Journal of Internal Medicine 1997; **242**: 61–66

Hyaluronan in skin

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Abstract. Juhlin L (University Hospital, Uppsala, Sweden). Hyaluronan in skin (Minisymposium: Hyaluronan) *J Intern Med* 1997; **242:** 61–6.

Because of the abundance of hyaluronan in skin, interest was early focused on variation in the content of the polysaccharide in various pathological conditions of this tissue. A large amount of early work utilized histological techniques of insufficient specificity but recent developments of specific analytical and staining methods for hyaluronan have supplied new data on its presence and possible role in skin disorders.

Keywords: ageing, cancer, mucinosis, psoriasis, systemic sclerosis, wound healing.

Introduction

Skin is the organ which contains most of the body's hyaluronan (HYA). It is also easily accessible to biopsies which has led to numerous microscopical studies of HYA in clinical material. However, the specificity in analytical and histochemical techniques have been considerably increased in the last decade and I will therefore mainly cite papers which have appeared in the last 10 years; for references to earlier papers using indirect methods the readers should consult the excellent review of Tammi *et al*. [1].

Normal skin

Hyaluronan can be localized histologically using hyaluronan-binding protein (HABP) isolated from cartilage. In normal skin HYA is found in the intercellular spaces of epidermis except in the upper granular layer and the stratum corneum. In the dermis the HABP shows a diffuse staining which is increased below the basement membrane and around the skin appendages [2]. The synthesis of HYA occurs in the plasma membrane and is influenced by various factors such as hormones and inflammatory mediators [1]. Certain forms of the lymphocyte homing receptor CD44 can act as a receptor for HYA on cell membranes. CD44 can be detected by monoclonal antibodies on most leukocytes, fibroblasts and epithelial cells [2].

Besides being a matrix in which cells are embedded, HYA has been found to have an increasing number of functions in skin. It can immobilize water in the tissue and thereby change dermal volume and compressibility. It can influence cell proliferation, differentiation and tissue repair [3]. The changes of HYA found in ageing, wound healing and diseases further indicate its importance.

Ageing skin

Healthy subjects

Hyaluronan and dermatan sulphate are the dominating glycosaminoglycans in skin. Although an age-related decrease of HYA and water has been described in earlier literature more advanced methods have not confirmed this. Using an ELISA assay

and histochemical techniques Meyer & Stern [4] found no significant difference in HYA concentration in 22-week-old fetuses, 31–32-year-old adults and 81 and 89-year-old subjects and the distribution of hyaluronan polymer sizes in various extracts did not change. However, with advancing age HYA became more firmly bound to HYA binding proteins (hyaladherins) and its extractability decreased. Significant histochemical differences could also be detected in foetal and postnatal development. Thus at 28 weeks of gestation the epidermis did not stain for HYA, only the dermis [4]. At this time wounds heal without scars, which correlates with elevated levels of HYA in wound fluid [5]. At 34 weeks, when scars can develop after wounding, intradermal staining is seen for the first time. From the third month of postnatal life there is marked staining for HYA in the basal epidermal layer and the upper dermis with an intermediate degree of staining in the spinal layer. With increasing age a steady decline of HYA occurs in the upper epidermal layers with the highest concentration in the papillary dermis. In the elderly HYA is only present in the upper dermis [4] which helps explain the dryness and minimal scarring in aged skin.

Werner's syndrome or Pangeria

This is an inherited disease characterized by accelerated ageing from an age between 15 and 30. These patients have subnormal levels of HYA in their atrophic skin [6], but they have increased urinary excretion of HYA [1] and their fibroblasts in tissue culture synthesize HYA at a higher rate than normal [7]. These findings indicate an increased turnover of HYA [7] in these patients.

Acrogeria

Acrogeria is premature ageing of the extremities starting the first 6 years of life. Skin atrophy and subcutaneous wasting of face and extremities dominates. Hyaluronan content of atrophic involved skin is 50% of that in non-involved skin [8].

