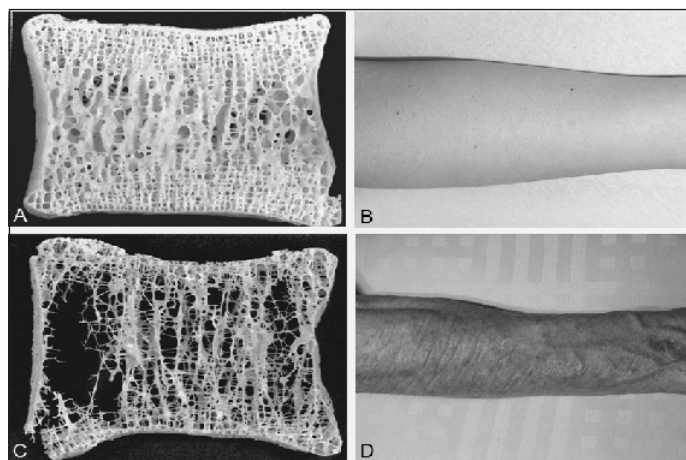
Skin atrophy is seen clinically as extreme thinning of the skin with the appearance of wrinkles (Figure 1D). Echographic analysis of the skin shows a significant decrease of the skin thickness (epidermis and dermis) (0.5-0.8 mm) when compared to that of the normal skin (1.2-1.5 mm).

Senile purpura

Senile purpura (or purpura of Bateman) is a superficial non-inflammatory hemorrhagic lesion histologically characterized by extravasation of erythrocytes in an atrophic dermis due to vascular fragility (Figure 1D).

Figure 1

As in osteoporosis where the bone mass is progressively decreased (A, C), the dermatoporosis is caused by the progressive decrease of the main structural elements of the skin (B, D)



Pseudoscar

Pseudoscar is a superficial scar due to the spontaneous laceration of the skin secondary to minor trauma (Figure 1D). Histologically these lesions correspond to a hypocellular

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sclerotic and fibrotic zone covered by an atrophic epidermis.

Prevalence of dermatoporosis

According to a French study conducted on 202 subjects ranging between 60 and 80 years, the prevalence of dermatoporosis is as high as 32%. The frequency of dermatoporosis is about 22% in females and 38% in males (4).

Topography of dermatoporosis

The zones of predilection of dermatoporosis are the following (according to frequency): posterior side of forearms, pretibial area, dorsum of hands, presternal area and scalp (Figure 2). This suggests different internal and external etiological factors.

Clinical stages of dermatoporosis

Four stages of dermatoporosis have been proposed (2):

Stage I: In this stage we find the abovementioned morphological markers of dermatoporosis i.e. skin atrophy, senile purpura and pseudocicatrices. This is the most common stage of dermatoporosis.

Stage IIa: Localized and small superficial lacerations (<3 cm) due to skin fragility.

Stage IIb: Larger lacerations (>3 cm).

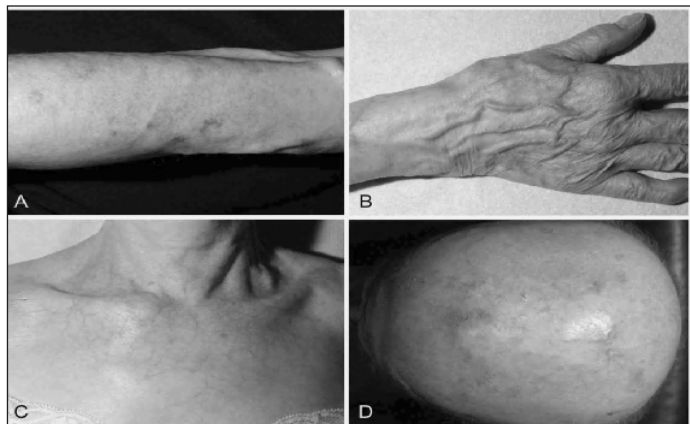
Stage IIIa: Superficial hematomas.

Stage IIIb: Deep dissecting hematomas without skin necrosis.

Stage IV: Large areas of skin necrosis with potential lethal complications.

Figure 2

The zones of predilection of dermatoporosis are posterior side of forearms (A), dorsum of hands (B), presternal area (C) and scalp (D)



Deep dissecting hematoma (DDH)

DDH is an emerging severe complication of dermatoporosis (5). Following a minor traumatic event, laceration of deep skin

vessels exposed to skin surface due to the extreme thinning of epidermis and dermis and to the loss of viscoelastic capacity of the dermatoporotic skin seems to be the pathogenetic mechanism. Prompt diagnosis by clinical evaluation and imaging procedures and early drainage of the hematoma by surgical intervention are critical to save the life of the patient.