Wound healing

The fact that wound healing occurs without scarring in the fetus has now been linked to the HYA tissue level [5]. This has focused interest on the effect of HYA on wound healing in adults. In a trephine biopsy wound model, coating of collagen matrices with HYA showed no significant effect [9] whereas 0.2% HYA in alginate vehicle aided the healing process [10]. Further studies are needed to establish the clinical relevance of HYA in wound healing.

Inflammatory skin diseases

Eczematous reactions

Skin reactions to dinitro-1-fluorobenzene can be inhibited in animals treated with antibodies to CD44 [11]. This may indicate an interplay between HYA and its receptor in contact dermatitis.

It has been reported that topical toxic irritants decrease the HYA level in skin the first 3 days and that it stays subnormal for a week [12]. In contrast the level of HYA in blister fluid is elevated after UVB or retinoic acid-treatment of various inflammatory skin lesions such as atopic dermatitis, eczema, mycosis fungoides and prurigo nodularis [13]. If the first observation is correct an explanation could be that HYA leaks out of the tissue faster than it can be replaced in these conditions.

Systemic sclerosis

In systemic sclerosis, which is characterized by an excessive accumulation of collagen in the skin and other organs, an increase of dermatan sulphate and a decrease of HYA has been reported [14–16]. The increase in dermatan sulphate was correlated with the clinical severity and the extent of sclerosis [14, 16]. In localized scleroderma HYA is increased at the active edges of the lesions but is reduced in the centre where dermatan sulphate and chondroitin sulphate are elevated instead. An increased level of HYA in skin blister fluid and serum was found in scleroderma [13, 18] suggesting that HYA is mobilized from the sclerotic skin into the circulation and that the tissue synthesis cannot replace it locally. Higher serum levels were seen in the patients with lung and internal organ involvement [19]. HYA was mainly increased in new active lesions of patients with lichen sclerosus and scleroderma [13].

Psoriasis

Patients with psoriasis have elevated levels of HYA in serum and in suction blisters raised on the lesions [13, 20, 21]. With the aid of HABP, the HYA has been localized especially to the dermal papillae and

around appendages [2, 22]. CD44 has a similar distribution [2].

A cell surface HYA receptor on liver endothelial cells (HARLEC) was earlier identified as the intercellular adhesion molecule-1 (ICAM-1) [23]. Although the HA binding sites on LEC are not specific for HA but recognize also other ligands, e.g. chondroitin sulphate and dextran sulphate *in vitro* as well as *in vivo* (S. Gustafson & T. Björkman, 1997, in press), a histological study was performed on ICAM-1 in psoriasis. Previous studies have shown very little ICAM-1 expression in normal skin [25, 26] whilst a high expression of ICAM-1 has been reported, especially around vessels in skin from psoriatic plaques [24, 25]. We found only low amounts of ICAM-1 in normal appearing skin of patients with psoriasis but after hyaluronidase treatment of the histological sections prior to staining, an increased expression was observed. The treatment of psoriatic skin sections with hyaluronidase increased the staining only slightly [24]. The results may be interpreted as a binding of HYA to normal endothelium blocking the ICAM-1 staining but not to the vessels in psoriatic skin.

Mucinosis

In old terminology glycosaminoglycans were incorrectly regarded as mucins and, unfortunately, the clinical terms mucinosis and mucopolysaccharidosis have been continued to be used for an accumulation of connective tissue polysaccharides. Histochemical stains, like toluidine blue and alcian blue, can be used for the diagnoses of these conditions. An increase of HYA in skin has been reported in the diffuse form termed mucinosis whilst other glycosaminoglycans are accumulated intracellularly in mucopolysaccharidoses.

Localized myxoedema

Localized myxoedema is often pretibial and considered to be an autoimmune complication of Grave's disease. Skin biopsies from the lesions show high amounts of glycosaminoglycans, especially HYA [27]. It has been suggested that a circulating IgG antibody stimulates the local skin fibroblasts to produce HYA [28].