Mechanisms of skin fragility in dermatoporosis

Hyalurosomes dysfunction

We have recently proposed a membrane organelle, hyalurosomes, composed of molecules involved in hyaluronate (HA) metabolism and cell signaling in the keratinocytes, such as CD44, heparin-binding epidermal growth factor (HB-EGF) and HB-EGF receptor erbB1, which is functionally defective in dermatoporosis (6) (Figure 3). Hyalurosomes mainly functions as a HA factory. The production and the degradation of HA are realized by hyaluronate synthase (HAS) and hyaluronidase (Hyal) enzymes, respectively. There are three mammalian HASs (HAS1, HAS2 and HAS3) which synthesize HA of different sizes at the cell membrane and release it to the extracellular area where it binds to proteoglycans such as CD44. CD44 is a transmembrane glycoprotein and the major cell surface receptor of HA (7). There are several isoforms of CD44 resulting from alternative splicing and post-translational modifications. The standard form and the variant forms are designated as CD44s and CD44v, respectively. A particular isoform of CD44, CD44v3, is expressed by keratinocytes. We have recently shown that HAS3 is co-localized with CD44v3 and HA in human epidermis, suggesting that hyalurosomes is found on the keratinocyte membrane (8). In fact, HAS3 transfection of cultured epithelial cells induces filopodia formation with consequential HA accumulation around filopodia (9). This observation implies that hyalurosomes is located on the filopodia. Filopodia are thin, actin-rich plasma membrane protrusions and among the distinct leading edge structures implicated in cell motility (10, 11). We have recently demonstrated that CD44v3, HA and actin were co-localized in the filopodia of cultured keratinocytes (manuscript in preparation). Suppression of keratinocyte CD44 results in skin atrophy, which is very similar to dermatoporosis, in mouse (12). We have shown that CD44 and HA expressions are decreased in human dermatoporotic skin (2). We have also shown that the expression of hyalurosomes molecules is diminished in dermatoporosis patients (manuscript in preparation). We also observed a decrease in HA production, in the expression of hyalurosomes molecules and in filopodia formation in cultured keratinocytes treated with corticosteroids (manuscript in preparation).

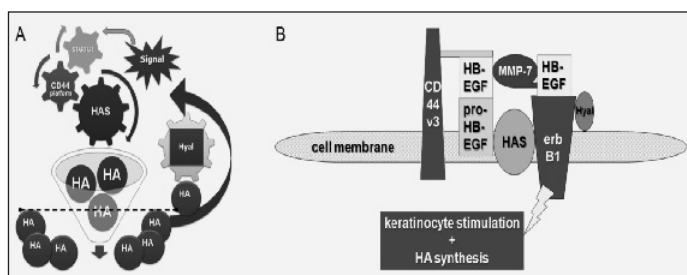
Ultraviolet (UV) irradiation

We have previously shown that UVA and UVB irradiation decrease the expression of CD44 and HA in mouse skin, suggesting the deleterious effect of UV on hyalurosomes

molecules (13).

Figure 3

Schematic representation of hyalurosomes composed of CD44 platform molecules (CD44v3, HB-EGF, matrix metalloproteinase-7 (MMP-7) and erbB1), HAS and Hyal. The activity of hyalurosomes is triggered by an exogenous or endogenous signal which, through a starter molecule, induces the CD44 platform molecules and HAS. The synthesis of HA molecules is controlled by Hyal (A). Induction of CD44 platform molecules localized at the cell membrane results in keratinocyte stimulation and HA synthesis (B)



Topical corticosteroids

We have recently shown that topical steroids induce a severe skin atrophy and decrease the expression of CD44, CD44v3 and pro-HB-EGF as well as the epidermal and dermal HA content in mouse skin in only 5 days (14).

Correction of hyalurosomes deficiency in dermatoporosis

HA is a high molecular weight polysaccharide (10^6 Da) which is the major component of the extracellular matrix (ECM) of the skin. HA has structural and mechanical functions such as viscoelasticity, filling and hydration (15). HA fragments (HAF) ($>8 \times 10^3$ Da - $<10^6$ Da) are generated in case of stress and injury and have signaling functions playing a role in cell renewal and angiogenesis (16). We hypothesized that activation of hyalurosomes by HAF might be an option to reverse skin atrophy in dermatoporosis.

Effect of HAF of defined size on keratinocytes

In order to understand the effect of HAF on keratinocytes, we generated HAF of small (HAFs), intermediate (HAFi) and large size (HAFI). HAFi, but not HAFs or HAFI, induced in vitro mouse keratinocyte proliferation (17). HAFi increased HA production in vitro in human keratinocytes. HAFi, but not HAFs or HAFI, also increased the number and size of filopodia in cultured keratinocytes (manuscript in preparation).