Lichen myxoedematosus

Lichen myxoedematosus (scleromyxoedema, papular mucinosis, lichen fibromucinosis) is another rare cutaneous disease with increased HYA deposition in the papillary dermis. A benign monoclonal gammopathy is usually present and the patients show discrete lichenoid papules, which can be confluent or generalized [29]. Serum from these patients can stimulate proliferation in cultured fibroblasts [30]. Fibroblasts from the lesions produce more glycosaminoglycans and the ratio of HYA to sulphated glycosaminoglycans is increased [31]. The patients' serum enhances the glycosaminoglycan production, including HYA, in normal cultured fibroblasts, and even more when it is added to the patients' own cultured fibroblasts [32].

Reticular erythematous mucinosis

Reticular erythematous mucinosis is mainly seen in middle-aged women as a pink reticulate erythema on the sternum or upper back. The areas become infiltrated and slowly increase in size. Alcian blue staining, that disappears after hyaluronidase, favours the involvement of HYA [33]. In suction blisters from lesional skin we found an eight-fold increase of HYA compared with normal looking skin of the same patient [13].

Scleredema

Scleredema was first described by Buschke in 1901. The onset is often sudden with nonpitting wooden-like induration of the skin, usually on the posterior aspects of the upper back spreading to the face and shoulders. The ground substance often stains positive with alcian blue at pH 2.5 but not at pH 0.5 and the positive material can be removed by hyaluronidase suggesting an increase of HYA [34]. The increase of HYA has since then been confirmed [35] and fibroblasts cultured from involved skin were found to produce more HYA than cells from uninvolved skin [36].

Tumours

Basal cell carcinoma

This shows histologically a high concentration of HYA in the stromal tissue but no or little HYA around the cancer cells [37]. These results were confirmed by Tammi *et al.* [1]. Hyaluronan receptors have been connected with metastasis of tumour cells and the presence of CD44 was therefore studied by Ysaka *et al.* [38] who did not find CD44 expressed on the

tumour cells but in dendritic cells which could be melanocytes in the tumour islands. Similar results were reported by Baum *et al.* [39] although when studying splice variants of CD44, they found one, which does not bind HYA, heterogeneously distributed and especially accentuated in peripheral tumour cells. The low expression of standard CD44 could be the factor that blocks metastasis in basal cell carcinoma.

Squamous cell carcinoma

In contrast this shows strong positive staining for HYA both around the cells and in the stroma [1]. The expression of standard CD44 is strong as in normal skin [38, 40] whilst the isoform, which does not bind HYA, is down-regulated suggesting a linkage to invasive growth.

Pseudoangiosarcomatous carcinoma

This mimics angiosarcoma and is poorly differentiated with complex channels and spaces containing HYA [48]. The tumour appears to be a variant of acantholytic squamous cell carcinoma.

Melanoma

Melanoma is the tumour in which HYA and HYA-recognition has been most closely linked to malignancy. Melanomas produce HYA both in the tumour cells and in the stroma [41, 42]. *In vitro* the cells secrete factors increasing fibroblast HYA synthesis [41]. The rate of HYA synthesis in cultured murine melanoma cells correlates with the metastatic capacity of the cells [41]. CD44 is expressed on normal melanocytes both in culture and *in situ* [43] but a cell surface chondroitin sulphate proteoglycan immunologically related to CD44 was found to be involved in melanoma cell motility and invasion [44]. Migration studies of CD44 positive melanoma cell lines show a dramatic dose-dependent increase in migration rate on HYA substrate but not on chondroitin 6-sulphate [43] and soluble CD44 receptor globulin inhibits migration of CD44 positive cells. The degree of expression of CD44 on the cells correlates with migration and invasiveness on HYA substrates *in vitro*. Those CD44 isoforms which contain the v5 segment are of special interest since they have been found to be related to progression of melanoma [45].

Juvenile hyaline fibromatosis

Juvenile hyaline fibromatosis or Puretic syndrome is a hereditary disease with skin lesions starting in early childhood as papules, which later become nodules and progressively larger tumours. The patients may also display gingival and osteolytic lesions. The early lesions contain fibroblast-like cells embedded in carbohydrate material, mainly HYA [46]. In the larger lesions the matrix is mainly composed of chondroitin 4- and 6-sulphate.

Merkel cell carcinoma

This often appears as reddish-blue nodules at any body site. The prognosis is poor. The risk for metastasis seemed to be correlated to the expression of CD44 in 25 cases studied retrospectively [47]. In tumours which proved on follow-up to be restricted to a primary lesion or local recurrence at the most, none expressed CD44; whereas CD44 was found on the tumour cell membranes in three of six cases with documented metastases.

Neurofibroma

Neurofibromas of various types have been examined for glycosaminoglycan content since mucoid degeneration is often seen in these tumours [49]. HYA was high and dermatan sulphate low in plexiform neurofibromas. In two patients with neurofibromatosis both cutaneous and senile type tumours showed slightly elevated HYA [50].

References

1 Tammi R, Ågren UM, Tuhkanen A-L, Tammi M. Hyaluronan metabolism in skin. In: *Progr Histochem Cytochem* 1994; **29:** 1–77. Stuttgart, New York: Gustav Fischer Verlag, 1994.
2 Tammi R, Paukkonen K, Wang C, Horsmanheimo M, Tammi M. Hyaluronan and CD44 in psoriatic skin. Intense staining for hyaluronan on dermal capillary loops and reduced expression of CD44 and hyaluronan in keratinocyte-leukocyte interfaces. *Arch Dermatol Res* 1994; **286:** 21–9.
3 Laurent TC, Fraser JRE. Hyaluronan. *FASEB J* 1992; **6:** 2397–404.
4 Meyer LJM, Stern R. Age-dependent changes of hyaluronan in human skin. *J Invest Dermatol* 1994; **102:** 385–9.
5 Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR, Stern R. Studies in fetal wound healing: V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. *Ann Surgery* 1991; **213:** 292–8.
6 Higuchi T, Ishikawa O, Hayashi H, Ohnishi K, Miyachi Y. Disaccharide analysis of the skin glycosaminoglycans in patients with Werner's syndrome. *Clin Exp Dermatol* 1994; **19:** 487–91.

7 Nakamura T, Takagaki K, Kubo K, Saito T, Endo M, Mori S *et al.* Hyaluronate synthesized by cultured skin fibroblasts derived from patients with Werner's syndrome. *Biochim Biophys Acta* 1992; **1139:** 84–90.

8 Konohana A, Miyakawa S, Tajima S, Nishikawa T. Decreased collagen and hyaluronic acid content in lesional skin of acrogeria. *Dermatologica* 1986; **172:** 241–4.

9 Vries HJC de, Zeegelaar JE, Middelkoop E, Gijsbers G, Marle J van, Wildevuur CHR, Westerhof W. Reduced wound contraction and scar formation in punch biopsy wounds. Native collagen dermal substitutes. A clinical study. *Br J Dermatol* 1995; **132:** 690–7.

10 Borgognoni L, Reali UM, Santucci M. Low molecular weight hyaluronic acid induces angiogenesis and modulation of the cellular infiltrate in primary and secondary healing wounds. *Eur J Dermatol* 1996; **6:** 127–31.

11 Camp RL, Scheynius A, Johansson C, Puré E. CD44 is necessary for optimal contact allergic responses but is not required for normal leukocyte extravasation. *J Exp Med* 1993; **178:** 497–507.

12 Poulsen JH, Cramers MK. Dermal glycosaminoglycans characterise the primary irritant dermatitis in psoriatics and healthy individuals. *Clin Chim Acta* 1983; **130:** 305–15.

13 Juhlin L, Tengblad A, Ortonne JP, Lacour JP. Hyaluronate in suction blisters from patients with scleroderma and various skin disorders. *Acta Derm Venereol (Stockh)* 1986; **66:** 409–13.

14 Levesque H, Baudot N, Delpech B, Vayssairat M, Gancel A, Lauret PH, Courtois H. Clinical correlation and prognosis based on hyaluronic acid serum levels in patients with progressive systemic sclerosis. *Br J Derm* 1991; **124:** 423–8.