Effect of HAFi on mouse skin

HAFi result in a CD44-dependent epidermal hyperplasia and increase the quantity of epidermal and dermal HA in mouse skin. HAFi also increase the protein expression of CD44v3, pro- and active HB-EGF in mouse skin (17). Topical HAFi

prevent the skin atrophy induced by topical corticosteroids in mice without interfering with their anti-inflammatory effect. Topical HAFi also restore diminished expression of CD44, CD44v3 and pro-HB-EGF, and augment the epidermal and dermal HA content in mouse skin treated with topical corticosteroids (14).

Effect of HAFi on the skin of dermatoporosis patients

Topical treatment with HAFi 1% of atrophic forearm skin of dermatoporosis patients for 1 month resulted in a significant clinical improvement with decrease of purpuric lesions and atrophic aspect of the skin (17). Histologically, epidermal atrophy was corrected and the dermal cellularity was increased. Echographic analysis of the skin showed that in elderly patients (74-86 years-old) the cutaneous thickness was significantly increased with HAFi treatment. In patients aged between 55 and 65 years where the skin atrophy was less pronounced, the effect of HAFi was minimal. In young controls (29-32 years-old) with no skin atrophy, there was no effect of HAFi. HA-Binding Protein (HABP) staining of dermatoporotic skin revealed a significant increase of HA content after HAFi treatment (manuscript in preparation). HAFi increased also the collagen, elastin and vascular content in the dermis of dermatoporotic skin after 1 month of treatment (17). Topical HAFi induced the expression of hyalurosomes molecules in dermatoporotic skin (manuscript in preparation).

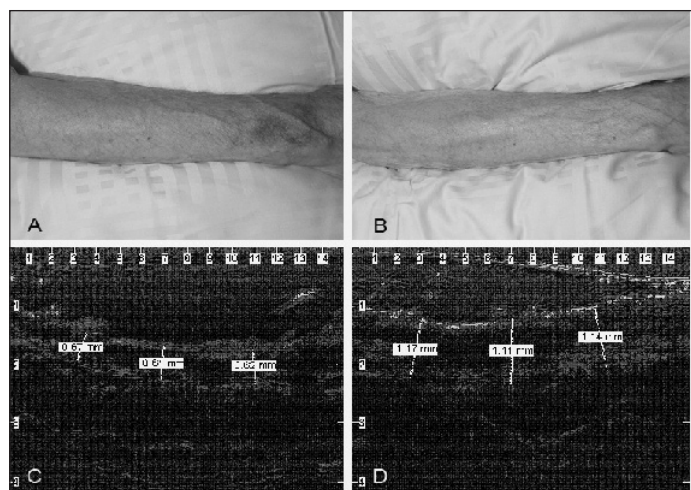
Synergistic effect of HAFi and retinoids

Topical retinoids are known to cause an epidermal hyperplasia by inducing HB-EGF (18). We have previously shown that topical retinaldehyde (RAL) increases the expression of CD44v3 and that the hyperplasia-inducing effect of RAL was abolished in CD44KO mice (19). RAL augments the epidermal and dermal content of HA by inducing the HAS (20). Topical RAL also prevents UV-induced decrease of CD44 and HA expression in mouse skin (13). Treatment of primary mouse keratinocyte cultures with RAL resulted in the most significant increase in keratinocyte proliferation when compared with other retinoids, retinoic acid, retinol or retinoyl palmitate. RAL and HAFi showed a more significant increase in keratinocyte proliferation than RAL or HAFi alone (6). We observed a synergy between RAL and HAFi in HA production and pro-HB-EGF expression in mouse skin and in the correction of skin atrophy in dermatoporosis patients (Figure 4) (6). RAL and HAFi also increase the HA and collagen content and decrease the number of senile purpura in dermatoporotic skin (manuscript in preparation).

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Figure 4

Clinical aspect of dermatoporotic forearm skin before (A) and 1 month after topical treatment with RAL and HAFi (B). Note the disappearance of senile purpura and improvement of atrophy. Echographic analysis performed using a skin ultrasound system (Episcan®; Longport Inc., Glen Mills, PA, USA) shows the correction of skin atrophy (average of 3 measurements between the top level of epidermis and dermal-subcutaneous fat junction). Average cutaneous thickness before treatment (0.63 mm; C) and after 1 month of topical treatment (1.14 mm; D)



Conclusion

Hyalurosomes seem to be a molecular target in dermatoporosis. We think that the dysfunction of hyalurosomes reflected by decreased HA synthesis and filopodia formation plays a central role in skin atrophy in dermatoporosis. We have identified HAFi as an anti-atrophic factor which acts on hyalurosomes to correct its dysfunction by stimulating HASS, CD44, HB-EGF and filopodia alone or in synergy with RAL. Further research is needed to shed more light on the understanding of other molecular pathways implicated in dermatoporosis and to develop therapeutic strategies targeting hyalurosomes.