15 Akimoto S, Hayashi H, Ishikawa H. Disaccharide analysis of the skin glycosaminoglycans in systemic sclerosis. *Br J Dermatol* 1992; **126:** 29–34.

16 Higuchi T, Ohnishi K, Hayashi H, Ishikawa O, Miyachi Y. Changes in skin disaccharide components correlate with the severity of sclerotic skin in systemic sclerosis. *Acta Derm Venereol (Stockh)* 1994; **74:** 179–82.

17 Møller R, Serup J, Ammitzbøll T. Glycosaminoglycans in localized scleroderma (morphoea). *Connect Tissue Res* 1985; **13:** 227–36.

18 Engström-Laurent A, Feltelius N, Hällgren R, Wasteson A. Raised serum hyaluronan levels in scleroderma: an effect of growth factor induced elevation of connective tissue cells. *Ann Rheum Dis* 1985; **44:** 614–20.

19 Freitas JP, Filipe PI, Emerit I, Meunier P, Manso CF, Guerra Rodrigo F. Hyaluronic acid in progressive systemic sclerosis. *Dermatol* 1996; **192:** 46–9.

20 Lundin Å, Engström-Laurent A, Hällgren R, Michaëlsson G. Circulating hyaluronate in psoriasis. *Br J Dermatol* 1985; **112:** 663–71.

21 Lundin Å, Engström-Laurent A, Michaëlsson G, Tengblad A. High levels of hyaluronate in suction blister fluid from active psoriatic lesions. *Br J Dermatol* 1987; **116:** 335–40.

22 Wells AF, Lundin Å, Michaëlsson G. Histochemical localization of hyaluronan in psoriasis, allergic contact dermatitis and normal skin. *Acta Derm Venereol (Stockh)* 1991; **71:** 232–8.

23 McCourt PAG, Ek B, Forsberg N, Gustafson S. ICAM-1 is a cell-surface receptor for hyaluronan. *J Biol Chem* 1994; **269:** 30081–4.

24 Gustafson S, Wikström T, Juhlin L. Histochemical studies of hyaluronan and the hyaluronan receptor ICAM-1 in psoriasis. *Int J Tissue React* 1995; **17:** 167–73.

25 Horrocks C, Duncan JI, Oliver AM, Thomson AW. Adhesion molecule expression in psoriatic skin lesions and the influence of cyclosporin A. *Clin Exp Immunol* 1991; **84:** 157–62.

26 Thomson AW, Nalesnik MA, Rilo HR, Woo J, Carroll PB, Van Thiel DH. ICAM-1 and E-selectin expression in lesional biopsies of psoriasis patients responding to systemic FK 506 therapy. *Autoimmunity* 1993; **15:** 215–23.

27 Kriss JP. Pathogenesis and treatment of pretibial myxedema. *Endocrinol Metab Clin North Am* 1987; **16:** 409–15.

28 Shishiba Y, Imai Y, Odajima R, Ozawa Y, Shimizu T. Immunoglobulin G of patients with circumscribed pretibial myxedema of Graves' disease stimulates proteoglycan synthesis in human skin fibroblasts in culture. *Acta Endocrinol* 1992; **127:** 44–51.

29 Taylor AL, Owen ET. Scleromyxoedema associated with synovitis and myopathy. *Br J Rheumatol* 1994; **33:** 872–4.

30 Harper RA, Rispler J. Lichen myxedematosus serum stimulates human skin fibroblast proliferation. *Science* 1978; **199:** 545–7.

31 Turakainen H, Välimäki M, Penttinen R. Synthesis of glycosaminoglycans and collagen in skin fibroblasts cultured from a patient with lichen myxedematosus. *Arch Dermatol Res* 1985; **277:** 55–9.

32 Pandya AG, Sontheimer RD, Cockerel CJ, Takashima A, Piepkorn M. Papulonodular mucinosis associated with systemic lupus erythematosus: possible mechanisms of increased glycosaminoglycan accumulation. *J Am Acad Dermatol* 1995; **32:** 199–205.