References

- Saurat JH (2007) Editorial: Dermatoporosis: The functional side of skin aging. *Dermatology* 215: 271-272.
- Kaya G, Saurat JH (2007) Dermatoporosis - A chronic cutaneous insufficiency/fragility syndrome: Clinico-pathological features, mechanisms, prevention and potential treatments. *Dermatology* 215: 284-294.
- Kaya G, Saurat JH (2010) Dermatoporosis: A new concept in skin aging. *Eur Ger Med J*: 216-219.
- Mengeaud V, Dautezac-Vieu C, Josse G, Vellas B, Schmitt AM (2012) Prevalence of dermatoporosis in elderly French hospital in-patients: a cross-sectional study. *Br J Dermatol* 166:442-443.
- Kaya G, Jacobs F, Prins C, Viero D, Kaya A, Saurat JH (2008) Deep dissecting hematoma: An emerging severe complication of dermatoporosis. *Arch Dermatol* 144: 1303-1308.
- Barnes L, Tran C, Sorg O, Hotz R, Grand D, Carraux P, Didierjean L, Stamenkovic I, Saurat JH, Kaya G (2010) Synergistic effect of hyaluronate fragments in retinaldehyde-induced skin hyperplasia which is a Cd44-dependent phenomenon. *PLoS One* 5: e14372.
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B (1990) CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61: 1303-1313.
- Barnes L, Carraux P, Saurat JH, Kaya G (2012) Increased expression of CD44 and hyaluronate synthase 3 is associated with accumulation of hyaluronate in spongiotic epidermis. *J Invest Dermatol* 132: 736-738.
- Kultti A, Rilla K, Tiihonen R, Spicer AP, Tammi RH, Tammi MI (2006) Hyaluronan synthesis induces microvillus-like cell surface protrusions. *J Biol Chem* 281:15821-15828.
- Ridley AJ (2011) Life at the leading edge. *Cell* 145: 1012-1022.
- Laitinen VM, Huang C, Sigal YJ, Jacobson K (2007) Filopodia are induced by aquaporin-9 expression. *Exp Cell Res* 313: 1295-1306.
- Kaya G, Rodriguez I, Jorcano JL, Vassalli P, Stamenkovic I (1997) Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. *Genes Dev* 11 : 996-1007.
- Calikoglu E, Sorg O, Tran C, Grand D, Carraux P, Saurat JH, Kaya G (2006) UVA and UVB decrease the expression of CD44 and hyaluronate in mouse epidermis which is counteracted by topical retinoids. *Photochem Photobiol* 82: 1342-1347.
- Kaya G, Tran C, Sorg O, Grand D, Hotz R, Carraux P, Saurat JH (2007) Topical hyaluronate fragments prevent corticosteroid-induced skin atrophy in mouse without interfering with anti-inflammatory effect. *J Invest Dermatol* 127: S55.
- Laurent TC, Fraser JR (1992) Hyaluronan. *FASEB J* 6 : 2397-2404.
- West DC, Hampson IN, Arnold F, Kumar S (1985) Angiogenesis induced by degradation products of hyaluronic acid. *Science* 228: 1324-1326.
- Kaya G, Tran C, Sorg O, Hotz R, Grand D, Carraux P, Didierjean L, Stamenkovic I, Saurat JH (2006) Hyaluronate fragments reverse skin atrophy by a CD44-dependent mechanism. *PLoS Med* 3: e493.
- Saurat JH, Didierjean L, Masgrau E, Piletta PA, Jaconi S, Chatellard-Gruaz D, Gumowski D, Masouyé I, Salomon D, Siegenthaler G (1994) Topical retinaldehyde on human skin : biologic effects and tolerance. *J Invest Dermatol* 103: 770-774.
- Kaya G, Tran C, Sorg O, Hotz R, Grand D, Carraux P, Didierjean L, Stamenkovic I, Saurat JH (2004) Retinaldehyde-induced epidermal hyperplasia via heparin binding epidermal growth factor is CD44-dependent. *J Invest Dermatol* 123: S189.
- Kaya G, Grand D, Hotz R, Augsburger E, Carraux P, Didierjean L, Saurat JH (2005) Upregulation of CD44 and hyaluronate synthases by topical retinoids in mouse skin. *J Invest Dermatol* 124: 284-287.