33 Braddock SW, Davis CS, Davis RB. Reticular erythematous mucinosis and thrombocytopenic purpura. Report of a case and review of the world literature, including plaque-like cutaneous mucinosis. *J Am Acad Dermatol* 1988; **19:** 859–68.

34 Fleischmajer R, Lara JV. Scleredema. A histochemical and biochemical study. *Arch Dermatol* 1965; **92:** 643–52.

35 Roupe G, Laurent TC, Malmström A, Suurküla M, Särnstrand B. Biochemical characterization and tissue distribution of the scleredema in a case of Buschke's disease. *Acta Derm Venereol (Stockh)* 1987; **67:** 193–8.

36 Christy W, Buckingham RB, Barnes EL, Prince RK. Scleredema adultorum of Buschke: a clinical, pathologic, and cell culture study of two patients. *J Rheumatol* 1983; **10:** 595–601.

37 Wells AF, Lundin Å, Michaëlsson G, Pontén F. Hyaluronan in basal cell carcinomas. *Acta Derm Venereol (Stockh)* 1991; **71:** 274–5.

38 Yasaka N, Furue M, Tamaki K. CD44 expression in normal human skin and skin tumours. *J Dermatol* 1995; **22:** 88–94.

39 Baum H-P, Schmid T, Schock G, Reichrath J. Expression of CD44 isoforms in basal cell carcinomas. *Br J Dermatol* 1996; **134:** 465–8.

40 Salmi M, Grön-Virta K, Sointu P, Grenman R, Kalimo H, Jalkanen S. Regulated expression of exon v6 containing isoforms of CD44 in man: Down regulation during malignant transformation of tumours of squamocellular origin. *J Cell Biol* 1993; **122:** 431–42.

41 Turley EA, Tretiak M. Glycosaminoglycan production by murine melanoma variants in vivo and in vitro. *Cancer Res* 1985; **45:** 5098–105.

42 Edward M, Grant AW, Mackie RM. Human melanoma cell-derived factor(s) stimulate fibroblast glycosaminoglycan synthesis. *Int J Cancer* 1992; **52:** 499–503.

43 Thomas L, Etoh T, Stamenkovic I, Mihm MC, Byers HR.

Migration of humn melanoma cells on hyaluronate is related to CD44 expression. *J Invest Dermatol* 1993; **100:** 115–20.
44 Faassen AE, Schrager JA, Klein DJ, Oegema TR, Couchman JR, McCarthy JB. A cell surface chondroitin sulfate proteoglycan, immunologically related to CD44, is involved in type-I collagen-mediated melanoma cell motility and invasion. *J Cell Biol* **1992;** 116: 521–31.
45 Manten-Horst E, Danen EH, Smit L, Snoek M, Le Poole IC, van Muijen GN *et al.* Expression of CD44 splice variants in human cutaneous melanoma and melanoma cell lines is related to tumour progression and metastatic potential. *Int J Cancer* 1995; **64:** 182–8.
46 Mayer-da-Silva A, Poiares-Baptista A, Guerra Rodrigo F, Teresa-Lopes M. Juvenile hyaline fibromatosis. A histologic and histochemical study. *Arch Pathol Lab Med* 1988; **112:** 928–31.
47 Penneys NS, Shapiro S. CD44 expression in Merkel cell carcinoma may correlate with risk of metastasis. *J Cutan Pathol* 1994; **21:** 22–6.
48 Banerjee SS, Eyden BP, Wells S, McWilliam LJ, Harris M. Pseudoangiosarcomatous carcinoma: a clinicopathological study of seven cases. *Histopathology* 1992; **21:** 13–23.
49 Sasaki T, Onodera S. Glycosaminoglycans in neurofibromas. *J Dermatol* 1990; **17:** 653–60.
50 Martelli H, Richard S, Moczar M, Godeau G, Pfister A, Pellerin D, Vekemans M. Congenital soft tissue dysplasias: a morphological and biochemical study. *Ped Pathol* 1994; **14:** 873–94.

Received 16 January 1997; accepted 13 February 1997.

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